Sigma® Life Science

Cell Culture Manual

2011–2014

Biointegrity.

Sigma® Life Science has been a trusted supplier of reliable, high-quality media, sera and reagents for more than 25 years.

Cultivating healthy cell growth means depending on a quality mix of media, sera and reagents. That's why we offer an unparalleled line of cell culture solutions. From classical and specialty media, to fetal bovine serum, supplements and reagents, our offering is extensive and comprehensive — exactly what you would expect from a leader in research and technology.

For more information, visit

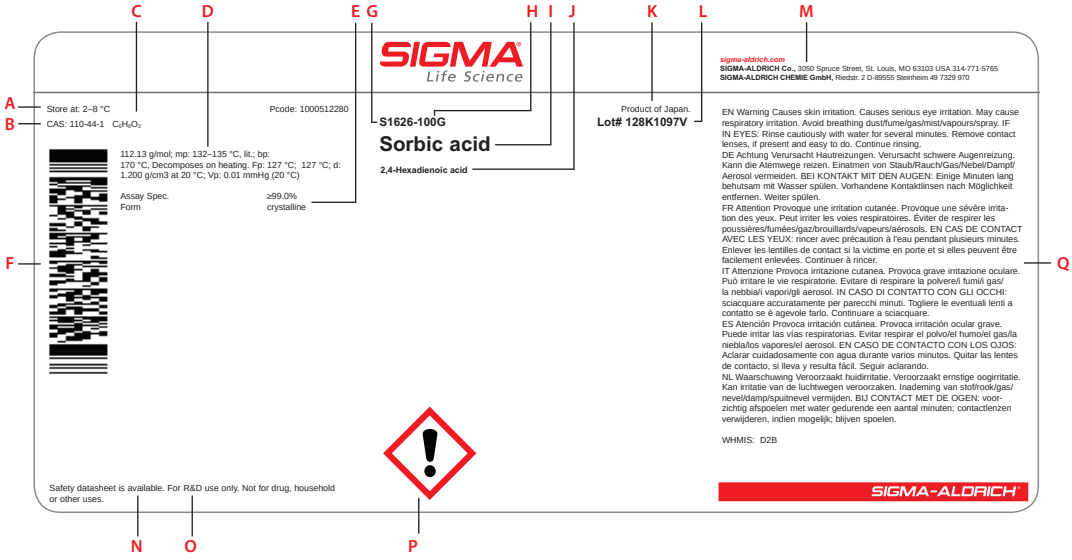
sigma.com/cellculture

Key to Product Labels

Sigma-Aldrich® labels provide the following information:

- Complete product name and description
- Hazard information
- Lot-specific analytical data on many types of products
- Pictograms for instant hazard recognition
- Useful data for reference, Chemical Abstracts Service (CAS®) registry number and chemical formula

For more information on understanding Sigma-Aldrich labels, visit sigma-aldrich.com/safetycenter. For product-specific hazard information, see the Catalog Number/Hazard Information Index.



Legend

A Recommendations on Handling and Storage
Storage temperatures indicated are for long-term storage of products. Products may be shipped under different conditions to reduce shipping costs, while still ensuring product quality.

B Chemical Abstract Service Registry Number (CAS® RN)
For a solution or suspension, the CAS® RN provided is for the principal component.

C Chemical Formula

D Physical Properties

E Specification(s)

F Barcode

G Catalog Number

H Package Size
Unless this material is described as pre-weighed, the package will normally contain at least the indicated quantity, usually somewhat more. For some products, the actual quantity at time of packaging is also shown. The user should always measure the amount needed from the container.

I Product Name

J Synonym(s)

K Country of Origin

L Lot Number

M Sigma-Aldrich Contact Address

N Material Safety Data Sheet (MSDS) Available
A MSDS is available for this product.

O Disclaimer

P Globally Harmonized System (GHS) Pictograms
Visual reference to safety hazards involved in the use of this product.

Q Hazard and Precautionary Codes
Descriptions of actual hazards, handling precautions, and emergency management procedures.

Globally Harmonized System (GHS) Pictograms

For more information on GHS regulatory information, visit sigma-aldrich.com/ghs

Select Products With the Quality Grade that Fits Your Research

Sigma® Life Science's simple grading system ensures you efficiently select the most appropriate and accurate product to support your research needs.

The well-defined and convenient grading system classifies life science products into three grades:

- BioUltra
- BioXtra
- BioReagent

Sigma Life Science has stringent quality standards for all products and while many products are not graded using this system, they are still appropriate for life science research. See individual product specifications to determine suitability for your specific application.

Contact your local Sigma Life Science office or sigma.com/techinfo for technical assistance or use our convenient, web-based Product Comparison feature.

Sigma Life Science Grading System

BioUltra	Finest quality products for life science analyzed using the most stringent testing protocols to ensure the highest purity. Superior manufacturing methods and product-specific testing protocols are designed to ensure product quality to our highest standard. Tests may include, but are not limited to purity by gel electrophoresis, trace metal testing and application testing.
BioXtra	Stringent testing protocols may include trace metal analysis and specialized suitability testing such as hybridoma or mouse embryo. Includes application-specific formulations such as tablets and prepared solutions.
BioReagent	Base-grade products, suitability-tested for specific applications such as molecular biology or cell culture.

Opening a Sigma-Aldrich® Account

When you are ready to place your order, call our Order/Customer Service number and supply the Sigma-Aldrich service representative with your shipping and billing information. The Sigma-Aldrich service representative will ask you some questions about your general business, along with taking the products for your order. Our New Account Department will then verify this information, as well as validate your intended use of our products. You may be contacted for further clarification; however, we will take quick and suitable action to establish your new account. To further expedite our process, please have a credit card available to prepay your first order. For information regarding the appropriate uses of Sigma-Aldrich products, please read the following.

Use of Products

Sigma-Aldrich's products are intended primarily for laboratory research purposes and, unless otherwise stated on product labels, in Sigma-Aldrich's catalogs, or in other literature furnished to Buyer, are not to be used for any other purposes, including but not limited to, *in vitro* diagnostic purposes, in foods, drugs, medical devices, or cosmetics for humans or animals, or for commercial purposes.

Buyer acknowledges that the products have not been tested by Sigma-Aldrich for safety and efficacy in food, drug, medical devices, cosmetic, commercial or any other use, unless otherwise stated in Sigma-Aldrich's literature furnished to Buyer. Buyer expressly represents and warrants to Sigma-Aldrich that Buyer will properly test, use, manufacture, and market any products purchased from Sigma-Aldrich and/or materials produced with products purchased from Sigma-Aldrich in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with all applicable laws and regulations, now and hereinafter enacted. Buyer further warrants to Sigma-Aldrich that any material produced with products from Sigma-Aldrich shall not be adulterated or misbranded within the meaning of the Federal Food, Drug and Cosmetic Act and shall not be materials which may not, under Sections 404, 505, or 512 of the Act, be introduced into interstate commerce.

Buyer realizes that, since Sigma-Aldrich's products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. Buyer assumes responsibility to assure that the products purchased from Sigma-Aldrich are approved for use under TSCA, if applicable.

Buyer has the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from Sigma-Aldrich. Buyer also has the duty to warn Buyer's customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

Buyer agrees to comply with instructions, if any, furnished by Sigma-Aldrich relating to the use of the products and not misuse the products in any manner. If the products purchased from Sigma-Aldrich are to be repackaged, relabeled or used as starting material or components of other products, Buyer will verify Sigma-Aldrich's assay of the products. No products purchased from Sigma-Aldrich shall, unless otherwise stated, be considered to be foods, drugs, medical devices, or cosmetics.

Pricing

Prices are subject to change. Please visit us at sigma-aldrich.com for current prices. We guarantee our written quotations for 90 days. When placing your order, please reference our quoted prices or our pro forma number. If you place your order by phone, we will confirm our current price at that time.

Price Changes

Shipment will be made promptly even if prices have been nominally increased. Price reductions will be automatically applied to your invoice.

Warranties and Disclaimers

Sigma-Aldrich® warrants that its products shall conform to the description of such products as provided by Sigma-Aldrich through Sigma-Aldrich's catalog, analytical data, or other literature. **THIS WARRANTY IS EXCLUSIVE, AND SIGMA-ALDRICH MAKES NO OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.**

Sigma-Aldrich's warranties made in connection with any sale shall not be effective if Sigma-Aldrich has determined, in its sole discretion, that Buyer has misused the products in any manner, has failed to use the products in accordance with industry standards and practices, or has failed to use the products in accordance with instructions, if any, furnished by Sigma-Aldrich.

Sigma-Aldrich's sole and exclusive liability and Buyer's exclusive remedy with respect to products proved to Sigma-Aldrich's satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, at Sigma-Aldrich's sole discretion, upon the return of such products in accordance with Sigma-Aldrich's instructions. **SIGMA-ALDRICH SHALL NOT IN ANY EVENT BE LIABLE FOR INCIDENTAL, CONSEQUENTIAL OR SPECIAL DAMAGES OF ANY KIND RESULTING FROM ANY USE OR FAILURE OF THE PRODUCTS, EVEN IF SIGMA-ALDRICH HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGE INCLUDING, WITHOUT LIMITATION, LIABILITY FOR LOSS OF USE, LOSS OF WORK IN PROGRESS, DOWN TIME, LOSS OF REVENUE OR PROFITS, FAILURE TO REALIZE SAVINGS, LOSS OF PRODUCTS OF BUYER OR OTHER USE OR ANY LIABILITY OF BUYER TO A THIRD PARTY ON ACCOUNT OF SUCH LOSS, OR FOR ANY LABOR OR ANY OTHER EXPENSE, DAMAGE OR LOSS OCCASIONED BY SUCH PRODUCT INCLUDING PERSONAL INJURY OR PROPERTY DAMAGE UNLESS SUCH PERSONAL INJURY OR PROPERTY DAMAGE IS CAUSED BY SIGMA-ALDRICH'S GROSS NEGLIGENCE.** All claims must be brought within one (1) year of shipment, regardless of their nature.

For more information about Sigma-Aldrich's terms and conditions of sale, you should review our invoices and packing slips, or you may obtain a copy of our terms and conditions by contacting our Customer Service Department.

Return Policy

Our Customer Service Department is available to assist you should a problem arise with your order. Please inspect your packages immediately upon receipt and notify us promptly of any damage or discrepancies. Should an item be shipped to you incorrectly, as the result of an error on our part, we will take quick and appropriate action to correct the problem.

Prior to returning any items, please contact the Customer Service Department to obtain a Return Material Authorization (RMA) and shipping instructions. A return authorization will ensure the safe and proper handling of material and enable us to expedite a resolution. Items returned without prior authorization may not be accepted. Shipment of authorized returns should be made within 30 days of the issuance of the RMA.

We will do our best to fulfill requests to return material. However, in order to maintain the quality of our products and continue to provide competitive prices, certain items may not be returned for credit. These items include: diagnostic reagents, refrigerated or frozen products; reagents and standards which have passed their expiration dates; custom products or special orders; products missing labels, parts, or instruction manuals; and books, computer software and equipment removed from their original packaging. Returns accepted for items ordered in error may be subject to a 20% processing fee.

Domestic Shipping Information

All orders are shipped FCA shipping point. Transportation charges will vary with the destination, weight, and content of each shipment. Special packaging, such as poison packs or metal over-pack cans, may be necessary for safe delivery of certain hazardous chemicals. All special packaging, administrative, and freight charges are available upon request at order entry and are indicated on our invoices.

We reserve the right to change a requested method of shipment if it does not comply with regulations or, in our judgment, will not deliver a product safely.

Any transportation and handling charges quoted or invoiced include charges in addition to actual freight costs. For collect shipments, please include your account number with your preferred carrier to ensure proper billing.

Backordered items are shipped and invoiced separately as they become available.

International Shipping Information

Numerous restrictions and regulations govern the international transport of chemical products. We strive to ship all orders without delay and to minimize delivery costs within these regulatory guidelines.

All orders prepared for shipment outside the contiguous USA are FCA shipping point. The volume of our international shipments results in very competitive rates from our freight forwarders. For orders shipped through our designated carriers, we pay the transportation and handling costs in advance. These costs are added to your invoice. You can choose other forwarders to handle your orders; however, such orders are delivered on a collect basis. Please note, we will only ship our products through an IATA-approved freight forwarder.

Hazardous items and items that require ice for shipment incur additional charges; a ◆ identifies these products. They are commonly shipped via air freight. In general, hazardous materials cannot be included with wet or dry ice air freight shipments. Orders containing hazardous and wet or dry ice items will incur two separate air freight charges. Whenever possible, Sigma-Aldrich consolidates air freight to minimize shipping costs. In some instances, Customs and other regulations may prevent us from doing this in your country.

Backordered items are shipped FCA shipping point when they become available, unless alternative instructions are provided when the original order is placed. To minimize freight costs, backorders can be consolidated with future shipments.

You can help us process your orders quickly and efficiently in a variety of ways:

- Include a contact name, telephone number, and fax number on your purchase order. If you use a broker or agent to clear your shipments through Customs, include the broker's name, telephone number, and fax number as well.
- Consolidate your orders. Minimize the number of shipments by consolidating the products that require air freight shipment.
- Consolidate orders from different departments within your institution. We can bill individual departments separately, if necessary. Please instruct us accordingly when placing your order.
- Confirm your acceptance of air freight costs on your order.
- For collect shipments, include your account number with your designated carrier.
- Place orders through your nearest Sigma-Aldrich office.
- Submit orders once to avoid duplication. If you place orders by mail, fax, or email, it is unnecessary to send a confirmation copy. Our service representatives are happy to assist you with the status of your order.

Wet Ice/Dry Ice Shipping Information

Recommended long-term storage is indicated on our website as follows:

2–8°C Refrigerator/cooler

–70°C Ultracold freezer at or below –70 °C

–20°C Freezer

–196°C Under liquid nitrogen

Not all items requiring long-term cold storage need to be shipped in ice. Shipping conditions may differ from our recommended storage temperatures. We will ship under conditions that ensure the quality of the product. We try to estimate the amount of dry or wet ice needed, but we cannot predict time lost in Customs offices or in route. The customer is responsible for prompt Customs clearance. If you cannot ensure prompt Customs clearance, be sure to order additional ice and specify which airport you prefer.

To help prevent delays in Customs, please include name and telephone or fax number of person to contact upon arrival.

Label Licenses for Biotechnology

ZFN License Agreement

This Product and its use are the subject of one or more of the following patents controlled by Sangamo BioSciences, Inc.: U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, 6,479,626, US20030232410, US20090203140 and corresponding foreign patent applications and patents.

For the complete ZFN license agreement, visit http://www.sigmaaldrich.com/life-science/zinc-finger-nuclease-technology/zfn-license-agreement.html

PCR License Agreement

1) Roche Enzyme Products Conveying Polymerase Patent Rights (Research Field)

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following U.S. patents and corresponding patent claims outside the U.S.: 5,789,224, 5,618,711, 6,127,155 and claims outside the U.S. corresponding to expired U.S. Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following U.S. patents and corresponding patent claims outside the U.S.: 5,789,224, 5,618,711, 6,127,155, 5,407,800, 5,322,770, and 5,310,652. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

4) Licensed dsDNA-Binding Dye Research Kits (Research Field)

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6) Authorized 5′ Nuclease Core Kit (i.e., without Licensed Probe) (Research Field)

NOTICE TO PURCHASER: LIMITED LICENSE

A license to perform the patented 5′ Nuclease Process for research is obtained by the purchase of (i) both Authorized 5′ Nuclease Core Kit and Licensed Probe, (ii) a Licensed 5′ Nuclease Kit, or (iii) license rights from Applied Biosystems. This product is an Authorized 5′ Nuclease Core Kit. Use of this product is covered by one or more of the following claims outside the U.S. corresponding to expired U.S. Patent Nos. 5,210,015 and 5,487,972. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. Separate purchase of a Licensed Probe would convey rights under U.S. Patents and corresponding patent claims outside the U.S.: 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569, 5,804,375 (claims 1-12 only), and claims outside the United States corresponding to U.S. Patent No. 6,214,979. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

11) For Products Subject to Section 2.17(d) That Could Be Confused with Licensed Royalty-Bearing Products and That Do Not Convey Necessary Patent Rights for the 5′ Nuclease Process and/or dsDNA-Binding Dye Process

NOTICE TO PURCHASER: DISCLAIMER OF LICENSE

No license is conveyed with the purchase of this product under any of U.S. Patents Nos., 5,804,375, 5,994,056, 6,171,785, 5,538,848, 5,723,591, 5,876,930, 6,030,787, and 6,258,569, and corresponding patents outside the United States, or any other patents or patent applications, relating to the 5′ Nuclease and dsDNA-Binding Dye Processes. For further information contact the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Cell Culture Essentials

Cell Culture Essentials

Abbreviations and Terminology

Sigma® Research Reagents are furnished **For Laboratory Research Use Only**.

They may not be used as Drugs, Cosmetics, Agriculture, or Pesticidal Products, Food Additives, or Household Chemicals.

Many products in this publication are expiration dated. We are not responsible for products whose expiration date has passed.

Sigma warrants that its products conform to the specifications cited in Sigma literature. Should a product fail to conform to its specifications, Sigma may choose to replace it at no charge or refund the purchase price. For more information about terms and conditions of sales please visit our Web site at sigma-aldrich.com.

Abbreviations	Terminology
ACF	Animal Component Free
BSA	Bovine Serum Albumin
BSE	Bovine Spongiform Encepalopathy
BVDV	Bovine Viral Diarrhea Virus
CD	Chemically Defined
DMF	Device/Drug Master File
EC	European Community
ECACC	European Collection of Cell Cultures
FBS	Fetal Bovine Serum
FCS	Fetal Calf Serum
FDA	Food and Drug Administration
HEPA	High Efficiency Particulate Air
IVD	*In Vitro* Diagnostics
PF	Protein Free
PPE	Personal Protective Equipment
SF	Serum Free
USDA	United States Department of Agriculture

Products Designated "For Manufacturing Use"

Comply with recognized compendia and are anticipated to be ready for use as an active drug entity, a recognized nutrient, a pharmaceutical ingredient, or component of a finished medical device. Prepared under cGMP conditions.

Certificates of Suitability

Sigma-Aldrich has the expertise available to obtain Certificates of Suitability granted by the Certification Secretariat of the European Directorate for the Quality of Medicines (EDQM) for products with the risk of transmitting agents of animal spongiform encephalopathies. Products are assessed upon request to ensure that appropriate controls exist to obtain these certificates. Sigma-Aldrich currently holds 18 Certificates of Suitability covering 33 products.

USP/EP/JP Grade Products

Tested according to Pharmacopeia criteria:

- USP – United States of America
- EP – European
- JP – Japanese

BPC Products – BioPerformance Certified Products

Products that have been tested and have met specifications for a number of applications. Testing performed on these products varies according to product applications but can include cell culture and molecular biology performance assays, ACS, and Pharmacopeia test monographs.

Drug or Device Master Files

DMFs are available on select products. For product-specific inquiries, contact technical support at sigma-aldrich.com/techinfo.

Cell Culture Quick Reference

This table contains the cell culture products that you use every day. It can be used as a quick-reference guide or as a shopping list for regularly purchased items.

Section	Name	Page	Cat. No.
Serum	Fetal Bovine Serum, USA origin, sterile-filtered, suitable for cell culture, suitable for hybridoma	*107*	F2442
	Fetal Bovine Serum, Heat Inactivated, USA origin, sterile-filtered, suitable for cell culture	*107*	F4135
	Fetal Bovine Serum, USDA approved, non-USA origin, sterile-filtered, suitable for cell culture	*108*	F0926
	Fetal Bovine Serum, USDA approved, non-USA origin, Heat Inactivated, sterile-filtered, suitable for cell culture	*108*	12306C
	Fetal Bovine Serum, Non-USA origin, sterile-filtered, suitable for cell culture	*108*	F7524
	Fetal Bovine Serum, Heat Inactivated, Non-USA origin, sterile-filtered, suitable for cell culture	*108*	F9665
Amino Acids	L-Glutamine solution, 200 mM, solution, sterile-filtered, suitable for cell culture	*129*	G7513
	MEM Non-essential Amino Acid Solution (100×), liquid, without L-glutamine, sterile-filtered, suitable for cell culture	*131*	M7145
Antibiotics	Amphotericin B solution, sterile-filtered, 250 µg/mL in deionized water, suitable for cell culture	*138*	A2942
	Amphotericin B solubilized, powder, γ-irradiated, suitable for cell culture	*138*	A9528
	Ampicillin sodium salt, suitable for cell culture, 91.0–100.5% (titration), powder	*138*	A0166
	Antibiotic-Antimycotic Stabilized, suspension, stabilized, sterile-filtered, suitable for cell culture	*139*	A5955
	G418 disulfate salt solution, 50 mg/mL solution, sterile-filtered, suitable for cell culture	*140*	G8168
	Gelatin solution, 2% in water, tissue culture grade, sterile, Type B, suitable for cell culture	*148*	G1393
	Gentamicin solution, 50 mg/mL in deionized water, liquid, sterile-filtered, suitable for cell culture	*140*	G1397
	Gentamicin solution, 10 mg/mL in deionized water, liquid, sterile-filtered, suitable for cell culture	*140*	G1272
	Hygromycin B from *Streptomyces hygroscopicus*, powder, suitable for cell culture, suitable for insect cell culture	*141*	H3274
	Mitomycin C from *Streptomyces caespitosus*, powder, suitable for cell culture	*142*	M4287
	Neomycin solution, liquid, sterile-filtered, suitable for cell culture	*143*	N1142
	Nystatin preparation, suspension, sterile; aseptically processed, suitable for cell culture	*144*	N1638
	Penicillin-Streptomycin, 100×, liquid, sterile-filtered, suitable for cell culture	*144*	P0781
	Penicillin-Streptomycin, 50×, liquid, stabilized, sterile-filtered, suitable for cell culture	*144*	P4458
	Penicillin-Streptomycin, 100×, liquid, stabilized, sterile-filtered, suitable for cell culture	*144*	P4333
	Puromycin dihydrochloride from *Streptomyces alboniger*, powder, ≥98% (TLC), suitable for cell culture	*145*	P8833
Attachment Factors	Fibronectin from bovine plasma, solution, 1 mg/mL (in 0.5 M NaCl, 0.05 M Tris, pH 7.5), suitable for cell culture, sterile	*147*	F1141
	Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane, 1 mg/mL in Tris buffered NaCl, sterile-filtered, suitable for cell culture	*148*	L2020
	Poly-D-lysine hydrobromide, average mol wt 30,000-70,000, γ-irradiated, lyophilized powder, suitable for cell culture	*148*	P7280
	Poly-D-lysine hydrobromide, mol wt 70,000-150,000, γ-irradiated, lyophilized powder, suitable for cell culture	*148*	P6407
	Poly-D-lysine hydrobromide, mol wt >300,000, γ-irradiated, lyophilized powder, suitable for cell culture	*148*	P7405
	Poly-L-lysine hydrobromide, mol wt 70,000-150,000, γ-irradiated, lyophilized powder, suitable for cell culture	*149*	P6282
	Poly-L-lysine solution, 0.01%, mol wt 70,000-150,000, sterile-filtered, suitable for cell culture	*149*	P4707
	Poly-L-lysine solution, 0.01%, mol wt 150,000-300,000, sterile-filtered, suitable for cell culture	*149*	P4832

Cell Culture Quick Reference

Section	Name	Page	Cat. No.
Balanced Salts	Dulbecco's Phosphate Buffered Saline, With $MgCl_2$ and $CaCl_2$, liquid, sterile-filtered, suitable for cell culture	*116*	D8662
	Dulbecco's Phosphate Buffered Saline, Modified, without calcium chloride and magnesium chloride, sterile-filtered, liquid, suitable for cell culture	*117*	D8537
	Dulbecco's Phosphate Buffered Saline, Without calcium chloride, powder, suitable for cell culture	*116*	D5773
	Dulbecco's Phosphate Buffered Saline, Modified, Without calcium chloride and magnesium chloride, powder, suitable for cell culture	*116*	D5652
	Dulbecco's Phosphate Buffered Saline 10×, Modified, without calcium chloride and magnesium chloride, liquid, sterile-filtered, suitable for cell culture	*116*	D1408
	Dulbecco's Phosphate Buffered Saline, Modified, with 1,000 mg/L glucose, sodium pyruvate, streptomycin sulfate, kanamycin monosulfate, $CaCl_2$, $MgCl_2$, liquid, sterile-filtered, suitable for cell culture	*116*	D4031
	Earle's Balanced Salts, With sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture	*117*	E2888
	Hanks' Balanced Salt solution, Modified, with sodium bicarbonate, without phenol red, calcium chloride and magnesium sulfate, liquid, sterile-filtered, suitable for cell culture	*117*	H6648
	Hanks' Balanced Salt solution, Modified, with sodium bicarbonate, without phenol red, liquid, sterile-filtered, suitable for cell culture	*117*	H8264
	Hanks' Balanced Salt solution, With sodium bicarbonate, sterile-filtered, suitable for cell culture	*118*	H9269
	Hanks' Balanced Salt solution, Modified, with sodium bicarbonate, without calcium chloride and magnesium sulfate, liquid, sterile-filtered, suitable for cell culture	*118*	H9394
	Krebs-Henseleit Buffer Modified, With 2000 mg/L glucose, without calcium chloride and sodium bicarbonate, powder, suitable for cell culture	*119*	K3753
Biochemicals	Heparin sodium salt from porcine intestinal mucosa, Grade I-A, suitable for cell culture, activity: ≥140 USP units/mg, powder	*245*	H3149
Biological Buffers	HEPES solution, 1 M, pH 7.0-7.6, sterile-filtered, suitable for cell culture	*160*	H0887
	Sodium bicarbonate, powder, ≥99.5%, suitable for cell culture, suitable for insect cell culture	*161*	S5761
	Sodium bicarbonate solution, solution (7.5%), sterile-filtered, suitable for cell culture	*161*	S8761
	Sodium chloride, solution (0.9%), sterile-filtered, suitable for cell culture	*234*	S8776
Cell Dissociation	Cell Dissociation Solution Non-enzymatic 1×, Prepared in Hanks' Balanced Salt Solution without calcium and magnesium, sterile-filtered, suitable for cell culture	*164*	C5789
	Cell Dissociation Solution Non-enzymatic 1×, Prepared in phosphate buffered saline without calcium and magnesium, sterile-filtered, suitable for cell culture	*164*	C5914
	Trypsin-EDTA solution, 0.25%, 2.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red, sterile-filtered, suitable for cell culture	*169*	T4049
	Trypsin-EDTA solution, 1×, 0.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red., sterile-filtered, suitable for cell culture	*169*	T3924
	Trypsin-EDTA solution, 10×, 5.0 g porcine trypsin and 2 g EDTA • 4Na per liter of 0.9% sodium chloride, sterile-filtered, suitable for cell culture	*169*	T4174
Growth Factors	Endothelial cell growth supplement from bovine neural tissue, suitable for cell culture	*191*	E2759
	Epidermal Growth Factor human, recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture	*189*	E9644
	Fibroblast Growth Factor-Basic human, recombinant, expressed in *Escherichia coli*, suitable for cell culture	*192*	F0291
	Insulin solution from bovine pancreas, 10 mg/mL insulin in 25 mM HEPES, pH 8.2, sterile-filtered, suitable for cell culture	*221*	I0516
Hormones	Gonadotropin from pregnant mare serum, lyophilized powder, suitable for mouse embryo cell culture	*233*	G4527
	Insulin from bovine pancreas, powder, suitable for cell culture	*22*	I6634
	Insulin from bovine pancreas, lyophilized powder, activity: ≥25 USP units/mg, secondary activity: 2500Â units (HPLC, /100 mg vial), γ-irradiated, suitable for cell culture	*22*	I1882
	Insulin solution human, Chemically defined, recombinant from *Saccharomyces cerevisiae*, solution, sterile-filtered, suitable for cell culture	*221*	I9278
Hormones & Misc. Reagents	ITS Liquid Media Supplement (100×), liquid, sterile-filtered, suitable for cell culture	*229*	I3146
	OPI Media Supplement, Hybri-Max™, γ-irradiated, lyophilized powder, suitable for hybridoma	*24*	O5003
	Red Blood Cell Lysing Buffer Hybri-Max™, liquid, sterile-filtered, suitable for hybridoma	*227*	R7757

Section	Name	Page	Cat. No.
Hybridoma	HAT Media Supplement (50×) Hybri-Max™, lyophilized powder, γ-irradiated, suitable for hybridoma	*226*	H0262
	Histopaque®-1077 Hybri-Max™, liquid, sterile-filtered, suitable for hybridoma	*180*	H8889
Lectins	Concanavalin A from *Canavalia ensiformis* (Jack bean), Type IV-S, lyophilized powder, aseptically processed, suitable for cell culture	*242*	C5275
Lipopolysaccharides	Lipopolysaccharides from *Escherichia coli* 0111:B4, suitable for cell culture, γ-irradiated	*250*	L4391
	Lipopolysaccharides from *Escherichia coli* 055:B5, suitable for cell culture, γ-irradiated	*250*	L6529
	Lipopolysaccharides from *Escherichia coli* 026:B6, suitable for cell culture, γ-irradiated	*250*	L2654
Media—See Media Selection Quick Reference Table on page 56	Dulbecco's Modified Eagle's Medium – high glucose, With 4500 mg/L glucose, L-glutamine, and sodium bicarbonate, without sodium pyruvate, liquid, sterile-filtered, suitable for cell culture	*59*	D5796
	Dulbecco's Modified Eagle's Medium – high glucose, With 4500 mg/L glucose and sodium bicarbonate, without L-glutamine and sodium pyruvate, liquid, sterile-filtered, suitable for cell culture, suitable for hybridoma	*59*	D5671
	Dulbecco's Modified Eagle's Medium – high glucose, With 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture	*59*	D6429
	Dulbecco's Modified Eagle's Medium – high glucose, With 4500 mg/L glucose, sodium pyruvate, and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*59*	D6546
	Dulbecco's Modified Eagle's Medium – high glucose, With 4500 mg/L glucose and sodium bicarbonate, without L-glutamine, sodium pyruvate, and phenol red, liquid, sterile-filtered, suitable for cell culture	*59*	D1145
	Dulbecco's Modified Eagle's Medium – high glucose, With 4500 mg/L glucose and L-glutamine, without sodium bicarbonate. Use at 13.4 g/L., powder, suitable for cell culture	*60*	D5648
	Dulbecco's Modified Eagle's Medium - low glucose, With 1000 mg/L glucose, L-glutamine, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture	*60*	D6046
	Dulbecco's Modified Eagle's Medium - low glucose, With 1000 mg/L glucose, and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*60*	D5546
	Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham, With 15 mM HEPES and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*61*	D6421
	Glasgow Minimum Essential Medium, With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*63*	G5154
	Iscove's Modified Dulbecco's Medium, With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture, suitable for hybridoma	*63*	I3390
	L-15 Medium (Leibovitz), Without L-glutamine., liquid, sterile-filtered, suitable for cell culture	*63*	L5520
	Medium 199, With Earle's salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture	*64*	M4530
	Minimum Essential Medium Eagle, HEPES Modification, with Earle's salts, 25 mM HEPES and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*68*	M7278
	Minimum Essential Medium Eagle, Joklik Modification, With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*68*	M8028
	Minimum Essential Medium Eagle, Joklik Modification, with L-glutamine, without calcium chloride and sodium bicarbonate, powder, suitable for cell culture	*68*	M0518
	Minimum Essential Medium Eagle, With Earle's salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture	*66*	M4655
	Minimum Essential Medium Eagle, With Earle's salts and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*66*	M2279
	Minimum Essential Medium Eagle, With Earle's salts, non-essential amino acids and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*66*	M5650
	Nutrient Mixture F-10 Ham, With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*69*	N6013
	RPMI-1640 Medium, With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture	*70*	R8758
	RPMI-1640 Medium, HEPES Modification, With 25 mM HEPES, without L-glutamine., liquid, sterile-filtered, suitable for cell culture	*70*	R5886
	RPMI-1640 Medium, With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture	*70*	R0883
	RPMI-1640 Medium, Modified, with sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture	*71*	R7509

Cell Culture Quick Reference

Section	Name	Page	Cat. No.
Miscellaneous Reagents	Sodium hydroxide solution, 1.0 N, suitable for cell culture	*254*	S2770
	Sodium pyruvate solution, 100 mM, sterile-filtered, suitable for cell culture	*24*	S8636
	Water, sterile-filtered, suitable for cell culture	*256*	W3500
Reagents	Albumin from bovine serum, powder, Fatty acid free, suitable for cell culture, ≥96% (agarose gel electrophoresis)	*124*	A8806
	Cell Freezing Medium-DMSO 1×, sterile-filtered, suitable for cell culture	*174*	C6164
Reagents & Biochemicals	Linoleic Acid-Albumin from bovine serum albumin, liquid, sterile-filtered, suitable for cell culture	*23*	L9530
Reagents & Hybridoma	Dimethyl sulfoxide, liquid (temperature dependent), ≥99.7%, Hybri-Max™, sterile-filtered, suitable for hybridoma	*175*	D2650
	Dimethyl sulfoxide, Biotechnology Performance Certified, sterile-filtered, meets EP, USP testing specifications suitable for hybridoma	*175*	D2438
Stains, Dyes, & Indicators	Thiazolyl Blue Tetrazolium Bromide, powder, suitable for cell culture, suitable for insect cell culture	*183*	M5655
	Trypan Blue solution, 0.4%, liquid, sterile-filtered, suitable for cell culture	*184*	T8154
Supplements	D-(+)-Glucose solution, 45% in water, tissue culture grade, autoclaved, suitable for cell culture	*244*	G8769

Serum-Free and Specialty Media

Serum-Free and Specialty Media

CHO Platform

Chinese Hamster Ovary (CHO) cells are of great interest for bioprocessing and pharmaceutical research and development. These cells are robust in culture and are able to produce a variety of recombinant glycoproteins at high levels on a large scale. However, different CHO cell clones often possess diverse nutritional requirements that are unique to each clone. As a result, medium optimization for CHO cells can be very challenging, often requiring the development of a custom medium for each particular clone.

The traditional approach to media development involves titrating each medium component individually to determine the optimal level of supplementation. This process involves extensive testing conditions and can take significant amounts of time. To address this, Sigma-Aldrich has developed a number of tools that allow you to develop optimized media formulations for your specific CHO clones faster and easier than ever before.

CHO Media

EX-CELL® 302 Serum-Free Medium for CHO Cells

for research or for further manufacturing use

suitable for cell culture

sterile-filtered

Do not freeze. Protect from light. Several components of the medium are light labile and should not be exposed to light for lengthy periods of time.

pH 7.1-7.7

without L-glutamine, liquid, sterile-filtered, suitable for cell culture

EX-CELL 302 is a serum-free medium which has been specifically developed for the long-term growth of transformed Chinese Hamster Ovary (CHO) cells in suspension for the expression of antibodies or protein products. EX-CELL 302 is an appropriate medium for use with the DHFR$^-$ or Glutamine Synthetase, or the GS System™, because it does not contain hypoxanthine, thymidine or L-glutamine. For use with the DHFR$^-$ selection system we recommend the addition of 4 mM L-glutamine for optimal growth. Due to current regulatory concerns about the sources of raw materials, EX-CELL 302 was developed using only recombinant human proteins that have molecular weights less than 10 kDa. The total protein concentration found in EX-CELL 302 is less than 1 mg/L. Pluronic® F68 has been added at a final concentration of 0.1% to EX-CELL 302 to protect against shear damage in sparged bioreactor systems.

ship: ambient store at: 2-8°C

14324C-500ML	500 mL
14324C-1000ML	1000 mL

without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture

EX-CELL 302 is a serum-free medium which has been specifically developed for the long-term growth of transformed Chinese Hamster Ovary (CHO) cells in suspension for the expression of antibodies or protein products. EX-CELL 302 is an appropriate medium for use with the DHFR$^-$ or Glutamine Synthetase, or the GS System™, because it does not contain hypoxanthine, thymidine or L-glutamine. For use with the DHFR$^-$ selection system we recommend the addition of 4 mM L-glutamine for optimal growth. Due to current regulatory concerns about the sources of raw materials, EX-CELL 302 was developed using only recombinant human proteins that have molecular weights less than 10 kDa. The total protein concentration found in EX-CELL 302 is less than 1 mg/L. Pluronic® F68 has been added at a final concentration of 0.1% to EX-CELL 302 to protect against shear damage in sparged bioreactor systems.

ship: ambient store at: 2-8°C

14326C-500ML	500 mL
14326C-1000ML	1000 mL

without L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

EX-CELL® 302 is a serum-free medium which has been specifically developed for the long-term growth of transformed Chinese Hamster Ovary (CHO) cells in suspension for the expression of antibodies or protein products. EX-CELL 302 is an appropriate medium for use with the DHFR$^-$ or Glutamine Synthetase, or the GS System™, because it does not contain hypoxanthine, thymidine or L-glutamine. For use with the DHFR$^-$ selection system we recommend the addition of 4 mM L-glutamine for optimal growth. Due to current regulatory concerns about the sources of raw materials, EX-CELL 302 was developed using only recombinant human proteins that have molecular weights less than 10 kDa. The total protein concentration found in EX-CELL 302 is less than 1 mg/L. Pluronic® F68 has been added at a final concentration of 0.1% to EX-CELL 302 to protect against shear damage in sparged bioreactor systems.

Drug Master File available

Formulated to contain 21.2 grams of powder per liter of medium.

Ex-Cell 302 has also been used successfully with BHK-21 cells and chicken embryo-related (CER) cell lines.

ship: ambient store at: 2-8°C

24324C-1L	1 L
24324C-5L	5 L
24324C-10L	10 L
24324C-50L	50 L
24324C-100L	100 L

without L-glutamine, without phenol red, without sodium bicarbonate, dry powder, suitable for cell culture NEW

EX-CELL® 302 is a serum-free medium, which has been specifically developed for the long-term growth of transformed Chinese Hamster Ovary (CHO) cells in suspension for the expression of antibodies or protein products. EX-CELL 302 is an appropriate medium for use with the DHFR$^-$ or Glutamine Synthetase, or GS System™ because it does not contain hypoxanthine, thymidine or L-glutamine. If EX-CELL 302 is to be used with CHO cells transformed using the GS selection system the media needs to be supplemented with GS Supplement 50X for the additional amino acids and nucelosides. For use with the DHFR$^-$ selection system we recommend the addition of 4 mM L-Glutamine for optimal growth. Due to current regulatory concerns about the sources of raw materials, EX-CELL 302 was developed using only recombinant human proteins that have molecular weights less than 10 kD. The total protein concentration found in EX-CELL 302 is less than 1 mg/L. Pluronic® F68 has been added at a final concentration of 0.1% to EX-CELL 302 to protect against shear damage in sparged bioreactor systems.

Formulated to contain 21.2 grams of powder per liter of medium
ship: ambient store at: 2-8°C

24326C-1L	1 L
24326C-5L	5 L
24326C-10L	10 L
24326C-50L	50 L

EX-CELL® 325 PF CHO Serum-Free Medium for CHO Cells

for research or for further manufacturing use

without L-glutamine
store at: 2-8°C

without L-glutamine, protein free, liquid, sterile-filtered, suitable for cell culture

EX-CELL® 325 PF CHO is a protein-free medium which has been developed for the growth of Chinese Hamster Ovary (CHO) cells. Because it contains no large macromolecules, EX-CELL 325 PF CHO facilitates the isolation and purification of secreted proteins from the cells. CHO cells propagated in EX-CELL 302 serum-free media (Catalog No. 14324C and 14326C) can be transferred directly into this protein-free medium without extensive weaning protocols. This medium is supplied without L-glutamine and does not contain purines or pyrimidines to provide an appropriate medium for specialized CHO cell lines (i.e., Glutamine Synthetase, or the GS System™, and DHFR⁻ selection systems).
ship: wet ice store at: 2-8°C

14340C-500ML	500 mL
14340C-1000ML	1000 mL

without L-glutamine, protein free, dry powder, suitable for cell culture NEW

EX-CELL® 325 PF CHO is a protein-free medium which has been developed for the growth of Chinese Hamster Ovary (CHO) cells. Because it contains no large macromolecules, EX-CELL 325 PF CHO facilitates the isolation and purification of secreted proteins from the cells. CHO cells propagated in EX-CELL 302 serum-free media can be transferred directly into this protein-free medium without extensive weaning protocols. This medium is supplied without L-glutamine and does not contain purines or pyrimidines to provide an appropriate medium for specialized CHO cell lines (i.e., Glutamine Synthetase, or the GS System™, and DHFR⁻ selection systems).

Formulated to contain 21.0 grams of powder per liter of medium.

Aseptically add 20-40 ml of 200 mM L-glutamine solution per liter of medium prior to use.
ship: ambient store at: 2-8°C

24340C-1L	1 L
24340C-5L	5 L
24340C-10L	10 L
24340C-50L	50 L

EX-CELL® ACF CHO Medium

CHO Medium

Animal component-free medium formulated to optimize cell growth and protein expression in Chinese hamster ovary (CHO) cells.

for research or for further manufacturing use

Drug/Device Master File available

Animal-component free, with HEPES, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Proprietary formulation containing inorganic salts, HEPES and sodium bicarbonate buffers, essential and non-essential amino acids, vitamins, recombinant human insulin, plant hydrolysates, other organic compounds, trace elements, and surfactants.
Does not contain antibiotics, antimycotics, L-glutamine, or transferrin.
Contains no animal-derived proteins or other components.

Features and Benefits

Developed to meet the needs of biotechnology and vaccine manufacturing, this medium supports rapid initial cell growth and high levels of protein expression in suspension cultures. It also supports high cell densities and maintenance of these densities of viable cells for extended periods resulting in increased productivity. Cell densities in excess of 8×10^6 cells/ml have been achieved in batch culture systems. Increases in protein productivity up to 500% per cell have been observed.

Aseptically add 20-40 ml of 200 mM L-glutamine solution per liter of medium prior to use.
ship: ambient store at: 2-8°C

C5467-1L	1 L
C5467-6X1L	6 × 1 L

Animal-component free, without L-glutamine, use at 21.5 g/L, dry powder, suitable for cell culture

Proprietary formulation containing inorganic salts, HEPES, essential and non-essential amino acids, vitamins, recombinant human insulin, plant hydrolysates, other organic compounds, trace elements, and surfactants.
Does not contain antibiotics, antimycotics, L-glutamine, or transferrin.
Contains no animal-derived proteins or other components.

Features and Benefits

Developed to meet the needs of biotechnology and vaccine manufacturing, this medium supports rapid initial cell growth and high levels of protein expression in suspension cultures. It also supports high cell densities and maintenance of these densities of viable cells for extended periods resulting in increased productivity.

concentration ..... 21.5 g/L
ship: ambient store at: 2-8°C

C9098-10L	10 L
C9098-100L	100 L

EX-CELL® CD CHO Fusion NEW

EX-CELL® CD CHO Serum-Free Medium for CHO Cells, Chemically Defined

Animal-component free, without L-glutamine, without hypoxanthine, without thymidine, liquid, sterile-filtered, suitable for cell culture

EX-CELL® CD CHO FUSION is a chemically defined, animal-component free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells. The absence of any large macromolecules allows for isolation and purification of secreted proteins from the cells.

This medium is supplied without L-glutamine to aid in media stability, to avoid L-glutamine degradation that causes ammonia build-up, and to provide an appropriate medium for the culture of CHO cells using the Glutamine Synthetase, or GS System™. This medium does not contain hypoxanthine or thymidine to allow for its use with dihydrofolate reductase (DHFR) gene amplification systems.
ship: ambient store at: 2-8°C

14365C-500ML	500 mL
14365C-1000ML	1000 mL

EX-CELL® CD CHO Fusion *(continued)*

Animal-component free, without L-glutamine, without hypoxanthine, without sodium bicarbonate, without thymidine, dry powder, suitable for cell culture

EX-CELL® CD CHO FUSION is a chemically defined, animal-component free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells. The absence of any large macromolecules allows for isolation and purification of secreted proteins from the cells.

This medium is supplied without L-glutamine to aid in media stability, to avoid L-glutamine degradation that causes ammonia build-up, and to provide an appropriate medium for the culture of CHO cells using the Glutamine Synthetase, or GS System™. This medium does not contain hypoxanthine or thymidine to allow for its use with dihydrofolate reductase (DHFR) gene amplification systems.

Formulated to contain 20.1 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24365C-1L	1 L
24365C-10L	10 L

EX-CELL® CD CHO-2 Medium

CHO Medium

Animal-component free, with sodium bicarbonate, without L-glutamine, without phenol red., liquid, sterile-filtered, suitable for cell culture

Proprietary formulation containing inorganic salts, HEPES and sodium bicarbonate buffers, essential and non-essential amino acids, vitamins, recombinant human insulin, other organic compounds, trace elements, and surfactants.
Does not contain antibiotics, antimycotics, L-glutamine, transferrin, hydrolysates, or other undefined nutrients or supplements. Contains no animal-derived components or components synthesized from animal-derived materials.

Complete, chemically-defined medium formulated to optimize cell growth and protein expression in Chinese hamster ovary (CHO) cells.

Features and Benefits

All components are completely defined to meet the needs of researchers studying nutritional and physiological control of cellular processes and manufacturers seeking to optimize downstream processing. This defined medium maintains the functional properties of good cell growth and protein expression in cultured cells.

for research or for further manufacturing use

Aseptically add 20-40 ml of 200 mM L-glutamine solution per liter of medium prior to use.

ship: ambient store at: 2-8°C

C4726-1L	1 L
C4726-6X1L	6 × 1 L

EX-CELL® CD CHO-3 Medium, Chemically Defined

Animal-component free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Proprietary formulation containing inorganic salts, HEPES and sodium bicarbonate buffers, essential and non-essential amino acids, vitamins, recombinant human insulin, other organic compounds, trace elements, and surfactants.
Does not contain antibiotics, antimycotics, L-glutamine, transferrin, hydrolysates, or other undefined nutrients or supplements. Contains no animal-derived components or components synthesized from animal-derived materials.

Complete, chemically-defined medium formulated to optimize cell growth and protein expression in Chinese hamster ovary (CHO) cells.

Features and Benefits

Developed to meet the needs of biotechnology and vaccine manufacturing, this medium supports rapid initial cell growth and high levels of protein expression in suspension cultures. This medium supports and maintains high cell densities for extended periods, resulting in increased productivity.

for research or for further manufacturing use

Aseptically add 20-40 mL of 200 mM L-glutamine solution per liter of medium prior to use.

Do not freeze. Protect from light. Several components of the medium are light labile and should not be exposed to light for lengthy periods of time.

endotoxin ..... tested

ship: ambient store at: 2-8°C

C1490-1L	1 L
C1490-6X1L	6 × 1 L

EX-CELL® CD CHO Serum-Free Medium for CHO Cells NEW

EX-CELL® CD CHO Serum-Free Medium for CHO Cells, Chemically Defined

Animal-component free, with hypoxanthine, with thymidine, without L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture

EX-CELL® CD CHO is a chemically defined, animal-component free, serum-free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells and expression of antibodies or protein products in suspension cell culture. CHO suspension cultures can be subcultured directly into EX-CELL CD CHO from serum-supplemented or serum-free medium with little or no adaptation. EX-CELL CD CHO is formulated without hypoxanthine, thymidine or L-glutamine, making it an appropriate medium for selection systems such as $DHFR^-$ and Glutamine Synthetase (GS System). For applications that do not require the selective pressure of a hypoxanthine/thymidine (HT)-deficient medium, we recommend the use of EX-CELL CD CHO with hypoxanthine and thymidine.

Formulated to contain 19.3 grams per liter of medium.

ship: ambient store at: 2-8°C

24361C-1L	1 L
24361C-5L	5 L
24361C-10L	10 L
24361C-50L	50 L

EX-CELL® CD CHO Serum-Free Medium for CHO Cells, Chemically Defined

Animal-component free, without L-glutamine, without hypoxanthine, without thymidine, liquid, sterile-filtered, suitable for cell culture

EX-CELL CD CHO is a chemically defined, animal-component free, serum-free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells and expression of antibodies or protein products in suspension cell culture. CHO suspension cultures can be subcultured directly into EX-CELL CD CHO from serum-supplemented or serum-free medium with little or no adaptation. EX-CELL CD CHO is formulated without hypoxanthine, thymidine or L-glutamine, making it an appropriate medium for selection systems such as DHFR$^-$ and Glutamine Synthetase (GS System™). For applications that do not require the selective pressure of a hypoxanthine/thymidine (HT)-deficient medium, we recommend the use of EX-CELL CD CHO with hypoxanthine and thymidine (Catalog No. 14361C).

ship: ambient store at: 2-8°C

14360C-500ML	500 mL
14360C-1000ML	1000 mL

Animal-component free, without L-glutamine, without hypoxanthine, without sodium bicarbonate, without thymidine, dry powder, suitable for cell culture NEW

EX-CELL® CD CHO is a chemically defined, animal-component free, serum-free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells and expression of antibodies or protein products in suspension cell culture CHO suspension cultures can be subcultured directly into EX-CELL CD CHO from serum-supplemented or serum-free medium with little or no adaptation. EX-CELL CD CHO is formulated without hypoxanthine, thymidine or L-glutamine, making it an appropriate medium for selection systems such as DHFR$^-$ and Glutamine Synthetase (GS System). For applications that do not require the selective pressure of a hypoxanthine/thymidine (HT)-deficient medium, we recommend the use of EX-CELL CD CHO with hypoxanthine and thymidine.

Formulated to contain 19.2 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24360C-1L	1 L
24360C-5L	5 L
24360C-10L	10 L
24360C-50L	50 L
24360C-100L	100 L

Animal-component free, with hypoxanthine, with thymidine, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

EX-CELL® CD CHO is a chemically defined, animal-component free, serum-free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells and expression of antibodies or protein products in suspension cell culture. CHO suspension cultures can be subcultured directly into EX-CELL CD CHO from serum-supplemented or serum-free medium with little or no adaptation. EX-CELL CD CHO (Catalog No. 14361C) is formulated with hypoxanthine and thymidine, making it an appropriate medium for applications that do not require the selective pressure of a hypoxanthine/thymidine (HT)-deficient medium, such as CHO-K1 and Glutamine Synthetase (GS System™) users.

for research or for further manufacturing use

ship: ambient store at: 2-8°C

14361C-500ML	500 mL
14361C-1000ML	1000 mL

EX-CELL® CD Hydrolysate Fusion NEW

EX-CELL® CD CHO Serum-Free Medium for CHO Cells, Chemically Defined

EX-CELL® CD Hydrolysate Fusion is a synthetic option to undefined hydrolysates used in cell culture processes, including both Batch and Fed-Batch modes. It is formulated to have minimal pH and osmolality impact upon addition. EX-CELL CD Hydrolysate Fusion is optimized for Chinese Hamster Ovary (CHO) cell lines but has also been shown to work well with other cell types (e.g. NS0 and Sp2/0). EX-CELL CD Hydrolysate Fusion is comprised only of animal-free, chemically defined components that were identified to be in hydrolysates (but synthetically produced) or are commonly found in cell culture media. In order to have broad applications, EX-CELL CD Hydrolysate Fusion was based on data generated from four hydrolysate types (soy, wheat gluten, yeast extract and meat).

for research or for further manufacturing use

Animal-component free, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

14700C-500ML	500 mL
14700C-1000ML	1000 mL

Animal-component free, powder, suitable for cell culture

ship: ambient store at: 2-8°C

24700C-100G	100 g
24700C-500G	500 g

EX-CELL® CHO Cloning Medium

Animal-component free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Proprietary formulation containing inorganic salts, sodium bicarbonate, essential and non-essential amino acids, vitamins, trace elements, plant hydrolysates, and other organic compounds.
Does not contain antibiotics, antimycotics, L-glutamine, insulin, or transferrin.

This animal component-free medium is designed to support clonal survival and growth of Chinese Hamster Ovary (CHO) cell lines, with results comparable to the traditional method using 10% fetal bovine serum.

Features and Benefits

Developed to meet the needs of the biotechnology industry, this animal component-free medium is designed for single-cell cloning of recombinant CHO cell lines adapted to serum-free suspension culture.

Aseptically add 10 mL of 200 mM L-glutamine solution per 500 mL of medium prior to use.

Do not freeze. Protect from light. Several components of the medium are light labile and should not be exposed to light for lengthy periods of time.

endotoxin ..... tested

ship: ambient store at: 2-8°C

C6366-500ML	500 mL

EX-CELL® CHO DHFR⁻ Medium

Animal-component free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Proprietary formulation containing inorganic salts, HEPES and sodium bicarbonate buffers, essential and non-essential amino acids, vitamins, recombinant human insulin, plant hydrolysates, other organic compounds, trace elements, and surfactants.
Does not contain antibiotics, antimycotics, L-glutamine, or transferrin.
Contains no animal-derived proteins or other components.

This animal component-free medium (containing no hypoxanthine or thymidine) is formulated to maximize cell growth and recombinant protein production using the Dihydrofolate Reductase (DHFR) gene amplification system in DHFR⁻ Chinese Hamster Ovary (CHO) cells.

Features and Benefits

Developed to meet the needs of biotechnology and vaccine manufacturing, this medium supports rapid initial cell growth and high levels of protein expression in suspension cultures. This medium supports and maintains high cell densities for extended periods, resulting in increased productivity.

for research or for further manufacturing use

Aseptically add 20-40 ml of 200 mM L-glutamine solution per liter of medium prior to use

Do not freeze. Protect from light. Several components of the medium are light labile and should not be exposed to light for lengthy periods of time.

endotoxin ..... tested
ship: ambient store at: 2-8°C

C8862-1L	1 L
C8862-6X1L	6 × 1 L

EX-CELL® EBx GRO-I Serum-Free Media NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, without L-glutamine and without sodium bicarbonate, dry powder, suitable for cell culture

Formulated to contain 19.1 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24530C-1L	1 L
24530C-5L	5 L
24530C-10L	10 L
24530C-50L	50 L
24530C-100L	100 L

EX-CELL® EBx™ GRO-I Serum-Free Media NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: wet ice store at: 2-8°C

14530C-500ML	500 mL
14530C-1000ML	1000 mL

Related Supplements

Antioxidant Supplement (1000×)

liquid, sterile-filtered, suitable for cell culture

Proprietary medium supplement specially formulated to boost cell growth for enhanced viral and protein expression. May be used in combination with Polyamine Supplement (P 8483).
One mL supplements 1 liter of medium.

endotoxin ..... tested
ship: ambient store at: 2-8°C

A1345-5ML	5 mL

CHO Feed Bioreactor Supplement

Animal Component-free, solution, sterile-filtered, suitable for cell culture

Feed concentrate formulated to optimize CHO cell growth and recombinant protein production in fed-batch bioreactor cultures.

endotoxin ..... tested
ship: ambient store at: 2-8°C

C1615-1L	1 L

Bioreactor pH Adjustment Solution

Bioreactor buffer solution
liquid, sterile-filtered, suitable for cell culture

Concentrated carbonate/bicarbonate solution designed specifically to adjust pH in bioreactor applications.

Features and Benefits

Using this buffered solution to adjust the pH in bioreactors minimizes detrimental localized pH effects and the osmolality impact compared to using NaOH.

endotoxin ..... tested
ship: ambient store at: room temp

B1185-1L	1 L

Fatty Acid Supplement

Animal-component free, liquid, sterile-filtered, suitable for cell culture

Animal-component free. Proprietary aqueous mixture of fatty acids that have been found to be optimal for cell growth and protein expression.

Recommended for use at 0.25 to 0.5 ml per liter with CHO cells but is considered to have broader application.

endotoxin ..... tested
ship: ambient store at: 2-8°C

F7050-5ML	5 mL
F7050-100ML	100 mL

GSEM Supplement

Glutamine synthetase expression medium supplement

▸ 50 ×, liquid, sterile-filtered, suitable for cell culture

The glutamine synthetase (GS System™) is used to express recombinant proteins from mammalian cells. Glutamine-dependent cells transfected with a glutamine synthetase gene can be selectively grown in a suitable glutamine-deficient medium.

One mL will supplement 50 mL of cell culture medium.

Contains (mg/L): L-alanine 450.0, L-asparagine · H_2O 4261.0, L-aspartic acid 650.0, L-glutamic acid 3750.0, L-proline 575.0, L-serine 500.0, adenosine 350.0, guanosine 350.0, cytidine 350.0, uridine 350.0, and thymidine 12.0

endotoxin ..... tested

ship: dry ice store at: –20°C

G9785-100ML	100 mL

Polyamine Supplement (1000×)

▸ liquid, sterile-filtered, BioReagent, suitable for cell culture

General medium supplement specially formulated to boost cell growth for enhanced viral and protein expression. May be used in combination with Antioxidant Supplement (A 1345).

One mL supplements 1 liter of medium.

endotoxin ..... tested

ship: ambient store at: 2-8°C

P8483-5ML	5 mL

GS Supplement 50X NEW

GSEM Supplement; Glutamine synthetase expression medium supplement

▸ 50 ×, liquid, sterile-filtered, suitable for cell culture

The glutamine synthetase (GS System™) is used to express recombinant proteins from mammalian cells. Glutamine-dependent cells transfected with a glutamine synthetase gene can be selectively grown in a suitable glutamine-deficient medium.

for research or for further manufacturing use

One mL will supplement 50 mL of cell culture medium.

Contains (mg/L): L-alanine 450.0, L-asparagine · H_2O 4261.0, L-aspartic acid 650.0, L-glutamic acid 3750.0, L-proline 575.0, L-serine 500.0, adenosine 350.0, guanosine 350.0, cytidine 350.0, uridine 350.0, and thymidine 12.0

ship: dry ice store at: –20°C

58672C-100ML	100 mL
58672C-1000ML	1000 mL

HyPep® 4601 Protein Hydrolysate from wheat gluten

Peptone from wheat gluten
[100684-25-1]

▸ Animal-component free, powder, suitable for cell culture

Animal-component free

Recommended for use as a rich source of L-glutamine for medium supplementation at 1 to 5 g/L of medium. It also provides amino acids that support the general nutritional requirements of cells in culture.

ship: ambient store at: room temp

H6784-100G	100 g
H6784-1KG	1 kg

Hybridoma Platform

The development of techniques to produce monoclonal antibodies in cell culture systems has greatly increased the specificity of antibodies available as well as permitted increased production of specific antibodies. Traditional techniques of monoclonal antibody production have relied upon the use of medium supplemented with serum of animal origin. Since the majority of these antibodies have been employed for research or *in vitro* diagnostic applications, the presence of serum did not pose significant regulatory concerns. For this reason, efforts to develop serum-free media have primarily focused on cost reduction without regard to the nature of the components used for serum replacement in the medium. However, as new technologies have been developed, an increasing number of antibodies are utilized in applications as *in vivo* therapeutics agents.

These changes in the use of such antibodies have led to reviews by regulatory agencies concerning the materials and methods employed for antibody production. Due to the threat of contamination by adventitious agents when using animal-derived components in medium, many agencies have strongly suggested that medium containing such components be discontinued for manufacturing therapeutic injectable products. In recognition of the changing regulatory requirements in the biotechnology industry for media used in the production of antibodies from hybridoma cell lines, Sigma is developing new serum-free media for both cost control during production and compliance with regulatory concerns. At the same time, Sigma continues to offer a broad product line of media designed for serum-supplemented or serum-free growth of hybridomas for antibody production.

Reference

1. Falkenberg, F.W., Monoclonal antibody production: problems and solutions. *Res. Immunol.*, **149**, 542-547, 1998.

Hybridoma Media

EX-CELL® 610-HSF Serum-Free Medium for Hybridoma Cells

EX-CELL 610-HSF is a low-protein (11 mg/L) serum-free medium. Originally developed to support the growth of hybrid cells in culture, it has been shown to support a wide range of cells including lymphoid and epithelial cells and B cell hybridomas of murine, rat and human origin. EX-CELL 610-HSF has been used in stationary culture systems and in large-scale bioreactors. In both types of culture, the production of cellular products, particularly monoclonal antibodies, has been shown to equal or exceed levels seen when the same cells are cultured in the presence of Fetal Bovine Serum (FBS).

for research or for further manufacturing use

Low-protein, with L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

Formulated to contain 15.1 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24610C-1L	1 L
24610C-5L	5 L
24610C-10L	10 L
24610C-50L	50 L

Low-protein, with L-glutamine, liquid, sterile-filtered, suitable for cell culture

Drug Master File available

ship: ambient store at: 2-8°C

14610C-500ML	500 mL
14610C-1000ML	1000 mL

EX-CELL® 620-HSF Serum-Free Medium for Hybridoma Cells

EX-CELL 620-HSF is a low-protein (approximately 11 mg/L) serum-free medium which has been specifically developed for the long-term growth of hybridoma and related cells capable of expressing monoclonal antibodies and other protein products. The following hybridoma, lymphoma and myeloma cell lines have demonstrated sustained long-term, serum-free growth (> 15 passages) in EX-CELL 620-HSF: Sp2/0, Ag14, L243, VD-10, Hut 78, K562. Additionally, transformed Chinese Hamster Ovary (CHO) cells have demonstrated excellent growth and productivity when grown in this medium.

for research or for further manufacturing use

Low-protein, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

14621C-500ML	500 mL
14621C-1000ML	1000 mL

Low-protein, without L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

Formulated to contain 17.2 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24621C-1L	1 L
24621C-5L	5 L
24621C-10L	10 L
24621C-50L	50 L

EX-CELL® Hybridoma Medium

Hybridoma Medium

without antibiotics, antimycotics, L-glutamine, and phenol red, with sodium bicarbonate, HEPES, essential and non-essential amino acids, liquid, sterile-filtered, suitable for hybridoma

This proprietary formulation includes inorganic salts, essential and non-essential amino acids, vitamins, sodium bicarbonate, HEPES, trace elements, fatty acids, and other organics. It contains low concentrations of bovine insulin, bovine serum albumin and human transferrin (source tested negative for HIV antibody and HbsAG). The medium does not contain L-glutamine, antibiotics, antimycotics, and phenol red.

Serum-free, low-protein medium formulated to support the growth of hybridomas used in the manufacture of monoclonal antibodies.

Features and Benefits

Designed to support the rapid growth of cells to high densities and the maintenance of viable cells for extended culture periods. This formulation reduces raw material cost and the low protein level reduces cost associated with downstream processing. Purified components in the product reduce the variability sometimes encountered with biological materials leading to improved consistency of the medium performance. Cells can be successfully transferred from serum-supplemented cultures to this medium with little or no adaptation.

endotoxin tested

for research or for further manufacturing use

Prior to use, medium should be warmed to 37°C and supplemented with 50 ml/L of 200 mM L-glutamine.

Do not freeze. Protect from light. Several components of the medium are light labile and should not be exposed to light for lengthy periods of time. When stored at 2-8°C in the dark, medium that has not been supplemented is stable until the expiration date on the label.

ship: ambient store at: 2-8°C

H4281-1L	1 L
H4281-6X1L	6 × 1 L

EX-CELL® NS0 Serum-Free Medium for NS0 Cells

EX-CELL NS0 is an animal-component free, protein-free, chemically defined, serum-free medium developed for the long-term growth of NS0-related cells in suspension culture. The NS0 cells are clonal derivatives of the parent NS1 cell line and are capable of growth in suspension culture. NS0 hybridoma suspension cultures can be subcultured direclty into EX-CELL NS0 from serum-supplemented or serum-free media. Suspension cultures in EX-CELL NS0 have been carried for more that 50 passages with no loss of growth or viability.

for research or for further manufacturing use

Animal-component free, Protein-free, Chemically defined, dry powder, without L-glutamine, without sodium bicarbonate, suitable for cell culture NEW

ship: ambient store at: 2-8°C

24651C-1L	1 L
24651C-5L	5 L
24651C-10L	10 L
24651C-50L	50 L

Animal-component free, Protein-free, Chemically defined, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

14650C-500ML	500 mL
14650C-1000ML	1000 mL

EX-CELL® Sp2/0 Serum-Free Medium for Sp2/0 Cells

for research or for further manufacturing use

Animal-component free, Protein free, Chemically defined, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

14660C-500ML	500 mL
14660C-1000ML	1000 mL

Animal-component free, Protein free, Chemically defined, without L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

Formulated to contain 17.3 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24660C-1L	1 L
24660C-5L	5 L
24660C-10L	10 L
24660C-50L	50 L

Hybridoma Media Supplements and Companion Products

Hybridoma monoclonal antibody expression systems are complex and are typically modified (or optimized) to meet the specific priorities and needs of the biotechnologist. In addition to offering the above varieties of hybridoma media, Sigma provides supplements that you may find useful in your culture system. The listings in this section detail some of the most useful supplements for your evaluation and use.

Other supplements potentially useful for the culture of hybridoma cells such as transferrin, albumin, and insulin are offered by Sigma in many varieties. These components are offered as individual reagents or in pre-formulated, ready-to-use mixtures. Please use the Sigma search engine to find these product families at *sigma-aldrich.com*.

Details for the use of the reagents and supplements may be found in literature published by Sigma which discusses the use of hybridoma cell lines for monoclonal antibody manufacturing applications.

Media Supplements

Antioxidant Supplement (1000×)

liquid, sterile-filtered, suitable for cell culture

Proprietary medium supplement specially formulated to boost cell growth for enhanced viral and protein expression. May be used in combination with Polyamine Supplement (P 8483).
One mL supplements 1 liter of medium.

endotoxin ..... tested

ship: ambient store at: 2-8°C

A1345-5ML	5 mL

Cholesterol solution

[57-88-5] $C_{27}H_{46}O$ FW 386.65

500 ×, Animal component-free, SyntheChol™ synthetic, aqueous solution, sterile-filtered, suitable for cell culture

Features and Benefits

Synthetic, non-animal cholesterol solution that is designed as a supplement to our hybridoma media formulations to promote excellent cell growth and protein productivity for NS0 mouse myeloma and other cholesterol-dependent cell lines.

ship: ambient store at: 2-8°C

S5442-2ML	2 mL
S5442-10ML	10 mL

L-Glutamine solution

[56-85-9]

Prepared in cell culture grade water.

endotoxin ..... tested

200 mM, solution, sterile-filtered, BioXtra, suitable for cell culture

ship: dry ice store at: −20°C

G7513-20ML	20 mL
G7513-100ML	100 mL

200 mM, Hybri-Max™, sterile-filtered, suitable for hybridoma

ship: dry ice store at: −20°C

G2150-20ML	20 mL

L-Glutamine Solution 200 mM

NEW

L-Glutamine solution
[56-85-9]

29.23 mg/mL in saline, solution, suitable for cell culture

for research or for further manufacturing use

ship: dry ice store at: –20°C

59202C-100ML	100 mL
59202C-500ML	500 mL

Hybridoma Enhancing Supplement from murine macrophage-like cell line

Conditioned Medium from murine macrophage-like cell line liquid, sterile-filtered, suitable for hybridoma

Conditioned medium from a proprietary murine macrophage-like cell line grown in IMDM with 2% FBS.

Use at a concentration of 10% in complete medium containing FBS (10%) or CPRS-3. Optimal conditions must be determined for specific applications.

Cell line viral testing:
Murine Antibody Production (map) testing for Sendai, MHV, PVM, Reo3, GDVII, Ectomelia, MVM, Polyoma, LDMV, M. Ad, EDIM, MCMV, LCM, Hantaan.
BVD virus by fluorescent antibody stain

Avoid repeated freezing and thawing. If entire contents are not used, store frozen in working aliquots. Precipitation may occur when stored. This is not detrimental to the product and may be reversed when warmed to 37 °C.

endotoxin ..... tested
Mycoplasma ..... tested

ship: dry ice store at: –20°C

H8142-50ML	50 mL

Hybridoma Enhancing Supplement from murine thymoma cell line

Conditioned Medium from murine thymoma cell line liquid, sterile-filtered, suitable for hybridoma

Conditioned medium from a proprietary murine thymoma cell line grown in RPMI-1640 with 15% FBS and IL-6.

Use at a concentration of 10% in complete medium containing FBS (10%) or CPRS-3. Optimal conditions must be determined for specific applications.

endotoxin tested, mycoplasma tested, hybridoma tested.

Avoid repeated freezing and thawing. If entire contents are not used, store frozen in working aliquots. Precipitation may occur when stored. This is not detrimental to the product and may be reversed when warmed to 37 °C.

ship: dry ice store at: –20°C

H2900-100ML	100 mL

Hybridoma Enhancing Supplement Hybri-Max™ from murine thymoma cell line

Conditioned Medium from murine thymoma cell line liquid, sterile-filtered, suitable for cell culture, suitable for hybridoma

Conditioned medium from a proprietary murine thymoma cell line grown in RPMI-1640 with 15% FBS.

Use at a concentration of 10% in complete medium containing FBS (10%) or CPSR-3. Optimal conditions must be determined for specific applications.

Cell line virus testing:
Murine Antibody Production (MAP) testing for Sendai, MHV, PVM, Reo 3, GDVII, Ectomelia, MVM, Polyoma, LDVM, M. Ad. EDIM MCMV, LCM, Hantaan.

endotoxin ..... tested
Mycoplasma ..... tested

ship: dry ice store at: –20°C

H6020-100ML	100 mL

Insulin from bovine pancreas

[11070-73-8] $C_{254}H_{377}N_{65}O_{75}S_6$ FW 5733.49

Two-chain polypeptide hormone produced by the β-cells of pancreatic islets. Its molecular weight is ~5800 Da. The α and β chains are joined by two interchain disulfide bonds. The α chain contains an intrachain disulfide bond. Insulin regulates the cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids and inhibits the breakdown of glycogen, protein, and fat.

powder, BioReagent, suitable for cell culture

Insulin is used as a growth factor in many mammalian cell culture systems.

Associated gene(s): INS (280829)

potency: ≥27 USP units per mg

Insulin has low solubility at neutral pH. It can be solubilized at 2 mg/ml in dilute acetic or hydrochloric acid, pH 2-3.

ship: ambient store at: –20°C

I6634-50MG	50 mg
I6634-100MG	100 mg
I6634-250MG	250 mg
I6634-500MG	500 mg
I6634-1G	1 g
I6634-5G	5 g

γ-irradiated, lyophilized powder, suitable for cell culture, activity: ≥25 USP units/mg

Insulin is used as a growth factor in many mammalian cell culture systems.

BioXtra

To prepare 10 mg/ml stock solution, add 10 ml of acidified H_2O (pH ≤2) - prepared by addition of glacial acetic acid (approx. 0.1 ml).

ship: ambient store at: –20°C

I1882-100MG	100 mg

Hybri-Max™, powder, suitable for hybridoma

potency: ≥27 USP units per mg

solubility
acidified water, pH 2.0 ..... 2 mg/mL
endotoxin ..... tested
Zn 0.4-1.08%

ship: ambient store at: –20°C

I4011-50MG	50 mg
I4011-100MG	100 mg
I4011-250MG	250 mg
I4011-500MG	500 mg

Insulin solution human

[11061-68-0]

Two-chain polypeptide hormone produced by the β-cells of pancreatic islets. Its molecular weight is ~5800 Da. The α and β chains are joined by two interchain disulfide bonds. The α chain contains an intrachain disulfide bond. Insulin regulates the cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids and inhibits the breakdown of glycogen, protein, and fat.

Chemically defined, recombinant from *Saccharomyces cerevisiae*, sterile-filtered, BioXtra, suitable for cell culture

Recommended for use in cell culture applications at 0.5 to 1 mL per liter of medium.

solution

concentration ..... 9.5-10.5 mg/mL
ship: ambient store at: 2-8°C

I9278-5ML	5 mL
I9278-10ML	10 mL

Insulin-transferrin-sodium selenite media supplement

ITS Supplement
γ-irradiated, lyophilized powder, BioXtra, suitable for cell culture

Each vial contains: ≥21 mg insulin from bovine pancreas; ≥19 mg human transferrin (substantially iron free), and 25 µg sodium selenite.

ITS is a mixture of bovine insulin, human transferrin, and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham?s F-12, DME/F-12, MEM) with sodium pyruvate.

Insulin-transferrin-sodium selenite supplement is used for serum-free cell culture applications.

Each vial sufficient to prepare 5 liters of medium.

ship: ambient store at: −20°C

I1884-1VL	1 vial
I1884-5X1VL	5 × 1 vial

ITS+1 Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Insulin-transferrin-sodium selenite, linoleic-BSA is used for serum-free cell culture applications.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 50 mg/ml bovine serum albumin and 470 µg/ml linoleic acid.

endotoxin ..... tested
ship: ambient store at: 2-8°C

I2521-5ML	5 mL

ITS+3 Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Insulin-transferrin-sodium selenite, linoleic;oleic-BSA is used for serum-free cell culture applications.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 470 µg/ml linoleic acid, 470 µg/ml oleic acid and 50 mg/ml bovine serum albumin.

Contains 2 moles each of linoleic acid and oleic acid per mole of albumin.

endotoxin ..... tested
ship: ambient store at: 2-8°C

I2771-5ML	5 mL

ITS Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

ITS is a mixture of bovine insulin, human transferrin, and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham?s F-12, DME/F-12, MEM) with sodium pyruvate.

Insulin-transferrin-sodium selenite supplement is used for serum-free cell culture applications.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), and 0.5 µg/ml sodium selenite.

endotoxin ..... tested
ship: ambient store at: 2-8°C

I3146-5ML	5 mL

Linoleic Acid-Albumin from bovine serum albumin

liquid, sterile-filtered, BioReagent, suitable for cell culture

Recommended usage: 1 ml/100 ml medium

Binding of linoleic acid improves its stability and solubility in culture media.

Contains 2 moles linoleic acid per mole BSA

concentration ..... 100 mg/mL BSA in DPBS
ship: ambient store at: 2-8°C

L9530-5ML	5 mL

Linoleic Acid-Oleic Acid-Albumin, 100x

liquid, sterile-filtered, BioReagent, suitable for cell culture

Binding of linoleic acid and oleic acid to BSA improves their stability and solubility in culture media.

Albumin from bovine

composition
linoleic acid and oleic acid 2 mol/mol albumin (each)

concentration ..... 100 mg/mL BSA in DPBS
endotoxin ..... tested
ship: ambient store at: 2-8°C

L9655-5ML	5 mL

Oleic Acid-Albumin from bovine serum

Oleic acid-BSA complex
liquid, sterile-filtered, BioReagent, suitable for cell culture

Use to deliver a solubilized form of oleic acid in cell culture application.

composition
Oleic acid 2 mol/mol albumin

concentration ..... 100 mg/mL BSA in DPBS
endotoxin ..... tested
ship: ambient store at: 2-8°C

O3008-5ML	5 mL

OPI Media Supplement

Hybri-Max™, γ-irradiated, lyophilized powder, suitable for hybridoma

Formulated with: 0.15 g oxaloacetate, 0.05 g pyruvate, and 0.0082 g bovine insulin.

Reconstitute contents of vial with 10 mL sterile water. Do not use cell culture medium for initial reconstitution. Each vial sufficient to prepare 1 liter of medium. Final working concentration: 1 mM oxaloacetate, 0.45 mM pyruvate, 0.2 U/ml insulin.

endotoxin ..... tested
ship: ambient store at: –20°C

O5003-1VL	1 vial

Oxaloacetic acid

Oxobutanedioic acid; 2-Oxosuccinic acid; Oxalacetic acid; Ketosuccinic acid
[328-42-7] $HOOCCH_2COCOOH$ $C_4H_4O_5$ FW 132.07

Hybri-Max™, powder, suitable for hybridoma

Use as a TCA (Krebs cycle) intermediate supplement in hybridoma cell culture applications. Enhances hybridoma growth and productivity.

≥97%
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: –20°C

O9504-5G	5 g
O9504-25G	25 g

Pluronic® F-68

Polyoxyethylene-polyoxypropylene block copolymer
[9003-11-6] $(C_3H_6O.C_2H_4O)_x$

Contains 100 ppm BHT

average mol wt 8350

CMC ..... 0.04 mM (20-25°C)
HLB 29

solid, BioReagent, suitable for cell culture, suitable for insect cell culture

A non-ionic detergent that protects cells from hydrodynamic damage.

non-ionic
ship: ambient store at: room temp

P1300-500G	500 g

Polyamine Supplement (1000×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

General medium supplement specially formulated to boost cell growth for enhanced viral and protein expression. May be used in combination with Antioxidant Supplement (A 1345).
One mL supplements 1 liter of medium.

endotoxin ..... tested
ship: ambient store at: 2-8°C

P8483-5ML	5 mL

SITE+3 Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 0.2 mg/ml ethanolamine, 470 µg/ml linoleic acid, 470 µg/ml oleic acid and 50 mg/ml bovine serum albumin. Contains 2 moles each of linoleic acid and oleic acid per mole of albumin.

endotoxin ..... tested
ship: ambient store at: 2-8°C

S5295-5ML	5 mL

SITE Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite and 0.2 mg/ml ethanolamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

S4920-5ML	5 mL

Sodium pyruvate

α-Ketopropionic acid sodium salt; 2-Oxopropanoic acid sodium salt; Pyruvic acid sodium salt
[113-24-6] $CH_3COCOONa$ $C_3H_3NaO_3$ FW 110.04

Hybri-Max™, powder, suitable for hybridoma

Sodium pyruvate is used by cells as an easily accessible carbohydrate source. Additionally, it is involved with amino acid metabolism and initiates the Kreb's cycle. The 100 mM solution should be diluted 1:100 for most cell culture applications.

≥99%
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: 2-8°C

P3662-25G	25 g
P3662-100G	100 g

Sodium pyruvate solution

[113-24-6]

Sodium pyruvate is used by cells as an easily accessible carbohydrate source. Additionally, it is involved with amino acid metabolism and initiates the Kreb's cycle. The 100 mM solution should be diluted 1:100 for most cell culture applications.

100 mM, sterile-filtered, BioReagent, suitable for cell culture

solution
endotoxin ..... tested
ship: ambient store at: 2-8°C

S8636-100ML	100 mL

Sodium selenite

[10102-18-8] Na_2SeO_3 Na_2O_3Se FW 172.94

γ-irradiated, lyophilized powder, BioXtra, suitable for cell culture

≥97.0%

ship: ambient store at: room temp

S9133-1MG	1 mg

SPITE Medium Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

SPITE is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, sodium pyruvate and ethanolamine. It is designed for cell cultures in which media without sodium pyruvate are used.

For suspension cell cultures

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 12 mg/ml pyruvate and 0.2 mg/ml ethanolamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

S5666-5ML	5 mL

Transferrin human

Siderophilin, partially saturated
[11096-37-0]

Purity by agarose electrophoresis.

powder, BioReagent, suitable for cell culture

Partially iron-saturated

Delivers iron to cells in a physiologically safe and effective form.

Associated gene(s): TF (7018)

≥98%

Heat treated at 60 °C for ten hours.

solubility
H_2O ..... 50 mg/mL

endotoxin ..... tested

hepatitis and HIV antibody ..... none detected

ship: ambient store at: −20°C

T8158-100MG	100 mg
T8158-1G	1 g

apo-Transferrin human

Human transferrin; Siderophilin
[11096-37-0]

Non-heme iron-transport protein.

Can be supplemented with iron or used to bind free iron present in media. Use to transport iron into mammalian cells.

mol wt 76-81 kDa

solubility
H_2O ..... 50 mg/mL

HIV and HBsAg ..... source material tested negative

powder, BioReagent, suitable for cell culture, ≥98% (agarose gel electrophoresis)

iron ..... ≤0.005%

ship: ambient store at: 2-8°C

T1147-100MG	100 mg
T1147-5X100MG	5 × 100 mg
T1147-500MG	500 mg
T1147-1G	1 g
T1147-5G	5 g

powder, BioReagent, suitable for cell culture, ≥98% (agarose gel electrophoresis)

Associated gene(s): TF (7018)

endotoxin ..... tested

Fe ..... ≤0.005%

ship: ambient store at: 2-8°C

T2036-100MG	100 mg
T2036-500MG	500 mg
T2036-1G	1 g

γ-irradiated, powder, BioXtra, suitable for cell culture, ≥98%

Associated gene(s): TF (7018)

iron ..... essentially free

ship: ambient store at: 2-8°C

T5391-10MG	10 mg

holo-Transferrin human

Siderophilin, iron-saturated
[11096-37-0]

Purity by agarose gel electrophoresis.

powder, BioReagent, suitable for cell culture, ≥97%

Provides iron to cells in a physiologically stable and safe form.

Iron-saturated

Cell Culture

Associated gene(s): TF (7018)

Heat treated at 60 °C (minimum) for a minimum of 10 hours.

Iron content by ICP reported on a lot-specific basis.

solubility
H_2O ..... 50 mg/mL

HIV and HBsAg ..... none detected

endotoxin ..... tested

ship: ambient store at: 2-8°C

T0665-50MG	50 mg
T0665-100MG	100 mg
T0665-500MG	500 mg
T0665-1G	1 g

Sera & Sera Substitutes

Fetal Bovine Serum

FBS

Endotoxin and hemoglobin tested

USA origin, sterile-filtered, suitable for cell culture, suitable for hybridoma

composition

Hemoglobin ≤20 mg/dL

endotoxin ..... ≤10 EU/mL

ship: dry ice store at: –20°C

F2442-100ML	100 mL
F2442-500ML	500 mL
F2442-6X500ML	6 × 500 mL
F2442-24X500ML	24 × 500 mL

Horse Serum

Donor herd, USA origin, sterile-filtered, suitable for cell culture, suitable for hybridoma

ship: dry ice store at: –20°C

H1270-100ML	100 mL
H1270-500ML	500 mL
H1270-6X500ML	6 × 500 mL

Antibiotics

Gentamicin solution

[1405-41-0]

Mode of Action: Inhibits protein synthesis by binding to L6 protein of 50S ribosomal subunit.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

Garamycin; Gentiomycin C
Hybri-Max™, 50 mg/mL gentamicin, sterile-filtered, BioReagent, suitable for hybridoma

Prepared in tissue culture grade water.

liquid

endotoxin ..... tested

ship: ambient store at: 2-8°C

G1522-10ML	10 mL

Kanamycin solution from *Streptomyces kanamyceticus*

[25389-94-0]

Mode of Action: Binds to 70S ribosomal subunit; inhibits translocation; elicits miscoding.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

50 mg/mL in 0.9% NaCl, BioReagent, liquid, sterile-filtered, suitable for cell culture

Recommended for use in cell culture applications at 2 ml/L.

Stable at 37 °C for 5 days.

endotoxin ..... tested

ship: ambient store at: 2-8°C

K0254-20ML	20 mL

Penicillin–Streptomycin Solution Hybri-Max™

Pen-Strep
with 10,000 units penicillin and 10mg/ml streptomycin in 0.9% NaCl, sterile-filtered, BioReagent, suitable for hybridoma

Penicillin-Streptomycin is used to supplement cell culture media to control bacterial contamination.

liquid

endotoxin ..... tested

ship: dry ice store at: –20°C

P7539-20ML	20 mL
P7539-100ML	100 mL

Reagents

AAT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 75 µM adenine, 0.8 µM aminopterin, 16 µM thymidine.

endotoxin ..... tested

ship: dry ice store at: –20°C

A5539-1VL	1 vial

Aminopterin

4-Amino-PGA; 4-Aminopteroyl-L-glutamic acid; 4-Aminofolic acid
[54-62-6] $C_{19}H_{20}N_8O_5$ FW 440.41

Folic acid antagonist. Aminopterin is actively transported into cells by the folate transporter. In the cell, it is converted to a high molecular weight polyglutamate metabolite by folylpolyglutamate synthase that, in turn, binds to dihydrofolate reductase and inhibits its activity. Aminopterin-polyglutamate is degraded intracellularly by γ-glutamyl hydrolase.

More potent, but more toxic, than methotrexate.

ε_{261nm}^{1M}, 0.1 M NaOH 25,700
ε_{282nm}^{1M}, 0.1 M NaOH 24,500
ε_{373nm}^{1M}, 0.1 M NaOH 8,100

Hybri-Max™, 50 ×, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Aminopterin concentrate supplement for hybridoma cell culture applications.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 0.4 µM aminopterin.

endotoxin ..... tested

ship: dry ice store at: –20°C

A5159-10VL	10 vials

AT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10ml sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration:75 µM adenine, 16 µM thymidine.

endotoxin ..... tested

ship: ambient store at: –20°C

A7422-1VL	1 vial

5-Azacytidine

Ladakamycin; 4-Amino-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one
[320-67-2] $C_8H_{12}N_4O_5$ FW 244.20

A potent growth inhibitor and cytotoxic agent; inhibits DNA methyltransferase, an important regulatory mechanism of gene expression, gene activation and silencing.

Hybri-Max™, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration: 10 µM 5-azacytidine.

endotoxin ..... tested
ship: ambient store at: −20°C

A1287-1VL	1 vial

8-Azaguanine

2-Amino-6-oxy-8-azapurine; 2-Amino-6-hydroxy-8-azapurine; Guanazolo
[134-58-7] $C_4H_4N_6O$ FW 152.11

8-AzaG
Hybri-Max™, γ-irradiated, powder, BioXtra, suitable for hybridoma

For use in hybridoma cell culture applications as a myeloma selection agent.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration for 10 mg/vial: 130 µM 8-azaguanine. Vial content provided on Certificate of Analysis.

ship: ambient store at: −20°C

A5284-10VL	10 vials

Azaserine

O-Diazoacetyl-L-serine
[115-02-6] $C_5H_7N_3O_4$ FW 173.13

Hybri-Max™, γ-irradiated, 50x, lyophilized powder, BioXtra, suitable for hybridoma

For use in hybridoma cell culture applications as a myeloma selection agent.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 5.7 µM azaserine.

endotoxin ..... tested
ship: ambient store at: −20°C

A1164-.5MG	0.5 mg

Azaserine-Hypoxanthine 50x

O-Diazoacetyl-L-serine-hypoxanthine
Hybri-Max™, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Qualified for use in hybridoma cell culture applications as a selection agent.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 5.7 µM azaserine, 100 µM hypoxanthine.

endotoxin ..... tested
ship: dry ice store at: −20°C

A9666-1VL	1 vial

Carboxyethyl-γ-aminobutyric acid

CEGABA
[4386-03-2] $C_7H_{13}NO_4$ FW 175.18

Cell growth promoter

Hybri-Max™, powder, γ-irradiated, BioXtra, suitable for hybridoma

A polyamine derivative that stimulates the growth of hybridoma cells.

Stimulates hybridoma growth.

solubility
H_2O ..... (Store solution at −20°C.)
cell culture medium ..... 1.5 mg/mL (Store solution at −20°C.)
endotoxin ..... tested
ship: ambient store at: room temp

C5556-15MG	15 mg

Demecolcine solution

N-Deacetyl-N-methylcolchicine solution; Colcemid™ solution
[477-30-5]

10 µg/mL in HBSS, ACF Qualified, BioXtra

Used for cell synchronization by arresting them at metaphase. Qualified for use in animal component free applications.

sterile-filtered

liquid

ship: ambient store at: 2-8°C

D1925-10ML	10 mL
D1925-100ML	100 mL

Dimethyl sulfoxide

DMSO; Methyl sulfoxide
[67-68-5] $(CH_3)_2SO$ C_2H_6OS FW 78.13

Supercools easily and remelts slowly at room temperature. Solidified product can be re-liquified by warming to room temperature without detriment to the product.

density ..... 1.10 g/mL
n_D^{20} 1.479
vd ..... 2.7 (vs air)
vp ..... 0.42 mmHg (20 °C)
ait ..... 573 °F
lel(63 °F) 42%

Hybri-Max™, sterile-filtered, BioReagent, suitable for hybridoma, ≥99.7%

This product is a Hybri-Max product. It is hybridoma tested and is assessed for suitability in cell freezing. This product is sterile filtered (0.2 micron) and tested for endotoxin levels.

DMSO is a polar aprotic solvent used in chemical reactions, in polymerase chain reactions (PCR) and as a cryoprotectant vitrification agent for the preservation of cells, tissues and organs. DMSO is used in cell freezing media to protect cells from ice crystal induced mechanical injury. It is used for frozen storage of primary, sub-cultured, and recombinant heteroploid and hybridoma cell lines; embryonic stem cells (ESC), and hematopoietic stem cells. DMSO is frequently used in the combinations with BSA or fetal bovine serum (FBS).

5 mL and 10 mL in flame sealed ampules, 100 mL in amber bottle

endotoxin ..... tested
ship: ambient store at: room temp

D2650-5X5ML	5 × 5 mL
D2650-5X10ML	5 × 10 mL
D2650-100ML	100 mL

D-(+)-Glucose

Dextrose
[50-99-7] $C_6H_{12}O_6$ FW 180.16

Hybri-Max™, powder, BioReagent, suitable for hybridoma

Provides the primary energy source for cell metabolism.

≥99.5%

endotoxin ..... tested
ship: ambient store at: room temp

G5146-1KG	1 kg
G5146-10KG	10 kg

HAT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 100 µM hypoxanthine, 0.4 µM aminopterin, 16 µM thymidine.

endotoxin ..... tested
ship: dry ice store at: −20°C

H0262-10VL	10 vials

Histopaque®-1077 Hybri-Max™

liquid, sterile-filtered, BioReagent, suitable for hybridoma

Used to create a density medium for the purification of lymphocytes and other mononuclear cells. Histopaque®-1077 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 +/- 0.001 g/ml. This medium facilitates rapid recovery of viable lymphocytes from small volumes of whole blood. This technique is suitable for use in cell mediated lympholysis and for human lymphocyte antigen (HLA)2 typing. It may also be employed as the initial isolation step prior to enumeration of T-, B-, and 'null' lymphocytes.

endotoxin ..... tested
density ..... 1.077 g/mL, 25 °C
ship: ambient store at: 2-8°C

H8889-100ML	100 mL
H8889-500ML	500 mL

HMT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

HMT Media Supplement is a cocktail of hypoxanthine, methotrexate and thymidine for use in hybridoma selection and cell culture applications.

Reconstitute contents of vial with 10ml sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration: 100 µM hypoxanthine, 0.4 µM methotrexate, 16 µM thymidine.

endotoxin ..... tested
ship: ambient store at: −20°C

H8016-10VL	10 vials

HT Media Supplement (50×) Hybri-Max™

lyophilized powder, γ-irradiated, BioXtra, suitable for hybridoma

HT Media supplement is a cocktail hypoxanthine and thymidine qualified for use in hybridoma selection and cell culture applications.

Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 100 µM hypoxanthine, 16 µM thymidine.

endotoxin ..... tested
ship: ambient store at: −20°C

H0137-10VL	10 vials

Poly(ethylene glycol)

PEG
[25322-68-3] $H(OCH_2CH_2)_nOH$

Recommended for cell fusion.

autoclaved

endotoxin ..... tested
vd ..... >1 (vs air) ait ..... 581 °F
vp ..... <0.01 mmHg (20 °C)

Hybri-Max™, mol wt 1,300-1,600, waxy solid, BioReagent, suitable for hybridoma

ship: ambient store at: room temp

P7777-5G	5 g

Hybri-Max™, mol wt 3,000-3,700, waxy solid, BioReagent, suitable for hybridoma

ship: ambient store at: room temp

P2906-5G	5 g

Polyethylene glycol solution

PEG solution
[25322-68-3]

Packaged in sealed ampules under nitrogen.

Solution is ready-to-use. If a less concentrated solution is desired, dilute with sterile DPBS without calcium (D5773). Some precipitate may appear after being exposed to cooler, but should disappear as the solution warms. Solution may be frozen if desired but should first be aliquotted to avoid repeated freeze/thaw cycles.

endotoxin ..... tested

Hybri-Max™, 50 % (w/v), average mol wt 1,450, sterile-filtered, BioReagent, suitable for hybridoma

Contains 50% (w/v) polyethylene glycol (Av. Mol. Wt. 1450) in DPBS without calcium.

Recommended for use in a normal fusion protocol requiring 50% PEG.

ship: ambient store at: 2-8°C

P7181-5X5ML	5 × 5 mL

Hybri-Max™, average mol wt 1,450, 50 % (w/v), sterile-filtered, BioReagent, suitable for hybridoma

Contains 50% (w/v) polyethylene glycol (Av. Mol. Wt. 1450) and 10% DMSO (v/v) in DPBS without Calcium.

Recommended for use in a normal fusion protocol requiring 50% PEG and 10% DMSO.

ship: ambient store at: 2-8°C

P7306-5X5ML	5 × 5 mL

Red Blood Cell Lysing Buffer Hybri-Max™

RBC Lysing Buffer
liquid, sterile-filtered, suitable for hybridoma

Red Blood Cell Lysing Buffer has been developed for use in hybridoma protocols to remove red blood cells from mouse splenocyte suspensions before fusion. It is also useful in systems where it may be desirable to remove red blood cells from cell suspensions, such as whole blood.

Recommended for use by adding 1 mL of buffer to a cell pellet (cell pellet = 1 spleen or 100-200 million cells). Gently mix for 1 minute. Dilute the buffer with 15-20 mL of medium or salt solution. Centrifuge at 250-500 × *g* for 7 minutes and decant the supernatant. Cells may be diluted and prepared for counting or fusion. If lysis is incomplete, steps 1-4 may be repeated.

Contains 8.3 g/L ammonium chloride in 0.01 M Tris-HCl buffer.

Note: This product is intended for the removal of red blood cells from mice. This product may not be appropriate for the lysis of red blood cells of other animals. The suitability of the product in any application other than mouse splenocytes must be determined by the researcher.

endotoxin ..... tested
ship: ambient store at: room temp

R7757-100ML	100 mL

Sodium bicarbonate

Sodium hydrogen carbonate
[144-55-8] $NaHCO_3$ $CHNaO_3$ FW 84.01
pKa (25 °C) ..... (1) 6.37, (2) 10.25 (carbonic acid)

Hybri-Max™, powder, suitable for hybridoma, ≥99.5%

endotoxin ..... tested
ship: ambient store at: room temp

S4019-500G	500 g
S4019-1KG	1 kg

6-Thioguanine

2-Amino-6-mercaptopurine; 2-Amino-6-purinethiol
[154-42-7] $C_5H_5N_5S$ FW 167.19

Hybri-Max™, 50 ×, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Used in hybridoma formation applications as a selection agent.

~98%

Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 30 µM 2-amino-6-mercaptopurine.

endotoxin ..... tested
ship: ambient store at: room temp

A4660-2.5MG	2.5 mg

Hybridoma Platform Technical Information

Demecolcine (Cat. No. D1925)

Product Description

Often in karyotyping and cell cycle research it is desirable to increase the yield of mitotic cells in a particular phase of the cell cycle. This can be achieved in a variety of ways with the most popular being the use of a cell cycle synchronizing agent such as demecolcine. Demecolcine will arrest cells in metaphase with no remarkable effect on the biochemical events in mitotic cells or in synchronized G_1 and S phase cells. White blood cells are often treated with demecolcine to arrest cells in metaphase.

Recommended Usage

1) Add reconstituted demecolcine to culture medium at a final concentration of 0.4 mg/ml.
2) Incubate cultures for 4-6 hours at 37 °C.
3) Remove medium containing demecolcine, replace with fresh medium.

Dimethylsulfoxide [DMSO] (Cat. Nos. D2650 and D2438)

Product Description
Human and animal cell lines grown in culture are generally stored frozen. Freezing protects the cell line from changes due to genetic drift and minimizes the risk of contamination. Liquid nitrogen used in conjunction with a cryoprotective agent such as DMSO has become a widely used method for preserving cells. Without the presence of a cryoprotective agent, freezing is lethal to most mammalian cells. Damage is caused by mechanical injury by ice crystals, concentration of electrolytes, dehydration, pH changes, and denaturization of proteins. These lethal effects are minimized by adding a cryoprotective agent which lowers the freezing point and allows for a slower cooling rate.

DMSO has also been used in some fusion protocols in which fusion is difficult to achieve.

Recommended Usage for Cell Freezing
DMSO is supplied as a sterile liquid. It may be used as a cryoprotective agent in conjunction with complete media for preservation of cell lines at -70 °C or below.
To prepare a solution for freezing cells:
1) Prepare freezing medium containing culture medium with 10-20% serum and 5-10% DMSO.
2) Remove adherent cells with trypsin or other appropriate means. (For optimal results cells should be in log phase of growth.)
3) Gently pellet the cells by centrifugation (10 minutes at 250 x *g*, 4 °C) and remove culture medium.
4) Resuspend the cells in the freezing medium at 10^6-10^7 cells/ml.
5) Aliquot into freezing vials.
6) Freeze cells according to standard freezing protocols. Store at -70 °C or below.

Recommended Usage for Fusion
1) Prepare 40-50% PEG solution.
2) Add 10% DMSO.
3) Follow normal fusion protocols.

Product Storage
Store at room temperature.

Methotrexate (Cat. No. M8407)

Product Description
Dihydrofolate analog which acts as an inhibitor of eukaryotic dihydrofolate reductase.

Recommended Usage:
Used as a selective agent to kill non-transformed cell by inhibition of endogenous dihydrofolate reductase. Cells transformed with a plasmid carrying the bacterial dihydrofolate reductase gene are rescued from the action of methotrexate. Recommended working concentration is 0.4 mM. Please refer to the literature for specific applications.

Polyethylene Glycol [PEG] (Cat. Nos. P2906, P7181, P7306, P7777)

Product Description
Polyethylene glycol (PEG) has been found to promote cell-cell membrane fusion by acting as a lipolytic agent in which the cellular membranes are sufficiently solubilized to form a fusion product. PEG has been used successfully with many different cell fusions, some of which include spleen-myeloma and plant protoplasts.

PEG as a fusing agent is offered in two different molecular weights. Determining which molecular weight to use will depend upon several factors such as cell types to be fused, difficulty in achieving a fusion product, use of dimethyl sulfoxide (DMSO), and temperature of the fusion.

Recommended Usage
(Cat. Nos. P2906, P7777):
PEG is supplied as a sterile, waxy substance that does not lend itself readily to forming aqueous solutions at room temperature. To prepare a solution for cell fusion:
1) Melt the PEG at 45 °C or higher in a waterbath. Cat. No. P2906 will require autoclaving at 121 °C for 5 minutes to melt.
2) Dilute to 30-50% using sterile, pre-warmed serum-free medium with phenol red (e.g. for a 50% solution add 5 ml to the 5 g vial of PEG).
3) The pH of the solution should be slightly alkaline (pink, not orange or purple). Adjustment of the pH with sterile NaOH may be required.

Recommended Usage
(Cat. Nos. P7306, P7181):
These solutions are ready-to-use in a normal fusion protocol requiring 50% PEG. If a less concentrated solution is desired, dilute with sterile DPBS without calcium (Cat. No. D5773). Some precipitation may appear after being exposed to cooler temperatures. This is normal and the precipitation should disappear as the solution warms.

Product Storage
(Cat. Nos. P2906, P7777):
Solid: Room Temperature
Solution: Below 0 °C. Prolonged storage or repeated freezing and thawing is not recommended.

Product Storage
(Cat. Nos. P7306, P7181):
Store ampules at 2-8 °C. Solution may be frozen if desired, but should first be aliquotted to avoid repeated freeze/thaw cycles.

Red Blood Cell Lysing Buffer (Cat. No. R7757)

Product Description

Red Blood Cell Lysing Buffer has been developed for use in hybridoma protocols to remove red blood cells from mouse splenocyte suspensions before fusion. It is also useful in systems where it may be desirable to remove red blood cells from cell suspensions, such as whole blood. Red Blood Cell Lysing Buffer is supplied as a sterile solution and contains 0.83% ammonium chloride in 0.01 M Tris buffer.

Recommended Usage

1) Add 1 ml of buffer to the cell pellet (cell pellet = 1 spleen or 1-2 x 10^8 million cells).
2) Gently mix for 1 minute.
3) Dilute the buffer with 15-20 ml of medium or salt solution.
4) Centrifuge at 250-500 x g for 7 minutes and decant the supernatant.
5) Cells may be diluted and prepared for counting or fusion.

Note: If lysis is incomplete, steps 1-4 may be repeated.

Product Storage

Red Blood Cell Lysing Buffer is stable at room temperature.

ITS, SITE, SPIT, SPITE, Fatty Acid-Albumin Supplements

Introduction

Most cells will not survive or exhibit optimal phenotypic properties for any length of time when cultured in basal medium alone. They require supplementation with additional growth and survival factors, such as hormones, transport proteins, trace elements, or ECM factors. Traditionally, serum has been the supplement of choice to provide these factors. However, many investigators prefer to work in a serum-free culture environment to avoid the variability and contaminants that can be introduced by serum.

Serum-free formulations that substitute a purified form of the factors normally supplied by serum are suitable for many *in vitro* growth and differentiation studies. These factors include insulin, transferrin, selenium, pyruvate, and ethanolamine. Addition of other components varies greatly, depending on the cell type being studied and the basal medium employed.

Role of Components

INSULIN is a polypeptide hormone that promotes the uptake of glucose and amino acids and may owe observed mitogenic effect to this property.

TRANSFERRIN is an iron-transport protein. Iron is an essential trace element, but can be toxic in the free form. To nourish cells in culture, it is supplied bound to transferrin in serum.

SELENIUM is an essential trace element normally provided by serum.

SODIUM PYRUVATE has been shown to be beneficial as an additional energy source in some instances.

ETHANOLAMINE is a highly polar alcohol that plays a significant role in the proliferation of hybridoma cells and frequently is added to supplements used for culturing these cells.

Medium Supplements

Nutritional studies indicate that the supplement components described are utilized by most mammalian cells. They enhance cell proliferation and decrease the serum requirement of many cell types. When the following supplements are used with 2 to 4% serum, proliferation is reported to be similar to medium supplemented with 10% serum.

ITS is a mixture of bovine insulin, human transferrin (partially iron-saturated), and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham's F-12, DME/F-12, MEM) with sodium pyruvate.

SITE is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, and ethanolamine. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e. g. Ham's F-12, DME/F-12, MEM) with sodium pyruvate.

SPIT is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, and sodium pyruvate. It is designed for cell cultures in which media without sodium pyruvate are used.

SPITE is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, sodium pyruvate, and ethanolamine. It is designed for cell cultures in which media without sodium pyruvate are used.

FATTY ACID-ALBUMIN complexes have been employed as alternative sources of lipids in the development of serum-free media. Fatty acids bind to serum proteins in high proportions. Such proteins may release beneficial fatty acids and bind those that are inhibitory. Oleic acid bound to BSA has been shown to be beneficial to the growth of a variety of cell types (e.g. BHK, hybridoma). Similar observations have been made regarding linoleic acid, a precursor of prostaglandins. A mixture of polyunsaturated and monosaturated fatty acids (i.e. linoleic acid and oleic acid) used as a medium supplement has been reported to exhibit a synergistic effect.

Hybri-Max™ Reagents

Name	Reconstitution	Form	Storage	Reference	Cat. No.
AAT Media Supplement (50×) Hybri-Max™	Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 75 µM adenine, 0.8 µM aminopterin, 16 µM thymidine.	lyophilized powder	–20°C	1,2,3,4 *p. 225*	A5539-1VL
Aminopterin	Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 0.4 µM aminopterin.	lyophilized powder	–20°C	1,10,11 *p. 225*	A5159-10VL
AT Media Supplement (50×) Hybri-Max™	Reconstitute contents of vial with 10ml sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration:75 µM adenine, 16 µM thymidine.	lyophilized powder	–20°C	1,2,3,4 *p. 225*	A7422-1VL
5-Azacytidine	Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration: 10 µM 5-azacytidine.	lyophilized powder	–20°C	13,14,15 *p. 226*	A1287-1VL
8-Azaguanine	Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration for 10 mg/vial: 130 µM 8-azaguanine. Vial content provided on Certificate of Analysis.	powder	–20°C	1,10,11 *p. 226*	A5284-10VL
Azaserine	Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 5.7 µM azaserine.	lyophilized powder	–20°C	1,16,17 *p. 27*	A1164-.5MG
Azaserine-Hypoxanthine 50x	Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 5.7 µM azaserine, 100 µM hypoxanthine.	lyophilized powder	–20°C	1,16,17 *p. 226*	A9666-1VL
HAT Media Supplement (50×) Hybri-Max™	Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 100 µM hypoxanthine, 0.4 µM aminopterin, 16 µM thymidine.	lyophilized powder	–20°C	1,5,11 *p. 226*	H0262-10VL
HMT Media Supplement (50×) Hybri-Max™	Reconstitute contents of vial with 10ml sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration: 100 µM hypoxanthine, 0.4 µM methotrexate, 16 µM thymidine.	lyophilized powder	–20°C	1,5,11,21,22 *p. 226*	H8016-10VL
HT Media Supplement (50×) Hybri-Max™	Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 100 µM hypoxanthine, 16 µM thymidine.	lyophilized powder	–20°C	1,5,11 *p. 226*	H0137-10VL
OPI Media Supplement	Reconstitute contents of vial with 10 mL sterile water. Do not use cell culture medium for initial reconstitution. Each vial sufficient to prepare 1 liter of medium. Final working concentration: 1 mM oxaloacetate, 0.45 mM pyruvate, 0.2 U/ml insulin.	lyophilized powder	–20°C	23 *p. 24*	O5003-1VL
6-Thioguanine	Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 30 µM 2-amino-6-mercaptopurine.	lyophilized powder	-	1,5,7,8,9 *p. 227*	A4660-2.5MG

References

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Insect Platform

The development of genetically engineered cell lines to express therapeutically valuable recombinant proteins has created a major break-through in health care. While the earliest methods of expressing recombinant proteins relied on bacterial systems, it is now recognized that the pharmacology and efficacy of recombinant proteins produced for therapeutic applications are affected by post-translational modifications such as glycosylation and phosphorylation. This has led to the use of higher order cell systems such as insect cell systems.

Insect cells have the ability to glycosylate and further modify proteins, and a number of recombinant proteins of potential therapeutic value have been expressed in insect cell systems. One of the most important insect cell expression systems for this type of application utilizes baculoviruses, propagated in the army worm, *Spodoptera frugiperda*, to produce recombinant proteins.

Because of the potential therapeutic applications of these proteins, there has been an increased emphasis on eliminating animal-source components from the media used to grow these cells. To address that need, Sigma-Aldrich has developed a number of serum-free media for insect cell culture applications.

Manufacturing and Testing Specifications

- All component chemicals are Sigma research biochemicals which meet our normal specifications.
- All media are produced in our controlled-environment facilities under systems designed to control air pressure, humidity, temperature, and particulate matter. To avoid the possibility of airborne cross-contamination, media dust is controlled with a specially engineered dust collector and air filtration system.
- Media are manufactured and tested in accordance with current Good Manufacturing Practices (cGMP).
- Powdered media are packaged in wide-mouth, amber glass bottles with torque-activated styrene inner seals. We find this method of packaging provides product stability and facilitates full recovery of material from the bottle.
- Classic media are produced according to original published formulas or accepted modifications.
- The water used in all production and testing is our USP-purified tissue culture grade water. It is deionized through mixed-bed resins and further filtered to remove particles >10,000 Da.
- To ensure in-lot homogeneity, minimal lot-to-lot variation, and formula integrity, several physical and chemical parameters are measured on finished powdered media. This includes amino acid analysis.
- Powdered media are preweighed to prepare the amount of liquid indicated in the listing and on the label.

Liquid Media Characteristics

Specification	Result
1. Appearance	Clear solution.
2. pH at Room Temperature (RT)	Specific to individual product.
3. Osmolality	Specific to individual product.
4. Sterility	This product is sterilized by filtration using a membrane with a porosity of 0.22 microns or less. Sterility is determined by protocols described in USP XXIII.
5. Endotoxin level	Specific to individual product.

Powdered Media Characteristics

Specification	Result
1. Appearance	White to off-white powder.
2. Moisture content	5.0% or less.
3. Solubility	Clear solution at 1× concentration
4. pH at room temperature (RH)—without sodium bicarbonate	Specific to individual product
5. pH at room temperature (RH)—with sodium bicarbonate	Specific to individual product
6. Osmolalityâ without sodium bicarbonate	Specific to individual product
7. Osmolality—with sodium bicarbonate	Specific to individual product

Biological Performance Protocol

The growth-promoting capacities of our insect cell culture media are assessed in a test medium supplemented with 10% heat-inactivated Fetal Bovine Serum using cell lines that represent a cross section of cells supported by the medium. Growth studies are carried through two subculture generations. Cells are counted, and growth is plotted as a logarithmic function of time in culture. Doubling times and final cell densities are determined. During the testing period, cultures are examined microscopically for atypical morphology and evidence of cytotoxicity.

Additional Information

- **Bulk Quantities:** Prices for powdered media are listed for up to 490 liters. We invite requests for quotation on orders of 500 liters or greater.
- **Lot Reservations:** To reserve specific lots of media for purchase on a standing order or as-needed basis, please contact your local Sigma office or representative.
- **Special Application/Packaging Needs:** Please call us to discuss your requirements.

Insect Media

EX-CELL® 405 Serum-Free Medium for Insect Cells

Baculoviruses are powerful expression systems for production of recombinant proteins. The most well known and highly investigated baculovirus vector expression system (BEVS) uses *Spodoptera frugiperda* cells (especially Sf9) as the host cell of choice for infection with recombinant *Autographa californica* nuclear polyhedrosis virus for heterologous protein expression. Recent investigations have shown that other cell lines may be better expression substrates than spodopteran cells. The BTI-TN-5B1-4 clone of *Trichoplusia ni*, commonly referred to as High Five™, is reported to be a superior host substrate for the expression of selected recombinant proteins using BEVS. This cell line portrays strong adherent characteristics, but can be adapted to suspension growth.

EX-CELL® 405 is a serum-free medium for optimal growth and viability of BTI-TN-5B1-4 (Tn5, High Five) cells. In addition, EX-CELL 405 supports recombinant protein production in High Five cells to higher levels than reported for Sf9 cells. EX-CELL 405 is a complete medium. No protein supplements need to be added to this medium prior to use.

for research or for further manufacturing use

with L-glutamine, liquid, sterile-filtered, suitable for insect cell culture

Drug Master File available

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

14405C-500ML	500 mL
14405C-1000ML	1000 mL

Protein-free, with L-glutamine, without sodium bicarbonate, dry powder, suitable for insect cell culture NEW

Formulated to contain 42.7 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24405C-1L	1 L
24405C-5L	5 L
24405C-10L	10 L
24405C-50L	50 L

EX-CELL® 420 Serum-Free Medium for Insect Cells

EX-CELL 420 is a complete medium developed and optimized for the serum-free growth of Sf9 and Sf21 insect cell lines. Cells can be subcultured directly into EX-CELL 420 from serum-free or seum-supplemented media without adaptation. Cultures in EX-CELL 420 routinely reach cell densities greater than 1×10^7 cells/mL with greater than 95% viability. Suspension cultures can be maintained, without refeeding, for more than 10 days. Sf9 and Sf21 cells have been carried for more than 20 passages in EX-CELL 420 with no loss of viability. Protein expression and virus production are improved over serum-containing media.

for research or for further manufacturing use

Protein-free, with L-glutamine, suitable for, sterile-filtered, suitable for insect cell culture

Drug Master File available

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

14420C-500ML	500 mL
14420C-1000ML	1000 mL

Protein-free, without L-glutamine, liquid, sterile-filtered, suitable for insect cell culture

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

14419C-500ML	500 mL
14419C-1000ML	1000 mL
14419C-10B	10 L

with L-glutamine, without sodium bicarbonate, dry powder, suitable for insect cell culture NEW

Formulated to contain 30.8 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24420C-1L	1 L
24420C-5L	5 L
24420C-10L	10 L
24420C-50L	50 L

EX-CELL® TiterHigh™ Medium

Animal-component free, liquid, sterile-filtered, suitable for insect cell culture

Animal component-free, protein-free formulation designed specifically for the *Sf*21 and *Sf*9 (*Spodoptera frugiperda*) insect cell lines

Features and Benefits

Designed to meet the needs of the biopharmaceutical industry, this animal component-free medium supports fast cell growth rates and high cell densities, while maintaining high cell viability and high recombinant protein production using the Baculovirus Expression Vector System (BEVS).

for research or for further manufacturing use

This formulation includes inorganic salts, sodium bicarbonate, essential and non-essential amino acids, vitamins, yeast extract, a proprietary lipid formulation, trace elements, and other organic compounds. Glutamine is supplied in the form of an alanine-glutamine dipeptide. It does not contain phenol red, antibiotics, antimycotics, transferrin, and products of animal origin.

Do not freeze. Protect from light. Several components of the medium are light labile and should not be exposed to light for lengthy periods of time.

ship: ambient store at: 2-8°C

I5408-1L	1 L
I5408-6X1L	6 × 1 L

Grace's Insect Medium

Grace's Insect Medium

Grace's medium was originally formulated to support the growth of cells derived from the Australian emperor gum moth, *Antherea eucalypti*. The medium is a modification of Wyatt's medium to more closely resemble *Antherea* hemolymph. The cell lines established by Grace using this medium were the first continuous lines developed. The basal medium, when properly supplemented, has been used to culture cells derived from a variety of insects including several species of lepidopterans as well as some dipterans. The medium is primarily used as a basal medium for the growth and maintenance of cell lines derived from lepidopterans.

With L-glutamine, without sodium bicarbonate, powder, suitable for insect cell culture

suitable for several dipteran and lepidopteran cell lines

ship: ambient store at: 2-8°C

G9771-1L	1 L
G9771-10X1L	10 × 1 L
G9771-2X5L	2 × 5 L
G9771-50L	50 L

With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for insect cell culture

ship: ambient store at: 2-8°C

G8142-100ML	100 mL
G8142-500ML	500 mL
G8142-6X500ML	6 × 500 mL
G8142-1L	1 L
G8142-6X1L	6 × 1 L

IPL-41 Insect Medium

IPL-41 Insect Medium

With L-glutamine and sodium bicarbonate., liquid, sterile-filtered, suitable for insect cell culture

IPL-41 is part of a series of media originally developed at the USDA Insect Pathology Laboratory by Weiss et al. for the large scale propagation of cells from the fall armyworm, *Spodoptera frugiperda*. The medium is primarily used for the growth and maintenance of cell lines derived from lepidopterans and the propagation of viruses in these cell lines. One of the most significant uses of the medium is the large scale culture of baculovirus infected *Spodoptera* cells. The expression of recombinant proteins is undertaken from *Spodoptera* cells utilizing the baculovirus expression system (BEVS). Attempts have also been made to obtain the expression of foreign genes in cells cultured in IPL-41 without serum supplementation.

ship: ambient store at: 2-8°C

I7760-500ML	500 mL

Schneider's Insect Medium

Schneider's Insect Medium

Originally developed for the culture of Drosophila; suitable for culture of other dipteran cell lines.

With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for insect cell culture

suitable for culture of other dipteran cell lines (Originally developed for the culture of Drosophila cells)

ship: ambient store at: 2-8°C

S0146-100ML	100 mL
S0146-500ML	500 mL
S0146-6X500ML	6 × 500 mL
S0146-1L	1 L
S0146-6X1L	6 × 1 L

With L-glutamine, without calcium chloride and sodium bicarbonate, powder, suitable for insect cell culture

concentration ..... 24.5 g/L

ship: ambient store at: 2-8°C

S9895-1L	1 L
S9895-10X1L	10 × 1 L
S9895-10L	10 L

Serum-free Insect Medium-1 Protein-free

liquid, sterile-filtered, With L-glutamine and sodium bicarbonate, suitable for insect cell culture

endotoxin ..... tested

ship: ambient store at: 2-8°C

S3777-500ML	500 mL
S3777-6X500ML	6 × 500 mL
S3777-1L	1 L
S3777-6X1L	6 × 1 L

Shields and Sang M3 Insect Medium

Shields and Sang M3 Insect Medium

Developed for the culture of Drosophila cell lines.

With L-glutamine and potassium bicarbonate., liquid, sterile-filtered, suitable for insect cell culture

ship: ambient store at: 2-8°C

S3652-500ML	500 mL
S3652-6X500ML	6 × 500 mL
S3652-1L	1 L
S3652-6X1L	6 × 1 L

With L-glutamine, without potassium bicarbonate, powder, suitable for insect cell culture

Use at 39.4 g/L.

ship: ambient store at: 2-8°C

S8398-1L	1 L
S8398-10X1L	10 × 1 L
S8398-2X5L	2 × 5 L

TC-100 Insect Medium

TC-100 Insect Medium

BML-TC/10
With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for insect cell culture

For baculovirus culturing in lepidoptera.

ship: ambient store at: 2-8°C

T3160-500ML	500 mL
T3160-6X500ML	6 × 500 mL
T3160-1L	1 L
T3160-6X1L	6 × 1 L

TNM-FH Insect Medium

Hink's TNM-FH Insect Medium NEW

TNM-FH insect medium

supplemented Grace's, with 4.1 mM L-glutamine, with 3.33 g/L lactalbumin hydrolysate (LAH), with 3.33 g/L ultrafiltered yeastolate, liquid, sterile-filtered, suitable for insect cell culture

Hink's TNM-FH medium was developed in 1970 for the culture of cabbagelooper, *Tricoplusi in* cell. Hink's TNM-FH medium is a modified Grace's formulation containing lactalbumin hydrolysate (LAH) and ultrafiltered yeastolate

for research or for further manufacturing use

ship: ambient store at: 2-8°C

51942C-500ML	500 mL
51942C-1000ML	1000 mL

TNM-FH insect medium

A number of modifications of Grace's medium were developed in several laboratories in the late 1960's and 1970's. Many of these modifications were developed by Dr. W.F. Hink for the growth of cells derived from the cabbage looper, *Tricoplusia ni*. This formula represents the modification known as TNM-FH. The medium when properly supplemented has been found to support the growth of cells derived from a variety of lepidopteran species.

With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for insect cell culture

ship: ambient store at: 2-8°C

T3285-500ML	500 mL
T3285-6X500ML	6 × 500 mL
T3285-1L	1 L
T3285-6X1L	6 × 1 L

Graces Modified Medium
With L-glutamine, without sodium bicarbonate, powder, suitable for insect cell culture

suitable for culture of other lepidopteran cell lines (Originally developed for the culture of *Trichoplusia ni* cells)

concentration ..... 50.6 g/L

ship: ambient store at: 2-8°C

T1032-1L	1 L
T1032-10X1L	10 × 1 L
T1032-10L	10 L
T1032-50L	50 L

Companion Products and Reagents

Agarose, low gelling temperature

2-Hydroxyethyl agarose
[39346-81-1]

BioReagent, suitable for cell culture, suitable for insect cell culture

A low gelling temperature derivative with unique gelling properties. Gels form at <30 °C, remelt at temperatures in excess of 60 °C. Gels exhibit excellent clarity and are particularly useful for the preparation of media containing heat-labile materials.

solubility
H_2O ..... 10 mg/mL (with heat)

ship: ambient store at: room temp

A9045-5G	5 g
A9045-10G	10 g
A9045-25G	25 g
A9045-50G	50 g
A9045-100G	100 g
A9045-250G	250 g

4% Agarose gel

sterile-filtered, suitable for insect cell culture

Matrix useful for protein MALDI-MS.

ship: ambient store at: room temp

A6689-40ML	40 mL

Ala-Gln

Alanyl-glutamine; Glutamine-S
[39537-23-0] $C_8H_{15}N_3O_4$ FW 217.22

BioReagent, suitable for cell culture, suitable for insect cell culture

Alanyl-glutamine (Ala-Gln) is a dipeptide that is used as a substitute for glutamine in mammalian cell culture media. Glutamine is unstable in solution and forms ammonia which can have deleterious effects on cells in culture. Ala-Glu is stable to heat sterilization and is less ammoniagenic than glutamine, which contributes to its advantages as a media component.

This product is cell culture tested (0.5 mg/ml) and insect cell culture tested (0.2 mg/ml). It is appropriate for use in cell culture and insect cell culture applications at 2-10 mM.

ship: ambient store at: room temp

A8185-5G	5 g
A8185-100G	100 g
A8185-1KG	1 kg

β-Alanine

β-Ala; 3-Aminopropionic acid
[107-95-9] $NH_2CH_2CH_2COOH$ $C_3H_7NO_2$ FW 89.09

Endogenous β-amino acid that is a nonselective agonist at glycine receptors and a ligand for the G protein-coupled orphan receptor, TGR7 (MrgD). β-alanine flux plays a cytoprotective role by supporting the osmotic stability of marine organisms, preimplantation mouse embryos and mammalian cells exposed to hypoxic stress.

BioReagent, suitable for cell culture, suitable for insect cell culture

An N-blocked form of alanine.

ship: ambient store at: room temp

A9920-10MG	10 mg
A9920-100G	100 g

Aluminum potassium sulfate dodecahydrate

Potassium aluminum sulfate dodecahydrate; Alum; Potassium alum

[7784-24-9] $AlK(SO_4)_2 \cdot 12H_2O$ $AlKO_8S_2 \cdot 12H_2O$ FW 474.39

density ..... 1.757 g/mL, 25 °C

BioReagent, suitable for insect cell culture

ship: ambient store at: room temp

A6435-100G	100 g

Ammonium molybdate tetrahydrate

Molybdic acid ammonium salt tetrahydrate; Ammonium heptamolybdate tetrahydrate

[12054-85-2] $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $H_{24}Mo_7N_6O_{24} \cdot 4H_2O$ FW 1235.86

density ..... 2.498 g/mL, 25 °C

BioReagent, suitable for cell culture, suitable for insect cell culture, 81.0-83.0% MoO_3 basis

ship: ambient store at: room temp

M1019-100G	100 g

L-Asparagine

L-Aspartic acid 4-amide; (*S*)-2-Aminosuccinic acid 4-amide

[70-47-3] $H_2NCOCH_2CH(NH_2)CO_2H$ $C_4H_8N_2O_3$ FW 132.12

BioReagent, suitable for cell culture, suitable for insect cell culture

≥98% (TLC)

ship: ambient store at: room temp

A4159-25G	25 g
A4159-100G	100 g
A4159-500G	500 g

L-Aspartic acid

(*S*)-(+)-Aminosuccinic acid; (*S*)-Aminobutanedioic acid

[56-84-8] $HO_2CCH_2CH(NH_2)CO_2H$ $C_4H_7NO_4$ FW 133.10

Principal neurotransmitter for fast synaptic excitation.

BioReagent, suitable for cell culture, suitable for insect cell culture

≥98% (TLC)

ship: ambient store at: room temp

A4534-100G	100 g
A4534-500G	500 g

Biotin

Vitamin H; D-Biotin; Bios II; Vitamin B7; Coenzyme R

[58-85-5] $C_{10}H_{16}N_2O_3S$ FW 244.31

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

Essential vitamin that is important for amino acid and energy metabolism, and fatty acid synthesis. It is a prosthetic group in four mammalian carboxylase families and facilitates the binding and transfer of carbon dioxide.

solubility

H_2O ..... 0.2 mg/mL (Solubility increases with addition of 1 N NaOH.)

ship: ambient store at: 2-8°C

B4639-500MG	500 mg
B4639-1G	1 g
B4639-5G	5 g

Calcium carbonate

[471-34-1] $CaCO_3$ $CCaO_3$ FW 100.09

density ..... 2.93 g/mL, 25 °C

BioReagent, suitable for insect cell culture, ~99%

ship: ambient store at: room temp

C5929-100G	100 g
C5929-500G	500 g

Calcium chloride

[10043-52-4] $CaCl_2$ FW 110.98

vp ..... 0.01 mmHg (20 °C)

anhydrous, BioReagent, suitable for insect cell culture, suitable for plant cell culture, ≥96.0%

ship: ambient store at: room temp

C5670-100G	100 g
C5670-500G	500 g

Calcium chloride dihydrate

[10035-04-8] $CaCl_2 \cdot 2H_2O$ FW 147.01

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

C7902-500G	500 g
C7902-1KG	1 kg

Casein from bovine milk

[9000-71-9]

Numerous experimental applications including use as a blocking agent in immunochemistry, recovery of enzyme activity from SDS extracted samples, and as a substrate for protease and kinase assays.

BioReagent, suitable for insect cell culture

ship: ambient store at: room temp

C6554-500G	500 g

vitamin free, BioReagent, suitable for insect cell culture

ship: ambient store at: room temp

C5679-500G	500 g

α-Cellulose

[9004-34-6]

BioReagent, suitable for insect cell culture

Non-nutritive bulk

ship: ambient store at: room temp

C6429-500G	500 g
C6429-5KG	5 kg

Choline chloride

(2-Hydroxyethyl)trimethylammonium chloride
[67-48-1] $(CH_3)_3N(Cl)CH_2CH_2OH$ $C_5H_{14}ClNO$ FW 139.62

Acyl group acceptor

Choline acetyltransferase substrate

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98%

A source of methyl groups for methyl-transfer reactions.

solubility
H_2O ..... 50 mg/mL

ship: ambient store at: room temp

C7527-100G	100 g
C7527-500G	500 g
C7527-1KG	1 kg

Cobalt(II) chloride hexahydrate

Cobaltous chloride hexahydrate
[7791-13-1] $CoCl_2 \cdot 6H_2O$ $Cl_2Co \cdot 6H_2O$ FW 237.93

vp ..... 40 mmHg (0 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture

ship: ambient store at: room temp

C8661-25G	25 g
C8661-100G	100 g

Cod liver oil fatty acid methyl esters

BioReagent, suitable for insect cell culture

Natural distribution of saturated and unsaturated fatty acids as methyl esters. With 15-25% ω-3 (octadecatetraenoic, eicosapentaenoic and docosahexaenoic) methyl esters.

Prepared by transmethylation of cod liver oil.

ship: ambient store at: 2-8°C

C2294-1G	1 g
C2294-5G	5 g

Fetal Bovine Serum

FBS

composition
Hemoglobin ≤20 mg/dL

Endotoxin and hemoglobin tested

endotoxin ..... ≤10 EU/mL

sterile-filtered, USA origin, suitable for insect cell culture

FBS is inherently variable in performance from lot-to-lot and is often tested and matched to specific cell culture applications. This FBS is tested and qualified for insect cell culture applications.

ship: dry ice store at: –20°C

F0643-100ML	100 mL
F0643-500ML	500 mL
F0643-6X500ML	6 × 500 mL

Heat Inactivated, USA origin, sterile-filtered, suitable for insect cell culture

Heat inactivated FBS is used in special cell culture applications where complement activity (normally present) would interfere with the cell culture objectives.

Prepared by heating at 56 °C for 30 minutes.

ship: dry ice store at: –20°C

F3018-500ML	500 mL

Folic acid

Vitamin M; PteGlu; Pteroyl-L-glutamic acid
[59-30-3] $C_{19}H_{19}N_7O_6$ FW 441.40

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥97%

A nutritional delivery form of folate. Folic acid and its derivatives are essential mediators of one-carbon metabolism within cells.

ship: ambient store at: room temp

F8758-5G	5 g
F8758-25G	25 g

D-(–)-Fructose

D-Levulose; Fruit sugar
[57-48-7] $C_6H_{12}O_6$ FW 180.16

BioReagent, suitable for cell culture, suitable for insect cell culture

Glucose ..... <0.05% (enzymatic)

ship: ambient store at: room temp

F3510-100G	100 g
F3510-500G	500 g
F3510-5KG	5 kg

Fumaric acid

[110-17-8] HOOCCH=CHCOOH $C_4H_4O_4$ FW 116.07
vp ..... 1.7 mmHg (165 °C) lel 40%
ait ..... 1364 °F

BioReagent, suitable for cell culture

A TCA (Krebs cycle) molecule used as a supplement in insect and mammalian cell culture.

ship: ambient store at: room temp

F8509-100G	100 g

D-(+)-Galactose

[59-23-4] $C_6H_{12}O_6$ FW 180.16

powder, anhydrous, BioReagent, suitable for cell culture, suitable for insect cell culture

solubility
H_2O ..... 100 mg/mL
Glucose ..... ≤2%

ship: ambient store at: room temp

G5388-100G	100 g
G5388-500G	500 g
G5388-1KG	1 kg

D-(+)-Glucose

Dextrose
[50-99-7] $C_6H_{12}O_6$ FW 180.16

powder, BioReagent, suitable for insect cell culture, ≥99.5%

Mixed anomers

Provides the primary energy source for cell metabolism.

solubility
H_2O ..... 0.13 g/mL

ship: ambient store at: room temp

G7021-100G	100 g
G7021-1KG	1 kg
G7021-5KG	5 kg
G7021-10KG	10 kg

L-Glutamic acid monosodium salt hydrate

Glu; (*S*)-2-Aminopentanedioic acid; Monosodium glutamate; MSG; L-Glutamic acid monosodium salt
$C_5H_8NNaO_4 \cdot xH_2O$ FW 169.11 (Anh)

Agonist at kainate, NMDA, and quisqualate receptors; an excitatory amino acid neurotransmitter.

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

G5889-100G	100 g
G5889-500G	500 g
G5889-1KG	1 kg

L-Glutamic acid potassium salt monohydrate

Potassium L-glutamate; Glu; L-α-Aminoglutaric acid potassium salt; L-2-Amino-pentanedioic acid potassium salt; (*S*)-2-Aminopentanedioic acid
[6382-01-0] $C_5H_8KNO_4 \cdot H_2O$ FW 203.23

Agonist at kainate, NMDA, and quisqualate receptors; an excitatory amino acid neurotransmitter.

BioReagent, suitable for insect cell culture, ≥99% (TLC)

ship: ambient store at: room temp

G1149-100G	100 g
G1149-500G	500 g

Glycerol

1,2,3-Propanetriol; Glycerin
[56-81-5] $HOCH_2CH(OH)CH_2OH$ $C_3H_8O_3$ FW 92.09

Glycerol is used both in sample preparation and gel formation for polyacrylamide gel electrophoresis. Glycerol (5-10%) increases the density of a sample so that the sample will layer at the bottom of a gel's sample well. Glycerol is also used to aid in casting gradient gels and as a protein stabilizer and storage buffer component.

density ..... 1.25 g/mL
n_D^{20} 1.474
vd ..... 3.1 (vs air)
vp ..... <1 mmHg (20 °C)
ait ..... 698 °F

BioReagent, suitable for cell culture, suitable for insect cell culture, ~99% (GC)

ship: ambient store at: room temp

G2025-100ML	100 mL
G2025-500ML	500 mL

Halocarbon oil 27

[9002-83-9]

ship: ambient store at: room temp

H8773-100ML	100 mL

Halocarbon oil 700

[9002-83-9]

ship: ambient store at: room temp

H8898-50ML	50 mL
H8898-100ML	100 mL

Hygromycin B from *Streptomyces hygroscopicus*

[31282-04-9] $C_{20}H_{37}N_3O_{13}$ FW 527.52

Mode of Action: Blocks polypeptide synthesis and inhibits elongation. For use in the selection and maintenance of prokaryotic and eukaryotic cells.

powder, BioReagent, suitable for cell culture, suitable for insect cell culture

Recommended for use as a selection agent at 100-800 µg/mL.

≥60% (HPAE)

Purified by ion exchange chromatography

solubility
H_2O .. 50 mg/mL (As a stock solution. Stock solutions should be stored at 2-8°C. Stable at 37°C for 30 days.)

ship: ambient store at: 2-8°C

H3274-50MG	50 mg
H3274-100MG	100 mg
H3274-5X100MG	5 × 100 mg
H3274-250MG	250 mg
H3274-1G	1 g

myo-Inositol

1,2,3,4,5,6-Hexahydroxycyclohexane; *i*-Inositol; *meso*-Inositol
[87-89-8] $C_6H_{12}O_6$ FW 180.16

A component of membrane phospholipids, glycosylphosphatidylinositol anchors that bind glycoproteins to cell membranes, and inositol phosphate second messengers.

vd ..... 6.2 (vs air)

BioReagent, suitable for cell culture, suitable for insect cell culture

ship: ambient store at: room temp

I7508-50G	50 g
I7508-100G	100 g
I7508-500G	500 g
I7508-1KG	1 kg

Insect Medium Supplement (10×)

liquid, sterile-filtered, BioReagent, suitable for insect cell culture

Proprietary mixture of amino acids, lipids, trace elements, vitamins and other growth enhancing components in an aqueous solution.

A serum replacement for general cell growth and maintenance.
May be used in the preparation of low protein medium. In many instances the level of serum can be reduced by 50-75% in cultures containing 10% medium supplement. The low protein content (<150 µg/ml) also makes the product suitable for use in preparing low protein serum-free medium. Serum-free medium can be prepared by adding the medium supplement to a final concentration of 10% to an appropriate cell culture medium. The final protein content of the medium will be dependent upon the level of proteins (if any) present in the basal medium. In many instances the adaptation of cells to growth in serum-free medium may be required. Also recommended for use with serum to improve cell growth and maintenance.

ship: ambient store at: 2-8°C

I7267-100ML	100 mL
I7267-500ML	500 mL

Iron(III) phosphate

Ferric phosphate
[58782-48-2] $FePO_4$ FeO_4P FW 150.82

BioReagent, suitable for insect cell culture

Compound is a mixture of ferric phosphate and sodium citrate.

Iron(II) sulfate heptahydrate is used in cell culture applications generally bound to transferrin, citrate or other iron transport molecules.

ship: ambient store at: room temp

F1523-500G	500 g

Iron(II) sulfate heptahydrate

Ferrous sulfate heptahydrate
[7782-63-0] $FeSO_4 \cdot 7H_2O$ $FeO_4S \cdot 7H_2O$ FW 278.01

density ..... 1.898 g/mL, 25 °C
vp ..... 14.6 mmHg (25 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

Iron(II) sulfate heptahydrate is used in cell culture applications generally bound to transferrin, citrate or other iron transport molecules.

ship: ambient store at: room temp

F8633-250G	250 g
F8633-1KG	1 kg

α-Ketoglutaric acid

2-Oxoglutaric acid; 2-Oxopentanedioic acid
[328-50-7] $HOOCCH_2CH_2COCOOH$ $C_5H_6O_5$ FW 146.10

BioReagent, suitable for cell culture, suitable for insect cell culture

A TCA (Krebs cycle) molecule used as a supplement in insect and mammalian cell culture.

ship: ambient store at: 2-8°C

K1128-5G	5 g
K1128-25G	25 g
K1128-100G	100 g

Lactalbumin enzymatic hydrolysate

powder, BioReagent, suitable for cell culture

Use as an amino acid, peptide rich supplement in mammalian and microbial cell culture applications.

nitrogen analysis ..... ~6.25% amino, ≥11.0% total

ship: ambient store at: 2-8°C

L9010-500G	500 g
L9010-1KG	1 kg

Lipid Medium Supplement

liquid, sterile-filtered, BioReagent, suitable for cell culture, suitable for insect cell culture

Originally developed for the serum-free growth of *Spodoptera frugiperda (Sf9)* cells in IPL-41 Insect Medium.

ship: ambient store at: 2-8°C

L2273-100ML	100 mL

Lipid Mixture (1000×)

liquid, sterile-filtered, BioReagent, suitable for insect cell culture

composition
cholesterol 4.5 g/L
cod liver oil fatty acids (methyl esters) 10 g/L
polyoxyethylenesorbitan monooleate 25 g/L
D-α-tocopherol acetate 2.0 g/L

ship: ambient store at: 2-8°C

L5146-100ML	100 mL

Lipids Cholesterol Rich from adult bovine serum

liquid, sterile-filtered, BioReagent, suitable for cell culture, suitable for insect cell culture

Aqueous lipoprotein solution

composition
Cholesterol 9.0-11.0 g/L
Protein 15.0-25.0 g/L

Adventitious viral agents ..... none detected (9 CFR; 113.53)
endotoxin ..... ≤6.0 EU/mg cholesterol
IgG ..... none detected (RID)
Microbial content ..... none detected (USP XXII)
Mycoplasma ..... none detected (Barile method)

ship: ambient store at: 2-8°C

L4646-20ML	20 mL
L4646-100ML	100 mL

Magnesium chloride

[7786-30-3] $MgCl_2$ Cl_2Mg FW 95.21

density ..... 2.32 g/mL, 25 °C

BioReagent, suitable for insect cell culture, ≥97.0%

ship: ambient store at: room temp

M4880-100G	100 g

Magnesium chloride hexahydrate

[7791-18-6] $MgCl_2 \cdot 6H_2O$ $Cl_2Mg \cdot 6H_2O$ FW 203.30

BioReagent, suitable for cell culture, suitable for insect cell culture

ship: ambient store at: room temp

M2393-100G	100 g
M2393-500G	500 g

Magnesium sulfate

[7487-88-9] $MgSO_4$ FW 120.37

vd ..... <0.01 (vs air)
vp ..... <0.1 mmHg (20 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture

ship: ambient store at: room temp

M2643-500G	500 g
M2643-1KG	1 kg

L-(−)-Malic acid

(*S*)-(−)-2-Hydroxysuccinic acid; L-Hydroxybutanedioic acid
[97-67-6] $HO_2CCH_2CH(OH)CO_2H$ $C_4H_6O_5$ FW 134.09

BioReagent, suitable for cell culture, suitable for insect cell culture

A TCA (Krebs cycle) intermediate and partner in the malic acid aspartate shuttle.

95-100% (enzymatic)

ship: ambient store at: room temp

M7397-25G	25 g
M7397-100G	100 g
M7397-500G	500 g

D-(+)-Maltose monohydrate

Maltobiose; 4-O-α-D-Glucopyranosyl-D-glucose
[6363-53-7] $C_{12}H_{22}O_{11} \cdot H_2O$ FW 360.31

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98%

solubility
H_2O ..... 50 mg/mL
Glucose ..... ≤0.3 mol %
Maltotriose ..... ≤1%

ship: ambient store at: room temp

M5895-500G	500 g
M5895-1KG	1 kg

Manganese(II) chloride tetrahydrate

[13446-34-9] $MnCl_2 \cdot 4H_2O$ $Cl_2Mn \cdot 4H_2O$ FW 197.91

BioReagent, suitable for insect cell culture

ship: ambient store at: room temp

M5005-100G	100 g

DL-Methionine

DL-2-Amino-4-(methylthio)butanoic acid; (±)-2-Amino-4-(methylmercapto)butyric acid
[59-51-8] $CH_3SCH_2CH_2CH(NH_2)COOH$ $C_5H_{11}NO_2S$ FW 149.21

BioReagent, suitable for cell culture, suitable for insect cell culture, ~99.5%

Use in mammalian and insect cell culture applications where mixed isomers are indicated.

ship: ambient store at: room temp

M2768-100G	100 g

Methotrexate hydrate

L-Amethopterin hydrate; L-4-Amino-N^{10}-methylpteroylglutamic acid hydrate; MTX hydrate; Methylaminopterin hydrate; 4-Amino-10-methylfolic acid hydrate; Antifolan hydrate
[133073-73-1] $C_{20}H_{22}N_8O_5 \cdot xH_2O$ FW 454.44 (Anh)

Potent inhibitor of dihydrofolate reductase[1] and agent for antitumor studies.[2,3] Use to inhibit dihydrofolate reductase in DHFR-based protein expression systems.

Lit. cited: 1. Sasso, S.P., et al., *Biochim. Biophys. Acta* **1207**, 74 (1994)
2. Huennekens, F.M., *Adv. Enzyme Regul.* **34**, 397 (1994)
3. Nagy, A., et al., *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6373 (1993)

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98% (HPLC)

Methotrexate used to inhibit dihyrofolate reductase and select for DHFR overexpresssion.

Recommended for use in cell culture and molecular biology applications at 0.01-300 µM in nucleoside-free cell culture medium to select for DHFR expression.

solubility
H_2O ..... insoluble

ship: ambient store at: −20°C

M8407-100MG	100 mg
M8407-500MG	500 mg

Methyl 4-hydroxybenzoate

Methyl paraben; *p*-Hydroxybenzoic acid methyl ester
[99-76-3] $HOC_6H_4CO_2CH_3$ $C_8H_8O_3$ FW 152.15

BioReagent, suitable for insect cell culture

Use in studies on sterol hormones such as testerone biosynthesis.

ship: ambient store at: room temp

H3647-100G	100 g
H3647-1KG	1 kg

Nicotinamide

Vitamin PP; Pyridine-3-carboxylic acid amide; Niacinamide; Nicotinic acid amide; Vitamin B_3
[98-92-0] $C_6H_6N_2O$ FW 122.12

BioReagent, suitable for cell culture, suitable for insect cell culture

ship: ambient store at: room temp

N0636-100G	100 g
N0636-500G	500 g

Nicotinic acid

Vitamin B_3; Niacin; Pyridine-3-carboxylic acid; 3-Picolinic acid; Pellagra preventive factor
[59-67-6] $C_6H_5NO_2$ FW 123.11

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98%

ship: ambient store at: room temp

N0761-100G	100 g
N0761-500G	500 g

Oxaloacetic acid

Oxobutanedioic acid; 2-Oxosuccinic acid; Oxalacetic acid; Ketosuccinic acid
[328-42-7] $HOOCCH_2COCOOH$ $C_4H_4O_5$ FW 132.07

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥97%

Use as a TCA (Krebs cycle) intermediate supplement in insect cell culture applications. Enhances insect cell growth and productivity.

solubility
H_2O ..... 100 mg/mL
ship: ambient store at: –20°C

O7753-5G	5 g
O7753-25G	25 g
O7753-100G	100 g

D-Pantothenic acid hemicalcium salt

Calcium D-pantothenate; (*R*)-(+)-N-(2,4-Dihydroxy-3,3-dimethyl-1-oxobutyl)-β-alanine hemicalcium salt; Vitamin B_5
[137-08-6] $HOCH_2C(CH_3)_2CH(OH)CONHCH_2CH_2CO_2 \cdot 1/2Ca$ $C_9H_{16}NO_5 \cdot 0.5Ca$
FW 238.27

Precursor in the biosynthesis of coenzyme A.

Due to the unstable, hygroscopic nature of the free acid, the calcium salt is employed.

BioReagent, suitable for cell culture, suitable for insect cell culture, suitable for plant cell culture

ship: ambient store at: 2-8°C

P5155-100G	100 g
P5155-500G	500 g

Phosphoric acid

Orthophosphoric acid
[7664-38-2] H_3PO_4 H_3O_4P FW 98.00

Concentrated phosphoric acid is approx. 85% H_3PO_4 (aqueous).

crystals
density ..... 1.685 g/mL, 25 °C vp ..... 2.2 mmHg (20 °C)
vd ..... 3.4 (vs air) vp ..... 5 mmHg (25 °C)

BioReagent, suitable for insect cell culture, 85%

Qualified for use as a titration acid in insect cell culture applications.

density ..... 1.69 g/mL, 25 °C
ship: ambient store at: room temp

P5811-100G	100 g
P5811-500G	500 g

Pluronic® F-68 solution

[106392-12-5]

solution, 10%, sterile-filtered, BioReagent, suitable for insect cell culture

Contains 100 g Pluronic F-68 per liter in cell culture grade water.

A nonionic copolymer surfactant

non-ionic
endotoxin ..... tested
ship: ambient store at: room temp

P5556-100ML	100 mL

Pluronic® F-68

Polyoxyethylene-polyoxypropylene block copolymer
[9003-11-6] $(C_3H_6O.C_2H_4O)_x$

Contains 100 ppm BHT

average mol wt 8350
CMC ..... 0.04 mM (20-25°C)
HLB 29

solid, BioReagent, suitable for cell culture, suitable for insect cell culture

A non-ionic detergent that protects cells from hydrodynamic damage.

non-ionic
ship: ambient store at: room temp

P1300-500G	500 g

Pluronic® F-127

[9003-11-6]

contains 100ppm BHT

powder, BioReagent, suitable for cell culture

A non-ionic copolymer surfactant qualified for use in insect cell culture applications as an antifoaming agent.

non-ionic
ship: ambient store at: room temp

P2443-250G	250 g
P2443-1KG	1 kg

Potassium chloride

[7447-40-7] KCl ClK FW 74.55

May be used for the preparation of phosphate buffered saline, and for the extraction and solubilization of proteins.

density ..... 1.98 g/mL, 25 °C

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.0%

solubility
H_2O ..... 133 mg/mL
ship: ambient store at: room temp

P5405-250G	250 g
P5405-500G	500 g
P5405-1KG	1 kg

Potassium phosphate monobasic

prim.-Potassium phosphate; Potassium dihydrogen phosphate; Monopotassium phosphate
[7778-77-0] KH_2PO_4 H_2KO_4P FW 136.09

pKa (25 °C) ..... (1) 2.15, (2) 6.82, (3) 12.38 (phosphoric acid)
density ..... 2.338 g/mL, 25 °C

powder, suitable for cell culture, suitable for insect cell culture, ≥99.0%

ship: ambient store at: room temp

P5655-100G	100 g
P5655-500G	500 g
P5655-1KG	1 kg

Propionic acid

Propanoic acid; Propanyl acid; Acid C_3
[79-09-4] CH_3CH_2COOH $C_3H_6O_2$ FW 74.08
density ..... 0.993 g/mL, 25 °C
n_D^{20} 1.386
vd ..... 2.55 (vs air)
vp ..... 2.4 mmHg (20 °C)
ait ..... 955 °F
lel 12.1%

BioReagent, suitable for insect cell culture, ~99%

Use in insect cell culture to inhibit mold growth.

density ..... 0.99 g/mL, 25 °C
ship: ambient store at: room temp

P5561-1L	1 L

Pyridoxal hydrochloride

PL HCl; 3-Hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridinecarboxaldehyde hydrochloride
[65-22-5] $C_8H_9NO_3 \cdot HCl$ FW 203.62

For the labeling of amino acids and their detection in picomolar amounts;[1] Coenzymes and Cofactors, vol. 1: Vitamin B_6, Pyridoxal Phosphate[2]

Lit. cited: 1. N. Lustenberger et al., *Angew. Chem.* **84**, 225 (1972)
2. Dolphin, D., Poulson, Avramovic, R.O., eds. , *Chemical, Biochemical and Medical Aspects* , John Wiley & Sons (New York: 1986), 725

BioReagent, suitable for cell culture, suitable for insect cell culture
ship: ambient store at: –20°C

P6155-5G	5 g
P6155-25G	25 g

Pyridoxine hydrochloride

PN HCl; Adermine hydrochloride; Pyridoxol hydrochloride; Vitamin B_6 hydrochloride
[58-56-0] $C_8H_{11}NO_3 \cdot HCl$ FW 205.64

BioReagent, suitable for cell culture, suitable for insect cell culture

Involved in a wide range of biochemical reactions that affect cell processes. Use as an alternative to pyridoxal which can degrade in media.

ship: ambient store at: room temp

P6280-10G	10 g
P6280-100G	100 g

(–)-Riboflavin

Lactoflavin; Vitamin G; Vitamin B_2
[83-88-5] $C_{17}H_{20}N_4O_6$ FW 376.36

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%
ship: ambient store at: room temp

R9504-25G	25 g
R9504-100G	100 g

Select Yeast Extract

[8013-01-2]

Water soluble portion of autolyzed yeast with intact B-complex vitamins. Yeast extract is a mixture of amino acids, peptides, water soluble vitamins and carbohydrates and can be used as additive for culture media.

For general bacteriological use with a variety of microorganisms.

powder, BioReagent, suitable for cell culture, suitable for insect cell culture

Spray dried, autolyzed yeast extract.
solubility
H_2O ..... 10% (remains clear after heating to 40°C.)
ship: ambient store at: room temp

Y1000-500G	500 g
Y1000-1KG	1 kg

DL-Serine

(±)-2-Amino-3-hydroxypropionic acid
[302-84-1] $HOCH_2CH(NH_2)COOH$ $C_3H_7NO_3$ FW 105.09

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98% (TLC)

For use in insect and mammalian cell culture applications where the pure L-form is not required.

ship: ambient store at: room temp

S5386-25G	25 g
S5386-100G	100 g

Sodium acetate

Acetic acid sodium salt
[127-09-3] CH_3COONa $C_2H_3NaO_2$ FW 82.03

solid

pKa ..... 4.76 (acetic acid)
ait ..... 1112 °F

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%
ship: ambient store at: room temp

S5636-500G	500 g

Sodium bicarbonate

Sodium hydrogen carbonate
[144-55-8] $NaHCO_3$ $CHNaO_3$ FW 84.01
pKa (25 °C) ..... (1) 6.37, (2) 10.25 (carbonic acid)

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, 99.5-100.5%

pH range ..... 6.8 - 7.4
ship: ambient store at: room temp

S5761-500G	500 g
S5761-1KG	1 kg
S5761-5KG	5 kg

Sodium chloride

[7647-14-5] NaCl ClNa FW 58.44

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.5% (titration)
ship: ambient store at: room temp

S5886-500G	500 g
S5886-1KG	1 kg
S5886-5KG	5 kg
S5886-10KG	10 kg

Sodium fluoride

[7681-49-4] NaF FNa FW 41.99
vp ..... 1.4 mmHg (0 °C)

BioReagent, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

S6776-100G	100 g
S6776-500G	500 g

Sodium phosphate dibasic

Disodium hydrogen phosphate; *sec*-Sodium phosphate; Disodium phosphate; Sodium hydrogenphosphate
[7558-79-4] Na_2HPO_4 HNa_2O_4P FW 141.96
pKa (25 °C) ..... (1) 2.15, (2) 6.82, (3) 12.38 (phosphoric acid)

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

S5136-100G	100 g
S5136-500G	500 g
S5136-1KG	1 kg
S5136-5KG	5 kg

Sodium phosphate monobasic

Monosodium phosphate; Sodium dihydrogen phosphate
[7558-80-7] NaH_2PO_4 H_2NaO_4P FW 119.98

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.0% (titration)

Use in insect cell culture to adjust pH.

ship: ambient store at: room temp

S5011-100G	100 g
S5011-500G	500 g
S5011-1KG	1 kg

Sodium propionate

Propionic acid sodium salt
[137-40-6] CH_3CH_2COONa $C_3H_5NaO_2$ FW 96.06

BioReagent, suitable for insect cell culture

Used as a mold inhibitor in insect cell culture applications.

ship: ambient store at: room temp

P5436-100G	100 g

Sodium pyruvate

α-Ketopropionic acid sodium salt; 2-Oxopropanoic acid sodium salt; Pyruvic acid sodium salt
[113-24-6] $CH_3COCOONa$ $C_3H_3NaO_3$ FW 110.04

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

solubility
H_2O ..... 100 mg/mL

ship: ambient store at: 2-8°C

P5280-25G	25 g
P5280-100G	100 g
P5280-500G	500 g

Sodium succinate dibasic hexahydrate

Succinic acid disodium salt; Butanedioic acid disodium salt
[6106-21-4] $NaOOCCH_2CH_2COONa \cdot 6H_2O$ $C_4H_4Na_2O_4 \cdot 6H_2O$ FW 270.14

BioReagent, suitable for cell culture, suitable for insect cell culture

Use as a TCA (Krebs cycle) supplement in cell culture applications.

ship: ambient store at: room temp

S9637-100G	100 g
S9637-500G	500 g

Succinic acid

Butanedioic acid
[110-15-6] $HOOCCH_2CH_2COOH$ $C_4H_6O_4$ FW 118.09

BioReagent, suitable for cell culture, suitable for insect cell culture

Use as a TCA (Krebs cycle) supplement in insect cell culture applications.

ship: ambient store at: room temp

S9512-100G	100 g
S9512-500G	500 g

Sucrose

α-D-Glucopyranosyl β-D-fructofuranoside; α-D-Glc-(1→2)-β-D-Fru; D(+)-Saccharose; Sugar; β-D-Fructofuranosyl-α-D-glucopyranoside
[57-50-1] $C_{12}H_{22}O_{11}$ FW 342.30

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.5% (GC)

Use to create sucrose gradients for purification of viruses and proteins.

solubility
H_2O ..... 500 mg/mL

ship: ambient store at: room temp

S1888-500G	500 g
S1888-1KG	1 kg
S1888-5KG	5 kg

Thiamine hydrochloride

Aneurine hydrochloride; Vitamin B_1 hydrochloride
[67-03-8] $C_{12}H_{17}ClN_4OS \cdot HCl$ FW 337.27

BioReagent, suitable for cell culture, suitable for insect cell culture

Required to support energy metabolism and amino acid synthesis in cultured cells. Present in many classical and serum-free formulations.

ship: ambient store at: room temp

T1270-25G	25 g
T1270-100G	100 g

Thiazolyl Blue Tetrazolium Bromide

MTT; 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; Methyl-thiazolyldiphenyl-tetrazolium bromide
[298-93-1] $C_{18}H_{16}BrN_5S$ FW 414.32

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥97.5% (TLC)

Use as a colorimetric metabolic activity indicator in cell viability assays.

solubility
H_2O ..... 5 mg/mL

ship: ambient store at: 2-8°C

M5655-100MG	100 mg
M5655-500MG	500 mg
M5655-1G	1 g

DL-Threonine

(±)-2-Amino-3-hydroxybutyric acid
[80-68-2] $CH_3CH(OH)CH(NH_2)COOH$ $C_4H_9NO_3$ FW 119.12

BioReagent, suitable for cell culture, suitable for insect cell culture

For use in insect and mammalian cell culture applications where the pure L-form is not required.

ship: ambient store at: room temp

T1520-100G	100 g

(+)-α-Tocopherol

5,7,8-Trimethyltocol; 2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol; Vitamin E; D-α-Tocopherol
[59-02-9] $C_{29}H_{50}O_2$ FW 430.71

density 0.95 g/mL, 25 °C
n_D^{20} 1.505

Type VI, from vegetable oil, neat (liquid, ≥0.88M based on potency, density and molecular wt.), BioReagent, suitable for insect cell culture, activity: ≥1000 IU/g

Mixed isomers

Use in insect cell culture applications as an antioxidant.

potency: ≥600 mg d-α-tocopherol per g

color clear yellow

ship: ambient store at: 2-8°C

T1539-25G	25 g
T1539-100G	100 g

(+)-α-Tocopherol acetate

Vitamin E acetate
[58-95-7] $C_{31}H_{52}O_3$ FW 472.74

Synthesized from natural α-tocopherol

BioReagent, suitable for insect cell culture, activity: ~1360 IU/g

Use in insect cell culture applications as an antioxidant.

purified by crystallization

Since the M.P. is about 25 °C this preparation is usually an oil. Does not air oxidize.

ship: ambient store at: 2-8°C

T1157-1G	1 g
T1157-10G	10 g

D-(+)-Trehalose dihydrate

α-D-Glucopyranosyl-α-D-glucopyranoside; α,α-Trehalose
[6138-23-4] $C_{12}H_{22}O_{11} \cdot 2H_2O$ FW 378.33

from *Saccharomyces cerevisiae*, powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

Use as a cryoprotectant in a variety of cell freezing media.

from *Saccharomyces cerevisiae*

solubility
H_2O 50 mg/mL

ship: ambient store at: room temp

T0167-10G	10 g
T0167-25G	25 g
T0167-100G	100 g

TWEEN® 80

Polyethylene glycol sorbitan monooleate; Polyoxyethylenesorbitan monooleate; Polysorbate 80; POE (20) sorbitan monooleate
[9005-65-6]

Non-ionic detergent used for selective protein extraction and isolation of nuclei from mammalian cell lines.

average mol wt 1310

viscous liquid, BioReagent, suitable for insect cell culture

non-ionic

composition
Oleic acid ~70% (balance primarily linoleic, palmitic, and stearic acids)

ship: ambient store at: room temp

P4675-100ML	100 mL

L-Tyrosine disodium salt hydrate

L-3-(4-Hydroxyphenyl)alanine disodium salt hydrate
$C_9H_9NNa_2O_3 \cdot xH_2O$ FW 225.15 (Anh)

Amino acid precursor of dopamine and other catecholamines

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98% (TLC)

Extremely hygroscopic.

ship: ambient store at: 2-8°C

T1145-25G	25 g
T1145-100G	100 g
T1145-500G	500 g

Vanderzant vitamin mixture for insects

Animal-component free, BioReagent, suitable for insect cell culture

Animal-derived component free vitamin mixture developed or use in insect cell culture.

ship: ambient store at: 2-8°C

V1007-100G	100 g
V1007-1KG	1 kg
V1007-5KG	5 kg

Vitamin B_{12}

CN-Cbl; α-(5,6-Dimethylbenzimidazolyl)cyanocobamide; Cyanocobalamin; Cyanocob(III)alamin
[68-19-9] $C_{63}H_{88}CoN_{14}O_{14}P$ FW 1355.37

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98.5%

Required for growth, genetic stability and survival of cells in vitro. Functions to support one-carbon metabolism. Present in many classical and serum-free formulations.

ship: ambient store at: 2-8°C

V6629-100MG	100 mg
V6629-250MG	250 mg
V6629-1G	1 g
V6629-5G	5 g

Yeast Brewers

Debittered, BioReagent, suitable for insect cell culture

ship: ambient store at: 2-8°C

Y4625-1KG	1 kg
Y4625-5KG	5 kg

Yeast Extract Ultrafiltrate (50×)

liquid, BioReagent, suitable for insect cell culture

Prepared using 200 g/L yeast extract in deionized water. Ultrafiltered using a 10,000 dalton cutoff filter.

ship: ambient store at: 2-8°C

Y4375-100ML	100 mL

Zinc chloride

[7646-85-7] $ZnCl_2$ Cl_2Zn FW 136.30

vp ..... 1 mmHg (428 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture

ship: ambient store at: room temp

Z0152-100G	100 g
Z0152-500G	500 g
Z0152-1KG	1 kg

Technical Information

Powdered Media Preparation

Powdered medium is extremely hygroscopic and should be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. Preparing medium in concentrated form is not recommended as precipitates may form.

Supplements can be added prior to filtration or introduced aseptically to sterile media. The nature of the supplement may affect the storage conditions and shelf life of the medium.

Procedure for Schneider's Powdered Insect Medium

- Measure out 90% of the final volume of tissue culture grade water. Water should be at room temperature.
- While gently stirring the water, add the powdered medium. Stir until dissolved. Do not heat.
- Rinse original package with a small amount of water to remove all traces of powder. Add to the solution.
- To the solution, add the required amount of sodium bicarbonate (or potassium bicarbonate) from the following chart for each liter of medium being prepared. Stir until dissolved.
- While stirring, adjust the pH 0.1-0.3 units below the desired pH with 1 N HCl or 1 N NaOH. The pH may rise during vacuum filtration.
- Add additional water to bring medium to final volume.
- Sterilize immediately by filtration using a membrane with a porosity of 0.22 µm or less.
- Aseptically dispense into sterile containers. Store liquid medium refrigerated at 2-8 °C and in the dark.

Reference

1. Inlow, D., Shaugar, A., and Maiorella, B., Insect cell culture and baculovirus propagation in protein-free medium. *J. Tissue Culture Method.*, **12**, 13-16, 1988.

Sodium Bicarbonate Addition Table

Recommended sodium bicarbonate ($NaHCO_3$) concentrations for 1x (single strength) powdered medium using 7.5% Sodium Bicarbonate Solution (**Cat. No. S8761**), or Cell Culture Tested Sodium Bicarbonate, Powder (**Cat. No. S5761**):

Product	Cat. No.	$NaHCO_3$ Solution mL/L of S8761	$NaHCO_3$ Powder g/L of S5761
Grace's	**G9771**	4.7	0.35
Schneider's	**S9895**	5.3	0.4
SS M3	**S8398**	None*	None*
TNM-FH	**T1032**	4.7	0.35

*Shields & Sang Media require the addition of potassium bicarbonate (Cat. No. P7682) at a concentration of 0.5 g/L.

Protein-Free Media for Insect Cell Culture

A procedure[1] has been described for the preparation and use of protein-free medium for the cultivation of Spodoptera frugiperda cells and the propagation of native or recombinant baculovirus. Preparation of 1 liter of serum-free medium consisting of a lipid supplement in Pluronic® F-68 and yeast extract ultrafiltrate:

Lipid Supplement

- Lipid Supplement (Cat. No. L2273) or prepare as follows:
- Use sterile 10% Pluronic F-68 (Cat. No. P5566) or prepare a 10% solution of Pluronic F-68 (Cat. No. P1300) in tissue culture grade water and sterilize by filtration through a membrane with a porosity of 0.22 µm or less.
- Warm 10 ml of the 10% Pluronic F-68 solution in a 37 °C waterbath.
- Aseptically add 1 ml of Lipid Mixture (Cat. No. L5146) to sterile 30 ml (or larger) screw capped tube and prewarm in 37 °C waterbath for 5 minutes.
- Aseptically add prewarmed Pluronic F-68 solution dropwise to the warm Lipid Mixture with rapid vortexing. The emulsion will first become milky, but should clear to slight opalescence. A cloudy white emulsion can result if the solutions were not adequately prewarmed or the rate of addition was too rapid or insufficient agitation was used during the mixing of the Pluronic F-68.

Note: The preparation of the lipid emulsion consists of the formation of liposomes in an appropriate aqueous solvent. The original citation describes the use of a 10% solution of Pluronic F-68 as the solvent for preparing the liquid emulsion. We have achieved success in using other products, e.g. yeast extract ultrafiltrate and medium, as the solvent for preparing the lipid emulsion.

Preparation of Medium

- Use liquid IPL-41 medium (Cat. No. I7760).
- Supplement medium with yeast extract ultrafiltrate (MW 10,000 Da) (Cat. No. Y4375) to a final concentration of 0.4% and prewarm to 27 °C.
- Prewarm 11 ml of lipid supplement in a 37 °C waterbath then transfer the supplement to the medium with a pipette and mix thoroughly.

Serum-Free Adaptation of Cells

Our serum-free insect media are based on IPL-41 as are other commercial serum-free insect media. Due to the fact that cells grown in Grace's medium or one of its derivatives (GTC-100, TC-100, or TNM-FH) are significantly stressed when passaged into IPL-41, we strongly suggest that cells be adapted to IPL-41 prior to attempting to culture them in serum-free medium. Cells cultured in IPL-41 supplemented with FBS have been successfully adapted to serum-free medium by both direct inoculation and by weaning.

Direct inoculation method:
Use a high initial inoculum of cells (usually about 10× the inoculum used for subculturing). This is necessary due to the high loss of cells as a result of selection for growth in serum-free medium. The cultures usually have a large population of selected cells after 7-10 days in culture which can be used to seed subcultures in serum-free medium. Because the growth rate is usually slower for several passages before returning to a growth rate similar to serum containing medium, initally the inoculation densities should be increased for subculturing.

Weaning methods:
1) The weaning process is initiated by diluting cells in serum containing medium 1:1 with serum-free medium. This process is repeated with culture to be weaned every 3 or 4 days until the serum content drops below 0.2%.
2) Alternatively, cells can be inoculated in serum-free medium supplemented with 10% serum. The level of serum supplementation is reduced by 50% during succeeding passages until the serum content drops below 0.2%. When the serum content of the medium is below 0.2%, the cells can then be inoculated directly into serum-free medium. As with the direct inoculation method the growth rate is usually slower for serveral passages before returning to a growth rate similar to serum containing medium and will initially require higher inoculation densities during subculturing.

EX-CELL® TiterHigh™ *Sf* Insect Medium, Animal Component-Free, Catalog Number I5408

Product Description
EX-CELL® TiterHigh™ *Sf* Insect Medium (Cat. No. I5408) is an animal component-free, protein-free formulation designed specifically for the *Sf*21 and *Sf*9 *(Spodoptera frugiperda)* insect cell lines. TiterHigh *Sf* supports fast cell growth rates (doubling times of 20-24 hours), high cell densities of >20 x 10^6 cells/ml for *Sf*21 and >10 x 10^6 cells/ml for *Sf*9, while maintaining high cell viability and high recombinant protein production using the Baculovirus Expression Vector System (BEVS).[1] TiterHigh *Sf* Insect Medium will also support high yields of wild-type AcMNPV (occluded and non-occluded forms) and recombinant virus, with and without the presence of 10% Fetal Bovine Serum.

As more recombinant proteins are being employed as therapeutic agents, the methods implemented in their production are coming under increasing regulatory scrutiny. A major area of concern is the presence of animal-derived components in media used to culture cells for recombinant protein expression. With the utilization of EX-CELL® TiterHigh™ *Sf* Insect Medium, regulatory concerns associated with the use of animal-derived components have been eliminated.

EX-CELL® TiterHigh™ *Sf* Insect Medium can be used to grow cells either attached (i.e. flasks and dishes) or in suspension (i.e. shaker flasks, spinner flasks, and bioreactors). It is also capable of supporting long-term cell growth of >50 passages.

Intended Use
For manufacturing, processing, or repacking.

Components
The formulation includes inorganic salts, sodium bicarbonate, essential and non-essential amino acids, vitamins, yeast extract, a proprietary lipid formulation, trace elements, and other organic compounds.

It does not contain phenol red, antibiotics, antimycotics, transferrin, and products of animal origin.

Preparation Instructions
This medium is supplied as a complete, sterile-filtered 1x liquid. No supplementation is necessary. The addition of a surfactant (such as Pluronic® F-68) is not required. Glutamine is supplied at 10 mM in the form of an Alanine-Glutamine dipeptide. This form is more stable relative to free L-glutamine and is readily accessible to the cells.

Storage/Stability
This medium is stable, when stored 2-8 °C and protected from light, until the indicated expiration date on the label. Do not freeze the medium.

Freezing and Thawing
Insect cells grown in EX-CELL® TiterHigh™ *Sf* Insect Medium have been successfully frozen in liquid nitrogen and recovered. Cells must be in the mid-logarithmic phase of growth with greater than 90% viability.

1. Pellet cells by centrifugation for 5 minutes at 200 x *g*. Re-suspend at a concentration of 1 x 10^7 cells/ml in a 1:1 mixture of fresh EX-CELL® TiterHigh™ *Sf* Insect Medium and conditioned EX-CELL® TiterHigh™ *Sf* Insect Medium supplemented with DMSO at a final concentration of 7.5%.
2. Freeze cells via controlled cooling method (1 °C decrease per minute) and store frozen cells in liquid nitrogen according to standard procedures.
3. Recover frozen cells by rapidly thawing the vial in a 37 °C water bath.
4. Dilute thawed cells 1:10 in fresh EX-CELL® TiterHigh™ *Sf* Insect Medium. Mix and centrifuge suspension at 200 x *g* for 5 minutes.
5. Re-suspend the pellet in 1 ml EX-CELL® TiterHigh™ *Sf* Insect Medium. Add 9 ml additional fresh TiterHigh™ *Sf* Insect Medium.
6. Transfer suspension to a T-75 flask containing fresh EX-CELL® TiterHigh™ *Sf* Insect Medium at a final volume of 20 ml. Suspension culture can be transferred to appropriate shaker culture after 2-3 days.

Adaptation to EX-CELL® TiterHigh™ *Sf* Insect Medium
Minimal time is required to adapt insect cells from serum-containing medium to EX-CELL® TiterHigh™ *Sf* Insect Medium. For good adaptation, it is critical that cell viability be >90% and the cells are in mid-logarithmic growth phase. Cells grown in serum-containing medium should be inoculated at a viable cell density of >3 x 10^5 cells/ml in a 1:1 mixture of serum-containing medium and TiterHigh™ *Sf* Insect Medium. Allow cells to reach a density of 1 to 3 x 10^6 cells/ml. Subculture to an initial density of 3 x 10^5 cells/ml into medium containing increasing proportions of TiterHigh™ *Sf* Insect Medium, first at a 1:3 ratio and then 1:7 ratio (serum-containing medium: serum-free medium). Cells are considered adapted when the cell density reaches at least 5 x 10^6 cells/ml in serum-free medium and cell doubling rates are <24 hours during log phase of growth. This usually occurs within 7 days after inoculation. The time interval required for adaptation will vary by individual insect cell lines. The direct adaptation method may also be used by transferring the cells growing in serum directly to EX-CELL® TiterHigh™ *Sf* Insect Medium. However, if sub-optimal performance is observed, the sequential (weaning) method should be used. All cultures should be incubated at 27 °C in a humidified atmosphere.

Normally, cells can be transferred directly from another serum-free medium to EX-CELL® TiterHigh™ *Sf* Insect Medium, but in some cases adaptation may be necessary. The same procedure outlined for adapting cells from serum can be followed.

Product Profile

EX-CELL® TiterHigh™ *Sf* Insect Medium was tested against the competition for cell growth and recombinant protein production (β-galactosidase) using a baculovirus, AcP1-57GAL.[3] All experiments were performed in duplicate in sterile 125 ml disposable Erlenmeyer shaker flasks (50 ml liquid volume) at 27 °C and 130 rpm shaker speed. Initial cell density was 3×10^5 viable cells/ml for the growth assays. The productivity assays were infected at an MOI of 5 at an initial cell density of 1×10^6 viable cells/ml. All cells were adapted to the respective medium prior to experimental set-up.

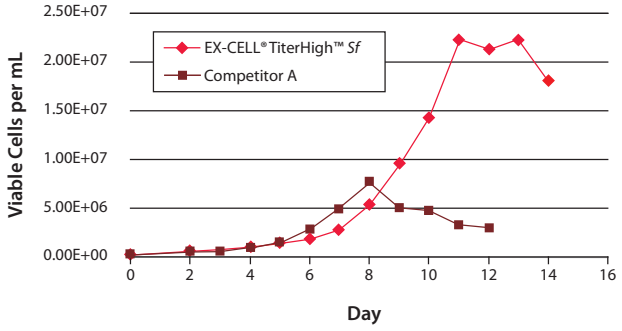

Figure 1. Growth Comparison — Sf21

β-gal was quantified using the Sigma® β-galactosidase Reporter Gene Activity Detection Kit (Catalog Number GALA). 1 ml samples were collected every day and the cells were washed with HBSS (Catalog Number H6648) after centrifugation. The cell lysates were diluted 1:2000.

Figure 1 demonstrates the excellent growth that is attainable in EX-CELL® TiterHigh™ *Sf* Insect Medium with the *Sf*21 cell line. **Figure 2** shows that TiterHigh™ *Sf* Insect Medium also supports excellent recombinant protein production after baculoviral infection.

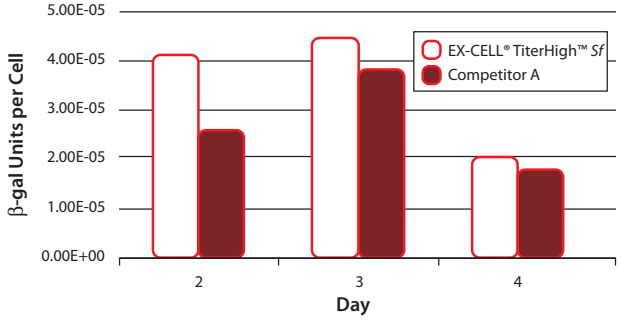

Figure 2. Productivity Comparison — Sf21

References

1. Smith, G.E., et al., Production of Human b-interferon in Insect Cells Infected with a Baculovirus Expression Vector. *J. Molecular and Cellular Biology,* **3,** 2156-2165, 1983.
2. Merten, O.W., Safety Issues of Animal Products Used in Serum-free Media. *Dev. Biol. Stand.,* **99,** 167-180, 1999.
3. Jarvis, D.L., et al., Requirements for Nuclear Localization and Supramolecular Assembly of a Baculovirus Polyhedrin Protein. *Virology,* **185,** 795-810, 1991.

Precautions and Disclaimer

MSDS is available upon request or at sigma-aldrich.com.
Pluronic is a registered trademark of BASF Corporation.

Vaccine Platform

Vaccine Media Overview

Cultured cells have long been known to serve as excellent hosts for propagation of many types of viruses. The ability of cell culture systems to produce large quantities of attenuated viral particles has served as the basis for the production of both human and veterinary vaccines. Traditional methods have relied on the production of viral agents in cells cultured in medium supplemented with serum, most commonly fetal bovine serum. The animal serum in cell cultures used in production processes can cause a number of problems for manufacturers. The increased costs of raw materials and post-production processing associated with serum has prompted interest in the development of serum-free media for vaccine production. More recently, the potential for contamination by adventitious agents present in serum has heightened regulatory concerns regarding the use of animal-derived components in media used for pharmaceutical manufacturing. With these factors in mind, Sigma has developed new media for vaccine production with reduced levels or completely devoid of animal-derived components. At the same time, we will continue our commitment to traditional manufacturing methods by maintaining and expanding the range of basal media for use with serum supplementation and serve as the basis for customization into serum-free formulations.

Gene Therapy

The ability to genetically engineer viral particles for use as therapeutic agents to treat genetic diseases (gene therapy) represents one exciting new avenue that science and technology are bringing to modern medicine. Cultured cells have long been known to serve as excellent hosts for the propagation of many types of viruses. Traditional methods have relied on the growth of viral agents in cells cultured in serum-supplemented media. A great concern is the potential for contamination by adventitious agents introduced into the manufacturing process through the use of animal-derived materials. This has heightened regulatory concerns regarding the use of such components in media employed in pharmaceutical manufacturing, particularly in the case of therapeutic injectables. These concerns have led to recommendations that all animal-derived components be avoided when therapeutic agents are manufactured. It is likely that these recommendations will become rigid requirements in the near future. With these factors in mind, Sigma has developed new media tailored to the needs of two of the more popular cell lines used for the propagation of viral particles employed in gene therapy. These media are formulated without the use of animal-derived components.

References

1. Benihoud, K., et al., Adenovirus vectors for gene delivery. *Curr. Opin. Biotech.*, **10**, 440-447, 1999.
2. Steel, M.P. and Roessler, B.J., Compliance with good manufacturing practices for facilities engaged in vector production, cell isolation, and genetic manipulations. *Curr. Opin. Biotech.*, **10**, 295-297, 1999.

Media

EX-CELL® 293 Serum-Free Medium for HEK 293 Cells

EX-CELL 293 is an animal-protein free, serum-free medium developed for the long-term growth of Human Embryonic Kidney 293 (HEK 293) and related cells. The cells, in a suspension culture, can be subcultured directly into EX-CELL 293 from serum-supplemented media with little or no adaptation. Suspension cultures can be maintained, without refeeding, for about 10 days and can be carried for more that 20 passages with no loss of viability.

for research or for further manufacturing use

Serum-free, Animal-protein free, without L-glutamine, liquid, suitable for cell culture

ship: ambient store at: 2-8°C

14571C-500ML	500 mL
14571C-1000ML	1000 mL

Animal-protein free, with L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

Formulated to contain 21.6 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24571C-1L	1 L
24571C-5L	5 L
24571C-10L	10 L
24571C-50L	50 L
24571C-100L	100 L

EX-CELL® EBx GRO-I Serum-Free Media

NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, without L-glutamine and without sodium bicarbonate, dry powder, suitable for cell culture

Formulated to contain 19.1 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24530C-1L	1 L
24530C-5L	5 L
24530C-10L	10 L
24530C-50L	50 L
24530C-100L	100 L

EX-CELL® EBx™ GRO-I Serum-Free Media

NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: wet ice store at: 2-8°C

14530C-500ML	500 mL
14530C-1000ML	1000 mL

EX-CELL® EBx PRO-II Serum-Free Media

NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, without L-glutamine, without phenol red, liquid, sterile-filtered, suitable for cell culture

ship: wet ice store at: 2-8°C

14533C-500ML	500 mL
14533C-1000ML	1000 mL

Animal-component free, Chemically defined, dry powder, suitable for cell culture

Formulated to contain 12.4 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24533C-1L	1 L
24533C-5L	5 L
24533C-10L	10 L
24533C-50L	50 L
24533C-100L	100 L

EX-CELL® EBx PRO-I Serum-Free Media

NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, dry powder, suitable for cell culture

Formulated to contain 20.7 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24531C-1L	1 L
24531C-5L	5 L
24531C-10L	10 L
24531C-50L	50 L
24531C-100L	100 L

EX-CELL® EBx™ PRO-I Serum-Free Media

NEW

EX-CELL® EBx™ Serum-Free Media

Animal-component-free, low protein

for research or for further manufacturing use

store at: 2-8°C

Animal-component free, without L-glutamine, without phenol red, liquid, suitable for cell culture

ship: wet ice store at: 2-8°C

14531C-500ML	500 mL
14531C-1000ML	1000 mL

EX-CELL® GTM-3

Gene Therapy Medium-3 for Adenovirus Production Animal-component free, liquid, sterile-filtered, suitable for cell culture

Features and Benefits

Complete, ready-to-use medium that requires only the addition of L-glutamine for the growth of HEK-293 cells, retinoblastoma-like cells and propagation of adenoviruses. Additionally, the medium is designed to support growth of cells in suspension culture with minimal levels of cell clumping. Developed to meet the needs of biotechnology and vaccine manufacturing.

for research or for further manufacturing use

Prior to use medium should be warmed to 37°C and supplemented with 20ml/L of 200mM L-glutamine.

Do not freeze.

endotoxin ..... tested

ship: ambient store at: 2-8°C

G9916-1L	1 L
G9916-6X1L	6 × 1 L

EX-CELL® HeLa Serum-Free Medium for HeLa Cells

EX-CELL HeLa is an animal-protein free, serum-free liquid medium developed for the long-term growth of HeLa cells in suspension culture. The HeLa cell line is a clonal derivative of the parent HeLa cell line and is capable of growth in suspension culture. HeLa suspension cultures can be subcultured directly into EX-CELL HeLa from serum-supplemented or serum-free media. Suspension cultures in EX-CELL HeLa have been carried for more than 25 passages with no loss of growth or viability.

serum-free

Animal-protein free, without L-glutamine, liquid, suitable for cell culture

for research or for further manufacturing use

ship: ambient store at: 2-8°C

14591C-500ML	500 mL
14591C-1000ML	1000 mL

EX-CELL® VPRO Serum-Free Medium for Retinoblast Cells

EX-CELL VPRO is an animal-protein free, serum-free medium developed for the long-term growth of Human Embryonic Retinoblast cells (PER.C6® and related cell lines). The cells can be subcultured directly into EX-CELL VPRO from serum-free media with little or no adaptation. PER.C6 cells can be grown as suspension cultures either in shaker flasks or roller bottles, with roller bottles being the preferred culture system. Suspension cultures can be maintained, without refeeding, for approximately 10 days and can be carried for more than 20 passages with no loss of viability.

for research or for further manufacturing use

Animal-protein free, without L-glutamine, liquid, suitable for cell culture

ship: ambient store at: 2-8°C

14561C-500ML	500 mL
14561C-1000ML	1000 mL

Animal-protein free, with L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

Formulated to contain 18.5 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24561C-1L	1 L
24561C-10L	10 L
24561C-50L	50 L

MDCK Media

MDCK Media

The Madin-Darby canine kidney cell line is an important, well established epithelial cell line. These cells have been used extensively as a model system for studies of renal function, as well as the host system for the growth of attenuated viruses for vaccine production.

Current manufacturing processes utilizing these cells rely on the use of serum-supplemented medium. However, the use of serum in a manufacturing process introduces a number of concerns such as material costs, increased down-stream purification complexity, and biological contamination risks. Sigma has developed serum-free and protein-free media that promote the growth of MDCK cells at rates and densities comparable to serum-supplemented cultures.

These media offer a number of advantages including improved medium definition, reduced cost, improved down-stream processing, and reduced biological safety risks associated with using traditional serum-supplemented media. In these media the use of animal-derived protein has been dramatically reduced. All hormones and growth factors used are recombinant proteins that were expressed in non-vertebrate systems.

MDCK Protein-Free Medium (M3678) exhibits growth performance in static cultures comparable to DMEM/F-12 (1:1) medium containing 5% fetal bovine serum (FBS). MDCK Serum-Free Medium (M3803) is supplemented with bovine serum albumin and it is used to facilitate the attachment of the MDCK cells to the surfaces of roller bottles and microcarrier beads in non-static cultures.

EX-CELL® MDCK Growth Medium

Madin-Darby Canine Kidney Low Protein Medium without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Complete serum-free medium formulated to support the growth of Madin-Darby Canine Kidney (MDCK) cells for the growth and production of viruses.

for research or for further manufacturing use

Contains inorganic salts, essential and non-essential amino acids, vitamins, recombinant human insulin and growth factors, other organic compounds, trace elements, and 100 µg/ml bovine serum albumin to facilitate cell attachment.

Aseptically add 16.5 mL of 200 mM L-glutamine solution per liter of medium prior to use.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M3803-1L	1 L
M3803-6X1L	6 × 1 L

EX-CELL® MDCK Production Medium

Madin-Darby Canine Kidney Protein-free Medium
Animal-component free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Complete protein-free medium formulated to support the growth of Madin-Darby Canine Kidney (MDCK) cells for the growth and production of viruses.

Designed and qualified for use in stringent BioManufacturing applications that requires the absence of both proteins and animal-derived components.

for research or for further manufacturing use

Contains inorganic salts, essential and non-essential amino acids, vitamins, recombinant human insulin and growth factors, other organic compounds, and trace elements.

Aseptically add 16.5 ml of 200 mM L-glutamine solution per liter of medium prior to use.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M3678-1L	1 L
M3678-6X1L	6 × 1 L

EX-CELL® MDCK Serum-Free Medium for MDCK Cells

EX-CELL MDCK is an animal-protein free, serum-free medium developed for the long-term growth of Madin Darby Canine Kidney (MDCK) and related cells. The cells, in an attachment culture, can be subcultured directly into EX-CELL MDCK from serum-supplemented media without adaptation. Cell densities and doubling times achieved under serum-free conditions are comparable to those achieved in a serum-supplemented culture.

for research or for further manufacturing use

Animal-protein free, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

14581C-500ML	500 mL
14581C-1000ML	1000 mL

Animal-protein free, with L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture NEW

Formulated to contain 21.6 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

24581C-1L	1 L
24581C-5L	5 L
24581C-10L	10 L

Stem Cell Biology Platform

Stem cells hold the key to a number of cellular processes from development to tissue regeneration and aging. They also hold the promise of cures for many diseases and injuries as well as offer an opportunity to bridge the 'animal to human gap' in ADMET and drug efficacy studies, a necessary step in providing therapeutic products for human use. The stem cell biology platform, as presented in this manual, highlights the most commonly used media, supplements, and reagents for stem cell discovery, culture, characterization and functional profiling .

The products listed in these section are commonly used in Stem Cell Biology. Many additional products can be found within this publication, listed in other publications, or at the Sigma-Aldrich Web site - sigma-aldrich.com

Stem Cell Media and Supplements

Claycomb Medium

▸ without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Claycomb Medium, named after Dr. William Claycomb who established the HL-1 cell line, is specifically designed for the growth of murine cardiomyocytes. HL-1 is the first cell line established that can maintain the differentiated cardiomyocyte phenotype and contractile activity *in vitro*. The HL-1 cell line can be used for the study of cardiac cell hypertrophy that follows myocardial infarction, the testing of novel cardiac therapeutic drugs and treatments, the production of high levels of cardiac proteins and the study of mature cardiomyocyte specific genes. Claycomb Medium, when supplemented with 100 µM norepinephrin, 10% fetal bovine serum (FBS) and 4 mM L-glutamine, will maintain the HL-1 cell line and the mature cardiomyocyte behavior. While observing the HL-1 cells under light microscopy, individual and groups of cells can be observed contracting, becoming more frequent as the cardiomyocytes reach confluency.

For research or for further manufacturing use.

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

51800C-500ML	500 mL

Stemline® Hematopoietic Stem Cell Expansion Medium

Proprietary formulation containing all non-animal derived components except human serum albumin. Contains no growth factors or cytokines.

Intended for the expansion of CD 34+ progenitors derived from bone marrow, mobilized peripheral blood and cord blood.

Features and Benefits

Contains only human albumin and no other animal-derived components to facilitate its use in clinical investigations. Medium has been tested extensively in development in 7 day expansion assays with a starting inoculum of 10,000; and with HPP-CFC's to demonstrate renewal of progenitors in CFU assays.

▸ Serum-free, Without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Developed to promote the optimal expansion of human hematopoietic stem cells (HSC) from bone marrow, mobilized peripheral blood, and cord blood, Stemline Hematopoietic Stem Cell Expansion Medium demonstrates higher total nucleated cell (TNC) fold increases than other commercially available serum-free media formulations. Additionally, colony-forming unit (CFU) assays indicate that CD34+ hematopoietic stem cells grown in Stemline media demonstrate significantly greater expansion of bother early (HPP-CFC) and late (GM-CFC) progenitor populations when compared to those grown in alternative media. Human cord blood cells expanded in Stemline Media demonstrate impressive self-revewal when transplanted into immunodeficient NOC/SCID mice, illustrating Stemline's utility in true functional trial.

Stemline Hematopoietic Stem Cell Expansion Medium free of serum and all other animal-derived components with the exception of human serum albumin. This exclusion increases performance consistency and elimates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Hematopoietic Stem Cell Expansion Medium is clearly an excellent choice for your HSC applications.

Drug/Device Master File available

Must be supplemented with growth factors and cytokines according to individual user protocols.

ship: wet ice store at: 2-8°C

S0189-500ML	500 mL
S0189-6X500ML	6 × 500 mL

Stemline® II Hematopoietic Stem Cell Expansion Medium

▸ Serum-free, contains L-glutamine, liquid, sterile-filtered, suitable for cell culture

The second generation of Sigma's hematopoietic stem cell expansion media family, Stemline II has been developed to optimize the balance of differientiated and undifferentiated cells while maximizing their expansion. Compatible with Hematopoietic Stem Cells (HSC) from bone marrow, cord blood, and mobilized peripheral blood, Stemline II has been shown to lead to significant increases in cell expansion from all three sources. Through flow cytometric analysis of clinical-scale expansions, Stemline II has also demonstrated a higher capacity than other commercially available media for the expansion of CD34+/CD38+ late progenitors required for short-term engraftment. Human cord blood cells expanded in Stemline Media demonstrate impressive self-revewal when transplanted into immunodeficient NOC/SCID mice, illustrating Stemline's utility in true functional trial.

Stemline Hematopoietic Stem Cell Expansion Medium free of serum and all other animal-derived components with the exception of human serum albumin. This exclusion increases performance consistency and elimates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Hematopoietic Stem Cell Expansion Medium is clearly an excellent choice for your HSC applications.

Drug/Device Master File available

Must be supplemented with growth factors and cytokines according to individual user protocols.

ship: wet ice store at: 2-8°C

S0192-500ML	500 mL
S0192-6X500ML	6 × 500 mL

Stemline® Mesenchymal Stem Cell Expansion Medium

MSC Medium
Without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Developed to promote the optimal expansion of human mesenchymal stem cells (MSC) from bone marrow, Stemline Mesenchymal Stem Cell Expansion Medium demonstrates greater total nucleated cell (TNC) fold increases than other commercially available formulations. Additionally, functional trials clearly demonstrated Stemline's capacity to promote differentiation into adipocytes, chondrocytes, and osteocytes.

Stemline Mesenchymal Stem Cell Expansion Medium requires supplementation with antibiotics, cytokines, L-glutamine and fetal bovine serum, as appropriate to individual research protocols. Known to be extremely sensitive during initial isolation and growth ex vivo, MSC proliferation depends highly on the composition of fetal bovine serum (FBS) used to supplement their medium. Pre-screening with FBS is recommended, as the specific FBS components that effect MSC growth have not been fully identified.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Mesenchymal Stem Cell Expansion Medium is clearly an excellent choice for your MSC applications.

Drug/Device Master File available

Medium must be supplemented with antibiotics, cytokines, 4 mM L-glutamine, and fetal bovine serum according to individual user protocols.

ship: wet ice store at: 2-8°C

S1569-1L	1 L

Stemline® T Cell Expansion Medium

Serum-free, Without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Developed to promote the optimal expansion of adult human T cells, Stemline T Cell Expansion Medium demonstrates significantly greater expansion (55%) when compared to alternative media, and viability greater than 95%. Additionally, flow cytometry confirms that with Stemline, a proper CD4/CD8 ratio is maintained. In an ex vivo functional assay (51Chromium Release Assay), T cells expanded in Stemline medium proved to be highly functional and possessed cytolytic potential greater than T cells expanded in serum-containing medium (RPMI with 10% fetal bovine serum). In an in vivo functional assay (GvHD Induction), human T lymphocytes expaned in Stemline medium were injected into NOD/SCIDb2M mice (n=12). Engraftment, perivascular infiltration, and lethal GvHD were observed by day 15 in 100% of mice, demonstrating excellent in vivo expansion and functionality.

Stemline T Cell Expansion Medium is free of serum and all other animal-derived components with the exceptions of human serum albumin, cholesterol, and transferrin. This exclusion increases performance consistency and elimates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline T-Cell Expansion Medium is clearly an excellent choice for your T-cell applications.

Drug/Device Master File available

The medium does not contain antibiotics or cytokines. Human serum albumin and human transferrin are the only human origin material and are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HB_sAg.

Add 20 mL of 200 mM L-glutamine solution or 0.584 g of γ-irradiated L-glutamine powder per liter of solution.

ship: wet ice store at: 2-8°C

S1694-1L	1 L

Stemline® Neural Stem Cell Expansion Medium

NSC Medium
without L-glutamine, growth factors, and antibiotics, liquid, sterile-filtered, suitable for cell culture

Developed to promote the optimal expansion of human neural stem cells (NSC, Stemline Neural Stem Cell Expansion Medium demonstrates rigorous expansion of human neural stem cells in both neurosphere and monolayer cultures.

Stemline Neural Stem Cell Expansion Medium is free of serum and all other animal components; this exclusion increases performance consistency and elimates safety risks associated with potential adventitious agents.

Produced in a GMP state-of-the-art facility with an available Device Master File (DMF), Stemline Neural Stem Cell Expansion Medium is clearly an excellent choice for your NSC applications.

Drug/Device Master File available

ship: wet ice store at: 2-8°C

S3194-500ML	500 mL

Keratinocyte Medium Supplement, 100x, BPE-free

Keratinocyte Medium Supplement

liquid, sterile-filtered, suitable for cell culture

This supplement is suitable for use with Stemline® Keratinocyte Medium II, S0196.

Produced in a GMP state-of-the-art facility, Stemline Keratinocyte Stem Cell Expansion Medium is clearly an excellent choice for your Keratinocyte applications.

Contains recombinant growth factors, hydrocortisone, bovine serum albumin, and bovine transferrin. Does not contain bovine pituitary extract (BPE).

endotoxin ..... tested

ship: ambient store at: −20°C

K3136-5ML	5 mL

Classical Media

Classical Media

Media Selection Quick Reference Tables

Dulbecco's Modified Eagle's Medium [DMEM] & Dulbecco's Modified Eagle's Medium: Ham's F12 Nutrient Mixture [DME F12]

Liquid/ Powder	w/Glutamine	w/$NaHCO_3$	w/HEPES	Glucose hi/lo	Phenol Red	Sodium Pyruvate	Cat. No.	Comment
L	no	yes	no	hi	no	no	**D1145**	Originally formulated to support mouse embryos
L	no	yes	no	hi	yes	no	**D5671**	
L	yes	yes	no	hi	yes	no	**D5796**	
L	no	yes	25 mM	hi	yes	no	**D6171**	HEPES modification
L	yes	yes	no	hi	yes	yes	**D6429**	
L	no	yes	no	hi	yes	yes	**D6546**	
L	no	no	no	lo	yes	yes	**D2429**	10× w/o folate
L	no	yes	no	lo	yes	no	**D5546**	
L	no	yes	no	lo	no	no	**D5921**	
L	yes	yes	no	lo	yes	yes	**D6046**	
P	yes	no	25 mM	hi	yes	no	**D1152**	HEPES modification
P	yes	no	no	hi	yes	no	**D5648**	
P	yes	no	no	hi	yes	yes	**D7777**	
P	yes	no	no	lo	no	no	**D2902**	
P	yes	no	no	lo	yes	no	**D5523**	
P	no	no	no	lo	no	no	**D5030**	
L	no	yes	15 mM	3.15 g/L	yes	0.055 g/L	**D6421**	1:1 DME:Ham's F12
L	yes	yes	no	3.15 g/L	yes	0.055 g/L	**D8062**	1:1 DME:Ham's F12
L	no	yes	15 mM	3.15 g/L	no	0.055 g/L	**D6434**	1:1 DME:Ham's F12
P	yes	no	no	3.15 g/L	yes	0.11 g/L	**D0547**	1:1 DME:Ham's F12
P	yes	no	15 mM	3.15 g/L	no	0.055 g/L	**D2906**	1:1 DME:Ham's F12
P	yes	no	15 mM	3.15 g/L	yes	0.055 g/L	**D8900**	1:1 DME:Ham's F12
P	no	no	15 mM	3.15 g/L	no	0.055 g/L	**D9785**	1:1 DME:Ham's F12

Various Media Selection Tables

Liquid/ Powder	w/Gluta-mine	w/$NaHCO_3$	w/HEPES	w/Earles salts (5% CO_2)	w/Hanks salts (2% CO_2)	Phenol Red	Sodium Pyruvate	Cat. No.	Comment
Medium 199									
L	no	no	no	yes		yes	no	**M0650**	10×
L	no	yes	no	yes		yes	no	**M2154**	
L	yes	yes	no	yes		yes	no	**M4530**	
L	no	yes	25 mM	yes		yes	no	**M7528**	
L	no	yes	no		yes	yes	no	**M7653**	
L	no	no	no		yes	yes	no	**M9163**	
P	yes	no	no		yes	yes	no	**M0393**	
P	yes	no	25 mM	yes		yes	no	**M2520**	
P	no	no	no	yes		no	no	**M3769**	
P	yes	no	no	yes		yes	no	**M5017**	
Minimal Essential Media [MEM] [alpha-MEM] [Joklik MEM] [Spinner MEM, S-MEM]									
L	no	no	no	yes		yes	no	**M0275**	10×
L	no	yes	no	yes		yes	no	**M2279**	
L	no	yes	no			yes	no	**M4526**	MEM-alpha (alpha-MEM), without ribonucleosides and deoxyribonucleosides
L	yes	yes	no	yes		yes	no	**M4655**	
L	yes	yes	no		yes	yes	no	**M4780**	
L	no	yes	no	yes		yes	no	**M5650**	with NEAA (non-essential amino acids)
L	no				yes	yes	no	**M5775**	
L	no	yes	25 mM	yes		yes	no	**M7278**	HEPES modification of media

Various Media Selection Tables

Liquid/ Powder	w/Gluta-mine	w/$NaHCO_3$	w/HEPES	w/Earles salts (5% CO_2)	w/Hanks salts (2% CO_2)	Phenol Red	Sodium Pyruvate	Cat. No.	Comment
L	no	yes	no			yes	no	M8042	MEM-alpha (alpha-MEM) with ribonucleo-sides and deoxyribonucleosides
L	no	yes	no	yes		yes	no	M8167	Spinner Modification(S-MEM) without cal-cium chloride
L	no	no	no		yes	yes	no	M9288	10×
P	yes	no	no	yes		yes	no	M0268	
P	yes	no	no			yes	no	M0518	Joklik (yoklik) Mod. For suspension, without calcium chloride
P	yes	no	no	yes		yes	no	M0643	with NEAA (non-essential amino acids)
P	yes	no	no			yes	no	M0644	MEM-alpha (alpha-MEM), with ribonucleo-sides and deoxyribonucleosides
P	no	no	no	yes		yes	no	M0769	Auto-Mod™ - autoclavable
P	yes	no	no			yes	no	M0894	MEM-alpha (alpha-MEM, without ribonu-cleosides and deoxyribonucleosides
P	yes	no	no		yes	yes	no	M1018	with NEAA (non-essential amino acids)
P	yes	no	25 mM	yes		yes	no	M2645	HEPES modification
P	no	no	no	yes		no	no	M3024	with NEAA (non-essential amino acids)
P	yes	no	no		yes	yes	no	M4642	
P	no	yes	no			yes	no	M8028	Joklik (yoklik) Mod. For suspension
Nutrient Mix F-10 Hams [Ham's-F10]									
L	no	no	20 mM			yes	0.11 g/L	N2147	HEPES modification of media
L	no	yes	no			yes	0.11 g/L	N6013	
L	yes	yes	no			yes	0.11 g/L	N6908	
P	yes	no	no			yes	0.055 g/L	N6635	
F-12 Hams [Ham's F-12]									
L	no	yes	no			yes	0.11 g/L	N4888	
L	no	yes	yes			yes	0.11 g/L	N8641	
P	yes	no	no			yes	0.22 g/L	N3520	
P	yes	no	no			yes	0.11 g/L	N6658	
P	yes	no	no			yes	0.11 g/L	N6760	
RPMI 1640									
L	no	yes	no			yes	no	R0883	very commonly used media
L	no	no	no			yes	no	R1145	10×, without folate
L	no	yes	25 mM		**	yes	no	R5886	**for use in non-CO_2 atm
L	yes	no	20 mM			yes	no	R7388	
L	no	yes	no			no	no	R7509	
L	no	yes	no			no	no	R7513	without methionine and cystine
L	no	yes	20 mM			yes	no	R7638	Dutch modification
L	yes	yes	no			yes	no	R8758	
P	yes	no	no			yes	no	R1383	without glucose
P	yes	no	25 mM			yes	no	R4130	
P	yes	no	no			yes	no	R6504	
P	no	no	no			yes	no	R7755	Auto-Mod™
P	yes	no	no			no	no	R8755	

Classical Media Formulations

Ames' Medium

Ames' Medium

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Use to maintain retinal tissues for *in vitro* studies on central nervous system.

Formulated to contain 8.8 grams of powder per liter of medium.

Supplement with 1.9 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

A1420-10X1L	10 × 1 L

Basal Medium Eagle (BME)

Basal Medium Eagle

BME

With Earle's salts and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

BME, when properly supplemented, has demonstrated wide applicability, for supporting monolayer growth of a wide variety of normal and transformed cell lines.

Formulated to contain 9.2 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

Eagle's Basal Medium or BME, developed by Harry Eagle, is one of the most widely used of all synthetic cell culture media.

ship: ambient store at: 2-8°C

B9638-10X1L	10 × 1 L
B9638-10L	10 L
B9638-50L	50 L

With Earle's salts and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

B1522-500ML	500 mL
B1522-6X500ML	6 × 500 mL

BME Vitamins 100x solution

BioReagent, sterile-filtered, suitable for cell culture

ship: dry ice store at: –20°C

B6891-100ML	100 mL

Claycomb

Claycomb Medium

without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Claycomb Medium, named after Dr. William Claycomb who established the HL-1 cell line, is specifically designed for the growth of murine cardiomyocytes. HL-1 is the first cell line established that can maintain the differentiated cardiomyocyte phenotype and contractile activity *in vitro*. The HL-1 cell line can be used for the study of cardiac cell hypertrophy that follows myocardial infarction, the testing of novel cardiac therapeutic drugs and treatments, the production of high levels of cardiac proteins and the study of mature cardiomyocyte specific genes. Claycomb Medium, when supplemented with 100 µM norepinephrin, 10% fetal bovine serum (FBS) and 4 mM L-glutamine, will maintain the HL-1 cell line and the mature cardiomycyte behavior. While observing the HL-1 cells under light microscopy, individual and groups of cells can be observed contracting, becoming more frequent as the cardiomyocytes reach confluency.

For research or for further manufacturing use.

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

51800C-500ML	500 mL

Click's Medium

Click's Medium

Eagle's Ham's amino acids; EHAA; Click's EHAA Medium
With sodium bicarbonate, without mercaptoethanol and L-glutamine, liquid, sterile-filtered, suitable for cell culture

Frequently used for the suspension, washing and culture of T-cells.

Supplement with 0.584 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

C5572-500ML	500 mL
C5572-6X500ML	6 × 500 mL

Dulbecco's Media

Dulbecco's Modified Eagle's Medium (DME)

Dulbecco's Modified Eagle's Medium

Without glucose, L-glutamine, phenol red, sodium pyruvate and sodium bicarbonate, powder, suitable for cell culture

The most basic formulation offered. This formulation is used by investigators who want to start with the essential components of DME, and have the flexibility to optimize the formula for their own application.

Formulated to contain 8.3 grams of powder per liter of medium.

Supplement with 1.0 g/L glucose, 0.584 g/L L-glutamine, 3.7 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D5030-10X1L	10 × 1 L
D5030-10L	10 L
D5030-50L	50 L

Dulbecco's Modified Eagle's Medium - high glucose

DME; DMEM

With 4500 mg/L glucose, L-glutamine, and sodium bicarbonate, without sodium pyruvate, liquid, sterile-filtered, suitable for cell culture

This DMEM-Hi glucose medium is a 1x complete medium with no added factors (common modifications) such as L-alanyl-L-glutamine, HEPES, or sodium pyruvate. It differs from the original DMEM-Hi formulation wherein pyridoxine is substituted for pyridoxal. Pyridoxal is an unstable component of media.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D5796-500ML	500 mL
D5796-6X500ML	6 × 500 mL
D5796-24X500ML	24 × 500 mL
D5796-1L	1 L
D5796-6X1L	6 × 1 L

With 4500 mg/L glucose and sodium bicarbonate, without L-glutamine and sodium pyruvate, liquid, sterile-filtered, suitable for cell culture, suitable for hybridoma

This DMEM-Hi glucose medium is a 1x complete medium with no added factors (common modifications) such as L-alanyl-L-glutamine, HEPES, or sodium pyruvate. It differs from the original DMEM-Hi formulation wherein pyridoxine is substituted for pyridoxal. Pyridoxal is an unstable component of media. This medium requires supplementation with L-glutamine or L-alanyl-L-glutamine.

Supplement with 0.584 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D5671-500ML	500 mL
D5671-6X500ML	6 × 500 mL
D5671-24X500ML	24 × 500 mL
D5671-1L	1 L
D5671-6X1L	6 × 1 L

With 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

This DMEM-Hi glucose medium is a 1x complete medium with sodium pyruvate added. It also differs from the original DMEM-Hi formulation wherein pyridoxine is substituted for pyridoxal. Pyridoxal is an unstable component of media.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D6429-500ML	500 mL
D6429-6X500ML	6 × 500 mL
D6429-24X500ML	24 × 500 mL
D6429-1L	1 L
D6429-6X1L	6 × 1 L

With 4500 mg/L glucose, sodium pyruvate, and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

This DMEM-Hi glucose medium is a 1x complete medium with sodium pyruvate added. It also differs from the original DMEM-Hi formulation wherein pyridoxine is substituted for pyridoxal. Pyridoxal is an unstable component of media. This medium requires supplementation with L-glutamine or L-alanyl-L-glutamine.

Supplement with 0.584 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D6546-500ML	500 mL
D6546-6X500ML	6 × 500 mL
D6546-24X500ML	24 × 500 mL

With 4500 mg/L glucose and sodium bicarbonate, without L-glutamine, sodium pyruvate, and phenol red, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.584 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D1145-500ML	500 mL
D1145-6X500ML	6 × 500 mL

HEPES modification, With 4500 mg/L glucose, 25 mM HEPES, and sodium bicarbonate, without L-glutamine and sodium pyruvate, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.584 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D6171-500ML	500 mL
D6171-6X500ML	6 × 500 mL
D6171-24X500ML	24 × 500 mL
D6171-1L	1 L

With 4500 mg/L glucose and sodium bicarbonate, without L-methionine, L-cystine and L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.584 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D0422-100ML	100 mL

Dulbecco's Modified Eagle's Medium - high glucose *(continued)*

With 4500 mg/L glucose and L-glutamine, without sodium bicarbonate., powder, suitable for cell culture

Formulated to contain 13.4 grams of powder per liter of medium.

Supplement with 3.7 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D5648-10X1L	10 × 1 L
D5648-2X5L	2 × 5 L
D5648-10L	10 L
D5648-50L	50 L

With 4500 mg/L glucose, L-glutamine, and sodium pyruvate, without sodium bicarbonate., powder, suitable for cell culture

Formulated to contain 13.5 grams of powder per liter of medium.

Supplement with 3.7 g/L sodium bicarbonate

ship: ambient store at: 2-8°C

D7777-10X1L	10 × 1 L
D7777-10L	10 L
D7777-50L	50 L

HEPES Modification, With 4500 mg/L glucose, L-glutamine, and 25 mM HEPES, without sodium bicarbonate and pyruvate, powder, suitable for cell culture

Formulated to contain 17.3 grams of powder per liter of medium.

Supplement with 3.7 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D1152-10X1L	10 × 1 L
D1152-10L	10 L
D1152-50L	50 L

Dulbecco's Modified Eagle's Medium - low glucose

DME

With 1000 mg/L glucose, L-glutamine, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Fully supplemented liquid form of the original Dulbecco's formulation, with one key exception. Pyridoxal, which is an aldehyde, has been replaced with pyridoxine. This modification has been shown to improve the stability of the medium.

This DMEM-Low glucose medium is a 1x complete medium with sodium pyruvate added. It also differs from the original DMEM-Hi formulation wherein pyridoxine is substituted for pyridoxal. Pyridoxal is an unstable component of media.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D6046-500ML	500 mL
D6046-6X500ML	6 × 500 mL
D6046-24X500ML	24 × 500 mL
D6046-1L	1 L
D6046-6X1L	6 × 1 L

With 1000 mg/L glucose, and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

This modification has been shown to improve the stability of the medium.

Supplement with 0.584 g/L glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D5546-500ML	500 mL
D5546-6X500ML	6 × 500 mL
D5546-24X500ML	24 × 500 mL
D5546-1L	1 L
D5546-6X1L	6 × 1 L

With 1000 mg/L glucose, and sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture

This modification has been shown to improve the stability of the medium.

Supplement with 0.584 g/L L-glutamine.

Investigators who work with stem cells or cell clones frequently prefer media without phenol red.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D5921-500ML	500 mL
D5921-6X500ML	6 × 500 mL

10 ×, With 1000 mg/L glucose (1x), without L-glutamine, sodium bicarbonate, and folic acid, liquid, sterile-filtered, suitable for cell culture

Supplement with 3.7 g/L sodium bicarbonate, 0.584 g/L L-glutamine, 0.004 g/L folic acid, at 1×.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D2429-100ML	100 mL

With 1000 mg/L glucose and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Provides investigator with the option to modify the buffering system.

Formulated to contain 10.0 grams of powder per liter of medium.

Supplement with 3.7 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D5523-10X1L	10 × 1 L
D5523-10L	10 L
D5523-50L	50 L

With 1000 mg/L glucose and L-glutamine, without sodium bicarbonate and phenol red, powder, suitable for cell culture

Formulated to contain 10.0 grams of powder per liter of medium.

Supplement with 3.7 g/L sodium bicarbonate.

Investigators who work with stem cells or cell clones frequently prefer media without phenol red.

ship: ambient store at: 2-8°C

D2902-10X1L	10 × 1 L
D2902-10L	10 L

With 1000mg/L L-glucose, L-glutamine, and sodium bicarbonate. Without arginine, leucine, lysine, sodium pyruvate, and phenol red, liquid, sterile-filtered, suitable for cell culture NEW

Designed for use in stable isotope labeling with amino acids in cell culture (SILAC) applications.

Supplement with 0.100 g/L L-Arginine-$^{13}C_6$, $^{15}N_4$ HCl (Product 608033), 0.100 g/L L-Leucine-$^{13}C_6$, ^{15}N (Product 608068), and 0.100 g/L L-Lysine-$^{13}C_6$, $^{15}N_2$ (Product 608041).

endotoxin ..... tested

ship: ambient store at: 2-8°C

D9443-500ML	500 mL

DME/Nutrient Mixture F-12 Ham

Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham

DME/F-12, 1:1 mixture

With L-glutamine, 15 mM HEPES, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: 2-8°C

D8437-500ML	500 mL
D8437-6X500ML	6 × 500 mL
D8437-24X500ML	24 × 500 mL

With 15 mM HEPES and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

DMEM:F12 is a 50:50 mixture of DMEM and Ham's F12 media that has proven to be useful in a wide range of cell culture applications, especially when supplemented with fetal bovine serum (FBS). Ham's Nutrient Mixture F12 was originally developed for the serum-free clonal growth of Chinese Hamster Ovary (CHO) cells, lung cells, and mouse L cells. It is frequently used with dialyzed serum, hormones, selenium, and other supplements for serum-free cultures. It is the medium of choice for supporting the growth of cells of rodent origin, particularly rabbbit and rat, and has been proven to be an excellent cloning medium for myeloma and hybridoma cells.

Supplement with 0.365 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

D6421-500ML	500 mL
D6421-6X500ML	6 × 500 mL
D6421-24X500ML	24 × 500 mL

With 15 mM HEPES and sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture

Pyridoxal, which is an aldehyde, has been replaced with pyridoxine. This modification has been shown to improve the stability of the medium. Suitable for growth of a wide range of cell types in low serum conditions. Absence of phenol red makes this a preferred medium for clonal and stem cell culture.

Supplement with 0.365 g/L L-glutamine

endotoxin ..... tested
ship: ambient store at: 2-8°C

D6434-500ML	500 mL
D6434-6X500ML	6 × 500 mL

With L-glutamine and sodium bicarbonate, without HEPES, liquid, sterile-filtered, suitable for cell culture

Allows the investigator to optimize the buffering system.

endotoxin ..... tested
ship: ambient store at: 2-8°C

D8062-500ML	500 mL
D8062-6X500ML	6 × 500 mL

With L-glutamine and 15 mM HEPES, without sodium bicarbonate, powder, suitable for cell culture

Suitable for growth of a wide range of cell types in low serum conditions.

Formulated to contain 15.6 grams of powder per liter of medium.

Supplement with 1.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D8900-10X1L	10 × 1 L
D8900-10L	10 L
D8900-50L	50 L

With L-glutamine and 15 mM HEPES, without sodium bicarbonate and phenol red, powder, suitable for cell culture

Suitable for growth of a wide range of cell types in low serum conditions. The removal of phenol red is often preferred by investigators working with clonal and stem cell cultures.

Formulated to contain 15.6 grams of powder per liter of medium.

Supplement with 1.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D2906-10X1L	10 × 1 L
D2906-10L	10 L

With L-glutamine and trace elements, without HEPES and sodium bicarbonate, powder, suitable for cell culture

Trace elements include ammonium molybdate, ammonium metavanadate, stannous chloride, sodium selenite, magnesium sulfate, nickel chloride, and sodium metasilicate

Formulated to contain 12.0 grams of powder per liter of medium.

Supplement with 1.2 g/L sodium bicarbonate

ship: ambient store at: 2-8°C

D0547-10X1L	10 × 1 L
D0547-10L	10 L

With 15 mM HEPES, without L-glutamine, L-leucine, L-lysine, L-methionine, $CaCl_2$, $MgCl_2$, $MgSO_4$, sodium bicarbonate, and phenol red, powder, suitable for cell culture

The most basic formulation offered. This formulation is used by investigators who want to start with the essential components of (1;1) DME/Ham's F-12, and have the flexibility to optimize the formula for their own application. This base formulation is particularly suitable for studies of cell protein metabolism with labeL-tagged amino acids.

Formulated to contain 14.8 grams of powder per liter of medium.

Supplement with 0.1545 g/L calcium chloride, 0.365 g/L L-glutamine, 0.05905 g/L L-leucine, 0.09125 g/L L-lysine, 0.0612 g/L magnesium chloride, 0.04884 g/L magnesium sulfate, 0.0172 g/L L-methionine, 1.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

D9785-10L	10 L

Dulbecco's Phosphate Buffered Saline

Dulbecco's Phosphate Buffered Saline

DPBS

With $MgCl_2$ and $CaCl_2$, liquid, sterile-filtered, suitable for cell culture

D-PBS is used to wash cells during preparation and serial transfer.

endotoxin ..... tested
ship: ambient store at: 2-8°C

D8662-100ML	100 mL
D8662-500ML	500 mL
D8662-6X500ML	6 × 500 mL
D8662-24X500ML	24 × 500 mL
D8662-1L	1 L
D8662-6X1L	6 × 1 L

Without calcium chloride, powder, suitable for cell culture

Use this formulation of DPBS when tissue or cell dissociation or release is the objective. Also, suitable for studies that involve calcium metabolism.

Formulated to contain 9.6 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

D5773-10X1L	10 × 1 L
D5773-10L	10 L
D5773-50L	50 L

Modified, with 36 mg sodium pyruvate, 50 mg streptomycin sulfate, 100 mg kanamycin monosulfate, 1000 mg glucose/L and $CaCl_2$, liquid, sterile-filtered, suitable for cell culture

Contains 50 mg/L streptomycin sulfate and 100 mg/L kanamycin monosulfate.

Fully supplemented isotonic solution. Contains energy sources and antibiotics. Frequently used with primary cells where risk of contamination may be high.

endotoxin ..... tested
ship: ambient store at: 2-8°C

D4031-100ML	100 mL
D4031-500ML	500 mL
D4031-6X500ML	6 × 500 mL
D4031-1L	1 L
D4031-6X1L	6 × 1 L

Modified, without calcium chloride and magnesium chloride, powder, suitable for cell culture

D-PBS is used to wash cells during preparation and serial transfer.

Formulated to contain 9.6 grams of powder per liter of medium.

This D-PBS is formulated without Ca or Mg. Formulations with Ca and Mg added are also available

ship: ambient store at: 2-8°C

D5652-10X1L	10 × 1 L
D5652-2X5L	2 × 5 L
D5652-10L	10 L
D5652-50L	50 L

With calcium chloride and magnesium chloride, 10×, liquid, sterile-filtered, suitable for cell culture

A general use, isotonic saline solution for washing cells and tissues.

ship: ambient store at: room temp

D1283-500ML	500 mL
D1283-6X500ML	6 × 500 mL

Modified, without calcium chloride and magnesium chloride, 10×, liquid, sterile-filtered, suitable for cell culture

Dulbecco's Phosphate Buffered Saline 10X is a stock solution used to prepare 1X D-PBS in cell culture grade water (W3500). D-PBS is used to wash cells during preparation and serial transfer.

endotoxin ..... tested
ship: ambient store at: room temp

D1408-100ML	100 mL
D1408-500ML	500 mL
D1408-6X500ML	6 × 500 mL
D1408-24X500ML	24 × 500 mL

Modified, without calcium chloride and magnesium chloride, liquid, sterile-filtered, suitable for cell culture

DPBS is a balanced salt solution (BSS) used for the handling and culturing of mammalian cells. DPBS is used to to irrigate, wash, and dilute mammalian cells. Phosphate buffering maintains the pH in the physiological range. Calcium and magnesium facilitate cell binding and clumping. DPBS without these ions can be used to wash and rinse suspended cells.

endotoxin ..... tested
ship: ambient store at: room temp

D8537-100ML	100 mL
D8537-500ML	500 mL
D8537-6X500ML	6 × 500 mL
D8537-24X500ML	24 × 500 mL
D8537-1L	1 L
D8537-6X1L	6 × 1 L

liquid, sterile-filtered, DPBS Modified, without calcium, without magnesium, suitable for cell culture NEW

for research or for further manufacturing use

ship: ambient store at: room temp

59321C-1000ML	1000 mL

liquid, sterile-filtered, DPBS Modified 10X, without calcium, without magnesium, suitable for cell culture NEW

for research or for further manufacturing use

ship: ambient store at: room temp

59331C-1000ML	1000 mL

Glasgow Minimum Essential Medium (GMEM)

Glasgow Minimum Essential Medium

GMEM

With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

For use with adherent kidney cell lines such as baby hamster kidney cells (BHK).

Supplement with 0.292 g/L L-glutamine and Tryptose phosphate broth solution (T 8159) at 100 ml/L of medium.

endotoxin ..... tested

ship: ambient store at: 2-8°C

G5154-500ML	500 mL
G5154-6X500ML	6 × 500 mL

With L-glutamine, without tryptose phosphate broth and sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 12.5 grams of powder per liter of medium.

Supplement with 2.75 g/L sodium bicarbonate and Tryptose phosphate broth solution (T 8159) at 100 ml/L of medium.

ship: ambient store at: 2-8°C

G6148-10X1L	10 × 1 L
G6148-10L	10 L
G6148-50L	50 L

Glasgow's Modified Eagle's Medium NEW

Glasgow Minimum Essential Medium

GMEM, with 800 mg/L glycine, with 2750 mg/L sodium bicarbonate, with 110 mg/L sodium pyruvate, without ferric nitrate, without L-glutamine

for research or for further manufacturing use

liquid

ship: ambient store at: 2-8°C

51492C-500ML	500 mL

Iscove's Modified Dulbecco's Medium

Iscove's Modified Dulbecco's Medium

IMDM

With L-glutamine and 25 mM HEPES, without sodium bicarbonate, powder, suitable for cell culture

Reported to support murine B lymphocytes, hematopoietic tissue from bone marrow, B cells stimulated with lipopolysaccharide, T lymphocytes, and a variety of hybrid cells.

Formulated to contain 17.7 grams of powder per liter of medium.

Supplement with 3.024 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

I7633-10X1L	10 × 1 L
I7633-10L	10 L
I7633-50L	50 L

liquid NEW

for research or for further manufacturing use

with 4.0 mM L-glutamine, with 25 mM HEPES

ship: ambient store at: 2-8°C

51471C-1000ML	1000 mL

liquid, sterile-filtered, With sodium bicarbonate, without L-glutamine, suitable for cell culture, suitable for hybridoma

Guilbert and Iscove demonstrated that precursor cells of erythrocytes and macrophages could be cultured in a totally defined serum free medium supplemented with albumin, transferrin, lecithin, and selenium. This medium is a modification of Dulbecco's Modified Eagle's Medium (DME) containing selenium, additional amino acids and vitamins, sodium pyruvate, HEPES buffer, and potassium nitrate instead of ferric nitrate. Further studies demonstrated that Iscove's Medium would support murine B lymphocytes, hemopoietic tissue from bone marrow, B cells stimulated with lipopolysaccharide, T lymphocytes, and a variety of hybrid cells.

Supplement with 0.584 g/L L-glutamine

endotoxin ..... tested

ship: ambient store at: 2-8°C

I3390-500ML	500 mL
I3390-6X500ML	6 × 500 mL
I3390-24X500ML	24 × 500 mL
I3390-1L	1 L
I3390-6X1L	6 × 1 L

With sodium bicarbonate and L-glutamine, liquid, sterile-filtered, suitable for cell culture, suitable for hybridoma

Reported to support murine B lymphocytes, hematopoietic tissue from bone marrow, B cells stimulated with lipopolysaccharide, T lymphocytes, and a variety of hybrid cells.

endotoxin ..... tested

ship: ambient store at: 2-8°C

I6529-500ML	500 mL

L-15 Medium (Lebovitz)

L-15 Medium (Leibovitz)

Originally formulated for use in carbon dioxide free systems requiring sodium bicarbonate supplement. L-15 supports established cell lines as well as primary explants of embryonic and adult human tissue.

endotoxin ..... tested

With L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

L1518-500ML	500 mL
L1518-6X500ML	6 × 500 mL

Without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.3 g/L L-glutamine.

ship: ambient store at: 2-8°C

L5520-100ML	100 mL
L5520-500ML	500 mL
L5520-6X500ML	6 × 500 mL
L5520-24X500ML	24 × 500 mL
L5520-1L	1 L
L5520-10L	10 L

With L-glutamine, powder, suitable for cell culture

Formulated to contain 13.8 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

L4386-10X1L	10 × 1 L
L4386-10L	10 L
L4386-50L	50 L

McCoy's 5A Modified Medium

McCoy's 5A Medium

Originally developed to support liver tumor cells by modification of the amino acids found in BME. This formulation has also be used in the culture of bone marrow, skin, gingiva, kidney, omentum, adrenals, lung, spleen, rat embryos, and other cell types.

Modified, with sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Used for the primary culture of cells from a wide range of tissues. Recommend for production of virus in primary cell cultures.

Supplement with 0.22 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M8403-500ML	500 mL
M8403-6X500ML	6 × 500 mL
M8403-24X500ML	24 × 500 mL

Modified, with L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested

ship: ambient store at: 2-8°C

M9309-500ML	500 mL
M9309-6X500ML	6 × 500 mL

Modified, with L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Used for the primary culture of cells from a wide range of tissues. Recommend use for production of virus in primary cell cultures.

Formulated to contain 11.9 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M4892-10X1L	10 × 1 L
M4892-10L	10 L

MCDB Medium

MCDB 105 Medium

With trace elements, L-glutamine and 25mM HEPES, powder, suitable for cell culture

Originally developed for long term growth of WI-38, MRC-5, and other human diploid fibroblast-like cells.

Formulated to contain 14.9 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

M6395-1L	1 L
M6395-10X1L	10 × 1 L
M6395-10L	10 L

MCDB 131 Medium

With trace elements and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Originally developed for the clonal growth of human microvascular endothelial cells (HMVEC) and has also been used to culture liver, smooth muscle, cardiac myocytes, and other cell types.

Formulated to contain 11.6 grams of powder per liter of medium.

Supplement with 1.18 g/L sodium bicarbonate.

concentration ..... 11.6 g/L

ship: ambient store at: 2-8°C

M8537-1L	1 L
M8537-10X1L	10 × 1 L
M8537-10L	10 L

MCDB 153 Medium

With trace elements, L-glutamine and 28mM HEPES, without sodium bicarbonate, powder, suitable for cell culture

Originally developed for clonal and long term growth of human epidermal keratinocytes under serum-free conditions.

Formulated to contain 17.6 grams of powder per liter of medium.

Supplement with 1.18 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M7403-1L	1 L
M7403-10X1L	10 × 1 L
M7403-10L	10 L

MCDB 201 Medium

With trace elements, L-glutamine and 30 mM HEPES, powder, suitable for cell culture

Originally developed for clonal growth of chicken embryo fibroblasts.

Formulated to contain 17.7 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

M6770-1L	1 L
M6770-10X1L	10 × 1 L

Medium 199

Medium 199

Originally developed as a completely defined media formulation for the culture of primary explants. These media have broad applicability, particularly for non-transformed cells and are widely used for vaccine production, primary pancreatic explants, and lens tissues.

With Earle's salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Complete liquid version of the original M 199 formulation published by Morgan.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M4530-100ML	100 mL
M4530-500ML	500 mL
M4530-6X500ML	6 × 500 mL
M4530-1L	1 L
M4530-6X1L	6 × 1 L

With Earle's salts and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.1 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M2154-500ML	500 mL
M2154-6X500ML	6 × 500 mL

HEPES Modification, with Earle's salts, 25 mM HEPES and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.1 g/L L-glutamine.

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

M7528-500ML	500 mL
M7528-6X500ML	6 × 500 mL

10 ×, With Earle's salts, without L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.1 g/L L-glutamine, 2.2 g/L sodium bicarbonate at 1×.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M0650-100ML	100 mL

With Earle's salts and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 9.5 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M5017-10X1L	10 × 1 L
M5017-50L	50 L

Modified, with Earle's salts, without L-glutamine, sodium bicarbonate, and phenol red, powder, suitable for cell culture

Formulated to contain 9.4 grams of powder per liter of medium.

Supplement with 0.1 g/L L-glutamine, 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M3769-10X1L	10 × 1 L
M3769-50L	50 L

HEPES Modification, with Earle's salts, L-glutamine and 25 mM HEPES, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 14.7 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M2520-10X1L	10 × 1 L

With Hanks' salts and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Modification of the original Medium 199.Earle's salts are replaced with Hanks' salts. Hanks' salts use a lower concentration of bicarbonate and different concentrations and salt forms of the essential inorganic ions.

Supplement with 0.1 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M7653-500ML	500 mL
M7653-6X500ML	6 × 500 mL

10 ×, With Hanks' salts, without L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.1 g/L L-glutamine, 0.35 g/L sodium bicarbonate at 1×.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M9163-500ML	500 mL
M9163-6X500ML	6 × 500 mL

With Hanks' salts and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 10.6 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M0393-10X1L	10 × 1 L
M0393-50L	50 L

Medium 199 Modified NEW

Medium 199

Originally developed as a completely defined media formulation for the culture of primary explants. These media have broad applicability, particularly for non-transformed cells and are widely used for vaccine production, primary pancreatic explants, and lens tissues.

for research or for further manufacturing use

Contains all L-form amino acids

with Hank's Balanced Salts, with 0.68 mM L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

51322C-1000ML	1000 mL

with Earle's Balanced Salts, with 0.68 mM L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture

Formulated to contain 9.5 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

56312C-10L	10 L
56312C-50L	50 L

Minimum Essential Medium Eagle (MEM)

Minimum Essential Medium NEW

Minimum Essential Medium Eagle; MEM

for research or for further manufacturing use

with Earle's Balanced Salts, with 2.0 mM L-glutamine, without sodium bicarbonate, dry powder, suitable for cell culture

Formulated to contain 9.5 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

56419C-10L	10 L
56419C-50L	50 L

with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate, dry powder, suitable for cell culture

Formulated to contain 9.6 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

56416C-1L	1 L
56416C-10L	10 L
56416C-50L	50 L
56416C-100L	100 L

with Earle's Balanced Salts, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

51412C-1000ML	1000 mL

Minimum Essential Medium *(continued)*

with Earle's Balanced Salts, with 2.0 mM L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

51411C-1000ML	1000 mL

with Earle's Balanced Salts, with non-essential amino acids, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

51416C-1000ML	1000 mL

with Earle's salts, with 2.0 mM L-glutamine, with 20.0 mM HEPES, with non-essential amino acids, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

51417C-1000ML	1000 mL

liquid, cell culture tested

with Earle's Balanced Salts, without L-glutamine, without phenol red

ship: ambient store at: 2-8°C

51414C-1000ML	1000 mL

with Earle's Balanced Salts, with 25mM HEPES, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: 2-8°C

51415C-1000ML	1000 mL

Minimum Essential Medium Eagle

MEM

With Earle's salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

This product lacks L-Ala; L-Asn; L-Glu; Gly; L-Pro; L-Ser.

Generally used to grow attached cell lines, such as fibroblasts, in the presence of FBS, calf or horse sera.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M4655-500ML	500 mL
M4655-6X500ML	6 × 500 mL
M4655-24X500ML	24 × 500 mL
M4655-1L	1 L
M4655-6X1L	6 × 1 L

With Earle's salts and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Generally used to grow attached cell lines, such as fibroblasts, in the presence of FBS, calf or horse sera.

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M2279-100ML	100 mL
M2279-500ML	500 mL
M2279-6X500ML	6 × 500 mL
M2279-24X500ML	24 × 500 mL
M2279-1L	1 L
M2279-6X1L	6 × 1 L

With Earle's salts, non-essential amino acids and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Contains 19 amino acids. The essential amino acids and the non-essential amino acids; L-ala; L-asn; L-asp; L-glu; L-gly; L-pro and L-ser. May be preferred for growth of adherent cells in the presence of reduced serum or as a base for development of a serum free medium.

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M5650-500ML	500 mL
M5650-6X500ML	6 × 500 mL
M5650-1L	1 L

10 ×, With Earle's salts, without L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Generally used to grow attached cell lines, such as fibroblasts, in the presence of FBS, calf or horse sera. The L-glutamine and sodium bicarbonate are added to the medium after dilution to 1x and pH adjustment.

Supplement with 0.292 g/L L-glutamine, 2.2 g/L sodium bicarbonate at 1×.

endotoxin ..... tested

ship: ambient store at: 2-8°C

M0275-100ML	100 mL
M0275-500ML	500 mL
M0275-6X500ML	6 × 500 mL

With Earle's salts and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Generally used to grow attached cell lines, such as fibroblasts, in the presence of FBS, calf or horse sera.

Formulated to contain 9.5 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

Essentially the same as the original formula, except it is provided as a powder.

ship: ambient store at: 2-8°C

M0268-10X1L	10 × 1 L
M0268-10L	10 L
M0268-50L	50 L

With Earle's salts, L-glutamine, and non-essential amino acids, without sodium bicarbonate, powder, suitable for cell culture

It contains 19 amino acids. The essential amino acids and the non-essential amino acids; L-ala; L-asn; L-asp; L-glu; L-gly; L-pro and L-ser. May be preferred for growth of adherent cells in the presence of reduced serum or as a base for development of a serum free medium.

Formulated to contain 9.6 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M0643-10X1L	10 × 1 L
M0643-10L	10 L
M0643-50L	50 L

With Hanks' salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Modification of the original MEM formulation. Earle's salts have been replaced with Hanks' salts. Since the bicarbonate concentration is substantially lower, this medium has a substantially reduced buffering capacity.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M4780-500ML	500 mL
M4780-6X500ML	6 × 500 mL
M4780-1L	1 L
M4780-6X1L	6 × 1 L

With Hanks' salts and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Modification of the original MEM formulation. Earle's salts have been replaced with Hanks' salts. Since the bicarbonate concentration is substantially lower, this medium has a substantially reduced buffering capacity.

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M5775-100ML	100 mL
M5775-500ML	500 mL
M5775-6X500ML	6 × 500 mL

10 ×, With Hanks' salts, without L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.292 g/L L-glutamine, 0.35 g/L sodium bicarbonate at 1×.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M9288-100ML	100 mL

With Hanks' salts and L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Modification of the original MEM formulation. Earle's salts have been replaced with Hanks' salts. Since the bicarbonate concentration is substantially lower, this medium has a substantially reduced buffering capacity.

Formulated to contain 10.6 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.
ship: ambient store at: 2-8°C

M4642-10L	10 L
M4642-50L	50 L

With Hanks' salts, L-glutamine and non-essential amino acids, without sodium bicarbonate, powder, suitable for cell culture

Modification of the original MEM formulation. Earle's salts have been replaced with Hanks' salts. Since the bicarbonate concentration is substantially lower, this medium has a substantially reduced buffering capacity. It contains 19 amino acids. The essential amino acids and the non-essential amino acids; L-ala; L-asn; L-asp; L-glu; L-gly; L-pro and L-ser. May be preferred for growth of adherent cells in the presence of reduced serum or as a base for development of a serum free medium.

Formulated to contain 10.7 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.
ship: ambient store at: 2-8°C

M1018-10L	10 L

αMEM
Alpha Modification, with sodium bicarbonate, without L-glutamine, ribonucleosides and deoxyribonucleosides, liquid, sterile-filtered, suitable for cell culture

This is the most enriched variation of the MEM formulation offered. It contains all 21 normal amino acids, some at increased concentrations. In addition, it contains 5 additional vitamins.

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M4526-500ML	500 mL
M4526-6X500ML	6 × 500 mL
M4526-24X500ML	24 × 500 mL

Alpha Modification, with ribonucleosides, deoxyribonucleosides and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

This is the most enriched variation of the MEM formulation offered. It contains all 21 normal amino acids, some at increased concentrations. In addition, it contains 5 additional vitamins. See formulation for details. Often used in cytogenetic analysis when supplemented with FBS.

Supplement with 0.292 g/L L-glutamine

endotoxin ..... tested
ship: ambient store at: 2-8°C

M8042-500ML	500 mL
M8042-6X500ML	6 × 500 mL
M8042-24X500ML	24 × 500 mL

Alpha Modification, with L-glutamine, ribonucleosides and deoxyribonucleosides, without sodium bicarbonate, powder, suitable for cell culture

This is the most enriched variation of the MEM formulation offered. It contains all 21 normal amino acids, some at increased concentrations. In addition, it contains 5 additional vitamins. Often used in cytogenetic analysis when supplemented with FBS.

Formulated to contain 10.1 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.
ship: ambient store at: 2-8°C

M0644-10X1L	10 × 1 L
M0644-10L	10 L

Alpha Modification, with L-glutamine, without ribonucleosides, deoxyribonucleosides and sodium bicarbonate, powder, suitable for cell culture

This is the most enriched variation of the MEM formulation offered. It contains all 21 normal amino acids, some at increased concentrations. In addition, it contains 5 additional vitamins.

Formulated to contain 10.1 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.
ship: ambient store at: 2-8°C

M0894-10X1L	10 × 1 L
M0894-10L	10 L

Minimum Essential Medium Eagle *(continued)*

Modified, with Earle's salts and reduced $NaHCO_3$. without L-glutamine, liquid, sterile-filtered, suitable for cell culture

This product lacks L-Ala; L-Asn; L-Glu; Gly; L-Pro; L-Ser and L-Gln.

Recommended for use in a carbon dioxide-free culture system.

Supplement with 0.292 g/L L-glutamine.

May also be supplemented with HEPES to raise the buffering range.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M2414-500ML	500 mL
M2414-6X500ML	6 × 500 mL

with Earle's salts and non-essential amino acids, without L-glutamine, phenol red and sodium bicarbonate, Modified, powder, suitable for cell culture

It contains 19 amino acids. The essential amino acids and the non-essential amino acids; L-ala; L-asn; L-asp; L-glu; L-gly; L-pro and L-ser. May be preferred for growth of adherent cells in the presence of reduced serum or as a base for development of a serum free medium.

Formulated to contain 9.3 grams of powder per liter of medium.

Supplement with 0.292 g/L L-glutamine, 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M3024-10X1L	10 × 1 L
M3024-10L	10 L

HEPES Modification, with Earle's salts, 25mM HEPES and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

M7278-100ML	100 mL
M7278-500ML	500 mL
M7278-6X500ML	6 × 500 mL

HEPES Modification, with Earle's salts, L-glutamine and 25 mM HEPES, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 14.2 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

Leaving the bicarbonate out gives the investigator greater control over the buffer system.

pH range ..... >7.2
ship: ambient store at: 2-8°C

M2645-10L	10 L

JMEM; Joklik Modified MEM
Joklik Modification, With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Recommended for suspension cultures.

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M8028-500ML	500 mL
M8028-6X500ML	6 × 500 mL

JMEM; Joklik Modified MEM
Joklik Modification, with L-glutamine, without calcium chloride and sodium bicarbonate, suitable for cell culture

Recommended for suspension cultures.

Formulated to contain 11.0 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M0518-10X1L	10 × 1 L
M0518-10L	10 L
M0518-50L	50 L

SMEM
Spinner Modification, with Earle's salts and sodium bicarbonate, without calcium chloride and L-glutamine, liquid, sterile-filtered, suitable for cell culture

Recommended for suspension cultures.

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M8167-500ML	500 mL
M8167-6X500ML	6 × 500 mL

AQmedia™, With Earle's salts, L-alanyl-glutamine, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture NEW

This product lacks L-Ala; L-Asn; L-Glu; Gly; L-Pro; L-Ser and L-Gln. It is supplemented with L-Ala-L-Gln dipeptide. This provides a more stable form of glutamine for cell culture. Free amino acid L-glutamine is known to be unstable in cell culture.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M0446-500ML	500 mL

Minimum Essential Medium Eagle, Auto-Mod™

Minimum Essential Medium Eagle; MEM

Auto-Mod™, with Earle's salts, without L-glutamine and sodium bicarbonate, powder, suitable for cell culture

Modified for autoclaving.

Modification of the original MEM formulation. Contains a succinate buffering system. See formulations table for specific information. Medium may be autoclaved.

Formulated to contain 9.4 grams of powder per liter of medium.

Supplement with 0.292 g/L L-glutamine, 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

M0769-10X1L	10 × 1 L
M0769-50L	50 L

Minimum Essential Medium Joklik's Modified NEW

Minimum Essential Medium Eagle; MEM

with Earle's Balanced Salts, with 2.0 mM L-glutamine, without calcium chloride, without sodium bicarbonate, dry powder, suitable for cell culture

for research or for further manufacturing use

Formulated to contain 11.0 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

56449C-10L	10 L
56449C-50L	50 L

NCTC Medium

NCTC 109 Medium

With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

NCTC-109 and the 135 modification were formulated to establish and maintain a strain of mouse cells (L929) in a chemically defined and serum free medium.

Supplement with 0.14 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

N1140-500ML	500 mL
N1140-6X500ML	6 × 500 mL

NCTC 135 Medium

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Originally developed for serum-free growth of mouse L-cells.

Formulated to contain 9.3 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

N3262-10X1L	10 × 1 L
N3262-10L	10 L

Nutrient Mixtures (Ham's)

Ham's Nutrient Mixture F12

NEW

Nutrient Mixture F12 Ham's

for further manufacturing use

liquid

Ham's Nutrient Mixture F12 was originally developed for the serum-free clonal growth of Chinese Hamster Ovary (CHO) cells, lung cells, and mouse L cells. It is frequently used with dialyzed serum, hormones, selenium, and other supplements for serum-free cultures. It is the medium of choice for supporting the growth of cells of rodent origin, particularly rabbbit and rat, and has been proven to be an excellent cloning medium for myeloma and hybridoma cells.

for research or for further manufacturing use

with 1.0 mM L-glutamine

ship: ambient store at: 2-8°C

51651C-1000ML	1000 mL

Nutrient Mixture F-10 Ham

Ham's F-10

With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: 2-8°C

N6908-500ML	500 mL
N6908-6X500ML	6 × 500 mL

With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supports the growth of human diploid cells, white blood cells for chromosomal analysis, primary explants of rat, rabbit and chicken tissues.

Supplement with 0.146 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

N6013-100ML	100 mL
N6013-500ML	500 mL
N6013-6X500ML	6 × 500 mL

With 20 mM HEPES, without sodium bicarbonate and L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.146 g/L L-glutamine.

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

N2147-100ML	100 mL
N2147-500ML	500 mL
N2147-6X500ML	6 × 500 mL

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 9.8 grams of powder per liter of medium.

Supplement with 1.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

N6635-10X1L	10 × 1 L
N6635-10L	10 L

Nutrient Mixture F-12 Ham

Ham's F-12

With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Developed for growth of primary rat hepatocytes and rat prostate epithelial cells. Also used in a clonal toxicity assay using CHO cells.

endotoxin ..... tested
ship: ambient store at: 2-8°C

N6658-500ML	500 mL
N6658-6X500ML	6 × 500 mL

With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Developed for growth of primary rat hepatocytes and rat prostate epithelial cells. Also used in a clonal toxicity assay using CHO cells.

Supplement with 0.146 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

N4888-500ML	500 mL
N4888-6X500ML	6 × 500 mL
N4888-24X500ML	24 × 500 mL

Nutrient Mixture F-12 Ham *(continued)*

HEPES Modification, with 25 mM HEPES and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.146 g/L L-glutamine.

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

N8641-100ML	100 mL
N8641-500ML	500 mL
N8641-6X500ML	6 × 500 mL

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Developed for growth of primary rat hepatocytes and rat prostate epithelial cells. Also used in a clonal toxicity assay using CHO cells.

Formulated to contain 10.6 grams of powder per liter of medium.

Supplement with 1.176 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

N6760-10X1L	10 × 1 L
N6760-10L	10 L
N6760-50L	50 L

powder, with L-glutamine and 0.863 mg/L zinc sulfate, without sodium bicarbonate, Coon's Modification, suitable for cell culture

Coon's modification of Ham's F-12 was developed for culturing hybrid cells that were produced by viral fusion. The modification consists of doubling the amino acids and pyruvate and including ascorbic acid. The salt concentrations have also been altered. The formula contains 0.863 mg/L zinc sulfate, which may render it unsuitable for culturing mouse L-cells.

Formulated to contain 11.5 grams of powder per liter of medium.

Supplement with 2.68 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

F6636-10X1L	10 × 1 L

Kaighn's Modification, with L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Kaighn's modification of Ham's F-12 and Coon's F-12 has increased concentrations of amino acids and pyruvate, as well as modified salts (Konigsbergs). This medium was designed to support the growth of differentiated rat and chicken cells, and primary human liver cells.

Formulated to contain 11.1 grams of powder per liter of medium.

Supplement with 2.5 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

N3520-10X1L	10 × 1 L
N3520-10L	10 L

RPMI-1640 Medium

RPMI-1640 Medium

With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: 2-8°C

R8758-100ML	100 mL
R8758-500ML	500 mL
R8758-6X500ML	6 × 500 mL
R8758-24X500ML	24 × 500 mL
R8758-1L	1 L
R8758-6X1L	6 × 1 L

HEPES Modification, With 25mM HEPES, without L-glutamine., liquid, sterile-filtered, suitable for cell culture

This RPMI-1640 medium is supplemented with HEPES. HEPES is a Good's physiological buffer that helps provide a more stable medium pH than bicarbonate buffers.

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

R5886-100ML	100 mL
R5886-500ML	500 mL
R5886-6X500ML	6 × 500 mL
R5886-24X500ML	24 × 500 mL

Modified, with 20mM HEPES and L-glutamine, without sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

R7388-100ML	100 mL
R7388-500ML	500 mL
R7388-6X500ML	6 × 500 mL

with 2.05 mM L-glutamine, with 25mM HEPES, liquid, sterile-filtered, suitable for cell culture NEW

for research or for further manufacturing use

ship: ambient store at: 2-8°C

51536C-1000ML	1000 mL

With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.3 g/L L-glutamine.

endotoxin ..... tested
ship: ambient store at: 2-8°C

R0883-100ML	100 mL
R0883-500ML	500 mL
R0883-6X500ML	6 × 500 mL
R0883-24X500ML	24 × 500 mL
R0883-1L	1 L
R0883-6X1L	6 × 1 L
R0883-10L	10 L

Dutch Modification, with sodium bicarbonate and 20mM HEPES, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.3 g/L L-glutamine.

endotoxin ..... tested
pH range ..... >7.2
ship: ambient store at: 2-8°C

R7638-500ML	500 mL
R7638-6X500ML	6 × 500 mL

10 ×, Without L-glutamine, folic acid and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.3 g/L L-glutamine, 2.0 g/L sodium bicarbonate, 0.001 g/L folic acid at 1×.

endotoxin ..... tested
ship: ambient store at: 2-8°C

R1145-500ML	500 mL
R1145-6X500ML	6 × 500 mL

Modified, with sodium bicarbonate, without methionine, cystine and L-glutamine, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: 2-8°C

R7513-100ML	100 mL

Modified, with sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture

Phenol red has been shown to interfere with the growth of some cells at clonal densities.
Use this medium when working with stem cells or when growing cells at low densities.
Also recommended for in vitro diagnostics use.

endotoxin ..... tested
ship: ambient store at: 2-8°C

R7509-500ML	500 mL
R7509-6X500ML	6 × 500 mL
R7509-24X500ML	24 × 500 mL
R7509-1L	1 L
R7509-6X1L	6 × 1 L

Auto-Mod™, without L-glutamine and sodium bicarbonate, powder, suitable for cell culture

Modified for autoclaving
Without L-glutamine and sodium bicarbonate.

Formulated to contain 10.3 grams of powder per liter of medium.

Supplement with 0.3 L-glutamine, 2.0 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

R7755-10L	10 L

HEPES Modification, with L-glutamine and 25mM HEPES, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 16.4 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

R4130-10X1L	10 × 1 L
R4130-10L	10 L
R4130-50L	50 L

With L-glutamine, without glucose and sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 8.4 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

R1383-10X1L	10 × 1 L

Modified, with L-glutamine, without phenol red and sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 10.4 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

Phenol red has been shown to interfere with the growth of some cells at clonal densities. Use this medium when working with stem cells or when growing cells at low densities. Also recommended for in vitro diagnostics use.

ship: ambient store at: 2-8°C

R8755-10X1L	10 × 1 L
R8755-10L	10 L

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 10.4 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

R6504-10X1L	10 × 1 L
R6504-2X5L	2 × 5 L
R6504-10L	10 L
R6504-50L	50 L

Hybri-Max™, Modified, with L-glutamine, 4500 mg/L glucose and 15mM HEPES, without sodium bicarbonate, powder, suitable for hybridoma

Formulated to contain 16.4 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

endotoxin ..... contains
ship: ambient store at: 2-8°C

R8005-10X1L	10 × 1 L
R8005-10L	10 L

With L-glutamine and sodium bicarbonate. Without arginine, leucine, lysine, and phenol red, liquid, sterile-filtered, suitable for cell culture NEW

Designed for use in stable isotope labeling with amino acids in cell culture (SILAC) applications.
Supplement with 0.100 g/L L-Arginine-$^{13}C_6$, $^{15}N_4$ HCl (Product 608033), 0.100 g/L L-Leucine-$^{13}C_6$, ^{15}N (Product 608068), and 0.100 g/L L-Lysine-$^{13}C_6$, $^{15}N_2$ (Product 608041).

endotoxin ..... tested
ship: ambient store at: room temp

R1780-500ML	500 mL

AQmedia™, With L-alanyl-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture NEW

This RPMI-1640 medium is supplemented with L-Ala-L-Gln dipeptide. This provides a more stable form of glutamine for cell culture. Free amino acid L-glutamine is known to be unstable in cell culture.

endotoxin ..... tested
ship: ambient store at: 2-8°C

R2405-500ML	500 mL
R2405-6X500ML	6 × 500 mL
R2405-24X500ML	24 × 500 mL

Waymouth Medium

Waymouth MB 752/1 Medium

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

For the cultivation of mouse L929 cells in a serum-free environment. Also used in whole organ culture, establishment of carcinoma cell lines from pleural effusions, and the growth of potentially tumorigenic cells prior to their assessment in vitro.

Formulated to contain 14.0 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

W1625-10X1L	10 × 1 L

Williams' Medium E

Williams' Medium E

With sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.292 g/L L-glutamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

W4128-500ML	500 mL
W4128-6X500ML	6 × 500 mL

With sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.292 g/L L-glutamine.

Phenol red has been shown to interfere with the growth of some cells at low or cloning densities. Use this version of William's E when working with stem cells or cells at low densities.

endotoxin ..... tested

ship: ambient store at: 2-8°C

W1878-500ML	500 mL
W1878-6X500ML	6 × 500 mL

With L-glutamine, without sodium bicarbonate, powder, suitable for cell culture

For long-term culture of adult liver epithelial cells.

Formulated to contain 10.8 grams of powder per liter of medium.

Supplement with 2.2 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

W4125-10X1L	10 × 1 L

Media Formulation Tables

Ames' Medium Formulation

Component	A1420 g/L
INORGANIC SALTS	
Calcium Chloride	0.1275
Magnesium Sulfate	0.1488
Potassium Chloride	0.231
Potassium Phosphate Monobasic (anhydrous)	0.068
Sodium Chloride	7.01
AMINO ACIDS	
L-Alanine	0.0024
L-Arginine • HCl	0.00421
L-Asparagine (anhydrous)	0.00084
L-Aspartic Acid	0.00012
L-Cystine • 2HCl	0.000065
L-Glutamine	0.073
L-Glutamic Acid (sodium)	0.001183
Glycine	0.00045
L-Histidine • HCl • H_2O	0.002513
L-Isoleucine	0.00058
L-Leucine	0.00144
L-Lysine • HCl	0.003648
L-Methionine	0.00039
L-Phenylalanine	0.00132
L-Proline	0.00007
L-Serine	0.00252
L-Taurine	0.00075
L-Threonine	0.00333
L-Tryptophan	0.00049
L-Tyrosine • 2Na • $2H_2O$	0.00211
L-Valine	0.00176
VITAMINS	
Ascorbic Acid • Na	0.01796
D-Biotin	0.0001
Choline Chloride	0.0007
Folic Acid	0.0001
myo-Inositol	0.0272
Niacinamide	0.0001
D-Pantothenic Acid (hemicalcium)	0.0001
Pyridoxal • HCl	0.0001
Riboflavin	0.00001
Thiamine • HCl	0.0001
OTHER	
Cytidine	0.00073
D-Glucose	1.081
Hypoxanthine	0.00082
Pyruvic Acid (sodium)	0.01333
Thymidine	0.00024
Uridine	0.00073
ADD	
Sodium Bicarbonate	1.9

Basal Medium Eagle

Component	B1522 [1×] g/L	B9638 g/L
INORGANIC SALTS		
Calcium Chloride	0.2	0.2
Magnesium Sulfate (anhydrous)	0.09767	0.09767
Potassium Chloride	0.4	0.4
Sodium Bicarbonate	2.2	—
Sodium Chloride	6.8	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122
AMINO ACIDS		
L-Arginine • HCl	0.021	0.021
L-Cystine • 2HCl	0.01565	0.01565
L-Glutamine	—	0.292
L-Histidine (free base)	0.008	0.008
L-Isoleucine	0.026	0.026
L-Leucine	0.026	0.026
L-Lysine • HCl	0.03647	0.03647
L-Methionine	0.0075	0.0075
L-Phenylalanine	0.0165	0.0165
L-Threonine	0.024	0.024
L-Tryptophan	0.004	0.004
L-Tyrosine • 2Na • $2H_2O$	0.02595	0.02595
L-Valine	0.0235	0.0235
VITAMINS		
D-Biotin	0.001	0.001
Choline Chloride	0.001	0.001
Folic Acid	0.001	0.001
myo-Inositol	0.002	0.002
Niacinamide	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.001
Pyridoxal • HCl	0.001	0.001
Riboflavin	0.0001	0.0001
Thiamine • HCl	0.001	0.001
OTHER		
D-Glucose	1.0	1.0
Phenol Red • Na	0.011	0.011
ADD		
L-Glutamine	0.292	—
Sodium Bicarbonate	—	2.2

Click's Medium

Component	C5572 [1×] g/L
INORGANIC SALTS	
Calcium Chloride	0.1854
Magnesium Chloride • $6H_2O$	0.1649
Potassium Chloride	0.4
Potassium Phosphate Dibasic	0.06
Sodium Bicarbonate	1.35
Sodium Chloride	8.0
Sodium Phosphate Dibasic	0.0479
AMINO ACIDS	
Alanine	0.0356
Arginine • HCl	0.3175
Asparagine • H_2O	0.06
L-Aspartic Acid	0.0532
Cystine • 2HCl	0.0782
Glutamic Acid	0.0588
Glycine	0.03
Histidine • HCl	0.1047
Isoleucine	0.13
Leucine	0.13
L-Lysine • HCl	0.1812
Methionine	0.0375
Phenylalanine	0.08
Proline	0.046
Serine	0.042
Threonine	0.12
Tryptophan	0.025
Tyrosine • 2Na	0.1189
Valine	0.115
VITAMINS	
D-Pantothenic Acid • ½Ca	0.002
Choline Chloride	0.002
Folic Acid	0.002
myo-Inositol	0.004
Niacinamide	0.002
Pyridoxal • HCl	0.002
Riboflavin	0.0002
Thiamine • HCl	0.002
OTHER	
Glucose	1.0
Phenol Red • Na	0.01
Sodium Pyruvate	0.275
ADD	
L-Glutamine	0.584

Dulbecco's Modified Eagle's Medium (DME)

Component	D0422 [1×] g/L	D1145 [1×] g/L
INORGANIC SALTS		
Calcium Chloride	0.2	0.2
Ferric Nitrate • $9H_2O$	0.0001	0.0001
Magnesium Sulfate (anhydrous)	0.09767	0.09767
Potassium Chloride	0.4	0.4
Sodium Bicarbonate	3.7	3.7
Sodium Chloride	6.4	6.4
Sodium Phosphate Monobasic (anhydrous)	0.109	0.109
AMINO ACIDS		
L-Arginine • HCl	0.084	0.084
L-Cystine • 2HCl	—	0.0626
Glycine	0.03	0.03
L-Histidine • HCl • H_2O	0.042	0.042
L-Isoleucine	0.105	0.105
L-Leucine	0.105	0.105
L-Lysine • HCl	1.46	0.146
L-Methionine	—	0.03
L-Phenylalanine	0.066	0.066
L-Serine	0.042	0.042
L-Threonine	0.095	0.095
L-Tryptophan	0.016	0.016
L-Tyrosine • 2Na •$2H_2O$	0.10379	0.6351
L-Valine	0.094	0.094
VITAMINS		
Choline Chloride	0.004	0.004
Folic Acid	0.004	0.004
myo-Inositol	0.0072	0.0072
Niacinamide	0.004	0.004
D-Pantothenic Acid (hemicalcium)	0.004	0.004
Pyridoxal • HCl	—	—
Pyridoxine • HCl	0.004	0.004
Riboflavin	0.0004	0.0004
Thiamine • HCl	0.004	0.004
OTHER		
D-Glucose	4.5	4.5
Phenol Red • Na	0.0159	—
Pyruvic Acid • Na	0.11	—
ADD		
L-Glutamine	0.584	0.584

Dulbecco's Modified Eagle's Medium (DME)

Component	D0819 [1×] g/L	D1152 [1×] g/L	D2429 [10×] g/L	D2902 g/L	D5030 g/L	D5523 g/L
INORGANIC SALTS						
Calcium Chloride	0.2	0.2	2	0.2	0.2	0.2
Ferric Nitrate • $9H_2O$	0.0001	0.0001	0.001	0.0001	0.0001	0.0001
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.9767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	0.4	4	0.4	0.4	0.4
Sodium Bicarbonate	3.7	—	—	—	—	—
Sodium Chloride	6.4	4.4	64	6.4	6.4	6.4
Sodium Phosphate Monobasic (anhydrous)	0.109	0.109	1.09	0.109	0.109	0.109
AMINO ACIDS						
L-Alanyl-L-Glutamine	0.869	—	—	—	—	—
L-Arginine • HCl	0.084	0.084	0.84	0.084	0.084	0.084
L-Cystine • 2HCl	0.0626	0.0626	0.626	0.0626	0.0626	0.0626
L-Glutamine	—	0.584	—	0.584	—	0.584
Glycine	0.03	0.03	0.3	0.03	0.03	0.03
L-Histidine • HCl • H_2O	0.042	0.042	0.42	0.042	0.042	0.042
L-Isoleucine	0.105	0.105	1.05	0.105	0.105	0.105
L-Leucine	0.105	0.105	1.05	0.105	0.105	0.105
L-Lysine • HCl	0.146	0.146	1.46	0.146	0.146	0.146
L-Methionine	0.03	0.03	0.3	0.03	0.03	0.03
L-Phenylalanine	0.066	0.066	0.66	0.066	0.066	0.066
L-Serine	0.042	0.042	0.42	0.042	0.042	0.042
L-Threonine	0.095	0.095	0.95	0.095	0.095	0.095
L-Tryptophan	0.016	0.016	0.16	0.016	0.016	0.016
L-Tyrosine • 2Na • $2H_2O$	0.10379	0.10379	1.0379	0.10379	0.10379	0.10379
L-Valine	0.094	0.094	0.94	0.094	0.094	0.094
VITAMINS						
Choline Chloride	0.004	0.004	0.04	0.004	0.004	0.004
Folic Acid	0.004	0.004	—	0.004	0.004	0.004
myo-Inositol	0.072	0.0072	0.072	0.0072	0.0072	0.0072
Niacinamide	0.004	0.004	0.04	0.004	0.004	0.004
D-Pantothenic Acid (hemicalcium)	0.004	0.004	0.04	0.004	0.004	0.004
Pyridoxal • HCl	—	0.004	—	0.004	0.004	0.004
Pyridoxine • HCl	0.004	—	0.04	—	—	—
Riboflavin	0.0004	0.0004	0.004	0.0004	0.0004	0.0004
Thiamine • HCl	0.004	0.004	0.04	0.004	0.004	0.004
OTHER						
D-Glucose	4.5	4.5	10	1.0	—	1.0
HEPES	—	5.958	—	—	—	—
Phenol Red • Na	0.0159	0.0159	0.159	—	—	0.0159
Pyruvic Acid • Na	—	—	1.1	0.11	—	0.11
ADD						
Glucose	—	—	—	—	1.0	—
L-Glutamine	—	—	0.584 at 1×	—	0.584	—
Sodium Bicarbonate	—	3.7	3.7 at 1×	3.7	3.7	3.7

Dulbecco's Modified Eagle's Medium (DME)

Component	D5546 [1×] g/L	D5648 g/L	D5671 [1×] g/L	D5796 [1×] g/L	D5921 [1×] g/L
INORGANIC SALTS					
Calcium Chloride	0.2	0.2	0.2	0.2	0.2
Ferric Nitrate • $9H_2O$	0.0001	0.0001	0.0001	0.0001	0.0001
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	0.4	0.4	0.4	0.4
Sodium Bicarbonate	3.7	—	3.7	3.7	3.7
Sodium Chloride	6.4	6.4	6.4	6.4	6.4
Sodium Phosphate Monobasic (anhydrous)	0.109	0.109	0.109	0.109	0.109
AMINO ACIDS					
L-Arginine • HCl	0.084	0.084	0.084	0.084	0.084
L-Cystine • 2HCl	0.0626	0.0626	0.0626	0.0626	0.0626
L-Glutamine	—	0.584	—	0.584	—
Glycine	0.03	0.03	0.03	0.03	0.03
L-Histidine • HCl • H_2O	0.042	0.042	0.042	0.042	0.042
L-Isoleucine	0.105	0.105	0.105	0.105	0.105
L-Leucine	0.105	0.105	0.105	0.105	0.105
L-Lysine • HCl	0.146	0.146	0.146	0.146	0.146
L-Methionine	0.03	0.03	0.03	0.03	0.03
L-Phenylalanine	0.066	0.066	0.066	0.066	0.066
L-Serine	0.042	0.042	0.042	0.042	0.042
L-Threonine	0.095	0.095	0.095	0.095	0.095
L-Tryptophan	0.016	0.016	0.016	0.016	0.016
L-Tyrosine • 2Na • $2H_2O$	0.10379	0.10379	0.10379	0.10379	0.10379
L-Valine	0.094	0.094	0.094	0.094	0.094
VITAMINS					
Choline Chloride	0.004	0.004	0.004	0.004	0.004
Folic Acid	0.004	0.004	0.004	0.004	0.004
myo-Inositol	0.0072	0.0072	0.0072	0.0072	0.0072
Niacinamide	0.004	0.004	0.004	0.004	0.004
D-Pantothenic Acid (hemicalcium)	0.004	0.004	0.004	0.004	0.004
Pyridoxal • HCl	—	0.004	—	—	—
Pyridoxine • HCl	0.004	—	0.004	0.004	0.004
Riboflavin	0.0004	0.0004	0.0004	0.0004	0.0004
Thiamine • HCl	0.004	0.004	0.004	0.004	0.004
OTHER					
D-Glucose	1.0	4.5	4.5	4.5	1.0
HEPES	—	—	—	—	—
Phenol Red • Na	0.0159	0.0159	0.0159	0.0159	—
Pyruvic Acid • Na	0.11	—	—	—	0.11
ADD					
Glucose	—	—	—	—	—
L-Glutamine	0.584	—	0.584	—	0.584
Sodium Bicarbonate	—	3.7	—	—	—

Dulbecco's Modified Eagle's Medium (DME)

Component	D6046 [1×] g/L	D6171 [1×] g/L	D6429 [1×] g/L	D6546 [1×] g/L	D7777 g/L	D9443 [1×] g/L
INORGANIC SALTS						
Calcium Chloride	0.2	0.2	0.2	0.2	0.2	0.2
Ferric Nitrate • $9H_2O$	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	0.4	0.4	0.4	0.4	0.4
Sodium Bicarbonate	3.7	3.7	3.7	3.7	—	3.7
Sodium Chloride	6.4	4.4	6.4	6.4	6.4	6.4
Sodium Phosphate Monobasic (anhydrous)	0.109	0.109	0.109	0.109	0.109	0.109
AMINO ACIDS						
L-Arginine • HCl	0.084	0.084	0.084	0.084	0.084	—
L-Cystine • 2HCl	0.0626	0.0626	0.0626	0.0626	0.0626	0.0626
L-Glutamine	0.584	—	0.584	—	0.584	0.584
Glycine	0.03	0.03	0.03	0.03	0.03	0.03
L-Histidine • HCl • H_2O	0.042	0.042	0.042	0.042	0.042	0.042
L-Isoleucine	0.105	0.105	0.105	0.105	0.105	0.105
L-Leucine	0.105	0.105	0.105	0.105	0.105	—
L-Lysine • HCl	0.146	0.146	0.146	0.146	0.146	—
L-Methionine	0.03	0.03	0.03	0.03	0.03	0.03
L-Phenylalanine	0.066	0.066	0.066	0.066	0.066	0.066
L-Serine	0.042	0.042	0.042	0.042	0.042	0.042
L-Threonine	0.095	0.095	0.095	0.095	0.095	0.095
L-Tryptophan	0.016	0.016	0.016	0.016	0.016	0.016
L-Tyrosine • 2Na • $2H_2O$	0.10379	0.10379	0.10379	0.10379	0.10379	0.10379
L-Valine	0.094	0.094	0.094	0.094	0.094	0.094
VITAMINS						
Choline Chloride	0.004	0.004	0.004	0.004	0.004	0.004
Folic Acid	0.004	0.004	0.004	0.004	0.004	0.004
myo-Inositol	0.0072	0.0072	0.0072	0.0072	0.0072	0.0072
Niacinamide	0.004	0.004	0.004	0.004	0.004	0.004
D-Pantothenic Acid (hemicalcium)	0.004	0.004	0.004	0.004	0.004	0.004
Pyridoxal • HCl	—	—	—	—	0.004	—
Pyridoxine • HCl	0.004	0.004	0.004	0.004	—	0.004
Riboflavin	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004
Thiamine • HCl	0.004	0.004	0.004	0.004	0.004	0.004
OTHER						
D-Glucose	1.0	4.5	4.5	4.5	4.5	1.0
HEPES	—	5.958	—	—	—	—
Phenol Red • Na	0.0159	0.0159	0.0159	0.0159	0.0159	—
Pyruvic Acid • Na	0.11	—	0.11	0.11	0.11	—
ADD						
Glucose	—	—	—	—	—	—
L-Glutamine	—	0.584	—	0.584	—	—
Sodium Bicarbonate	—	—	—	—	3.7	—

Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F-12 (DME/F12)

Component	D0547 g/L	D2906 g/L	D6421 [1×] g/L	D6434 [1×] g/L	D8062 [1×] g/L	D8437 [1×] g/L	D8900 g/L	D9785 g/L
INORGANIC SALTS								
Ammonium Molybdate • $4H_2O$	0.00000618	—	—	—	—	—	—	—
Ammonium Metavandate	0.00000058	—	—	—	—	—	—	—
Calcium Chloride	0.1166	0.1166	0.1166	0.1166	0.1166	0.1166	0.1166	—
Cupric Sulfate • $5H_2O$	0.0000013	0.0000013	0.0000013	0.0000013	0.0000013	0.0000013	0.0000013	0.0000013
Ferric Nitrate • $9H_2O$	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005
Ferrous Sulfate • $7H_2O$	0.000417	0.000417	0.000417	0.000417	0.000417	0.000417	0.000417	0.000417
Manganese Sulfate	0.000000151	—	—	—	—	—	—	—
Magnesium Chloride • $6H_2O$	0.0612	0.0612	0.0612	0.0612	0.0612	0.0612	0.0612	—
Magnesium Sulfate (anhydrous)	0.04884	0.04884	0.04884	0.04884	0.04884	0.04884	0.04884	—
Nickel Chloride	0.00000012	—	—	—	—	—	—	—
Potassium Chloride	0.3118	0.3118	0.3118	0.3118	0.3118	0.3118	0.3118	0.3118
Sodium Bicarbonate	—	—	1.2	1.2	1.2	1.2	—	—
Sodium Chloride	6.996	6.996	6.996	6.996	6.996	6.996	6.996	6.996
Sodium Metasilicate • $9H_2O$	0.0000142	—	—	—	—	—	—	—
Sodium Selenite	0.00000519	—	—	—	—	—	—	—
Sodium Phosphate Dibasic (anhydrous)	0.07102	0.07102	0.07102	0.07102	0.07102	0.07102	0.07102	0.07102
Sodium Phosphate Monobasic (anhydrous)	0.0543	0.0543	0.0543	0.0543	0.0543	0.0543	0.0543	0.0543
Stannous Chloride • $2H_2O$	0.00000011	—	—	—	—	—	—	—
Zinc Sulfate • $7H_2O$	0.000432	0.000432	0.000432	0.000432	0.000432	0.000432	0.000432	0.000432
AMINO ACIDS								
L-Alanine	0.00445	0.00445	0.00445	0.00445	0.00445	0.00445	0.00445	0.00445
L-Arginine • HCl	0.1475	0.1475	0.1475	0.1475	0.1475	0.1475	0.1475	0.1475
L-Asparagine • H_2O	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075	0.0075
L-Aspartic Acid	0.00665	0.00665	0.00665	0.00665	0.00665	0.00665	0.00665	0.00665
L-Cystine • 2HCl	0.01756	0.01756	0.01756	0.01756	0.01756	0.01756	0.01756	0.01756
L-Cystine • HCl • H_2O	0.03129	0.03129	0.03129	0.03129	0.03129	0.03129	0.03129	0.03129
L-Glutamic Acid	0.00735	0.00735	0.00735	0.00735	0.00735	0.00735	0.00735	0.00735
L-Glutamine	0.365	0.365	—	—	0.365	0.365	0.365	—
Glycine	0.01875	0.01875	0.01875	0.01875	0.01875	0.01875	0.01875	0.01875
L-Histidine • HCl • H_2O	0.03148	0.03148	0.03148	0.03148	0.03148	0.03148	0.03148	0.03148
L-Isoleucine	0.05447	0.05447	0.5447	0.05447	0.05447	0.05447	0.05447	0.05447
L-Leucine	0.05905	0.05905	0.05905	0.05905	0.05905	0.05905	0.05905	—
L-Lysine • HCl	0.09125	0.09125	0.09125	0.09125	0.09125	0.09125	0.09125	—
L-Methionine	0.01724	0.01724	0.01724	0.01724	0.01724	0.01724	0.01724	—
L-Phenylalanine	0.03548	0.03548	0.03548	0.03548	0.03548	0.03548	0.03458	0.03548
L-Proline	0.01725	0.01725	0.01725	0.01725	0.01725	0.01725	0.01725	0.01725
L-Serine	0.02625	0.02625	0.02625	0.02625	0.02625	0.02625	0.02625	0.02625
L-Threonine	0.05345	0.05345	0.05345	0.05345	0.05345	0.05345	0.05345	0.05345
L-Tryptophan	0.00902	0.00902	0.00902	0.00902	0.00902	0.00902	0.00902	0.00902
L-Tyrosine • 2Na • $2H_2O$	0.05579	0.05579	0.05579	0.05579	0.05579	0.05579	0.05579	0.05579
L-Valine	0.05285	0.05285	0.05285	0.05285	0.05285	0.05285	0.05285	0.05285
VITAMINS								
D-Biotin	0.0000035	0.0000035	0.0000035	0.0000035	0.0000035	0.0000035	0.0000035	0.0000035
Choline Chloride	0.00898	0.00898	0.00898	0.00898	0.00898	0.00898	0.00898	0.00898
Folic Acid	0.00266	0.00266	0.00265	0.00266	0.00266	0.00266	0.00266	0.00266
myo-Inositol	0.0126	0.0126	0.0126	0.0126	0.0126	0.0126	0.0126	0.0126
Niacinamide	0.00202	0.00202	0.00202	0.00202	0.00202	0.00202	0.00202	0.00202
D-Pantothenic Acid (hemicalcium)	0.00224	0.00224	0.00224	0.00224	0.00224	0.00224	0.00224	0.00224
Pyridoxal • HCl	0.002	0.002	—	0.002	0.002	0.002	0.002	0.002
Pyridoxine • HCl	0.000031	0.000031	0.002031	—	—	0.000031	0.000031	0.000031
Riboflavin	0.000219	0.000219	0.000219	0.000219	0.000219	0.000219	0.000219	0.000219
Thiamine • HCl	0.00217	0.00217	0.00217	0.00217	0.00217	0.00217	0.00217	0.00217
Vitamin B_{12}	0.00068	0.00068	0.00068	0.00068	0.00068	0.00068	0.00068	0.00068

Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F-12 (DME/F12)

Component	D0547 g/L	D2906 g/L	D6421 [1×] g/L	D6434 [1×] g/L	D8062 [1×] g/L	D8437 [1×] g/L	D8900 g/L	D9785 g/L
OTHER								
D-Glucose	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15
HEPES	—	3.5745	3.5745	3.5745	—	3.5745	3.5745	3.5745
Hypoxanthine	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021
Linoleic Acid	0.000042	0.000042	0.000042	0.000042	0.000042	0.000042	0.000042	0.000042
Phenol Red • Na	0.00863	—	0.00863	—	0.00863	0.00863	0.00863	—
Putrescine • HCl	0.000081	0.000081	0.000081	0.000081	0.000081	0.000081	0.000081	0.000081
Pyruvic Acid • Na	0.11	0.055	0.055	0.055	0.055	0.055	0.055	0.055
DL-Thioctic Acid	0.000105	0.000105	0.000105	0.000105	0.000105	0.000105	0.000105	0.000105
Thymidine	0.000365	0.000365	0.000365	0.000365	0.000365	0.000365	0.000365	0.000365
ADD								
Sodium Bicarbonate	1.2	1.2	—	—	—	—	1.2	1.2
Calcium Chloride • $2H_2O$	—	—	—	—	—	—	—	0.1545
L-Glutamine	—	—	0.365	0.365	—	—	—	0.365
L-Leucine	—	—	—	—	—	—	—	0.05905
L-Lysine • HCl	—	—	—	—	—	—	—	0.09125
Magnesium Chloride • $6H_2O$	—	—	—	—	—	—	—	0.0612
Magnesium Sulfate	—	—	—	—	—	—	—	0.04884
L-Methionine	—	—	—	—	—	—	—	0.0172

F-12 Coon's Modification

Component	F6636 g/L
INORGANIC SALTS	
Calcium Chloride	0.1245
Cupric Sulfate • $5H_2O$	0.0000025
Ferrrous Sulfate • $7H_2O$	0.000834
Magnesium Chloride • $6H_2O$	0.046659
Magnesium Sulfate (anhydrous)	0.02528
Potassium Chloride	0.305
Potassium Phosphate Monobasic	0.06124
Sodium Chloride	7.517
Sodium Phosphate Dibasic (anhydrous)	0.1324
Zinc Sulfate • $7H_2O$	0.000863
AMINO ACIDS	
L-Alanine	0.018
L-Arginine • HCl	0.422
L-Asparagine • H_2O	0.03
L-Aspartic Acid	0.026
L-Cysteine • HCl • H_2O	0.07026
L-Glutamic Acid	0.03
L-Glutamine	0.292
Glycine	0.016
L-Histidine • HCl • H_2O	0.042
L-Isoleucine	0.0078
L-Leucine	0.0262
L-Lysine • HCl	0.073
L-Methionine	0.009
L-Phenylalanine	0.01
L-Proline	0.07
L-Serine	0.021
L-Threonine	0.0238
L-Tryptophan	0.004
L-Tyrosine • 2Na • $2H_2O$	0.01586
L-Valine	0.0234

F-12 Coon's Modification

Component	F6636 g/L
VITAMINS	
L-Ascorbic Acid	0.15
D-Biotin	0.0000073
Choline Chloride	0.01396
Folic Acid	0.00132
myo-Inositol	0.01802
Niacinamide	0.00004
D-Pantothenic Acid (hemicalcium)	0.000238
Pyridoxine • HCl	0.00006
Riboflavin	0.00004
Thiamine • HCl	0.000337
Vitamin B_{12}	0.00136
OTHER	
D-Glucose	1.802
Hypoxanthine	0.00404
Linoleic Acid	0.00009
Phenol Red • Na	0.00125
Putrescine • 2HCl	0.000161
Pyruvic Acid • Na	0.22
Thioctic Acid	0.000206
Thymidine	0.0007
ADD	
Sodium Bicarbonate	2.676

Glasgow Minimum Essential Medium

Component	G5154 [1×] g/L	G6148 g/L
INORGANIC SALTS		
Calcium Chloride	0.2	0.2
Ferric Nitrate • $9H_2O$	0.0001	0.0001
Magnesium Sulfate (anhydrous)	0.09767	0.09767
Potassium Chloride	0.4	0.4
Sodium Bicarbonate	2.75	—
Sodium Chloride	6.4	6.4
Sodium Phosphate Monobasic (anhydrous)	0.1078	0.1078
AMINO ACIDS		
L-Arginine • HCl	0.042	0.042
L-Cystine • 2HCl	0.03129	0.03129
L-Glutamine	—	0.292
L-Histidine • HCl • H_2O	0.021	0.021
L-Isoleucine	0.0524	0.0524
L-Leucine	0.0524	0.0524
L-Lysine • HCl	0.0731	0.0731
L-Methionine	0.015	0.015
L-Phenylalanine	0.033	0.033
L-Threonine	0.0476	0.0476
L-Tryptophan	0.008	0.008
L-Tyrosine • 2Na • H_2O	0.05219	0.05219
L-Valine	0.0468	0.0468
VITAMINS		
Choline Chloride	0.002	0.002
Folic Acid	0.002	0.002
myo-Inositol	0.0036	0.0036
Niacinamide	0.002	0.002
D-Pantothenic Acid (hemicalcium)	0.002	0.002
Pyridoxal • HCl	0.002	0.002
Riboflavin	0.0002	0.0002
Thiamine • HCl	0.002	0.002
OTHER		
D-Glucose	4.5	4.5
Phenol Red • Na	0.016	0.016
ADD		
Sodium Bicarbonate	—	2.75
L-Glutamine	0.292	—

Iscove's Modified Dulbecco's Medium (IMDM)

Component	I3390 [1×] g/L	I6529 [1×] g/L	I7633 g/L
INORGANIC SALTS			
Calcium Chloride	0.1653	0.219	0.1653
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.9767
Potassium Chloride	0.33	0.33	0.33
Potassium Nitrate	0.000076	0.000076	0.000076
Sodium Bicarbonate	3.024	3.024	—
Sodium Chloride	4.505	4.505	4.505
Sodium Phosphate Monobasic (anhydrous)	0.109	0.109	0.109
Sodium Selenite	0.000017	0.0000173	0.000017
AMINO ACIDS			
L-Alanine	0.025	0.025	0.025
L-Arginine • HCl	0.084	0.084	0.084
L-Asparagine • H_2O	0.0284	0.0284	0.0284
L-Aspartic Acid	0.03	0.03	0.03
L-Cystine • 2HCl	0.09124	0.09124	0.09124
L-Glutamic Acid	0.075	0.075	0.075
L-Glutamine	—	0.584	0.584
Glycine	0.03	0.03	0.03
L-Histidine • HCl • H_2O	0.042	0.042	0.042
L-Isoleucine	0.105	0.105	0.105
L-Leucine	0.105	0.105	0.105
L-Lysine • HCl	0.146	0.146	0.146
L-Methionine	0.03	0.03	0.03
L-Phenylalanine	0.066	0.066	0.066
L-Proline	0.04	0.04	0.04
L-Serine	0.042	0.042	0.042
L-Threonine	0.095	0.095	0.095
L-Tryptophan	0.016	0.016	0.016
L-Tyrosine • 2Na • $2H_2O$	0.10379	0.10379	0.10379
L-Valine	0.094	0.094	0.094
VITAMINS			
D-Biotin	0.000013	0.000013	0.000013
Choline Chloride	0.004	0.004	0.004
Folic Acid	0.004	0.004	0.004
myo-Inositol	0.0072	0.0072	0.0072
Niacinamide	0.004	0.004	0.004
D-Pantothenic Acid (hemicalcium)	0.004	0.004	0.004
Pyridoxal • HCl	0.004	0.004	0.004
Riboflavin	0.0004	0.0004	0.0004
Thiamine • HCl	0.004	0.004	0.004
Vitamin B_{12}	0.000013	0.000013	0.000013
OTHER			
D-Glucose	4.5	2.25	4.5
HEPES	5.958	0	5.958
Phenol Red • Na	0.016	0	0.016
Pyruvic Acid • Na	0.11	0	0.11
ADD			
Sodium Bicarbonate	—	0	3.024
L-Glutamine	0.584	0	—

L-15 Medium Leibovitz

Component	L1518 [1×] g/L	L4386 g/L	L5520 [1×] g/L
INORGANIC SALTS			
Calcium Chloride	0.1396	0.1396	0.1396
Magnesium Chloride	0.0937	0.0937	0.0937
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767
Potassium Chloride	0.4	0.4	0.4
Potassium Phosphate Monobasic (anhydrous)	0.06	0.06	0.06
Sodium Chloride	8.0	8.0	8.0
Sodium Phosphate Dibasic (anhydrous)	0.19	0.19	0.19
AMINO ACIDS			
L-Alanine	0.225	0.225	0.225
L-Arginine (free base)	0.5	0.5	0.5
L-Asparagine (anhydrous)	0.25	0.25	0.25
L-Cysteine (free base)	0.12	0.12	0.12
L-Glutamine	0.3	0.3	—
Glycine	0.2	0.2	0.2
L-Histidine	0.25	0.25	0.25
L-Isoleucine	0.125	0.125	0.125
L-Leucine	0.125	0.125	0.125
L-Lysine	0.0937	0.0937	0.0937
L-Methionine	0.075	0.075	0.075
L-Phenylalanine	0.125	0.125	0.125
L-Serine	0.2	0.2	0.2
L-Threonine	0.3	0.3	0.3
L-Tryptophan	0.02	0.02	0.02
L-Tyrosine (free base)	0.3	0.3	0.3
L-Valine	0.1	0.1	0.1
VITAMINS			
Choline Chloride	0.001	0.001	0.001
Flavin Mononucleotide • Na	0.0001	0.0001	0.0001
Folic Acid	0.001	0.001	0.001
myo-Inositol	0.002	0.002	0.002
Niacinamide	0.001	0.001	0.001
DL-Pantothenic Acid (hemicalcium)	0.001	0.001	0.001
Pyridoxine • HCl	0.001	0.001	0.001
Thiamine Monophosphate • HCl	0.001	0.001	0.001
OTHER			
D-Galactose	0.9	0.9	0.9
Phenol Red • Na	0.011	0.011	0.011
Pyruvic Acid • Na	0.55	0.55	0.55
ADD			
L-Glutamine	—	—	0.3

McCoy's 5A Modified Medium

Component	M4892 g/L	M8403 [1×] g/L	M9309 [1×] g/L
INORGANIC SALTS			
Calcium Chloride	0.09995	0.09995	0.1324324
Magnesium Sulfate (anhydrous)	0.0976876	0.0976876	0.0976876
Potassium Chloride	0.4	0.4	0.4
Sodium Bicarbonate	—	2.2	2.2
Sodium Chloride	6.46	6.46	6.46
Sodium Phosphate Monobasic (anhydrous)	0.504	0.504	0.504
AMINO ACIDS			
L-Alanine	0.01336	0.01336	0.01336
L-Arginine • HCl	0.04214	0.04214	0.04214
L-Asparagine • H_2O	0.04503	0.04503	0.04503
L-Aspartic Acid	0.01997	0.01997	0.01997
L-Cysteine	0.02424	0.02424	0.02424
L-Glutamic Acid	0.02207	0.02207	0.02207
L-Glutamine	0.21915	—	0.2192
Glycine	0.00751	0.00751	0.00751
L-Histidine • HCl • H_2O	0.02096	0.02096	0.02096
Hydroxy-l-proline	0.01967	0.01967	0.01967
L-Isoleucine	0.03936	0.03936	0.03936
L-Leucine	0.03936	0.03936	0.03936
L-Lysine • HCl	0.03654	0.03654	0.03654
L-Methionine	0.01492	0.01492	0.01492
L-Phenylalanine	0.01652	0.01652	0.01652
L-Proline	0.01727	0.01727	0.01727
L-Serine	0.02628	0.02628	0.02628
L-Threonine	0.01787	0.01787	0.01787
L-Tryptophan	0.00306	0.00306	0.00306
L-Tyrosine • 2Na • $2H_2O$	0.0261	0.0261	0.0261
L-Valine	0.01757	0.01757	0.01757
VITAMINS			
Ascorbic Acid	0.0005625	0.0005625	0.0005625
p-Aminobenzoic Acid	0.001	0.001	0.001
D-Biotin	0.0002	0.0002	0.0002
Choline Chloride	0.005	0.005	0.005
Folic Acid	0.01	0.01	0.01
myo-Inositol	0.036	0.036	0.036
Niacinamide	0.0005	0.0005	0.0005
Nicotinic Acid	0.0005	0.0005	0.0005
D-Pantothenic Acid (hemicalcium)	0.0002	0.0002	0.0002
Pyridoxal • HCl	0.0005	0.0005	0.0005
Pyridoxine • HCl	0.0005	0.0005	0.0005
Riboflavin	0.0002	0.0002	0.0002
Thiamine • HCl	0.0002	0.0002	0.0002
Vitamin B_{12}	0.002	0.002	0.002
OTHER			
Peptone	0.6	0.6	0.6
D-Glucose	3	3	3
Glutathione (reduced)	0.0005	0.0005	0.0005
Phenol Red • Na	0.011	0.011	0.01062
ADD			
Sodium Bicarbonate	2.2	—	—
L-Glutamine	—	0.21915	—

Medium 199

Component	M0393 g/L	M0650 [10×] g/L	M2154 [1×] g/L	M2520 g/L	M3769 g/L
INORGANIC SALTS					
Calcium Chloride	0.1396	2	0.2	0.2	0.2
Ferric Nitrate • $9H_2O$	0.00072	0.0072	0.00072	0.00072	0.00072
Magnesium Sulfate (anhydrous)	0.09767	0.9767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	4.0	0.4	0.4	0.4
Potassium Phosphate Monobasic	0.06	—	—	—	—
Sodium Acetate (anhydrous)	0.05	0.5	0.05	0.05	0.05
Sodium Bicarbonate	—	—	2.2	—	—
Sodium Chloride	8.0	68.0	6.8	6.0	6.8
Sodium Phosphate Dibasic (anhydrous)	0.04788	—	—	—	—
Sodium Phosphate Monobasic (anhydrous)	—	1.22	0.122	0.122	0.122
AMINO ACIDS					
L-Alanine	0.025	0.25	0.025	0.025	0.025
L-Arginine • HCl	0.07	0.7	0.07	0.07	0.07
L-Aspartic Acid	0.03	0.3	0.03	0.03	0.03
L-Cysteine • HCl • H_2O	0.00011	0.0011	0.00011	0.00011	0.00011
L-Cystine • 2HCl	0.026	0.26	0.026	0.026	0.026
L-Glutamic Acid	0.0668	0.668	0.0668	0.0668	0.0668
L-Glutamine	0.1	—	—	0.1	—
Glycine	0.05	0.5	0.05	0.05	0.05
L-Histidine • HCl • H_2O	0.02188	0.2188	0.02188	0.02188	0.02188
Hydroxy-L-Proline	0.01	0.1	0.01	0.01	0.01
L-Isoleucine	0.02	0.2	0.02	0.02	0.02
L-Leucine	0.06	0.6	0.06	0.06	0.06
L-Lysine • HCl	0.07	0.7	0.07	0.07	0.07
L-Methionine	0.015	0.15	0.015	0.015	0.015
L-Phenylalanine	0.025	0.25	0.025	0.025	0.025
L-Proline	0.04	0.4	0.04	0.04	0.04
L-Serine	0.025	0.25	0.025	0.025	0.025
L-Threonine	0.03	0.3	0.03	0.03	0.03
L-Tryptophan	0.01	0.1	0.01	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.05766	0.5766	0.05766	0.05766	0.05766
L-Valine	0.025	0.25	0.025	0.025	0.025
VITAMINS					
Ascorbic Acid • Na	0.0000566	0.0005625	0.0000566	0.0000566	0.0000566
D-Biotin	0.00001	0.0001	0.00001	0.00001	0.00001
Calciferol	0.0001	0.001	0.0001	0.0001	0.0001
Choline Chloride	0.0005	0.005	0.0005	0.0005	0.0005
Folic Acid	0.00001	0.0001	0.00001	0.00001	0.00001
Menadione (sodium bisulfite)	0.000016	0.00016	0.000016	0.000016	0.000016
myo-Inositol	0.00005	0.0005	0.00005	0.00005	0.00005
Niacinamide	0.000025	0.00025	0.000025	0.000025	0.000025
Nicotinic Acid	0.000025	0.00025	0.000025	0.000025	0.000025
p-Aminobenzoic Acid	0.00005	0.0005	0.00005	0.00005	0.00005
D-Pantothenic Acid (hemicalcium)	0.00001	0.0001	0.00001	0.00001	0.00001
Pyridoxal • HCl	0.000025	0.00025	0.000025	0.000025	0.000025
Pyridoxine • HCl	0.000025	0.00025	0.000025	0.000025	0.000025
Retinol Acetate	0.00014	0.0014	0.00014	0.00014	0.00014
Riboflavin	0.00001	0.0001	0.00001	0.00001	0.00001
DL-α-Tocopherol Phosphate • Na	0.00001	0.0001	0.00001	0.00001	0.00001
Thiamine • HCl	0.00001	0.0001	0.00001	0.00001	0.00001

Medium 199

Component	M0393 g/L	M0650 [10×] g/L	M2154 [1×] g/L	M2520 g/L	M3769 g/L
OTHER					
Adenine Sulfate	0.01	0.1	0.01	0.01	0.01
Adenosine Triphosphate • 2Na	0.001	0.01	0.001	0.001	0.001
Adenosine Monophosphate • Na	0.0002385	0.002385	0.0002385	0.0002385	0.0002385
Cholesterol	0.0002	0.002	0.0002	0.0002	0.0002
Deoxyribose	0.0005	0.005	0.0005	0.0005	0.0005
Glucose	1.0	10.0	1.0	1.0	1.0
Glutathione (reduced)	0.00005	0.0005	0.00005	0.00005	0.00005
Guanine • HCl	0.0003	0.003	0.0003	0.0003	0.0003
HEPES	—	—	—	5.958	—
Hypoxanthine	0.0003	0.003	0.0003	0.0003	0.0003
Phenol Red • Na	0.0213	0.213	0.0213	0.0213	—
Polyoxyethylenesorbitan Monooleate (TWEEN® 80)	0.02	0.2	0.02	0.02	0.02
Ribose	0.0005	0.005	0.0005	0.0005	0.0005
Thymine	0.0003	0.003	0.0003	0.0003	0.0003
Uracil	0.0003	0.003	0.0003	0.0003	0.0003
Xanthine • Na	0.000344	0.00344	0.000344	0.000344	0.000344
ADD					
L-Glutamine	—	0.1 at 1×	0.1	—	0.1
Sodium Bicarbonate	0.35	2.2 at 1×	—	2.2	2.2

Medium 199

Component	M4530 [1×] g/L	M5017 g/L	M7528 [1×] g/L	M7653 [1×] g/L	M9163 [10×] g/L
INORGANIC SALTS					
Calcium Chloride	0.2	0.2	0.2	0.1396	1.396
Ferric Nitrate • $9H_2O$	0.00072	0.00072	0.00072	0.00072	0.0072
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767	0.09767	0.9767
Potassium Chloride	0.4	0.4	0.4	0.4	4.0
Potassium Phosphate Monobasic	—	—	—	0.06	0.6
Sodium Acetate (anhydrous)	0.05	0.05	0.05	0.05	0.5
Sodium Bicarbonate	2.2	—	2.2	0.35	—
Sodium Chloride	6.8	6.8	6.0	8.0	80.0
Sodium Phosphate Dibasic (anhydrous)	—	—	—	0.04788	0.4788
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122	0.122	—	—
AMINO ACIDS					
L-Alanine	0.025	0.025	0.025	0.025	0.25
L-Arginine • HCl	0.07	0.07	0.07	0.07	0.7
L-Aspartic Acid	0.03	0.03	0.03	0.03	0.3
L-Cysteine • HCl • H_2O	0.00011	0.00011	0.00011	0.00011	0.0011
L-Cystine • 2HCl	0.026	0.026	0.026	0.026	0.26
L-Glutamic Acid	0.0668	0.0668	0.0668	0.0668	0.668
L-Glutamine	0.1	0.1	—	—	—
Glycine	0.05	0.05	0.05	0.05	0.5
L-Histidine • HCl • H_2O	0.02188	0.02188	0.02188	0.02188	0.2188
Hydroxy-L-Proline	0.01	0.01	0.01	0.01	0.1
L-Isoleucine	0.02	0.02	0.02	0.02	0.2
L-Leucine	0.06	0.06	0.06	0.06	0.6
L-Lysine • HCl	0.07	0.07	0.07	0.07	0.7
L-Methionine	0.015	0.015	0.015	0.015	0.15
L-Phenylalanine	0.025	0.025	0.025	0.025	0.25
L-Proline	0.04	0.04	0.04	0.04	0.4
L-Serine	0.025	0.025	0.025	0.025	0.25
L-Threonine	0.03	0.03	0.03	0.03	0.3
L-Tryptophan	0.01	0.01	0.01	0.01	0.1
L-Tyrosine • 2Na • $2H_2O$	0.05766	0.05766	0.05766	0.05766	0.5766
L-Valine	0.025	0.025	0.025	0.025	0.25

Medium 199

Component	M4530 [1×] g/L	M5017 g/L	M7528 [1×] g/L	M7653 [1×] g/L	M9163 [10×] g/L
VITAMINS					
Ascorbic Acid • Na	0.0000566	0.0000566	0.0000566	0.0000566	0.000566
D-Biotin	0.00001	0.00001	0.00001	0.00001	0.0001
Calciferol	0.0001	0.0001	0.0001	0.0001	0.001
Choline Chloride	0.0005	0.0005	0.0005	0.0005	0.005
Folic Acid	0.00001	0.00001	0.00001	0.00001	0.0001
Menadione (sodium bisulfite)	0.000016	0.000016	0.000016	0.000016	0.00016
myo-Inositol	0.00005	0.00005	0.00005	0.00005	0.0005
Niacinamide	0.000025	0.000025	0.000025	0.000025	0.00025
Nicotinic Acid	0.000025	0.000025	0.000025	0.000025	0.00025
p-Aminobenzoic Acid	0.00005	0.00005	0.00005	0.00005	0.0005
D-Pantothenic Acid (hemicalcium)	0.00001	0.00001	0.00001	0.00001	0.0001
Pyridoxal • HCl	0.000025	0.000025	0.000025	0.000025	0.00025
Pyridoxine • HCl	0.000025	0.000025	0.000025	0.000025	0.00025
Retinol Acetate	0.00014	0.00014	0.00014	0.00014	0.0014
Riboflavin	0.00001	0.00001	0.00001	0.00001	0.0001
DL-α-Tocopherol Phosphate • Na	0.00001	0.00001	0.00001	0.00001	0.0001
Thiamine • HCl	0.00001	0.00001	0.00001	0.00001	0.0001
OTHER					
Adenine Sulfate	0.01	0.01	0.01	0.01	0.1
Adenosine Triphosphate • 2Na	0.001	0.001	0.001	0.001	0.01
Adenosine Monophosphate • Na	0.0002385	0.0002385	0.0002385	0.0002385	0.002385
Cholesterol	0.0002	0.0002	0.0002	0.0002	0.002
Deoxyribose	0.0005	0.0005	0.0005	0.0005	0.005
Glucose	1.0	1.0	1.0	1.0	10.0
Glutathione (reduced)	0.00005	0.00005	0.00005	0.00005	0.0005
Guanine • HCl	0.0003	0.0003	0.0003	0.0003	0.003
HEPES	—	—	5.958	—	—
Hypoxanthine	0.0003	0.0003	0.0003	0.0003	0.003
Phenol Red • Na	0.0213	0.0213	0.0213	0.0213	0.0213
Polyoxyethylenesorbitan Monooleate (TWEEN® 80)	0.02	0.02	0.02	0.02	0.2
Ribose	0.0005	0.0005	0.0005	0.0005	0.005
Thymine	0.0003	0.0003	0.0003	0.0003	0.003
Uracil	0.0003	0.0003	0.0003	0.0003	—
Xanthine • Na	0.000344	0.000344	0.000344	0.000344	—
ADD					
L-Glutamine	—	—	0.1	0.1	0.1 at 1×
Sodium Bicarbonate	—	2.2	—	—	0.35 at 1×

Minimum Essential Medium Eagle (MEM)

Component	M0268 g/L	M0275 [10×] g/L	M0446 [1×] g/L	M0643 g/L	M1018 g/L
INORGANIC SALTS					
Calcium Chloride	0.2	2	0.2	0.2	0.1396
Magnesium Sulfate (anhydrous)	0.09767	0.9767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	4.0	0.4	0.4	0.4
Potassium Phosphate Monobasic (anhydrous)	—	—	—	—	0.06
Sodium Bicarbonate	—	—	2.2	—	—
Sodium Chloride	6.8	68.0	6.8	6.8	8.0
Sodium Phosphate Dibasic (anhydrous)	—	—	—	—	0.04788
Sodium Phosphate Monobasic (anhydrous)	0.122	1.22	0.122	0.122	—
AMINO ACIDS					
L-Alanine	—	—	—	0.0089	0.0089
L-Alanyl-L-Glutamine	—	—	0.4344	—	—
L-Arginine • HCl	0.126	1.26	0.126	0.126	0.126
L-Asparagine • H_2O	—	—	—	0.015	0.015
L-Aspartic Acid	—	—	—	0.0133	0.0133
L-Cystine • 2HCl	0.0313	0.313	0.0313	0.0313	0.0313
L-Glutamic Acid	—	—	—	0.0147	0.0147
L-Glutamine	0.292	—	0.292	0.292	0.292
Glycine	—	—	—	0.0075	0.0075
L-Histidine • HCl • H_2O	0.042	0.42	0.042	0.042	0.042
L-Isoleucine	0.052	0.52	0.052	0.052	0.052
L-Leucine	0.052	0.52	0.052	0.052	0.052
L-Lysine • HCl	0.0725	0.725	0.0725	0.0725	0.0725
L-Methionine	0.015	0.15	0.015	0.015	0.015
L-Phenylalanine	0.032	0.32	0.032	0.032	0.032
L-Proline	—	—	—	0.0115	0.0115
L-Serine	—	—	—	0.0105	0.0105
L-Threonine	0.048	0.48	0.048	0.048	0.048
L-Tryptophan	0.01	0.1	0.01	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.0519	0.519	0.0519	0.0519	0.0519
L-Valine	0.046	0.46	0.046	0.046	0.046
VITAMINS					
Choline Chloride	0.001	0.01	0.001	0.001	0.001
Folic Acid	0.001	0.01	0.001	0.001	0.001
myo-Inositol	0.002	0.02	0.002	0.002	0.002
Niacinamide	0.001	0.01	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.01	0.001	0.001	0.001
Pyridoxal • HCl	0.001	0.01	0.001	0.001	0.001
Riboflavin	0.0001	0.001	0.0001	0.0001	0.0001
Thiamine • HCl	0.001	0.01	0.001	0.001	0.001
OTHER					
Glucose	1.0	10.0	1.0	1.0	1.0
Phenol Red • Na	0.011	0.11	0.011	0.011	0.011
ADD					
L-Glutamine	—	0.292 at 1×	—	—	—
Sodium Bicarbonate	2.2	2.2 at 1×	—	2.2	0.35

Minimum Essential Medium Eagle (MEM)

Component	M2279 [1×] g/L	M2414 [1×] g/L	M4642 g/L	M4655 [1×] g/L
INORGANIC SALTS				
Calcium Chloride	0.2	0.2	0.2	0.2
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	0.4	0.4	0.4
Potassium Phosphate Monobasic (anhydrous)	—	—	0.06	—
Sodium Bicarbonate	2.2	0.85	—	2.2
Sodium Chloride	6.8	6.8	8.0	6.8
Sodium Phosphate Dibasic (anhydrous)	—	—	0.04788	—
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122	—	0.122
AMINO ACIDS				
L-Alanine	—	—	—	—
L-Arginine • HCl	0.126	0.126	0.126	0.126
L-Asparagine • H_2O	—	—	—	—
L-Aspartic Acid	—	—	—	—
L-Cystine • 2HCl	0.0313	0.0313	0.0313	0.0313
L-Glutamic Acid	—	—	—	—
L-Glutamine	—	—	0.292	0.292
Glycine	—	—	—	—
L-Histidine • HCl • H_2O	0.042	0.042	0.042	0.042
L-Isoleucine	0.052	0.052	0.052	0.052
L-Leucine	0.052	0.052	0.052	0.052
L-Lysine • HCl	0.0725	0.0725	0.0725	0.0725
L-Methionine	0.015	0.015	0.015	0.015
L-Phenylalanine	0.032	0.032	0.032	0.032
L-Proline	—	—	—	—
L-Serine	—	—	—	—
L-Threonine	0.048	0.048	0.048	0.048
L-Tryptophan	0.01	0.01	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.0519	0.0519	0.0519	0.0519
L-Valine	0.046	0.046	0.046	0.046
VITAMINS				
Choline Chloride	0.001	0.001	0.001	0.001
Folic Acid	0.001	0.001	0.001	0.001
myo-Inositol	0.002	0.002	0.002	0.002
Niacinamide	0.001	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.001	0.001	0.001
Pyridoxal • HCl	0.001	0.001	0.001	0.001
Riboflavin	0.0001	0.0001	0.0001	0.0001
Thiamine • HCl	0.001	0.001	0.001	0.001
OTHER				
Glucose	1.0	1.0	1.0	1.0
Phenol Red • Na	0.011	0.011	0.011	0.011
ADD				
L-Glutamine	0.292	0.292	—	—
Sodium Bicarbonate	—	—	0.35	—

Minimum Essential Medium Eagle (MEM)

Component	M4780 [1×] g/L	M5650 [1×] g/L	M5775 [1×] g/L	M9288 [10×] g/L
INORGANIC SALTS				
Calcium Chloride	0.1396	0.2	0.1396	1.396
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767	0.9767
Potassium Chloride	0.4	0.4	0.4	4.0
Potassium Phosphate Monobasic (anhydrous)	0.06	—	0.06	0.6
Sodium Bicarbonate	0.35	2.2	0.35	—
Sodium Chloride	8.0	6.8	8.0	80.0
Sodium Phosphate Dibasic (anhydrous)	0.04788	—	0.04788	0.4788
Sodium Phosphate Monobasic (anhydrous)	—	0.122	—	—
AMINO ACIDS				
L-Alanine	—	0.0089	—	—
L-Arginine • HCl	0.126	0.126	0.126	1.26
L-Asparagine • H_2O	—	0.015	—	—
L-Aspartic Acid	—	0.0133	—	—
L-Cystine • 2HCl	0.0313	0.0313	0.0313	0.313
L-Glutamic Acid	—	0.0147	—	—
L-Glutamine	0.292	—	—	—
Glycine	—	0.0075	—	—
L-Histidine • HCl • H_2O	0.042	0.042	0.042	0.42
L-Isoleucine	0.052	0.052	0.052	0.52
L-Leucine	0.052	0.052	0.052	0.52
L-Lysine • HCl	0.0725	0.0725	0.0725	0.725
L-Methionine	0.015	0.015	0.015	0.15
L-Phenylalanine	0.032	0.032	0.032	0.32
L-Proline	—	0.0115	—	—
L-Serine	—	0.0105	—	—
L-Threonine	0.048	0.048	0.048	0.48
L-Tryptophan	0.01	0.01	0.01	0.1
L-Tyrosine • 2Na • $2H_2O$	0.0519	0.0519	0.0519	0.519
L-Valine	0.046	0.046	0.046	0.46
VITAMINS				
Choline Chloride	0.001	0.001	0.001	0.01
Folic Acid	0.001	0.001	0.001	0.01
myo-Inositol	0.002	0.002	0.002	0.02
Niacinamide	0.001	0.001	0.001	0.01
D-Pantothenic Acid (hemicalcium)	0.001	0.001	0.001	0.01
Pyridoxal • HCl	0.001	0.001	0.001	0.01
Riboflavin	0.0001	0.0001	0.0001	0.001
Thiamine • HCl	0.001	0.001	0.001	0.01
OTHER				
Glucose	1.0	1.0	1.0	10.0
Phenol Red • Na	0.011	0.011	0.011	0.11
ADD				
L-Glutamine	—	0.292	0.292	0.292 at 1×
Sodium Bicarbonate	—	—	—	0.35 at 1×

Minimum Essential Medium Eagle (MEM) Alpha Modification

Component	M0644 g/L	M0894 g/L	M4526 [1×] g/L	M8042 [1×] g/L
INORGANIC SALTS				
Calcium Chloride	0.2	0.2	0.2	0.2
Magnesium Sulfate (anhydrous)	0.09767	0.09767	0.09767	0.09767
Potassium Chloride	0.4	0.4	0.4	0.4
Sodium Bicarbonate	—	—	2.2	2.2
Sodium Chloride	6.8	6.8	6.8	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122	0.122	0.122
AMINO ACIDS				
L-Alanine	0.025	0.025	0.025	0.025
L-Arginine • HCl	0.126	0.126	0.126	0.126
L-Asparagine • H_2O	0.05	0.05	0.05	0.05
L-Aspartic Acid	0.03	0.03	0.03	0.03
L-Cysteine • HCl • H_2O	0.1	0.1	0.1	0.1
L-Cystine • 2HCl	0.0313	0.0313	0.0313	0.0313
L-Glutamic Acid	0.075	0.075	0.075	0.075
L-Glutamine	0.292	0.292	—	—
Glycine	0.05	0.05	0.05	0.05
L-Histidine • HCl • H_2O	0.042	0.042	0.042	0.042
L-Isoleucine	0.052	0.052	0.052	0.052
L-Leucine	0.052	0.052	0.052	0.052
L-Lysine • HCl	0.0725	0.0725	0.0725	0.0725
L-Methionine	0.015	0.015	0.015	0.015
L-Phenylalanine	0.032	0.032	0.032	0.032
L-Proline	0.04	0.04	0.04	0.04
L-Serine	0.025	0.025	0.025	0.025
L-Threonine	0.048	0.048	0.048	0.048
L-Tryptophan	0.01	0.01	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.0519	0.0519	0.0519	0.0519
L-Valine	0.046	0.046	0.046	0.046
VITAMINS				
L-Ascorbic Acid • Na	0.05	0.05	0.05	0.05
Biotin	0.0001	0.0001	0.0001	0.0001
Choline Chloride	0.001	0.001	0.001	0.001
Folic Acid	0.001	0.001	0.001	0.001
myo-Inositol	0.002	0.002	0.002	0.002
Lipoic Acid	—	—	0.0002	—
Niacinamide	0.001	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.001	0.001	0.001
Pyridoxal • HCl	0.001	0.001	0.001	0.001
Riboflavin	0.0001	0.0001	0.0001	0.0001
Thiamine • HCl	0.001	0.001	0.001	0.001
Vitamin B_{12}	0.00136	0.00136	0.00136	0.00136

Minimum Essential Medium Eagle (MEM) Alpha Modification

Component	M0644 g/L	M0894 g/L	M4526 [1×] g/L	M8042 [1×] g/L
OTHER				
Adenosine	0.01	—	—	0.01
Cytidine	0.01	—	—	0.01
2′-Deoxyadenosine	0.01	—	—	0.01
2′-Deoxycytidine • HCl	0.011	—	—	0.011
2′-Deoxyguanosine	0.01	—	—	0.01
Glucose	1.0	1.0	1.0	1.0
Guanosine	0.01	—	—	0.01
Phenol Red • Na	0.011	0.011	0.011	0.011
Pyruvic Acid • Na	0.11	0.11	0.11	0.11
Thioctic Acid	0.0002	0.0002	—	0.0002
Thymidine	0.01	—	—	0.01
Uridine	0.01	—	—	0.01
ADD				
L-Glutamine	—	—	0.292	0.292
Sodium Bicarbonate	2.2	2.2	—	—

Minimum Essential Medium Eagle (MEM) HEPES Modification

Component	M2645 g/L	M7278 [1×] g/L
INORGANIC SALTS		
Calcium Chloride	0.2	0.2
Magnesium Sulfate (anhydrous)	0.09767	0.09767
Potassium Chloride	0.4	0.4
Sodium Bicarbonate	—	2.2
Sodium Chloride	5.5	5.5
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122
AMINO ACIDS		
L-Arginine • HCl	0.126	0.126
L-Cystine • 2HCl	0.0313	0.0313
L-Glutamine	0.292	—
L-Histidine • HCl • H_2O	0.042	0.042
L-Isoleucine	0.052	0.052
L-Leucine	0.052	0.052
L-Lysine • HCl	0.0725	0.0725
L-Methionine	0.015	0.015
L-Phenylalanine	0.032	0.032
L-Threonine	0.048	0.048
L-Tryptophan	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.0519	0.0519
L-Valine	0.046	0.046
VITAMINS		
Choline Chloride	0.001	0.001
Folic Acid	0.001	0.001
myo-Inositol	0.002	0.002
Niacinamide	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.001
Pyridoxal • HCl	0.001	0.001
Riboflavin	0.0001	0.0001
Thiamine • HCl	0.001	0.001

Minimum Essential Medium Eagle (MEM) HEPES Modification

Component	M2645 g/L	M7278 [1×] g/L
OTHER		
Glucose	1.0	1.0
HEPES	5.958	5.958
Phenol Red • Na	0.011	0.011
ADD		
L-Glutamine	—	0.292
Sodium Bicarbonate	2.2	—

Minimum Essential Medium Eagle (MEM) Joklik Modification for Suspension Cultures

Component	M0518 g/L	M8028 [1×] g/L
INORGANIC SALTS		
Magnesium Chloride • $6H_2O$	0.2	0.2
Potassium Chloride	0.4	0.4
Sodium Bicarbonate	—	2.0
Sodium Chloride	6.5	6.5
Sodium Phosphate Monobasic (anhydrous)	1.154	1.154
AMINO ACIDS		
L-Arginine • HCl	0.126	1.26
L-Cystine • 2HCl	0.0324	0.0324
L-Glutamine	0.292	—
L-Histidine • HCl • H_2O	0.042	0.042
L-Isoleucine	0.052	0.052
L-Leucine	0.052	0.052
L-Lysine • HCl	0.0725	0.0725
L-Methionine	0.015	0.015
L-Phenylalanine	0.032	0.032
L-Threonine	0.048	0.048
L-Tryptophan	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.05452	0.05452
L-Valine	0.046	0.046

Minimum Essential Medium Eagle (MEM) Joklik Modification for Suspension Cultures

Component	M0518 g/L	M8028 [1×] g/L
VITAMINS		
Choline Chloride	0.001	0.001
Folic Acid	0.001	0.001
myo-Inositol	0.002	0.002
Niacinamide	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.001
Pyridoxal • HCl	0.001	0.001
Riboflavin	0.0001	0.0001
Thiamine • HCl	0.001	0.001
OTHER		
Glucose	2.0	2.0
Phenol Red • Na	0.011	0.011
ADD		
L-Glutamine	—	0.292
Sodium Bicarbonate	2.0	—

Minimum Essential Medium Eagle (MEM) Modified

Component	M3024 g/L
INORGANIC SALTS	
Calcium Chloride	0.2
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.4
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122
AMINO ACIDS	
L-Alanine	0.0089
L-Arginine • HCl	0.126
L-Asparagine • H_2O	0.015
L-Aspartic Acid	0.0133
L-Cystine • 2HCl	0.0313
L-Glutamic Acid	0.0147
Glycine	0.0075
L-Histidine • HCl • H_2O	0.042
L-Isoleucine	0.052
L-Leucine	0.052
L-Lysine • HCl	0.0725
L-Methionine	0.015
L-Phenylalanine	0.032
L-Proline	0.0115
L-Serine	0.0105
L-Threonine	0.048
L-Tryptophan	0.01
L-Tyrosine • 2Na • $2H_2O$	0.0519
L-Valine	0.046
VITAMINS	
Choline Chloride	0.001
Folic Acid	0.001
myo-Inositol	0.002
Niacinamide	0.001
D-Pantothenic Acid (hemicalcium)	0.001
Pyridoxal • HCl	0.001
Riboflavin	0.0001
Thiamine • HCl	0.001

Minimum Essential Medium Eagle (MEM) Modified

Component	M3024 g/L
OTHER	
Glucose	1
ADD	
L-Glutamine	0.292
Sodium Bicarbonate	2.2

Minimum Essential Medium Eagle (MEM) Auto-Mod™ Modified for Autoclaving

Component	M0769 g/L
INORGANIC SALTS	
Calcium Chloride	0.2
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.4
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122
Sodium Succinate • $6H_2O$	0.1
Succinic Acid (free acid)	0.075
AMINO ACIDS	
L-Arginine • HCl	0.126
L-Cystine • 2HCl	0.0313
L-Histidine • HCl • H_2O	0.042
L-Isoleucine	0.052
L-Leucine	0.052
L-Lysine • HCl	0.0725
L-Methionine	0.015
L-Phenylalanine	0.032
L-Threonine	0.048
L-Tryptophan	0.01
L-Tyrosine (free base)	0.036
L-Valine	0.046
VITAMINS	
Choline Bitartrate	0.0018
Folic Acid	0.001
myo-Inositol	0.002
Niacinamide	0.001
D-Pantothenic Acid (hemicalcium)	0.001
Pyridoxal • HCl	0.001
Riboflavin	0.0001
Thiamine • HCl	0.001
OTHER	
Glucose	1
Phenol Red • Na	0.0064
ADD	
L-Glutamine	0.292
Sodium Bicarbonate	2.2

Minimum Essential Medium Eagle (S-MEM) Spinner Modification

Component	M8167 [1×] g/L
INORGANIC SALTS	
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.4
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122
AMINO ACIDS	
L-Arginine • HCl	0.126
L-Cystine • 2HCl	0.0313
L-Histidine • HCl • H_2O	0.042
L-Isoleucine	0.052
L-Leucine	0.052
L-Lysine • HCl	0.0725
L-Methionine	0.015
L-Phenylalanine	0.032
L-Threonine	0.048
L-Tryptophan	0.01
L-Tyrosine • 2Na • $2H_2O$	0.0519
L-Valine	0.046
VITAMINS	
Choline Chloride	0.001
Folic Acid	0.001
myo-Inositol	0.002
Niacinamide	0.001
D-Pantothenic Acid (hemicalcium)	0.001
Pyridoxal • HCl	0.001
Riboflavin	0.0001
Thiamine • HCl	0.001
OTHER	
Glucose	1
Phenol Red • Na	0.011
Sodium Bicarbonate	2.2
ADD	
L-Glutamine	0.292

NCTC 109 Medium

Component	N1140 [1×] g/L	N3262 g/L
INORGANIC SALTS		
Calcium Chloride	0.2	0.2
Magnesium Sulfate (anhydrous)	0.1	0.1
Potassium Chloride	0.4	0.4
Sodium Acetate (anhydrous)	0.03	0.03
Sodium Bicarbonate	2.2	—
Sodium Chloride	6.8	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122
AMINO ACIDS		
L-Alanine	0.03148	0.03148
L-Arginine • HCl	0.03116	0.03116
L-Asparagine • H_2O	0.00919	0.00919
L-Aspartic Acid	0.00991	0.00991
L-Cysteine • HCl • H_2O	0.28971	—
L-Cystine • 2HCl	0.01368	0.01368
L-Glutamic Acid	0.00826	0.00826
L-Glutamine	—	0.13573
Glycine	0.01351	0.01351
L-Histidine • HCl • H_2O	0.02665	0.02665

NCTC 109 Medium

Component	N1140 [1×] g/L	N3262 g/L
Hydroxy-L-Proline	0.00409	0.00409
L-Isoleucine	0.01804	0.01804
L-Leucine	0.02044	0.02044
L-Lysine • HCl	0.03843	0.03843
L-Methionine	0.00444	0.00444
L-Ornithine • HCl	0.00941	0.00941
L-Phenylalanine	0.01653	0.01653
L-Proline	0.00613	0.00613
L-Serine	0.01075	0.01075
L-Threonine	0.01893	0.01893
L-Tryptophan	0.0175	0.0175
L-Tyrosine • 2Na • $2H_2O$	0.0237	0.0237
L-Valine	0.025	0.025
VITAMINS		
L-Ascorbic Acid	0.05	0.05
D-Biotin	0.000025	0.000025
Calciferol	0.00025	0.00025
Choline Chloride	0.00125	0.00125
Folic Acid	0.000025	0.000025
myo-Inositol	0.000125	0.000125
Menadione (sodium bisulfite)	0.00004	0.00004
Niacinamide	0.0000625	0.0000625
Nicotinic Acid	0.0000625	0.0000625
p-Aminobenzoic Acid	0.000125	0.000125
D-Pantothenic Acid (hemicalcium)	0.000025	0.000025
Pyridoxal • HCl	0.0000625	0.0000625
Pyridoxine • HCl	0.0000625	0.0000625
Retinol Acetate	0.00025	0.00025
Riboflavin	0.000025	0.000025
Thiamine • HCl	0.000025	0.000025
DL-α-Tocopherol Phosphate • 2Na	0.000025	0.000025
Vitamin B_{12}	0.01	0.01
OTHER		
L-Amino-n-Butyric Acid	0.00551	0.00551
Cocarboxylase	0.001	0.0001
Coenzyme A • Na	0.0025	0.0025
2′-Deoxyadenosine	0.01	0.01
2′-Deoxycytidine • HCl	0.01	0.01
2′-Deoxyguanosine • HCl	0.01	0.01
Flavin Adenine Dinucleotide • 2Na	0.001	0.001
D-Glucosamine • HCl	0.00385	0.00385
D-Glucose	1	1
Glucuronate • Na	0.0018	0.0018
D-Glucuronolactone	0.0018	0.0018
Glutathione • Na	0.02	0.02
5′-Methylcytosine • HCl	0.0001	0.0001
β-NAD	0.007	0.007
β-NADP • Na	0.001	0.001
Phenol Red • Na	0.02	0.02
L-Taurine	0.00418	0.00418
Thymidine	0.01	0.01
TWEEN® 80	0.0125	0.0125
Uridine Triphosphate • Na	0.001	0.001
ADD		
L-Glutamine	0.13573	—

Nutrient Mixture F-10 Ham

Component	N2147 [1×] g/L	N6013 [1×] g/L
INORGANIC SALTS		
Calcium Chloride	0.0333	0.0333
Cupric Sulfate • $5H_2O$	0.0000025	0.0000025
Ferrous Sulfate • $7H_2O$	0.000834	0.000834
Magnesium Sulfate (anhydrous)	0.07464	0.07464
Potassium Chloride	0.285	0.285
Potassium Phosphate Monobasic (anhydrous)	0.083	0.083
Sodium Bicarbonate	—	1.2
Sodium Chloride	7.4	7.4
Sodium Phosphate Dibasic (anhydrous)	0.1537	0.1537
Zinc Sulfate • $7H_2O$	0.0000288	0.0000288
AMINO ACIDS		
L-Alanine	0.009	0.009
L-Arginine • HCl	0.211	0.211
L-Asparagine • H_2O	0.01501	0.01501
L-Aspartic Acid	0.0133	0.0133
L-Cysteine • HCl • H_2O	0.035	0.035
L-Glutamic Acid	0.0147	0.0147
Glycine	0.00751	0.00751
L-Histidine • 3HCl • H_2O	0.021	0.021
L-Isoleucine	0.0026	0.0026
L-Leucine	0.0131	0.0131
L-Lysine • HCl	0.0293	0.0293
L-Methionine	0.00448	0.00448
L-Phenylalanine	0.00496	0.00496
L-Proline	0.0115	0.0115
L-Serine	0.0105	0.0105
L-Threonine	0.00357	0.00357
L-Tryptophan	0.0006	0.0006
L-Tyrosine • 2Na • $2H_2O$	0.00261	0.00261
L-Valine	0.0035	0.0035
VITAMINS		
D-Biotin	0.000024	0.000024
Choline Chloride	0.000698	0.000698
Folic Acid	0.00132	0.00132
myo-Inositol	0.000541	0.000541
Niacinamide	0.000615	0.000615
D-Pantothenic Acid (hemicalcium)	0.000715	0.000715
Pyridoxine • HCl	0.000206	0.000206
Riboflavin	0.000376	0.000376
Thiamine • HCl	0.001	0.001
Vitamin B_{12}	0.00136	0.00136
OTHER		
D-Glucose	1.1	1.1
HEPES	4.77	—
Hypoxanthine	0.00408	0.00408
Phenol Red (sodium)	0.0013	0.0013
Pyruvic Acid (sodium)	0.11	0.11
Thioctic Acid	0.00021	0.00021
Thymidine	0.00073	0.00073
ADD		
L-Glutamine	0.146	0.146

Nutrient Mixture F-10 Ham

Component	N6635 g/L	N6908 [1×] g/L
INORGANIC SALTS		
Calcium Chloride	0.0333	0.0333
Cupric Sulfate • $5H_2O$	0.0000025	0.000025
Ferrous Sulfate • $7H_2O$	0.000834	0.000834
Magnesium Sulfate (anhydrous)	0.07464	0.07464
Potassium Chloride	0.285	0.285
Potassium Phosphate Monobasic (anhydrous)	0.083	0.083
Sodium Bicarbonate	—	1.2
Sodium Chloride	7.4	7.4
Sodium Phosphate Dibasic (anhydrous)	0.1537	0.1537
Zinc Sulfate • $7H_2O$	0.0000288	0.0000288
AMINO ACIDS		
L-Alanine	0.009	0.009
L-Arginine • HCl	0.211	0.211
L-Asparagine • H_2O	0.01501	0.01501
L-Aspartic Acid	0.0133	0.0133
L-Cysteine • HCl • H_2O	0.035	0.035
L-Glutamic Acid	0.0147	0.0147
L-Glutamine	0.146	0.146
Glycine	0.00751	0.00751
L-Histidine • HCl • H_2O	0.021	0.021
L-Isoleucine	0.0026	0.0026
L-Leucine	0.0131	0.0131
L-Lysine • HCl	0.0293	0.0293
L-Methionine	0.00448	0.00488
L-Phenylalanine	0.00496	0.00496
L-Proline	0.0115	0.0115
L-Serine	0.0105	0.0105
L-Threonine	0.00357	0.00357
L-Tryptophan	0.0006	0.0006
L-Tyrosine • 2Na • $2H_2O$	0.00261	0.00261
L-Valine	0.0035	0.0035
VITAMINS		
D-Biotin	0.000024	0.000024
Choline Chloride	0.000698	0.000698
Folic Acid	0.00132	0.00132
myo-Inositol	0.000541	0.000541
Niacinamide	0.000615	0.000615
D-Pantothenic Acid (hemicalcium)	0.000715	0.000715
Pyridoxine • HCl	0.000206	0.000206
Riboflavin	0.000376	0.000376
Thiamine • HCl	0.001	0.001
Vitamin B_{12}	0.00136	0.00136
OTHER		
D-Glucose	1.1	1.1
Hypoxanthine	0.00408	0.00408
Phenol Red • Na	0.0013	0.0013
Pyruvic Acid • Na	0.11	0.11
Thioctic Acid	0.00021	0.00021
Thymidine	0.00073	0.00073
ADD		
Sodium Bicarbonate	1.2	—

Nutrient Mixture F-12 Ham

Component	N4888 [1×] g/L	N6658 [1×] g/L	N6760 g/L
INORGANIC SALTS			
Calcium Chloride	0.0333	0.0333	0.0333
Cupric Sulfate • $5H_2O$	0.0000025	0.0000025	0.0000025
Ferrous Sulfate • $7H_2O$	0.000834	0.000834	0.000834
Magnesium Chloride	0.0576	0.0576	0.0576
Potassium Chloride	0.224	0.224	0.224
Sodium Bicarbonate	1.176	1.176	—
Sodium Chloride	7.599	7.599	7.599
Sodium Phosphate Dibasic (anhydrous)	0.14204	0.14204	0.14204
Zinc Sulfate • $7H_2O$	0.000863	0.000863	0.000863
AMINO ACIDS			
L-Alanine	0.009	0.009	0.009
L-Arginine • HCl	0.211	0.211	0.211
L-Asparagine • H_2O	0.01501	0.01501	0.01501
L-Aspartic Acid	0.0133	0.0133	0.0133
L-Cysteine • HCl • H_2O	0.035	0.035	0.035
L-Glutamic Acid	0.0147	0.0147	0.0147
L-Glutamine	—	0.146	0.146
Glycine	0.00751	0.00751	0.00751
L-Histidine • 3HCl • H_2O	0.02096	0.02096	0.02096
L-Isoleucine	0.00394	0.00394	0.00394
L-Leucine	0.0131	0.0131	0.0131
L-Lysine • HCl	0.0365	0.0365	0.0365
L-Methionine	0.00448	0.00448	0.00448
L-Phenylalanine	0.00496	0.00496	0.00496
L-Proline	0.0345	0.0345	0.345
L-Serine	0.0105	0.0105	0.0105
L-Threonine	0.0119	0.0119	0.0119
L-Tryptophan	0.00204	0.00204	0.00204
L-Tyrosine • 2Na • $2H_2O$	0.00778	0.00778	0.00778
L-Valine	0.0117	0.0117	0.0117
VITAMINS			
D-Biotin	0.0000073	0.0000073	0.000073
Choline Chloride	0.01396	0.01396	0.01396
Folic Acid	0.00132	0.00132	0.00132
myo-Inositol	0.018	0.018	0.018
Niacinamide	0.000037	0.000037	0.000037
D-Pantothenic Acid (hemicalcium)	0.00048	0.00048	0.00048
Pyridoxine • HCl	0.000062	0.000062	0.000062
Riboflavin	0.000038	0.000038	0.000038
Thiamine • HCl	0.00034	0.00034	0.00034
Vitamin B_{12}	0.00136	0.00136	0.00136
OTHER			
D-Glucose	1.802	1.802	1.802
Hypoxanthine	0.00408	0.00408	0.00408
Linoleic Acid	0.000084	0.000084	0.000084
Phenol Red • Na	0.0013	0.0013	0.0013
Putrescine • HCl	0.000161	0.000161	0.000161
Pyruvic Acid • Na	0.11	0.11	0.11
Thioctic Acid	0.00021	0.00021	0.00021
Thymidine	0.00073	0.00073	0.00073
ADD			
L-Glutamine	0.146	—	—
Sodium Bicarbonate	—	—	1.176

Nutrient Mixture F-12 Ham HEPES Modification

Component	N8641 [1×] g/L
INORGANIC SALTS	
Calcium Chloride	0.0333
Cupric Sulfate • $5H_2O$	0.0000025
Ferrous Sulfate • $7H_2O$	0.000834
Magnesium Chloride	0.0576
Potassium Chloride	0.224
Sodium Bicarbonate	1.176
Sodium Chloride	7.1
Sodium Phosphate Dibasic (anhydrous)	0.14204
Zinc Sulfate • $7H_2O$	0.000863
AMINO ACIDS	
L-Alanine	0.009
L-Arginine • HCl	0.211
L-Asparagine • H_2O	0.01501
L-Aspartic Acid	0.0133
L-Cysteine • HCl • H_2O	0.035
L-Glutamic Acid	0.0147
Glycine	0.00751
L-Histidine • 3HCl • H_2O	0.02096
L-Isoleucine	0.00394
L-Leucine	0.0131
L-Lysine • HCl	0.0365
L-Methionine	0.00448
L-Phenylalanine	0.00496
L-Proline	0.0345
L-Serine	0.0105
L-Threonine	0.0119
L-Tryptophan	0.00204
L-Tyrosine • 2Na • $2H_2O$	0.00778
L-Valine	0.0117
VITAMINS	
D-Biotin	0.0000073
Choline Chloride	0.01396
Folic Acid	0.00132
myo-Inositol	0.018
Niacinamide	0.000037
D-Pantothenic Acid (hemicalcium)	0.000238
Pyridoxine • HCl	0.000062
Riboflavin	0.000038
Thiamine • HCl	0.00034
Vitamin B_{12}	0.00136
OTHER	
D-Glucose	1.802
HEPES	5.958
Hypoxanthine	0.00408
Linoleic Acid	0.000084
Phenol Red • Na	0.0013
Putrescine • HCl	1.000161
Pyruvic Acid • Na	0.11
Thioctic Acid	0.00021
Thymidine	0.00073
ADD	
L-Glutamine	0.146

Nutrient Mixture F-12 Ham Kaighn's Modification

Component	N3520 g/L
INORGANIC SALTS	
Calcium Chloride	0.10207
Cupric Sulfate • $5H_2O$	0.0000025
Ferrous Sulfate • $7H_2O$	0.000834
Magnesium Chloride	0.04961
Magnesium Sulfate	0.19264
Potassium Chloride	0.28329
Potassium Phosphate Monobasic	0.058523
Sodium Phosphate Dibasic	0.11502
Sodium Chloride	7.5972
Zinc Sulfate • $7H_2O$	0.00014375
AMINO ACIDS	
L-Alanine	0.017818
L-Arginine	0.4214
L-Asparagine • H_2O	0.03002
L-Aspartic Acid	0.02662
L-Cysteine • HCl • H_2O	0.07024
L-Glutamic Acid	0.02942
L-Glutamine	0.2922
Glycine	0.015014
L-Histidine • HCl • H_2O	0.04192
L-Isoleucine	0.007872
L-Leucine	0.02624
L-Lysine • HCl	0.07304
L-Methionine	0.008952
L-Phenylalanine	0.009912
L-Proline	0.06906
L-Serine	0.02102
L-Threonine	0.02382
L-Tryptophan	0.004084
L-Tyrosine	0.010872
L-Valine	0.02342
VITAMINS	
D-Biotin	0.00007329
Choline Chloride	0.01396
Folic Acid	0.0013242
Hypoxanthine	0.004083
myo-Inositol	0.01802
Niacinamide	0.00003663
D-Pantothenic Acid (hemicalcium)	0.000477
Putrescine • 2HCl	0.0003222
Pyridoxine • HCl	0.00006168
Riboflavin	0.00003764
Thiamine • HCl	0.0003373
Thymidine	0.0007266
Vitamin B_{12}	0.0013554
OTHER	
D+-Glucose	1.26
Phenol Red • Na	0.00331806
Pyruvic Acid • Na	0.22
DL-6,8-Thioctic Acid	0.0002063
ADD	
Sodium Bicarbonate	2.5

RPMI-1640 Medium

Component	R0883 [1×] g/L	R1145 [10×] g/L	R1383 g/L	R1780 [1×] g/L	R2405 [1×] g/L	R6504 g/L	R8758 [1×] g/L
INORGANIC SALTS							
Calcium Nitrate • $4H_2O$	0.1	1	0.1	0.1	0.1	0.1	0.1
Magnesium Sulfate (anhydrous)	0.04884	0.4884	0.04884	0.04884	0.04884	0.04884	0.04884
Potassium Chloride	0.4	4	0.4	0.4	0.4	0.4	0.4
Sodium Bicarbonate	2	—	—	2	2	—	2
Sodium Chloride	6	60	6	6	6	6	6
Sodium Phosphate Dibasic (anhydrous)	0.8	8	0.8	0.8	0.8	0.8	0.8
AMINO ACIDS							
L-Alanyl-L-Glutamine	—	—	—	—	0.4344	—	—
L-Arginine	0.2	2	0.2	—	0.2	0.2	0.2
L-Asparagine (anhydrous)	0.05	0.5	0.05	0.05	0.05	0.05	0.05
L-Aspartic Acid	0.02	0.2	0.02	0.02	0.02	0.02	0.02
L-Cystine • 2HCl	0.0652	0.652	0.0652	0.0652	0.0652	0.0652	0.0652
L-Glutamic Acid	0.02	0.2	0.02	0.02	0.02	0.02	0.02
L-Glutamine	—	—	0.3	0.3	—	0.3	0.3
Glycine	0.01	0.1	0.01	0.01	0.01	0.01	0.01
L-Histidine	0.015	0.15	0.015	0.015	0.015	0.015	0.015
Hydroxy-L-Proline	0.02	0.2	0.02	0.02	0.02	0.02	0.02
L-Isoleucine	0.05	0.5	0.05	0.05	0.05	0.05	0.05
L-Leucine	0.05	0.5	0.05	—	0.05	0.05	0.05
L-Lysine • HCl	0.04	0.4	0.04	—	0.04	0.04	0.04
L-Methionine	0.015	0.15	0.015	0.015	0.015	0.015	0.015
L-Phenylalanine	0.015	0.15	0.015	0.015	0.015	0.015	0.015
L-Proline	0.02	0.2	0.02	0.02	0.02	0.02	0.02
L-Serine	0.03	0.3	0.03	0.03	0.03	0.03	0.03
L-Threonine	0.02	0.2	0.02	0.02	0.02	0.02	0.02
L-Tryptophan	0.005	0.05	0.005	0.005	0.005	0.005	0.005
L-Tyrosine • 2Na • $2H_2O$	0.02883	0.2883	0.02883	0.02883	0.02883	0.02883	0.02883
L-Valine	0.02	0.2	0.02	0.02	0.02	0.02	0.02
VITAMINS							
D-Biotin	0.0002	0.002	0.0002	0.002	0.002	0.0002	0.0002
Choline Chloride	0.003	0.03	0.003	0.003	0.003	0.003	0.003
Folic Acid	0.001	—	0.001	0.001	0.001	0.001	0.001
myo-Inositol	0.035	0.35	0.035	0.035	0.035	0.035	0.035
Niacinamide	0.001	0.01	0.001	0.001	0.001	0.001	0.001
p-Aminobenzoic Acid	0.001	0.01	0.001	0.001	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.00025	0.0025	0.00025	0.00025	0.00025	0.00025	0.00025
Pyridoxine • HCl	0.001	0.01	0.001	0.001	0.001	0.001	0.001
Riboflavin	0.0002	0.002	0.0002	0.0002	0.0002	0.0002	0.0002
Thiamine • HCl	0.001	0.01	0.001	0.001	0.001	0.001	0.001
Vitamin B_{12}	0.000005	0.00005	0.000005	0.000005	0.000005	0.000005	0.000005
OTHER							
D-Glucose	2	20	—	2	2	2	2
Glutathione (reduced)	0.001	0.01	0.001	0.001	0.001	0.001	0.001
Phenol Red • Na	0.0053	0.053	0.0053	—	0.0053	0.0053	0.0053
ADD							
L-Glutamine	0.3	0.3 at 1×	—	—	—	—	—
Sodium Bicarbonate	—	2.0 at 1×	2	—	—	2	—

RPMI-1640 Medium Dutch Modification

Component	R7638 [1×] g/L
INORGANIC SALTS	
Calcium Nitrate • $4H_2O$	0.1
Magnesium Sulfate (anhydrous)	0.04884
Potassium Chloride	0.4
Sodium Bicarbonate	1
Sodium Chloride	6.4
Sodium Phosphate Dibasic (anhydrous)	0.8
AMINO ACIDS	
L-Arginine	0.2
L-Asparagine (anhydrous)	0.05
L-Aspartic Acid	0.02
L-Cystine • 2HCl	0.0652
L-Glutamic Acid	0.02
Glycine	0.01
L-Histidine	0.015
Hydroxy-L-Proline	0.02
L-Isoleucine	0.05
L-Leucine	0.05
L-Lysine • HCl	0.04
L-Methionine	0.015
L-Phenylalanine	0.015
L-Proline	0.02
L-Serine	0.03
L-Threonine	0.02
L-Tryptophan	0.005
L-Tyrosine • 2Na • $2H_2O$	0.02883
L-Valine	0.02
VITAMINS	
D-Biotin	0.0002
Choline Chloride	0.003
Folic Acid	0.001
myo-Inositol	0.035
Niacinamide	0.001
p-Aminobenzoic Acid	0.001
D-Pantothenic Acid (hemicalcium)	0.00025
Pyridoxine • HCl	0.001
Riboflavin	0.0002
Thiamine • HCl	0.001
Vitamin B_{12}	0.000005
OTHER	
D-Glucose	2
Glutathione (reduced)	0.001
HEPES	4.77
Phenol Red • Na	0.0053
ADD	
L-Glutamine	0.3

RPMI-1640 Medium HEPES Modification

Component	R4130 g/L	R5886 [1×] g/L	R8005 g/L
INORGANIC SALTS			
Calcium Nitrate • $4H_2O$	0.1	0.1	0.1
Magnesium Sulfate (anhydrous)	0.04884	0.04884	0.04884
Potassium Chloride	0.4	0.4	0.4
Sodium Bicarbonate	—	2	—
Sodium Chloride	6	6	5.9
Sodium Phosphate Dibasic (anhydrous)	0.8	0.8	0.8
AMINO ACIDS			
L-Arginine	0.2	0.2	0.2
L-Asparagine (anhydrous)	0.05	0.05	0.05
L-Aspartic Acid	0.02	0.02	0.02
L-Cystine • 2HCl	0.0652	0.0652	0.0652
L-Glutamic Acid	0.02	0.02	0.02
L-Glutamine	0.3	—	0.3
Glycine	0.01	0.01	0.01
L-Histidine	0.015	0.015	0.015
Hydroxy-L-Proline	0.02	0.02	0.02
L-Isoleucine	0.05	0.05	0.05
L-Leucine	0.05	0.05	0.05
L-Lysine • HCl	0.04	0.04	0.04
L-Methionine	0.015	0.015	0.015
L-Phenylalanine	0.015	0.015	0.015
L-Proline	0.02	0.02	0.02
L-Serine	0.03	0.03	0.03
L-Threonine	0.02	0.02	0.02
L-Tryptophan	0.005	0.005	0.005
L-Tyrosine • 2Na • $2H_2O$	0.02883	0.02883	0.02883
L-Valine	0.02	0.02	0.02
VITAMINS			
D-Biotin	0.0002	0.0002	0.0002
Choline Chloride	0.003	0.003	0.003
Folic Acid	0.001	0.001	0.001
myo-Inositol	0.035	0.035	0.035
Niacinamide	0.001	0.001	0.001
p-Aminobenzoic Acid	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.00025	0.00025	0.00025
Pyridoxine • HCl	0.001	0.001	0.001
Riboflavin	0.0002	0.0002	0.0002
Thiamine • HCl	0.001	0.001	0.001
Vitamin B_{12}	0.000005	0.000005	0.000005
OTHER			
D-Glucose	2	2	4.5
Glutathione (reduced)	0.001	0.001	0.001
HEPES	5.96	5.96	3.5745
Phenol Red • Na	0.0053	0.0053	0.0053
ADD			
L-Glutamine	—	0.3	—
Sodium Bicarbonate	2	—	2

RPMI-1640 Medium Modified

Component	R7388 [1×] g/L	R7509 [1×] g/L	R7513 [1×] g/L	R8755 g/L
INORGANIC SALTS				
Calcium Nitrate • $4H_2O$	0.1	0.1	0.1	0.1
Magnesium Sulfate (anhydrous)	0.04884	0.04884	0.04884	0.04884
Potassium Chloride	0.4	0.4	0.4	0.4
Sodium Bicarbonate	—	2	2	—
Sodium Chloride	6	6	6	6
Sodium Phosphate Dibasic (anhydrous)	0.8	0.8	0.8	0.8
AMINO ACIDS				
L-Arginine	0.2	0.2	0.2	0.2
L-Asparagine (anhydrous)	0.05	0.05	0.05	0.05
L-Aspartic Acid	0.02	0.02	0.02	0.02
L-Cystine • 2HCl	0.0652	0.0652	—	0.0652
L-Glutamic Acid	0.02	0.02	0.02	0.02
L-Glutamine	0.3	—	—	0.3
Glycine	0.01	0.01	0.01	0.01
L-Histidine	0.015	0.015	0.015	0.015
Hydroxy-L-Proline	0.02	0.02	0.02	0.02
L-Isoleucine	0.05	0.05	0.05	0.05
L-Leucine	0.05	0.05	0.05	0.05
L-Lysine • HCl	0.04	0.04	0.04	0.04
L-Methionine	0.015	0.015	—	0.015
L-Phenylalanine	0.015	0.015	0.015	0.015
L-Proline	0.02	0.02	0.02	0.02
L-Serine	0.03	0.03	0.03	0.03
L-Threonine	0.02	0.02	0.02	0.02
L-Tryptophan	0.005	0.005	0.005	0.005
L-Tyrosine • 2Na • $2H_2O$	0.02883	0.02883	0.02883	0.02883
L-Valine	0.02	0.02	0.02	0.02
VITAMINS				
D-Biotin	0.0002	0.0002	0.0002	0.0002
Choline Chloride	0.003	0.003	0.003	0.003
Folic Acid	0.001	0.001	0.001	0.001
myo-Inositol	0.035	0.035	0.035	0.035
Niacinamide	0.001	0.001	0.001	0.001
p-Aminobenzoic Acid	0.001	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.00025	0.00025	0.00025	0.00025
Pyridoxine • HCl	0.001	0.001	0.001	0.001
Riboflavin	0.0002	0.0002	0.0002	0.0002
Thiamine • HCl	0.001	0.001	0.001	0.001
Vitamin B_{12}	0.000005	0.000005	0.000005	0.000005
OTHER				
D-Glucose	2	2	2	2
Glutathione (reduced)	0.001	0.001	0.001	0.001
HEPES	4.77	—	—	—
Phenol Red • Na	0.0053	—	0.0053	—
ADD				
L-Cystine • 2HCl	—	—	0.0652	—
L-Glutamine	—	0.3	0.3	—
L-Methionine	—	—	0.015	—
Sodium Bicarbonate	2	—	—	2

RPMI-1640 Medium Auto-Mod™ Modified for Autoclaving

Component	R7755 g/L
INORGANIC SALTS	
Calcium Nitrate • $4H_2O$	0.1
Magnesium Sulfate (anhydrous)	0.04884
Potassium Chloride	0.4
Sodium Chloride	6
Sodium Phosphate Dibasic (anhydrous)	0.8
Succinic Acid • $6H_2O$ • Na	0.1
Succinic Acid (free acid)	0.075
AMINO ACIDS	
L-Arginine	0.2
L-Asparagine (anhydrous)	0.05
L-Aspartic Acid	0.02
L-Cystine • 2HCl	0.0652
L-Glutamic Acid	0.02
Glycine	0.01
L-Histidine	0.015
Hydroxy-L-Proline	0.02
L-Isoleucine	0.05
L-Leucine	0.05
L-Lysine • HCl	0.04
L-Methionine	0.015
L-Phenylalanine	0.015
L-Proline	0.02
L-Serine	0.03
L-Threonine	0.02
L-Tryptophan	0.005
L-Tyrosine	0.02
L-Valine	0.02
VITAMINS	
D-Biotin	0.0002
Choline Bitartrate	0.00544
Folic Acid	0.001
myo-Inositol	0.035
Niacinamide	0.001
p-Aminobenzoic Acid	0.001
D-Pantothenic Acid (hemicalcium)	0.00025
Pyridoxine • HCl	0.001
Riboflavin	0.0002
Thiamine • HCl	0.001
Vitamin B_{12}	0.000005
OTHER	
D-Glucose	2
Glutathione (reduced)	0.001
Phenol Red • Na	0.00318
ADD	
L-Glutamine	0.3
Sodium Bicarbonate	2

Waymouth MB 752/1 Medium

Component	W1625 g/L
INORGANIC SALTS	
Calcium Chloride	0.0906
Magnesium Chloride	0.11239
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.15
Potassium Phosphate Monobasic (anhydrous)	0.08
Sodium Chloride	6
Sodium Phosphate Dibasic (anhydrous)	0.3
AMINO ACIDS	
L-Arginine • HCl	0.075
L-Aspartic Acid	0.06
L-Cysteine • HCl • H_2O	0.10029
L-Cystine • 2HCl	0.01955
L-Glutamic Acid	0.15
L-Glutamine	0.35
Glycine	0.05
L-Histidine • HCl • H_2O	0.1641
L-Isoleucine	0.025
L-Leucine	0.05
L-Lysine • HCl	0.24
L-Methionine	0.05
L-Phenylalanine	0.05
L-Proline	0.05
L-Threonine	0.075
L-Tryptophan	0.04
L-Tyrosine • 2Na • $2H_2O$	0.0577
L-Valine	0.065
VITAMINS	
Ascorbic Acid • Na	0.0198
D-Biotin	0.00002
Choline Bitartrate	0.45352
Folic Acid	0.0004
myo-Inositol	0.001
Niacinamide	0.001
D-Pantothenic Acid (hemicalcium)	0.001
Pyridoxine • HCl	0.001
Riboflavin	0.001
Thiamine • HCl	0.01
Vitamin B_{12}	0.0002
OTHER	
D-Glucose	5
Glutathione (reduced)	0.015
Hypoxanthine	0.025
Phenol Red • Na	0.011
ADD	
Sodium Bicarbonate	2.24

Williams' Medium E

Component	W1878 [1×] g/L	W4125 g/L	W4128 [1×] g/L
INORGANIC SALTS			
Calcium Chloride	0.2	0.2	0.2
Cupric Sulfate • 5H2O	0.0000001	0.0000001	0.0000001
Ferric Nitrate • $9H_2O$	0.0000001	0.0000001	0.0000001
Magnesium Chloride • $4H_2O$	0.0000001	0.0000001	0.0000001
Magnesium Sulfate (anhydrous)	0.0977	0.0977	0.0977
Potassium Chloride	0.4	0.4	0.4
Sodium Bicarbonate	2.2	—	2.2
Sodium Chloride	6.8	6.8	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122	0.122
Zinc Sulfate • $7H_2O$	0.0000002	0.0000002	0.0000002
AMINO ACIDS			
L-Alanine	0.09	0.09	0.09
L-Arginine (free base)	0.05	0.05	0.05
L-Asparagine • H_2O	0.02	0.02	0.02
L-Aspartic Acid	0.03	0.03	0.03
L-Cysteine (free acid)	0.04	0.04	0.04
L-Cystine	0.02	0.02	0.02
L-Glutamic Acid	0.0445	0.0445	0.0445
L-Glutamine	—	0.292	—
Glycine	0.05	0.05	0.05
L-Histidine (free base)	0.015	0.015	0.015
L-Isoleucine	0.05	0.05	0.05
L-Leucine	0.075	0.075	0.075
L-Lysine • HCl	0.08746	0.08746	0.08746
L-Methionine	0.015	0.015	0.015
L-Phenylalanine	0.025	0.025	0.025
L-Proline	0.03	0.03	0.03
L-Serine	0.01	0.01	0.01
L-Threonine	0.04	0.04	0.04
L-Tryptophan	0.01	0.01	0.01
L-Tyrosine • 2Na • $2H_2O$	0.05045	0.05045	0.05045
L-Valine	0.05	0.05	0.05
VITAMINS			
Ascorbic Acid • Na	0.00227	0.00227	0.00227
D-Biotin	0.0005	0.0005	0.0005
Calciferol	0.0001	0.0001	0.0001
Choline Chloride	0.0015	0.0015	0.0015
Folic Acid	0.001	0.001	0.001
myo-Inositol	0.002	0.002	0.002
Menadione (sodium bisulfite)	0.00001	0.00001	0.00001
Niacinamide	0.001	0.001	0.001
D-Pantothenic Acid (hemicalcium)	0.001	0.001	0.001
Pyridoxal • HCl	0.001	0.001	0.001
Retinol Acetate	0.0001	0.0001	0.0001
Riboflavin	0.0001	0.0001	0.0001
Thiamine • HCl	0.001	0.001	0.001
DL-α-Tocopherol Phosphate • Na	0.00001	0.00001	0.00001
Vitamin B_{12}	0.002	0.0002	0.0002

Williams' Medium E

Component	W1878 [1×] g/L	W4125 g/L	W4128 [1×] g/L
OTHER			
D-Glucose	2	2	2
Glutathione (reduced)	0.00005	0.00005	0.00005
Methyl Linoleate	0.00003	0.00003	0.00003
Phenol Red • Na	—	0.0107	0.0107
Pyruvic Acid • Na	0.025	0.025	0.025
ADD			
L-Glutamine	0.292	—	0.292
Sodium Bicarbonate	—	2.2	—

Powdered Media Preparation Instructions

Preparation of Media for Filtration

Powdered media and salt mixtures are extremely hygroscopic and should be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. Preparing medium in concentrated form is not recommended as precipitates may form. Supplements can be added prior to filtration or introduced aseptically to sterile medium. The nature of the supplement may affect the storage conditions and shelf life of the medium.

Procedure

- Measure out 90% of final volume of tissue culture grade water. Water should be at room temperature.
- While gently stirring the water, add the powdered medium or salt mixture. Stir until dissolved. Do not heat water.
- Rinse original package with a small amount of water to remove all traces of powder. Add to solution.
- To the solution, add the required amount of sodium bicarbonate from the chart below for each liter of final volume of medium being prepared. Stir until dissolved.
- While stirring, adjust the pH to 0.1-0.3 pH units below the desired pH since the pH may rise during vacuum filtration. The use of 1 N HCl or 1 N NaOH is recommended.
- Bring medium to final volume with tissue culture grade water.
- Sterilize immediately by filtration using a membrane with a porosity of 0.2 micrometers or less.
- Aseptically dispense into sterile containers. Store liquid medium refrigerated at 2-8 °C and in the dark.
- Osmolality will vary with the addition of sodium or potassium chloride, approximately 10 mOsm for 300 mg/L NaCl or 400 mg/L of KCl

For more specific information regarding powdered media or salt mixtures, see the product insert that accompanies each package.

10× Liquid Medium Concentrates

Since certain components in 10× medium concentrates precipitate at pH 7.0, it is necessary for Sigma to adjust the pH of these solutions to maintain solubility. Therefore, it may be necessary for the user to adjust the pH of the 1× preparation with sterile 1 N NaOH or 1 N HCl.

Procedure For Dilution to 1× (1 Liter)

(**NOTE**: Dilution of 10× solutions should be performed with sterile containers, components, and equipment.)

- Aseptically measure out approximately 850 ml of tissue culture grade water into an appropriate size container.
- While gently stirring the water, add 100 ml of 10× medium.
- To the solution, add the required amount of sodium bicarbonate (see table below).
- Supplements (e.g., L-glutamine, antibiotics, sera) may be added aseptically to the solution. The nature of the supplement will affect the storage conditions and the shelf life of the medium.
- While stirring, adjust the solution to desired pH with 1 N NaOH or 1 N HCl.
- Bring medium to final volume with additional tissue culture grade water.
- Aseptically dispense into sterile containers. Store refrigerated at 2-8 °C.

Sodium Bicarbonate and L-Glutamine Addition Table

The following are recommended sodium bicarbonate and L-Glutamine concentrations for 1x (single strength) liquid media using 7.5% Sodium Bicarbonate Solution (Cat. No. S8761) and 200 mM L-Glutamine Solution (Cat. No. G7513) or L-Glutamine powder (Cat. No. G6392).

10× Liquid Medium	Cat. No.	$NaHCO_3$ 7.5% Solution (mL/L)	L-Glutamine 200 mM (mL/L)	L-Glutamine Powder (g/L)
M199 HBSS	**M9163**	4.7	3.4	0.1
MEM HBSS	**M9288**	4.7	10	0.292
MEM EBSS	**M0275**	29.3	10	0.292
M199 EBSS	**M0650**	29.3	3.4	0.1
DPBS	**D1283**	—	—	—
DPBS w/o Ca^{2+} and Mg^{2+}	**D1408**	—	—	—
EBSS	**E7510**	29.3	—	—
HBSS	**H1641**	84.7	—	—
HBSS w/o Ca^{2+} and Mg^{2+}	**H4641**	84.7	—	—

(—) = not applicable

Sodium Bicarbonate Addition Table

The following are recommended sodium bicarbonate concentrations for 1x (single strength) powdered media using 7.5% Sodium Bicarbonate ($NaHCO_3$) Solution (Cat. No. S8761) or Cell Culture Tested Sodium Bicarbonate Powder (Cat. No. S5761):

Powdered Media	Cat. No.	$NaHCO_3$ 7.5% Solution (mL/L)	$NaHCO_3$ Powder (g/L)
Ames' Medium	**A1420**	25.7	1.932
BME EBSS	**B9638**	29.3	2.2
DME/F-12 w/o phenol red	**D2906**	16	1.2
DME/F-12	**D9785**	16	1.2
DME/F-12	**D8900**	16	1.2
DME/F-12	**D0547**	16	1.2
DME HEPES	**D1152**	49.3	3.7
DME w/o phenol red	**D2902**	49.3	3.7
DME Deficient	**D5030**	49.3	3.7
DME	**D5523**	49.3	3.7
DME	**D5648**	49.3	3.7
DME	**D7777**	49.3	3.7
F-12 (Coon's Modification)	**F6636**	35.7	2.676
Glasgow MEM	**G6148**	36.7	2.75
HBSS w/o phenol red	**H1387**	84.7	0.35
HBSS	**H2387**	84.7	0.35
HBSS w/o phenol red	**H4891**	84.7	0.35
HBSS	**H6136**	84.7	0.35
IMDM	**I7633**	40.3	3.024
KREBS-HENSELEIT	**K3753**	28	2.1
KREBS-RINGER	**K4002**	16.8	1.26
McCOY'S 5A	**M4892**	29.3	2.2
MCDB-131	**M8537**	15.7	1.176
MCDB-153	**M7403**	15.7	1.176
M199 EBSS HEPES	**M2520**	29.3	2.2
M199 EBSS w/o phenol red	**M3769**	29.3	2.2
M199 EBSS	**M5017**	29.3	2.2

Classical Media Formulations: Sodium Bicarbonate Addition Table

Powdered Media	Cat. No.	$NaHCO_3$ 7.5% Solution (mL/L)	$NaHCO_3$ Powder (g/L)
MEM EBSS	M0268	29.3	2.2
MEM JOKLIK	M0518	26.7	2
MEM EBSS NEAA	M0643	29.3	2.2
MEM Alpha	M0644	29.3	2.2
MEM EBSS AUTO-MOD™	M0769	29.3	2.2
MEM Alpha	M0894	29.3	2.2
MEM HBSS NEAA	M1018	84.7	0.35
MEM EBSS HEPES	M2645	29.3	2.2
MEM EBSS NEAA, w/o phenol red	M3024	29.3	2.2
MEM HBSS	M4642	84.7	0.35
NCTC 135	N3262	29.3	2.2
Nutrient Mixture F-10	N6635	16	1.2
Nutrient Mixture F-12	N6760	15.7	1.176
Nutrient Mixture F-12K	N3520	33.3	2.5
RPMI-1640 Deficient	R1383	26.7	2
RPMI-1640 HEPES	R4130	26.7	2
RPMI-1640	R6504	26.7	2
RPMI-1640 w/o phenol red	R8755	26.7	2
Tyrode's Salts	T2145	13.3	1
Waymouth MB752/1	W1625	29.9	2.24
Williams' Medium E	W4125	29.3	2.2

Preparation of Auto-Mod™ Powdered Media

Cat. Nos. M0769 and R7755

Sigma's Auto-Mod™ powdered media are specially formulated to withstand the temperatures and conditions required for autoclaving. The same recommendations as with all powdered media for protection against atmospheric moisture and the preparation of concentrated media apply to Auto-Mod™ media.

Procedure

- Medium supplements can be added on the basis of their thermostability, i.e., heat stable supplements can be added prior to autoclaving and heat labile supplements must be added aseptically after autoclaving. The storage conditions and shelf life of the supplemented medium will be determined by the type of supplement added.
- Measure out 90% of final volume of tissue culture grade water.
- While gently stirring the water, add the powdered Auto-Mod™ medium. Stir until dissolved.
- Rinse original package with a small amount of water to remove all traces of powder. Add to solution.
- The pH for RPMI-1640, (Cat. No. R7755), should be adjusted to 4.0 before autoclaving.
- Add final volume of tissue culture grade water. Since sodium bicarbonate and L-glutamine solutions will be added after autoclaving, subtract these volumes from the final volume before adding to medium.
- Autoclave medium at 121 °C (250 °F) at 15 psi for 15 minutes.
  Note: Autoclaves vary in performance. Validation of each system is recommended.
- Medium should be promptly removed from the autoclave to avoid extended heating and evaporation.
- After cooling medium to 15-20 °C, add required amounts of sterile Sodium Bicarbonate Solution, 7.5% w/v (Cat. No. S8761) and of L-Glutamine, using either a sterile 200 mM solution (Cat. No. G7513) or γ-irradiated L-glutamine (Cat. No. G6392) for each liter of final volume of medium being prepared. See the following table for appropriate amounts.
- If necessary, adjust the pH using sterile 1 N NaOH or 1 N HCl.
- Store liquid medium refrigerated at 2-8 °C and in the dark.

Sodium Bicarbonate and L-Glutamine Addition Table

Recommended sodium bicarbonate and L-glutamine concentrations for 1× (single strength) Auto-Mod™ powdered media using 7.5% Sodium Bicarbonate (NaHCO3) Solution, (Cat. No. S8761), and 200 mM sterile L-Glutamine Solution, (Cat. No. G7513):

Auto-Mod™ Medium	Cat. No.	$NaHCO_3$ 7.5% Solution (mL/L)	L-Glutamine 200 mM (mL/L)	L-Glutamine γ-Irradiated (g/L)
MEM	M0769	29.3	10	0.292
RPMI-1640	R7755	26.7	10.25	0.3

Stabilized Glutamine - AQmedia™ Formulations

L-Glutamine is an essential amino acid that is required by virtually all mammalian cells in culture. Unfortunately however, unlike other amino acids L-glutamine is labile in solution, and will spontaneously break down in cell culture media to form ammonia and pyrrolidone carboxylic acid [PCA]. The rate at which this breakdown occurs is temperature and pH dependent, and is detailed in the L-Glutamine Stability Study that can be found at the end of the Amino Acids section of this manual.

Because of this breakdown, researchers will typically supplement their cultures with L-glutamine periodically to ensure sufficient glutamine is available for their growing cells. However, this supplementation brings risks, including the possibility of culture contamination as well as the development of potentially toxic levels of ammonia in long-term cultures.

To address these problems, we now offer a line of AQmedia classical media, which contain the stable glutamine dipeptide alanyl-glutamine (Ala-Gln). Ala-Gln provides a stable source of L-glutamine for cells, eliminating the need to supplement cultures with glutamine and reducing the potential buildup of ammonia in culture.

AQMedia

Dulbecco's Modified Eagle's Medium - high glucose NEW

DME; DMEM

AQmedia™, With 4500 mg/L glucose, L-alanyl-glutamine, and sodium bicarbonate, without sodium pyruvate., liquid, sterile-filtered, suitable for cell culture

This DMEM-Hi glucose medium differs from the original formula, because it is supplemented with L-alanyl-L-glutamine dipeptide. Glutamine is notoriously unstable in cell culture. This dipeptide provides a more stable form of glutamine for use in cell cultures. It also differs from the original DMEM-Hi formulation wherein pyridoxine is substituted for pyridoxal. Pyridoxal is an unstable component of media.

endotoxin ..... tested

ship: ambient store at: 2-8°C

D0819-500ML 500 mL

Minimum Essential Medium Eagle

NEW

MEM

AQmedia™, With Earle's salts, L-alanyl-glutamine, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

This product lacks L-Ala; L-Asn; L-Glu; Gly; L-Pro; L-Ser and L-Gln. It is supplemented with L-Ala-L-Gln dipeptide. This provides a more stable form of glutamine for cell culture. Free amino acid L-glutamine is known to be unstable in cell culture.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M0446-500ML	500 mL

RPMI-1640 Medium

NEW

AQmedia™, With L-alanyl-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

This RPMI-1640 medium is supplemented with L-Ala-L-Gln dipeptide. This provides a more stable form of glutamine for cell culture. Free amino acid L-glutamine is known to be unstable in cell culture.

endotoxin ..... tested
ship: ambient store at: 2-8°C

R2405-500ML	500 mL
R2405-6X500ML	6 × 500 mL
R2405-24X500ML	24 × 500 mL

MegaCell™ Fortified Media

MegaCell™ is a line of versatile media, formulated to significantly reduce the amount of serum required for cultivating mammalian cells

MegaCell™ Dulbecco's Modified Eagle's Medium

liquid, sterile-filtered, suitable for cell culture

Reduces FBS or other serum usage from 10% to 3%.

Supplement with 4 mM L-glutamine (20 ml of 200 mM solution, G7513)

Sigma-Aldrich uses the MegaCell trademark pursuant to an agreement with Promega Corporation.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M3942-500ML	500 mL

MegaCell™ Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham

without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Reduces FBS or other serum usage from 10% to 3%.

Supplement with 4 mM L-glutamine (20 ml of 200 mM solution, G7513)

Sigma-Aldrich uses the MegaCell trademark pursuant to an agreement with Promega Corporation.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M4192-500ML	500 mL

MegaCell™ RPMI-1640 Medium

without L-glutamine, liquid, sterile-filtered, suitable for cell culture

Reduces FBS or other serum usage from 10% to 3%.

Supplement with 4 mM L-glutamine (20 ml of 200 mM solution, G7513)

Sigma-Aldrich uses the MegaCell trademark pursuant to an agreement with Promega Corporation.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M3817-500ML	500 mL

Sera

Sera

Sera

Collection, handling, processing and storing are conducted in a manner to protect and maintain the quality of the serum. All sera are tested as described and a certificate of analysis for each lot is available upon request.

- **Source:** Sera are derived from clotted whole blood or plasma, from donor source or abattoir.
- **Collection method:** Aseptically collected via cardiac puncture or venipuncture, or by open collection.
- **Filtration:** All serum is processed through filters down to at least 0.2 µm pore size. FBS products are filtered through 0.1 µm filters.
- **Sterility:** All sera are tested for the absence of aerobic and anaerobic bacteria, molds, and fungi according to the methods of sampling and testing described in the current United States Pharmacopeia Code of Federal Regulations (9CFR).
- **Mycoplasma:** All sera are tested for mycoplasma by the large batch method (Barile) for 28 days. Positive controls are inoculated and grown in parallel.
- **Bacteriophage:** All sera are screened by plaque assay using sensitive strains of E. coli known to be good hosts for bacteriophages. Testing includes positive controls using bacteriophages.
- **Endotoxin:** All sera are tested to determine levels of endotoxin. Testing is done by Gel-Clot LAL method or the Quantitative Chromogenic LAL method. The endotoxin standard used in both methods is standardized against USP Reference Endotoxin Standard EC-5.
- **Hemoglobin:** Serum hemoglobin concentration is determined by a spectrophotometric assay using Trizma buffer at 125 mM.
- **Electrophoretic identity:** Examined for characteristic protein patterns on an agarose gel in Trizma-Barbital buffer at pH 8.8.
- **Cell culture testing:** Biological performance on all sera except human is assessed using Sigma® cell culture medium supplemented with the serum and tested at a final concentration of 10%. Testing is performed using an MTT test method which compares absorbance on day 4 of a growth period against the absorbance of a satisfactory serum control.
- **Cloning efficiency assay:** A cloning assay is performed on many FBS products. The assay is performed at a concentration of 10% to verify that each lot will support clonal growth of myeloma cells and/or fusion products.
- **Total protein:** Determined by Biuret.
- **SMA-24:** Routinely performed on all sera.
- **Chemical analysis:** Routinely performed on all sera.
- **Osmolality:** Determined by freeze depression.
- **Hybridoma testing:** Hybridoma-tested sera have been shown to support mouse myeloma cells and hybridoma cells in culture.
- **Heat inactivation:** Heat-inactivated sera have been heated at 56°C for 30 minutes.
- **Gamma-Irradiation:** Gamma-irradiated sera have been exposed to 2.5-3.5 mRads (25-35 kGy) of radiation from a ^{60}Co source.
- **Dialyzed sera:** Serum is dialyzed against 0.15 M NaCl. Dialysis is done using a 10,000 molecular weight cut-off cartridge.
- **Usage:** Products are labeled for research use only, except where indicated in individual listings.

Bovine Sera

Virus testing: All US-origin sera are tested for the absence of the indicated viruses using the protocols stated in the Code of Federal Regulations for ingredients of animal origin, 9CFR. All tests are conducted on cultures that are grown for a minimum of 21 days in medium supplemented with 15% test serum. They are subcultured at least twice during this period. Positive control cultures that have been inoculated with virus are tested in parallel. Cytopathic observations, fluorescent antibody staining, inclusion body staining, and hemadsorption must result in non-detection in test results.

Viruses:
Blue tongue [BT]
Bovine Adenovirus - Type I, Type V
Bovine Parvovirus [BPV]
Bovine respiratory syncytial virus [BRSV]
Bovine viral diarrhea [BVD]
Infectious bovine rhinotracheitis [IBR]
Parainfluenza type 3 [PI3]
Rabies
Reovirus

Tetracycline screening: Item F2442 and F6178, Fetal Bovine Serum US Origin, are tetracycline screened. This assay is a service that is available for our other sera products for a nominal fee.

Note: The information on this page applies to the majority of our sera products. For further testing details regarding specific products, please review the tables found on the following pages of this section.

Human Sera

Human Serum Testing: Before pooling, each individual donor unit is tested and found negative for Hepatitis B [HBsAg], Hepatitis C [Anti-HCV], and Human Immunodeficiency Virus [HIV] antibodies by ELISA. Final product is tested and found negative for HBsAg, Hepatitis A, and HIV. Biological performance is assessed using cell culture medium supplemented with the serum and tested at a final concentration of 10%. All human sera are tested for 7 days in culture and observed microscopically for normal morphology and cell growth. All virus testing is conducted by outside clinical laboratories that are licensed under the Clinical Laboratory Improvement Act (CLIA). **Nevertheless, products of human origin should be considered potentially infectious and handled accordingly.**

Equine Sera

Equine Serum Testing: All grades of horse sera are tested for EIA (equine infectious anemia) by the Coggins test. This is an immunodiffusion test that detects antibodies to EIA virus. All donor herd horse serum is collected from a controlled herd. These animals are routinely screened for glanders, dourine, pyroplasmosis, *Brucella abortus*, and EIA. Testing for all animal pathogens is conducted in a diagnostic laboratory approved by the United States Department of Agriculture (USDA) Veterinary Services.

Shipping

Sigma ships serum products:
- In insulated containers
- Packed in dry ice
- Via air courier as applicable
- Freight charges and dry ice charges appear as a separate line item on your invoice.

Shipping these products under these conditions maintains sera in a frozen state, which ensures maximum quality. Contact us if you want to have your order shipped without dry ice.

Storage

- If the entire bottle will not be used within three to four weeks after thawing, appropriate aliquots should be prepared using sterile techniques and sterile, pyrogen-free containers. Convenient 100 ml package sizes are available.
- Serum should be stored at -20 °C. DO NOT STORE SERUM IN A FROST-FREE FREEZER, since temperature cycling may cause bottles to crack and may contribute to deterioration of product. We also recommend avoiding multiple freeze-thaws because it may cause denaturization of proteins.

Fetal Bovine Sera (FBS)

Fetal Bovine Sera - USA Origin

Fetal Bovine Serum

FBS

Endotoxin and hemoglobin tested

USA origin, sterile-filtered, suitable for cell culture, suitable for hybridoma

composition
Hemoglobin ≤20 mg/dL

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F2442-100ML	100 mL
F2442-500ML	500 mL
F2442-6X500ML	6 × 500 mL
F2442-24X500ML	24 × 500 mL

USA origin, sterile-filtered, suitable for cell culture

FBS sourced in the United States is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

composition
Hemoglobin ≤25 mg/dL

endotoxin ..... ≤25 EU/mL
ship: dry ice store at: –20°C

F6178-50ML	50 mL
F6178-100ML	100 mL
F6178-500ML	500 mL

USA origin, Heat Inactivated, sterile-filtered, suitable for cell culture, suitable for insect cell culture

FBS sourced in the United States is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

composition
Hemoglobin ≤20 mg/dL

Prepared by heating at 56°C for 30 minutes.

This product is prepared by heating at 56°C for 30 minutes. Heating serum to 56°C for 30 minutes inactivates the complement system for immunoassays. Heat activation also inactivates other undetermined inhibitors of cell growth in culture.

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F4135-100ML	100 mL
F4135-500ML	500 mL
F4135-6X500ML	6 × 500 mL
F4135-24X500ML	24 × 500 mL

USA origin, γ-irradiated by SER-TAIN® process, sterile-filtered, suitable for cell culture

FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

Gamma irradiation by SER-TAIN® process (25-35kGy).

(Formerly product number F3885)

ship: dry ice store at: –20°C

12107C-500ML	500 mL
12107C-1000ML	1000 mL

USA origin, Dialyzed by ultrafiltration against 0.15 M NaCl, sterile-filtered, suitable for cell culture

FBS sourced in the United States is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

composition
Hemoglobin ≤20 mg/dL

10,000 Molecular Weight Cut-off

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F0392-100ML	100 mL
F0392-500ML	500 mL

USA origin, Charcoal Stripped, sterile-filtered, suitable for cell culture

Charcoal Stripped Fetal Bovine Serum (FBS) is available for researchers requiring decreased levels of various hormones in their studies. Dextran treated Charcoal is used to selectively remove hormones without nonspecific loss of other serum components. The effectiveness of hormone removal is assessed on the basis of reductions in the levels of T3, T4, and Progesterone pre and post treatment.

This product is designed for researchers involved in studying processes influenced by steroid hormones such as estrogen stimulation and the obesity process and other areas that benefit from reduced hormone levels such as certain types of viral infections.

Fetal Bovine Serum *(continued)*

FBS sourced in the United States is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture. Charcoal stripped FBS is often used instead of FCS to minimize the level of androgen and other hormones provided in 10% serum supplemented media.

composition
Hemoglobin ≤20 mg/dL

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F6765-100ML	100 mL
F6765-500ML	500 mL

Fetal Bovine Sera - Non-USA Origin

Fetal Bovine Serum

FBS

Endotoxin and hemoglobin tested

non-USA origin, sterile-filtered, suitable for cell culture

FBS is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

FBS remains a popular media supplement because it provides a wide array of functions in cell culture. FBS delivers nutrients, growth and attachment factors and protects cells from oxidative damage and apoptosis by mechanisms that are difficult to reproduce in serum-free media (SFM) systems.

suitable for

composition
Hemoglobin ≤20 mg/dL

Not available in USA or Canada. Please check with your local supplier regarding availability and import restrictions in your country.

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F7524-50ML	50 mL
F7524-100ML	100 mL
F7524-500ML	500 mL

Heat Inactivated, non-USA origin, sterile-filtered, suitable for cell culture

FBS is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

suitable for

composition
Hemoglobin ≤20 mg/dL

Prepared by heating at 56°C for 30 minutes. Heating serum to 56°C for 30 minutes is used to inactivate the complement system for immunoassays. Heat inactivation also inactivates other undetermined inhibitors of cell growth in culture.

Not available in USA or Canada. Please check with your local supplier regarding availability and import restrictions in your country.

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F9665-50ML	50 mL
F9665-100ML	100 mL
F9665-500ML	500 mL

Canada origin, sterile-filtered, suitable for cell culture

FBS sourced from Canada is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

composition
Hemoglobin ≤20 mg/dL

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F1051-100ML	100 mL
F1051-500ML	500 mL
F1051-6X500ML	6 × 500 mL

USDA approved, sterile-filtered, suitable for cell culture

FBS sourced from USDA approved non-USA countries is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

USDA Approved Sourcing refers to FBS produced from blood collected in countries outside of the United States that have been approved by the USDA (United States Department of Agriculture) for export of beef products into the United States. Eligible countries for export into the U.S. include Australia, Canada, Chile, Costa Rica, Honduras, Iceland, Japan, Mexico, New Zealand, Nicaragua, Uruguay. Foreign serum is tested to ensure the absence of exotic viruses before release by the USDA.

composition
Hemoglobin ≤25 mg/dL

endotoxin ..... ≤10 EU/mL
ship: dry ice store at: –20°C

F0926-50ML	50 mL
F0926-100ML	100 mL
F0926-500ML	500 mL

non-USA origin, from USDA approved countries, Heat Inactivated, sterile-filtered, suitable for cell culture

FBS sourced from USDA approved non-USA countries is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

USDA Approved Sourcing refers to FBS produced from blood collected in countries outside of the United States that have been approved by the USDA (United States Department of Agriculture) for export of beef products into the United States. Eligible countries for export into the U.S. include Australia, Canada, Chile, Costa Rica, Honduras, Iceland, Japan, Mexico, New Zealand, Nicaragua, Uruguay. Foreign serum is tested to ensure the absence of exotic viruses before release by the USDA.

composition
Hemoglobin ≤10 EU/mL

endotoxin ..... ≤25 mg/dL
ship: ambient store at: –20°C

12306C-50ML	50 mL
12306C-100ML	100 mL
12306C-500ML	500 mL

Australia origin, USDA approved, sterile-filtered, suitable for cell culture, suitable for hybridoma

FBS sourced from Australia is use in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture. Australia carries the best possible rating in relation to Geographical Bovine Spongiform Encephalopathy (BSE) Risk (GBR) as designated by the Scientific Steering Committee on the Geographical Risk of Bovine Spongiform Encephalopathy. The classification for Australia is GBR Level I.

composition

Hemoglobin ≤20 mg/dL

available only in Japan

endotoxin ..... ≤10 EU/mL

ship: dry ice store at: –20°C

F9423-100ML	100 mL
F9423-500ML	500 mL

Australia origin, USDA approved, sterile-filtered, suitable for cell culture

FBS sourced in Australia is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

composition

Hemoglobin ≤25 mg/dL

endotoxin ..... ≤10 EU/mL

ship: dry ice store at: –20°C

12003C-100ML	100 mL
12003C-500ML	500 mL
12003C-1000ML	1000 mL

Australia origin, γ-irradiated, sterile-filtered, suitable for cell culture NEW

FBS sourced from Australia is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture. Australia carries the best possible rating in relation to Geographical Bovine Spongiform Encephalopathy (BSE) Risk (GBR) as designated by the Scientific Steering Committee on the Geographical Risk of Bovine Spongiform Encephalopathy. The classification for Australia is GBR Level I.

Gamma irradiation by SER-TAIN® process (25-35kGy).

ship: dry ice store at: –20°C

12007C-100ML	100 mL
12007C-500ML	500 mL
12007C-1000ML	1000 mL

New Zealand origin, sterile-filtered, suitable for cell culture

FBS sourced from New Zealand is used in a broad range of cell culture applications. FBS provides many non-defined growth promoting and survival enhancing factor to cells in culture.

ship: dry ice store at: –20°C

12203C-50ML	50 mL
12203C-100ML	100 mL
12203C-500ML	500 mL
12203C-1000ML	1000 mL

Product Testing for Fetal Bovine Sera (USA Origin Products)

	F2442	F6178	F4135	F0392	12107C	F6765
Source	bovine	bovine	bovine	bovine	bovine	bovine
Country	USA	USA	USA	USA	USA	USA
Sterility	✓	✓	✓	✓	✓	✓
Performance	✓	✓	✓	✓	✓	✓
Cloning Assay	✓	✓	✓	✓	✓	✓
Virus (raw material)	✓	✓	✓	✓	✓	✓
Mycoplasma	✓	✓	✓	✓	✓	✓
Bacteriophage	✓	✓	✓	✓	✓	✓
Endotoxin (EU/mL)	≤10	≤25	≤10	≤10	≤10	≤10
Hemoglobin (mg%)	≤20	≤25	≤20	≤20	≤20	≤20
Total Protein (g%)	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.3	3.0 – 4.5
Electrophorectic Pattern	✓	✓	✓	✓	✓	✓
IgG	✓	✓	✓	✓	✓	✓
Hormone Testing	✓	✓	✓	✓	✓	✓
pH at RT	6.7 – 8.0	6.7 – 8.0	6.7 – 8.0	6.7 – 8.0	6.8 – 8.1	6.7 – 8.0
Osmolality (mOsm/Kg H_2O)	260 – 340	260 – 340	260 – 340	260 – 340	280 – 330	260 – 340
Chemical Analysis	✓	✓	✓	✓	✓	✓
Tetracyline	✓	✓	•	•	•	✓

✓ – Indicates testing is performed and product meets specification.
• – Test is not performed.
[a] – Available in Europe only. Please check with your local supplier regarding availability.
[b] – Available in Canada only.

Product Testing for Fetal Bovine Sera (Non-USA Origin Products)

	F7524[a]	F9665[a]	F1051[b]	F9423[c]	12003C	12007C	12203C	12306C	F0926
Source	bovine	bovine	bovine	bovine	bovine	bovine	bovine	bovine	bovine
Country	Not of USA Origin	Not of USA Origin	Canada	Australia	Australia	Australia	New Zealand	USDA Approved	USDA Approved
Sterility	✓	✓	✓	✓	✓	✓	✓	✓	✓
Performance	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cloning Assay	✓	✓	✓	✓	✓	✓	✓	✓	✓
Virus (raw material)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mycoplasma	✓	✓	✓	✓	✓	✓	✓	✓	✓
Bacteriophage	•	•	•	✓	✓	✓	✓	•	•
Endotoxin (EU/mL)	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10
Hemoglobin (mg%)	≤20	≤20	≤20	≤20	≤25	≤25	≤25	≤25	≤25
Total Protein (g%)	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5	3.0 – 4.5
Electrophoretic Pattern	✓	✓	✓	✓	✓	✓	✓	✓	✓
IgG	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hormone Testing	•	•	✓	✓	•	•	•	•	•
pH at RT	6.7 – 8.0	6.7 – 8.0	6.7 – 8.0	6.7 – 8.0	6.8 – 8.1	6.8 – 8.1	6.8 – 8.1	6.8 – 8.1	6.8 – 8.1
Osmolality (mOsm/Kg H_2O)	260 – 340	260 – 340	260 – 340	260 – 340	260 – 330	260 – 330	260 – 330	260 – 330	260 – 330
Chemical Analysis	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tetracycline	•	•	•	•	•	•	•	•	✓

✓ – Indicates testing is performed and product meets specification.
• – Test is not performed.
[a] – Available in Europe only. Please check with your local supplier regarding availability.
[b] – Available in Canada only.
[c] – Available in Japan only.

Newborn Calf, Iron Supplemented Calf and Adult Bovine Sera

Adult Bovine Sera

Bovine Serum, Adult

USA Origin, sterile-filtered, suitable for cell culture

Adult bovine serum may be used for the cultivation of Helicobacter pylori and in various low-serum media for diploid cell culture.

ship: dry ice store at: –20°C

B9433-100ML	100 mL
B9433-500ML	500 mL
B9433-6X500ML	6 × 500 mL

Calf Serum

Endotoxin tested

from formula-fed bovine calves, iron supplemented, USA origin, sterile-filtered, suitable for cell culture

Iron-supplemented bovine serum may be used as an alternative to fetal bovine serum in the CHO/HGPRT mutation assay, Oberly, et. al. Development of a more rapid, reduced serum culture system for Caco-2 monolayers and application to the biopharmaceutics

Each lot is supplemented to a constant iron concentration.

ship: dry ice store at: –20°C

C8056-100ML	100 mL
C8056-500ML	500 mL
C8056-6X500ML	6 × 500 mL

Newborn Calf Serum

Endotoxin tested

USA origin, sterile-filtered, suitable for cell culture

Newborn calf serum (NCS) is collected within 10 days post partum. NCS is useful in a wide range of cell culture and organ preservation applications. For example, it is used in cornea preservation and cryopreservation of bovine embryos. NCS is used in cell culture to support the maturation of oocytes; to study the function of tubular epithelial cells; and to develop primary cultures of cells such as visceral adipocytes.

Collected from calves that are 10 days old or less.

ship: dry ice store at: –20°C

N4637-100ML	100 mL
N4637-500ML	500 mL
N4637-6X500ML	6 × 500 mL

USA origin, Heat Inactivated, sterile-filtered, suitable for cell culture

NCS is useful in a wide range of cell culture and organ preservation applications. For example, it is used in cornea preservation and cryopreservation of bovine embryos. NCS is used in cell culture to support the maturation of oocytes; to study the function of tubular epithelial cells; and to develop primary cultures of cells such as visceral adipocytes.

Collected from calves that are 10 days old or less.
Prepared by heating at 56°C for 30 minutes.

ship: dry ice store at: –20°C

N4762-100ML	100 mL
N4762-500ML	500 mL
N4762-6X500ML	6 × 500 mL

USA origin, γ-irradiated, sterile-filtered, suitable for cell culture

Newborn calf serum (NCS) is collected within 10 days post partum calves. NCS is useful in a wide range of cell culture and organ preservation applications. For example, it is used in cornea preservation and cryopreservation of bovine embryos. NCS is used in cell culture to support the maturation of oocytes; to study the function of tubular epithelial cells; and to develop primary cultures of cells such as visceral adipocytes.

Collected from calves that are 10 days old or less.
Exposed to 25-35 kGy of irradiation.

ship: dry ice store at: –20°C

N4887-100ML	100 mL
N4887-500ML	500 mL
N4887-6X500ML	6 × 500 mL

Product Testing for Newborn Calf and Adult Bovine Serum

	N4637	N4762	N4887	C8056	12133C	B9433
Source	bovine	bovine	bovine	bovine	bovine	bovine
Country	USA	USA	USA	USA	USA	USA
Sterility	✓	✓	✓	✓	✓	✓
Performance	✓	✓	✓	✓	✓	✓
Virus (raw material)	✓	✓	✓	✓	✓	✓
Mycoplasma	✓	✓	✓	✓	✓	✓
Endotoxin (EU/mL)	≤100	≤100	≤100	≤100	≤10	≤150
Hemoglobin (mg%)	≤20	≤20	≤20	≤20	≤35	≤30
Total Protein (g%)	4.5 – 6.0	4.5 – 6.0	4.5 – 6.0	5.0 – 8.0	✓	5.0 – 9.5
Electrophorectic Pattern	✓	✓	✓	✓	✓	✓
pH at RT	7.0 – 8.0	7.0 – 8.0	7.0 – 8.0	7.0 – 8.0	7.0 – 8.1	7.0 – 8.0
Osmolality (mOsm/Kg H_2O)	240 – 340	240 – 340	240 – 340	240 – 340	260 – 330	260 – 340

✓ – Indicates testing is performed and product meets specification.

Other Sera

Alternative Sera

Chicken Serum

USA origin, sterile-filtered, cell culture tested

Chicken serum is used in parasitology to study excystation, growth, survival and egg production of the microphallid trematode Maritrema novaezealandensis. Chicken serum is used for the serial cultivation of chicken keratinocytes.

Endotoxin tested

ship: dry ice store at: –20°C

C5405-100ML	100 mL
C5405-500ML	500 mL
C5405-6X500ML	6 × 500 mL

Goat Serum Donor Herd

USA origin, sterile-filtered, suitable for cell culture

Goat serum is a suitable alternative to fetal bovine serum in a wide range of cell culture applications such as the isolation and identification of viruses produced in a variety of primary cell lines.

Collected from a controlled donor herd.

Endotoxin tested

ship: dry ice store at: –20°C

G6767-100ML	100 mL
G6767-500ML	500 mL
G6767-6X500ML	6 × 500 mL

Porcine Serum

USA origin, sterile-filtered, suitable for cell culture

Pig serum is used for the culture of procine islet cells.

Collected from adult animals.

Endotoxin tested

ship: dry ice store at: –20°C

P9783-500ML	500 mL
P9783-6X500ML	6 × 500 mL

Rabbit Serum

Serum from rabbit

USA origin, sterile-filtered, suitable for cell culture

Rabbit serum is used to support cultures of Borrelia burgdorferi, the causative agent of Lyme disease.

Rabbit Serum *(continued)*

Endotoxin tested
ship: dry ice store at: –20°C

R4505-20ML	20 mL
R4505-100ML	100 mL
R4505-500ML	500 mL
R4505-6X500ML	6 × 500 mL

USA origin, sterile-filtered, Suitable for *Borrelia* culture

Selected for ability to support growth of *Borrelia burgdorferi* in BSK-H medium (B3528). Recommended for use at 6 ml/100 mL in B3528.

Endotoxin tested.
ship: dry ice store at: –20°C

R7136-30ML	30 mL
R7136-60ML	60 mL

Sheep Serum

USA origin, sterile-filtered, suitable for cell culture

Sheep serum is useful for the propagation of long-term cultures of erythrocytic stages of the Theileria uilenbergi intracellular protozoan parasite.

Collected from adult animals.

Endotoxin tested
ship: dry ice store at: –20°C

S2263-100ML	100 mL
S2263-500ML	500 mL
S2263-6X500ML	6 × 500 mL

Product Testing for Alternative Sera

	C5405	G6767	P9783	R4505	R7136	S2263
Country	USA	USA	USA	USA	USA	USA
Sterility	✓	✓	✓	✓	✓	✓
Performance	✓	✓	✓	✓	✓	✓
Virus (raw material)	✓	✓	✓	•	•	✓
Mycoplasma	✓	✓	✓	✓	✓	✓
Endotoxin (EU/mL)	≤50	≤20	≤20	report result	≤100	≤100
Hemoglobin (mg%)	≤60	≤20	≤50	report result	report result	≤50
Total Protein (g%)	2.0 – 4.3	6.0 – 9.0	7.0 – 9.5	5.0 – 7.0	5.0 – 7.0	6.0 – 7.0
Electrophorectic Pattern	✓	✓	✓	✓	✓	✓
pH at RT	7.0 – 8.2	7.0 – 8.0	7.2 – 8.0	7.0 – 8.0	7.0 – 8.0	7.0 – 8.5
Osmolality (mOsm/Kg H_2O)	285 – 340	260 – 340	280 – 345	260 – 340	260 – 340	260 – 340

✓ – Indicates testing is performed and product meets specification.
• – Test is not performed.

Horse Sera

Horse Serum

Donor herd, USA origin, sterile-filtered, suitable for cell culture, suitable for hybridoma
ship: dry ice store at: –20°C

H1270-100ML	100 mL
H1270-500ML	500 mL
H1270-6X500ML	6 × 500 mL

Donor Herd, USA origin, Heat inactivated, sterile-filtered, suitable for cell culture
ship: dry ice store at: –20°C

H1138-100ML	100 mL
H1138-500ML	500 mL
H1138-6X500ML	6 × 500 mL

USA origin, Donor Herd, From platelet-poor plasma, sterile-filtered, suitable for cell culture
ship: dry ice store at: –20°C

P5552-500ML	500 mL
P5552-6X500ML	6 × 500 mL

Product Testing for Horse Sera

	H1270	H1138	P5552
Source	donor herd	donor herd	donor herd
Origin of Source	USA	USA	USA
Sterility	✓	✓	✓
Virus (raw material)	✓	✓	✓
Mycoplasma	✓	✓	✓
Endotoxin (EU/mL)	≤10	≤10	≤10
Hemoglobin (mg%)	≤20	≤20	≤20
Total Protein (g%)	6.0 – 7.5	6.0 – 7.5	5.0 – 6.5
pH at RT	7.0 – 8.2	7.0 – 8.2	7.0 – 8.2
Osmolality (mOsm/Kg H_2O)	260 – 340	260 – 340	260 – 340

✓ – Indicates testing is performed and product meets specification.

Human Sera

Human Serum

Endotoxin tested

from human male AB plasma, sterile-filtered

Human AB serum is used in tissue engineering, transplantation and cell therapy applications for the expansion of mesenchymal stem cells (MSC) from adipose tissue or mesenchymal stromal cells from human bone marrow; for standardized limbal epithelial stem cell graft generation and transplantation; for ex vivo expansion of NK cells from peripheral blood in Hematopoeitic Stem Cell Expansion Medium; and for upgrading pretransplant human islet culture technology.

Each donor is tested for and found non-reactive for Hepatitis B & C and non-reactive for Human Immunodeficiency Virus (HIV) antibody by ELISA. Nevertheless, products of human origin should be considered potentially infectious and handled accordingly.

ship: dry ice store at: −20°C

H4522-20ML	20 mL
H4522-100ML	100 mL

from platelet poor human plasma, sterile-filtered, total impurities (mycoplasma tested, virus tested)

Human serum from platelet poor plasma (PPP) is useful for studies of the effects of platelet lysates on the growth of cells such as articular chondrocytes and human skin fibroblasts. It has also been used in platelet derived growth factor (PDGF) purification assays.

ship: dry ice store at: −20°C

P2918-20ML	20 mL
P2918-100ML	100 mL

(from male AB clotted whole blood), sterile-filtered NEW

Human AB serum is used in tissue engineering, transplantation and cell therapy applications for the expansion of mesenchymal stem cells (MSC) from adipose tissue or mesenchymal stromal cells from human bone marrow; for standardized limbal epithelial stem cell graft generation and transplantation; for ex vivo expansion of NK cells from peripheral blood in Hematopoeitic Stem Cell Expansion Medium; and for upgrading pretransplant human islet culture technology.

Each donor is tested for and found non-reactive for Hepatitis B & C and non-reactive for Human Immunodeficiency Virus (HIV) antibody by ELISA. Nevertheless, products of human origin should be considered potentially infectious and handled accordingly.

ship: ambient store at: room temp

H6914-20ML	20 mL
H6914-100ML	100 mL

Product Testing for Human Sera

	H4522	H6914	P2918
Source	male/plasma	male/plasma	donor/plasma
Sterility	✓	✓	✓
Virus (raw material)	✓	✓	✓
Mycoplasma	✓	✓	✓
Endotoxin (EU/mL)	≤10	≤10	✓
Total Protein (g%)	4.0 – 9.0	4.0 – 9.0	4.0 – 6.5
pH at RT	7.0 – 9.0	7.0 – 8.0	7.0 – 9.0
Osmolality (mOsm/Kg H_2O)	260 – 340	260 – 340	260 – 340
Hemoglobin (mg%)	≤20	≤20	•

✓ – Indicates testing is performed and product meets specification.
• – Test is not performed.

Serum Replacements

Serum Replacement 1 (50×)

liquid, sterile-filtered, suitable for cell culture

Defined, multi-purpose serum replacement useful in studying the effects of growth factors and the production of proteins. Contains highly purified, heat-treated bovine serum albumin, heat-treated bovine transferrin and bovine insulin. Does not contain growth factors, steroid hormones, glucocorticoids, cell adhesion factors, detectable Ig and mitogens. May be used with anchorage-dependent and suspension cells.

Stable for 30 days when diluted in cell culture medium.

endotoxin ..... tested
ship: ambient store at: 2-8°C

S0638-10ML	10 mL
S0638-100ML	100 mL

Serum Replacement 2 (50×)

liquid, sterile-filtered, suitable for cell culture

Defined, multi-purpose serum replacement with additional components for cells requiring higher concentrations of serum than 10%. Contains highly purified, heat-treated bovine serum albumin, heat-treated bovine transferrin and bovine insulin. Does not contain growth factors, steroid hormones, glucocorticoids, cell adhesion factors, detectable Ig and mitogens. May be used with anchorage-dependent and suspension cells.

Stable for 30 days when diluted in cell culture medium.

endotoxin ..... tested
ship: ambient store at: 2-8°C

S9388-10ML	10 mL
S9388-100ML	100 mL

Serum Replacement 3 (50×)

liquid, sterile-filtered, suitable for cell culture

Defined serum replacement designed primarily for the long-term growth of human cells or other mammalian cells. Recommended for use in the production of cell-secreted proteins. Contains only human proteins (i.e., human serum albumin, human transferrin, human recombinant insulin). Does not contain growth factors, steroid hormones, glucocorticoids, cell adhesion factors, detectable Ig and mitogens. May be used with anchorage-dependent and suspension cells.

Stable for 30 days when diluted in cell culture medium.

This product is tested and found non-reactive for Hepatitis B Surface Antigen (HBsAg) and non-reactive for Human Immunodeficiency Virus (HIV) antibody by ELISA. Nevertheless, products of human origin should be considered potentially infectious and handled accordingly.

endotoxin ..... tested
ship: ambient store at: 2-8°C

S2640-10ML	10 mL
S2640-100ML	100 mL

Serum Replacements

These concentrated serum replacements are defined, multi-purpose serum replacements designed to eliminate the use of serum in culture. They are effective culturing anchorage dependent and suspension cells.

Serum Replacement 1
Useful for the study of the effects of growth factors or the production of biologicals (viruses, enzymes, monoclonal antibodies).

Serum Replacement 2
Contains additional components necessary for cells requiring higher concentrations of serum. It does not contain steroid hormones (i.e. estrogen or testosterone) or growth factors such as EGF, FGF, or TGF.

Serum Replacement 3

Designed primarily for the growth of human cells and the production of proteins. It contains only human proteins (i.e. human serum albumin and transferrin). The product is tested for and found non-reactive for Hepatitis B Surface Antigen (HbsAG), and non- reactive for Human Immunodeficiency Virus (HIV) antibody by ELISA. Product should still be considered potentially infectious and handled accordingly.

Product Testing for Serum Replacements

	S0638	S9388	S2640
Virus	•	•	✓
Sterility	✓	✓	✓
Mycoplasma	✓	✓	✓
Endotoxin (EU/mL)	≤0.5 (50×)	≤0.5 (50×)	≤0.5 (50×)
Hemoglobin (mg%)	•	•	•
Total Protein (g%)	•	•	•
pH at RT	6.9 – 7.2	6.9 – 7.2	6.9 – 7.2
Osmolality (mOsm/Kg H_2O)	280 – 340	295 – 350	240 – 340

✓ – Indicates testing is performed and product meets specification.
• – Test is not performed.

Balanced Salt Mixtures

Balanced Salt Mixtures

Balanced Salts

The use of a balanced salt solution (BSS) in cell and tissue culture is generally attributed to early workers in the field. In 1885, Sydney Ringer developed a solution of inorganic salts designed to maintain contractility of mammalian heart tissue. A less specific salt solution was designed by Tyrode for use in work with primary mammalian cells. Tyrode's salt solution became the accepted fluid for diluting protein components of media of natural origin. Since that time, many other balanced salt solutions have been developed for use in cell and tissue culture. The current role of a balanced salt solution in cell culture is multifaceted and can be divided into four principal functions: serves as an irrigating, transporting and diluting fluid while maintaining intra- and extracellular osmotic balance; provides cells with water and certain bulk inorganic ions essential for normal cell metabolism; combined with a carbohydrate, such as glucose, provides the principal energy source for cell metabolism; and provides a buffering system to maintain the medium within the physiological pH range (7.2-7.6).

References

1. Dulbecco, R. and Vogt, M., Plaque formation and isolation of pure lines with Poliomyelitis viruses. *J. Exp. Med.*, **106,** 167-169, 1957.
2. Earle, W., Production of malignancy *in vitro*. IV. The mouse fibroblast cultures and changes seen in the living cells. *J.N.C.I.*, **4,** 165-169, 1943.
3 Eagle, H., Amino acid metabolism in mammalian cell cultures. *Science*, **130,** 432-437, 1959.
4. Hanks, J., Hanks' balanced salt solution and pH control. *Tissue Culture Association Manual*, 3, 3, 1976.

Balanced Salt Mixtures and Solutions

Dulbecco's Phosphate Buffered Saline

Dulbecco's Phosphate Buffered Saline

DPBS

With $MgCl_2$ and $CaCl_2$, liquid, sterile-filtered, suitable for cell culture

D-PBS is used to wash cells during preparation and serial transfer.

endotoxin ..... tested
ship: ambient store at: 2-8°C

D8662-100ML	100 mL
D8662-500ML	500 mL
D8662-6X500ML	6 × 500 mL
D8662-24X500ML	24 × 500 mL
D8662-1L	1 L
D8662-6X1L	6 × 1 L

Without calcium chloride, powder, suitable for cell culture

Use this formulation of DPBS when tissue or cell dissociation or release is the objective. Also, suitable for studies that involve calcium metabolism.

Formulated to contain 9.6 grams of powder per liter of medium.

ship: ambient store at: 2-8°C

D5773-10X1L	10 × 1 L
D5773-10L	10 L
D5773-50L	50 L

Modified, with 36 mg sodium pyruvate, 50 mg streptomycin sulfate, 100 mg kanamycin monosulfate, 1000 mg glucose/L and $CaCl_2$, liquid, sterile-filtered, suitable for cell culture

Contains 50 mg/L streptomycin sulfate and 100 mg/L kanamycin monosulfate.

Fully supplemented isotonic solution. Contains energy sources and antibiotics. Frequently used with primary cells where risk of contamination may be high.

endotoxin ..... tested
ship: ambient store at: 2-8°C

D4031-100ML	100 mL
D4031-500ML	500 mL
D4031-6X500ML	6 × 500 mL
D4031-1L	1 L
D4031-6X1L	6 × 1 L

Modified, without calcium chloride and magnesium chloride, powder, suitable for cell culture

D-PBS is used to wash cells during preparation and serial transfer.

Formulated to contain 9.6 grams of powder per liter of medium.

This D-PBS is formulated without Ca or Mg. Formulations with Ca and Mg added are also available

ship: ambient store at: 2-8°C

D5652-10X1L	10 × 1 L
D5652-2X5L	2 × 5 L
D5652-10L	10 L
D5652-50L	50 L

With calcium chloride and magnesium chloride, 10×, liquid, sterile-filtered, suitable for cell culture

A general use, isotonic saline solution for washing cells and tissues.

ship: ambient store at: room temp

D1283-500ML	500 mL
D1283-6X500ML	6 × 500 mL

Modified, without calcium chloride and magnesium chloride, 10×, liquid, sterile-filtered, suitable for cell culture

Dulbecco's Phosphate Buffered Saline 10X is a stock solution used to prepare 1X D-PBS in cell culture grade water (W3500). D-PBS is used to wash cells during preparation and serial transfer.

endotoxin ..... tested
ship: ambient store at: room temp

D1408-100ML	100 mL
D1408-500ML	500 mL
D1408-6X500ML	6 × 500 mL
D1408-24X500ML	24 × 500 mL

Modified, without calcium chloride and magnesium chloride, liquid, sterile-filtered, suitable for cell culture

DPBS is a balanced salt solution (BSS) used for the handling and culturing of mammalian cells. DPBS is used to to irrigate, wash, and dilute mammalian cells. Phosphate buffering maintains the pH in the physiological range. Calcium and magnesium facilitate cell binding and clumping. DPBS without these ions can be used to wash and rinse suspended cells.

endotoxin ..... tested
ship: ambient store at: room temp

D8537-100ML	100 mL
D8537-500ML	500 mL
D8537-6X500ML	6 × 500 mL
D8537-24X500ML	24 × 500 mL
D8537-1L	1 L
D8537-6X1L	6 × 1 L

dry powder, DPBS Modified, without calcium, without magnesium, suitable for cell culture NEW

for research or for further manufacturing use

Formulated to contain 9.6 grams of powder per liter of medium.

ship: ambient store at: room temp

56064C-10L	10 L
56064C-50L	50 L
56064C-100L	100 L

liquid, sterile-filtered, DPBS Modified, without calcium, without magnesium, suitable for cell culture NEW

for research or for further manufacturing use

ship: ambient store at: room temp

59321C-1000ML	1000 mL

liquid, sterile-filtered, DPBS Modified 10X, without calcium, without magnesium, suitable for cell culture NEW

for research or for further manufacturing use

ship: ambient store at: room temp

59331C-1000ML	1000 mL

Earle's Balance Salts

Earle's Balanced Salts

EBSS

endotoxin ..... tested

With sodium bicarbonate, without phenol red, liquid, sterile-filtered, suitable for cell culture

Phenol red has been shown to interfere with the growth of some cells at cloning densities. Use this medium when working with stem cells or when growing cells at low densities.

ship: ambient store at: room temp

E3024-500ML	500 mL
E3024-6X500ML	6 × 500 mL

With sodium bicarbonate, without calcium chloride and magnesium sulfate, liquid, sterile-filtered, suitable for cell culture

Recommended for suspension cultures or whenever cell clumping is a problem.

ship: ambient store at: room temp

E6267-500ML	500 mL
E6267-6X500ML	6 × 500 mL

With sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

ship: ambient store at: room temp

E2888-100ML	100 mL
E2888-500ML	500 mL
E2888-6X500ML	6 × 500 mL

Earle's Balanced Salt Solution 10x

Earle's Balanced Salts

EBSS
Without sodium bicarbonate, 10 ×, liquid, sterile-filtered, suitable for cell culture

Supplement with 2.2 g/L sodium bicarbonate at 1×.

endotoxin ..... tested
ship: ambient store at: room temp

E7510-100ML	100 mL
E7510-500ML	500 mL
E7510-6X500ML	6 × 500 mL

Hank's Balanced Salts

Hanks' Balanced Salt solution

Modified, with sodium bicarbonate, without phenol red, calcium chloride and magnesium sulfate, liquid, sterile-filtered, suitable for cell culture

Calcium and magnesium support cell adhesion. Use to minimize cell attachment or aggregation.

Phenol red has been shown to interfere with the growth of some cells at cloning densities. Use this medium when working with stem cells or when growing cells at low densities.

endotoxin ..... tested
ship: ambient store at: room temp

H6648-100ML	100 mL
H6648-500ML	500 mL
H6648-6X500ML	6 × 500 mL
H6648-1L	1 L
H6648-6X1L	6 × 1 L

Modified, with sodium bicarbonate, without phenol red, liquid, sterile-filtered, suitable for cell culture

Phenol red has been shown to interfere with the growth of some cells at cloning densities. Use this medium when working with stem cells or when growing cells at low densities.

endotoxin ..... tested
ship: ambient store at: room temp

H8264-100ML	100 mL
H8264-500ML	500 mL
H8264-6X500ML	6 × 500 mL
H8264-1L	1 L
H8264-6X1L	6 × 1 L

Hanks' Balanced Salt *(continued)*

With sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: room temp

H9269-100ML	100 mL
H9269-500ML	500 mL
H9269-6X500ML	6 × 500 mL
H9269-1L	1 L
H9269-6X1L	6 × 1 L

Modified, with sodium bicarbonate, without calcium chloride and magnesium sulfate, liquid, sterile-filtered, suitable for cell culture

Recommended for suspension cultures or whenever cell clumping is a problem.

endotoxin ..... tested
ship: ambient store at: room temp

H9394-100ML	100 mL
H9394-500ML	500 mL
H9394-6X500ML	6 × 500 mL
H9394-24X500ML	24 × 500 mL
H9394-1L	1 L
H9394-6X1L	6 × 1 L

HBSS Modified, with phenol red, without calcium, without magnesium, liquid NEW

Hanks' Balanced Salt Solution (HBSS) is designed for use with cells maintained in non-CO_2 atmospheric conditions. For cell dissociation procedures, use HBSS Modified, which is formulated without calcium and magnesium salts.

for research or for further manufacturing use
ship: ambient store at: room temp

55021C-1000ML	1000 mL

Hanks' Balanced Salts

Modified, without phenol red and sodium bicarbonate, powder, suitable for cell culture

Use this medium when working with stem cells or when growing cells at low densities.

Formulated to contain 9.7 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.

The pH is adjusted after addition of sodium bicarbonate. Investigators who work with stem cells or cell clones frequently prefer media without phenol red.

ship: ambient store at: 2-8°C

H1387-10X1L	10 × 1 L
H1387-10L	10 L

Modified, without calcium chloride, magnesium sulfate and sodium bicarbonate, powder, suitable for cell culture

The pH is adjusted after addition of sodium bicarbonate. Calcium and magnesium support cell adhesion. Recommended for suspension cultures or whenever cell clumping is a problem.

Formulated to contain 9.5 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.

The pH is adjusted after addition of sodium bicarbonate.

ship: ambient store at: 2-8°C

H2387-10X1L	10 × 1 L

Modified, without calcium chloride, magnesium sulfate, phenol red and sodium bicarbonate, powder, suitable for cell culture

Use this medium when working with stem cells or when growing cells at low densities. Use to minimize cell attachment or aggregation.

Formulated to contain 9.5 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.

The pH is adjusted after addition of sodium bicarbonate. Investigators who work with stem cells or cell clones frequently prefer media without phenol red.

ship: ambient store at: 2-8°C

H4891-10X1L	10 × 1 L
H4891-50L	50 L

Without phenol red and sodium bicarbonate, liquid, sterile-filtered

For use in Mycoplasma Stain Kit.

The pH is adjusted after addition of sodium bicarbonate. Phenol red has been shown to interfere with the growth of some cells at cloning densities. Use this medium when working with stem cells or when growing cells at low densities.

ship: ambient store at: room temp

H5899-35ML	35 mL

Without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 9.8 grams of powder per liter of medium.

Supplement with 0.35 g/L sodium bicarbonate.

The pH is adjusted after addition of sodium bicarbonate.

ship: ambient store at: 2-8°C

H6136-10X1L	10 × 1 L

Hanks' Balanced Salt Solution 10x

Hanks' Balanced Salt solution

Without calcium chloride, magnesium sulfate and sodium bicarbonate, 10 ×, liquid, sterile-filtered, suitable for cell culture

Calcium and magnesium support cell adhesion. Recommended for suspension cultures or whenever cell clumping is a problem.

Supplement with 0.35 g/L sodium bicarbonate at 1X.

ship: ambient store at: room temp

H4641-100ML	100 mL
H4641-500ML	500 mL
H4641-6X500ML	6 × 500 mL

Without sodium bicarbonate, 10 ×, liquid, sterile-filtered, suitable for cell culture

Supplement with 0.35 g/L sodium bicarbonate at 1×.

endotoxin ..... tested
ship: ambient store at: room temp

H1641-500ML	500 mL
H1641-6X500ML	6 × 500 mL

Other Salt Mixtures

Alsever's Solution

liquid, sterile-filtered, suitable for cell culture

An isotonic, balanced salt solution. It is routinely used as an anti-coagulant/blood preservative, which permits the storage of whole blood at refrigerator temperatures for approximately 10 weeks.

endotoxin ..... tested
ship: ambient store at: room temp

A3551-100ML	100 mL
A3551-500ML	500 mL
A3551-6X500ML	6 × 500 mL
A3551-1L	1 L
A3551-6X1L	6 × 1 L

Gey's Balanced Salt Solution

GBSS
liquid, sterile-filtered, suitable for cell culture

Gey's Balanced Salt Solution is a phosphate bicarbonate buffered cell suspension and washing solution. A complete balanced salt solution with a unique combination of ""salt forms"". Contains the same five basic ions as EBSS, HBSS and DPBS.

endotoxin ..... tested
ship: ambient store at: 2-8°C

G9779-500ML	500 mL
G9779-6X500ML	6 × 500 mL

Krebs-Henseleit Buffer Modified

With 2000 mg/L glucose, without calcium chloride and sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 9.6 grams of powder per liter of medium.

Supplement with 2.1 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

K3753-10X1L	10 × 1 L
K3753-10L	10 L

Krebs-Ringer Bicarbonate Buffer

With 1800 mg/L glucose, without calcium chloride and sodium bicarbonate, powder, suitable for cell culture

Bicarbonate buffered isotonic saline containing magnesium, potassium, sodium and phosphate ions.

Formulated to contain 9.5 grams of powder per liter of medium.

Supplement with 1.26 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

K4002-10X1L	10 × 1 L

Tyrode's Salts

Tyrode's Salts

Complete and ready to use formulation of Tyrode's salt solution for cell washing.

With sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: room temp

T2397-100ML	100 mL
T2397-500ML	500 mL
T2397-6X500ML	6 × 500 mL
T2397-1L	1 L
T2397-6X1L	6 × 1 L

Without sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 9.6 grams of powder per liter of medium.

Supplement with 1.0 g/L sodium bicarbonate.

ship: ambient store at: 2-8°C

T2145-10X1L	10 × 1 L
T2145-10L	10 L

Tyrode's Solution, Acidic

liquid, sterile-filtered, suitable for mouse embryo

For removal of the zona pellucida.

endotoxin ..... tested
pH ..... 2.5±0.3
ship: dry ice store at: –20°C

T1788-100ML	100 mL

Balanced Salts Formulation Tables

Alsever's Solution

Component	A3551 [1×] g/L
INORGANIC SALTS	
Citric Acid • 3Na • $2H_2O$	8
Citric Acid • H_2O	0.55
Sodium Chloride	4.2
OTHER	
D-Glucose	20.5

Dulbecco's Phosphate Buffered Saline (DPBS)

Component	D1283 [10×] g/L	D1408 [10×] g/L	D8537 [1×] g/L	D8662 [1×] g/L	D5652 g/L
INORGANIC SALTS					
Calcium Chloride	1.0	—	—	0.1	—
Magnesium Chloride • $6H_2O$	1	—	—	0.1	—
Potassium Chloride	2	2	0.2	0.2	0.2
Potasium Phosphate Monobasic (anhydrous)	2	2	0.2	0.2	0.2
Sodium Chloride	80	80	8	8	8
Sodium Phosphate Monobasic (anhydrous)	11.5	11.5	1.15	1.15	1.15

Dulbecco's Phosphate Buffered Saline (DPBS) Modified

Component	D4031 [1×] g/L	D5773 g/L
INORGANIC SALTS		
Calcium Chloride	0.1	—
Magnesium Chloride • $6H_2O$	0.1	0.1
Potassium Chloride	0.2	0.2
Potasium Phosphate Monobasic (anhydrous)	0.2	0.2
Sodium Chloride	8	8
Sodium Phosphate Monobasic (anhydrous)	1.15	1.15
OTHER		
D-Glucose	1	—
Kanamycin Sulfate	0.1	—
Pyruvic Acid • Na	0.036	—
Streptomycin Sulfate	0.05	—

Earle's Balanced Salt Solution (EBSS)

Component	E2888 [1×] g/L	E6267 [1×] g/L	E7510 [10×] g/L	E3024 [1×] g/L
INORGANIC SALTS				
Calcium Chloride	0.2	—	2.0	0.2
Magnesium Sulfate (anhydrous)	0.09767	—	0.09767	0.09767
Potassium Chloride	0.4	0.4	4	0.4
Sodium Bicarbonate	2.2	2.2	—	2.2
Sodium Chloride	6.8	6.8	68	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122	0.122	1.22	0.122
OTHER				
D-Glucose	1	1	10	1
Phenol Red • Na	0.011	0.011	0.11	—
ADD				
Sodium Bicarbonate	—	—	2.2 at 1×	—

Hanks' Balanced Salts (HBSS) Modified

Component	H1387 g/L	H1641 [10×] g/L	H2387 g/L	H4641 [10×] g/L	H4891 g/L
INORGANIC SALTS					
Calcium Chloride	0.1396	1.396	—	—	—
Magnesium Sulfate (anhydrous)	0.09767	0.9767	—	—	—
Potassium Chloride	0.4	4	0.4	4	0.4
Potasium Phosphate Monobasic (anhydrous)	0.06	0.6	0.06	0.6	0.06
Sodium Chloride	8	80	8	80	8
Sodium Phosphate Dibasic (anhydrous)	0.04788	0.4788	0.04788	0.4788	0.04788
OTHER					
D-Glucose	1	10	1	10	1
Phenol Red • Na	—	0.11	0.011	0.11	—
ADD					
Sodium Bicarbonate	0.35	0.35 at 1×	0.35	0.35 at 1×	0.35

Hanks' Balanced Salts (HBSS) Modified

Component	H6136 g/L	H6648 [1×] g/L	H8264 [1×] g/L	H9269 [1×] g/L	H9394 [1×] g/L
INORGANIC SALTS					
Calcium Chloride	0.1396	—	0.1396	0.1396	—
Magnesium Sulfate (anhydrous)	0.09767	—	0.09767	0.09767	—
Potassium Chloride	0.4	0.4	0.4	0.4	0.4
Potasium Phosphate Monobasic (anhydrous)	0.06	0.06	0.06	0.06	0.06
Sodium Bicarbonate	—	0.35	0.35	0.35	0.35
Sodium Chloride	8	8	8	8	8
Sodium Phosphate Dibasic (anhydrous)	0.04788	0.4788	0.04788	0.04788	0.04788
OTHER					
D-Glucose	1	1	1	1	1
Phenol Red • Na	0.011	—	—	0.011	0.011
ADD					
Sodium Bicarbonate	0.35	—	—	—	—

Krebs-Henseleit Buffer

Component	K3753 g/L
INORGANIC SALTS	
Magnesium Sulfate (anhydrous)	0.141
Potassium Chloride	0.35
Potasium Phosphate Monobasic (anhydrous)	0.16
Sodium Chloride	6.9
OTHER	
D-Glucose	2
ADD	
Sodium Bicarbonate	2.1

Krebs-Ringer Bicarbonate Buffer

Component	K4002 g/L
INORGANIC SALTS	
Magnesium Chloride (anhydrous)	0.0468
Potassium Chloride	0.34
Sodium Chloride	7
Sodium Phosphate Dibasic (anhydrous)	0.1
Sodium Phosphate Monobasic (anhydrous)	0.18
OTHER	
D-Glucose	1.8
ADD	
Sodium Bicarbonate	1.26

Tyrode's Salts

Component	T2145 g/L	T2397 [1×] g/L
INORGANIC SALTS		
Calcium Chloride	0.2	0.2
Magnesium Chloride • $6H_2O$	0.214	0.214
Potassium Chloride	0.2	0.2
Sodium Bicarbonate	—	1
Sodium Chloride	8	8
Sodium Phosphate Monobasic (anhydrous)	0.05	0.05
OTHER		
D-Glucose	1	1
ADD		
Sodium Bicarbonate	1	—

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Reagents and Supplements

Reagents and Supplements

Cell Culture Tested Reagents and Supplements

The Cell Culture and Insect Cell Culture Tested reagents and supplements listed in this section differ from Sigma's research grade compounds in that they undergo additional testing in a cell culture system. This testing is designed to eliminate the need for screening biochemicals prior to use in a cell culture application.

Biological Performance Protocol

Sigma's reagents and supplements are evaluated for their ability to promote or maintain cell growth while demonstrating no cytotoxic effects.

Biochemicals that are used as medium components are added to a basal medium at a concentration consistent with their biochemical activity or nutritional importance. The medium is supplemented with 10% fetal bovine serum, and growth performance is assessed using appropriate cell lines. Biochemicals that are not medium components are application tested. The cell lines chosen represent a cross section of cells with a broad spectrum of nutritional requirements.

The cells are counted and growth is plotted as a logarithmic function of time in culture. Doubling time and final cell densities are determined. Cultures are examined microscopically for any morphological abnormalities that may indicate cytotoxicity from the biochemical being tested. All growth data must be consistent with expected values for the cell lines used in the test system.

Biochemicals labeled "Insect Cell Culture Tested" are tested in a basal medium at a concentration consistent with their biochemical activity or nutritional importance. The medium is supplemented with 10% heat-inactivated fetal bovine serum (Catalog Number F3018). Growth performance is assessed using appropriate insect cell lines.

Albumins and Transport Proteins

Albumin

Albumin from bovine serum

Bovine albumin; BSA
[9048-46-8]

mol wt ~66 kDa

lyophilized powder, BioReagent, suitable for cell culture, ≥96% (agarose gel electrophoresis)

Bovine serum albumin is broadly used as an additive to cell culture media, especially serum-free media. It provides a range of benefits including protection from oxidative damage and stabilization of other media components such as fatty acids and pyridoxal.

BSA is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates. At pH 5-7 it contains 17 intrachain disulfide bridges and 1 sulfhydryl group.

Often referred to as Cohn fraction V; this product is prepared by a modified method of the Cohn cold ethanol fractionation method.

solubility
H_2O ..... 40 mg/mL
ship: ambient store at: 2-8°C

A9418-5G	5 g
A9418-10G	10 g
A9418-50G	50 g
A9418-100G	100 g
A9418-500G	500 g

fatty acid free, low endotoxin, lyophilized powder, BioReagent, suitable for cell culture, ≥96% (agarose gel electrophoresis)

Often referred to as Cohn fraction V; this product is prepared by a modified method of the Cohn cold ethanol fractionation method.

endotoxin ..... ≤0.1 ng/mg
fatty acid ..... ≤0.02% (GC)
ship: ambient store at: 2-8°C

A8806-1G	1 g
A8806-5G	5 g

low endotoxin, lyophilized powder, BioReagent, suitable for cell culture

Prepared using heat shock fractionation

endotoxin ..... ≤1.0 EU/mg
ship: ambient store at: 2-8°C

A4919-1G	1 g
A4919-5G	5 g
A4919-25G	25 g
A4919-100G	100 g

lyophilized powder, essentially IgG-free, low endotoxin, BioReagent, suitable for cell culture

≥97% (agarose gel electrophoresis)

New Zealand origin

Prepared by salt fractionation, ion exchange, and gel filtration chromatography.

endotoxin ..... ≤1.0 EU/mg
IgG ..... ≤0.05% (immunodetection)
ship: ambient store at: 2-8°C

A2058-1G	1 g
A2058-5G	5 g
A2058-25G	25 g

lyophilized powder, low endotoxin, BioReagent, suitable for cell culture, ≥98% (agarose gel electrophoresis)

New Zealand origin

Prepared by salt fractionation, ion exchange, and gel filtration chromatography.

endotoxin ..... ≤1.0 EU/mg

ship: ambient store at: 2-8°C

A1933-1G	1 g
A1933-5G	5 g
A1933-25G	25 g

lyophilized powder, essentially globulin free, BioReagent, suitable for cell culture

Purified by a combination of heat shock and ethanol fractionation

ship: ambient store at: 2-8°C

A4161-250MG	250 mg
A4161-1G	1 g
A4161-5G	5 g
A4161-10G	10 g

lyophilized powder, γ-irradiated, Globulin Free, BioXtra, suitable for cell culture

Qualified for use in cell culture applications.

Purified by a combination of heat shock and ethanol fractionation

ship: ambient store at: 2-8°C

A3156-5G	5 g

low endotoxin, lyophilized powder, Cohn Analog™, BioReagent, suitable for cell culture, ≥98% (agarose gel electrophoresis)

Recommended as a growth-enhancing supplement for cell culture.

Manufactured by a novel process that maintains the lipid and growth factor characteristics of cold-ethanol precipitated albumins.

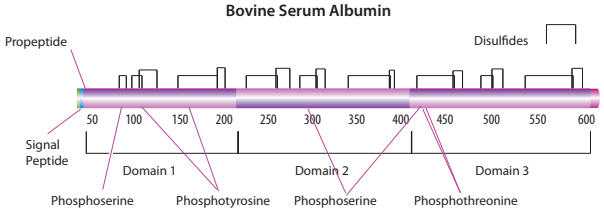
ship: ambient store at: 2-8°C

A1470-10G	10 g
A1470-25G	25 g
A1470-100G	100 g
A1470-5KG	5 kg
A1470-10KG	10 kg

Albumin solution from bovine serum

BSA solution

[9048-46-8]



35% in DPBS, sterile-filtered, BioXtra, suitable for cell culture

endotoxin tested

ship: ambient store at: 2-8°C

A7979-50ML	50 mL
A7979-12X50ML	12 × 50 mL

30% in DPBS, sterile-filtered, BioXtra, suitable for cell culture

Prepared from A9418 in DPBS (D5652).

endotoxin ..... tested

ship: ambient store at: 2-8°C

A9576-50ML	50 mL

7.5% in DPBS, sterile-filtered, BioXtra, suitable for cell culture

Qualified for use in cell culture applications.

low endotoxin

ship: ambient store at: 2-8°C

A8412-100ML	100 mL

10% in DPBS, sterile-filtered, BioReagent, suitable for cell culture

Recommended for use as a base for the preparation of defined, fatty acid supplements. Can be loaded with up to 4 moles of fatty acids per mole of albumin.

Prepared with fatty acid-free albumin in DPBS (D 5652).

endotoxin ..... tested

ship: ambient store at: 2-8°C

A1595-50ML	50 mL

Conalbumin from chicken egg white

Ovotransferrin

[1391-06-6]

BioReagent, suitable for cell culture

Conablumin (aka ovotransferrin) is a glycoprotein derived from egg white with a sequence similar to serum transferrin, but with a different glycosylation pattern. It is a strong iron binder with antiviral and antibacterial activities.

Fe ..... ≤0.02%

ship: ambient store at: −20°C

C7786-100MG	100 mg
C7786-1G	1 g
C7786-5G	5 g

Fetuin

Fetuin from fetal calf serum

[9014-81-7]

lyophilized powder, BioReagent, suitable for cell culture

A glycoprotein derived from FBS that is used as Fetuin has been used at a concentration of 500 mg/ml to supplement serum free F12 medium (along with insulin, transferrin and 2-mercaptoethanol) in culture of embryonal carcinoma cells.

Prepared by ammonium sulfate fractionation of fetal bovine serum by the method of Pederson, K.O., *J. Phys. and Colloid Chem.*, 51, 164 (1947).

solubility

H_2O ..... 1 mg/mL

free N-acetylneuraminic acid ..... ≤0.3%

ship: ambient store at: 2-8°C

F3385-100MG	100 mg
F3385-1G	1 g
F3385-5G	5 g
F3385-25G	25 g

γ-irradiated, BioXtra, suitable for cell culture

This product is cell culture tested (0.5 g/L) and is appropriate for use in cell culture applications.

ship: ambient store at: 2-8°C

F6131-250MG	250 mg

Transferrin and Transferrin Replacements

Iron supplement, chemically defined

▶ 1,000 ×, sterile-filtered, BioReagent, suitable for cell culture

Proprietary product designed to replace transferrin for iron transport in a broad range of cell culture systems. Animal-component free

Iron is an essential element for cell growth and survival; however it is toxic in free form. Free iron undergoes oxidation and reduction and it plays a central role in oxidation processes, such as the Fenton reaction, that damage cells and media components. This product is a more stable form of iron. Recommended for use in cell culture applications at 0.5 to 2 ml/L of medium.

composition
Iron 222-334 µg/mL

endotoxin ..... tested
ship: ambient store at: 2-8°C

I3153	

apo-Transferrin bovine

All cells require iron for the proper uptake of oxygen from their environment. Transferrin, a protein isolated from serum, causes the uptake and transport of iron from culture medium to the cells. This allows proper oxygen uptake and also stimulates growth-related enzyme activity. Iron-saturated transferrin (holo transferrin) is continually recycled after releasing iron and becomes iron-deficient transferrin (apo-transferrin). Choosing which form to use depends upon the culture conditions. In media such as Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F12 (DMEM/F12) that contain high levels of iron salt, using apo-transferrin is preferred.

▶ BioReagent, suitable for cell culture, ≥98%

solubility
H_2O ..... 50 mg/mL
iron ..... essentially free
ship: ambient store at: 2-8°C

T1428-50MG	50 mg
T1428-100MG	100 mg
T1428-500MG	500 mg
T1428-5G	5 g
T1428-10G	10 g

holo-Transferrin bovine

Siderophilin, iron-saturated; Siderophilin
[11096-37-0]

Purity by agarose gel electrophoresis.

▶ Iron-saturated, BioReagent, suitable for cell culture

97-100% (agarose gel electrophoresis)

powder
solubility
H_2O ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: 2-8°C

T1283-50MG	50 mg
T1283-100MG	100 mg
T1283-500MG	500 mg
T1283-1G	1 g

Amino Acids and Vitamins

Amino Acids

Amino Acid Quick Reference Table

Product	Cat. No.	Mol. Wt.	Storage Temperature	Solubilize or Reconstitute In	Testing Concentration (g/L)
L-Asparagine	A4159	132.1	RT	1N NaOH or 1N HCl	0.25
L-Asparagine • H_2O	A4284	150.1	RT	1N NaOH or 1N HCl	0.29
DL-Aspartic Acid	A4409	133.1	RT	1N NaOH or 1N HCl	0.07
L-Aspartic Acid	A4534	133.1	RT	1N NaOH or 1N HCl	0.07
L-Cystine	C7602	240.3	RT	1N NaOH	0.02
DL-Glutamic Acid	G5513	147.1	RT	1N HCl	0.15
L-Glutamic Acid	G5889	147.1	RT	H_2O	0.15
Hypoxanthine	H9636	136.1	RT	1N NaOH	0.025
L-Isoleucine	I7403	131.2	RT	H_2O	0.262
L-Leucine	L8912	131.2	RT	1N NaOH	0.39
L-Methionine	M5308	149.2	RT	1N NaOH	0.149
L-Phenylalanine	P5482	165.2	RT	1N NaOH	0.248
DL-Tryptophan	T7425	204.2	RT	0.5 N HCl	0.02
L-Tryptophan	T8941	204.2	RT	1N NaOH	0.04
L-Tyrosine	T2025/T8566	217.7	RT	1N NaOH and Heat	0.37
L-Valine	V0513	117.1	RT	1N HCl	0.234

RT = Room Temperature

Ala-Gln

Alanyl-glutamine; Glutamine-S
[39537-23-0] $C_8H_{15}N_3O_4$ FW 217.22

BioReagent, suitable for cell culture, suitable for insect cell culture

Alanyl-glutamine (Ala-Gln) is a dipeptide that is used as a substitute for glutamine in mammalian cell culture media. Glutamine is unstable in solution and forms ammonia which can have deleterious effects on cells in culture. Ala-Glu is stable to heat sterilization and is less ammoniagenic than glutamine, which contributes to its advantages as a media component.

This product is cell culture tested (0.5 mg/ml) and insect cell culture tested (0.2 mg/ml). It is appropriate for use in cell culture and insect cell culture applications at 2-10 mM.

ship: ambient store at: room temp

A8185-5G	5 g
A8185-100G	100 g
A8185-1KG	1 kg

200 mM, solution, sterile-filtered, Biotechnology Performance Certified

Contains the dipeptide L-Alanyl-L-glutamine. 200 mM supplied in 0.85% NaCl.

This supplement provides a stable form of L-glutamine while in solution.

endotoxin ..... tested
ship: ambient store at: 2-8°C

G8541-20ML	20 mL
G8541-100ML	100 mL

L-Alanine

(*S*)-2-Aminopropionic acid; L-α-Aminopropionic acid
[56-41-7] $C_3H_7NO_2$ FW 89.09

from non-animal source, meets EP, USP testing specifications, suitable for cell culture, ≥98.5%

powder
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

A7469-10MG	10 mg
A7469-25G	25 g
A7469-100G	100 g
A7469-1KG	1 kg

L-Arginine

(*S*)-2-Amino-5-guanidinopentanoic acid
[74-79-3] $H_2NC(=NH)NH(CH_2)_3CH(NH_2)CO_2H$ $C_6H_{14}N_4O_2$ FW 174.20

Substrate of nitric oxide synthase, which is converted to citrulline and nitric oxide (NO). Induces insulin release by a nitric oxide-dependent mechanism.

from non-animal source, meets EP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

A8094-10MG	10 mg
A8094-25G	25 g
A8094-100G	100 g
A8094-1KG	1 kg

L-Arginine monohydrochloride

S-(+)-2-Amino-5-[(aminoiminomethyl)amino]pentanoic acid monohydrochloride
[1119-34-2] $C_6H_{14}N_4O_2 \cdot HCl$ FW 210.66

Substrate of nitric oxide synthase, which is converted to citrulline and nitric oxide (NO). Induces insulin release by a nitric oxide-dependent mechanism.

not synthetic, meets EP, JP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

A6969-25G	25 g
A6969-100G	100 g
A6969-1KG	1 kg
A6969-25KG	25 kg

L-Asparagine

L-Aspartic acid 4-amide; (*S*)-2-Aminosuccinic acid 4-amide
[70-47-3] $H_2NCOCH_2CH(NH_2)CO_2H$ $C_4H_8N_2O_3$ FW 132.12

BioReagent, suitable for cell culture, suitable for insect cell culture

≥98% (TLC)
ship: ambient store at: room temp

A4159-25G	25 g
A4159-100G	100 g
A4159-500G	500 g

L-Asparagine monohydrate

(*S*)-2-Aminosuccinic acid 4-amide; L-Aspartic acid 4-amide; (*S*)-(+)-2-Amino-succinamic acid
[5794-13-8] $NH_2COCH_2CH(NH_2)COOH \cdot H_2O$ $C_4H_8N_2O_3 \cdot H_2O$ FW 150.13

BioReagent, suitable for cell culture

ship: ambient store at: room temp

A4284-25G	25 g
A4284-100G	100 g
A4284-1KG	1 kg

from non-animal source, BioReagent, suitable for cell culture, ≥98.0%

powder
solubility
H_2O ..... 20 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

A7094-25G	25 g
A7094-100G	100 g
A7094-1KG	1 kg

L-Aspartic acid

(*S*)-(+)-Aminosuccinic acid; (*S*)-Aminobutanedioic acid
[56-84-8] $HO_2CCH_2CH(NH_2)CO_2H$ $C_4H_7NO_4$ FW 133.10

Principal neurotransmitter for fast synaptic excitation.

▶ from non-animal source, meets EP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
1 M HCl ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

A7219-100G	100 g
A7219-1KG	1 kg

▶ BioReagent, suitable for cell culture, suitable for insect cell culture

≥98% (TLC)
ship: ambient store at: room temp

A4534-100G	100 g
A4534-500G	500 g

BME Amino Acids Solution 50×

▶ Without L-glutamine, sterile-filtered, BioReagent, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: 2-8°C

B6766-100ML	100 mL

L-Cysteine

(*R*)-2-Amino-3-mercaptopropionic acid
[52-90-4] $HSCH_2CH(NH_2)CO_2H$ $C_3H_7NO_2S$ FW 121.16

NMDA glutamatergic receptor agonist.

▶ from non-animal source, BioReagent, suitable for cell culture, ≥98%

solubility
H_2O ..... 25 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

C7352-10MG	10 mg
C7352-25G	25 g
C7352-100G	100 g
C7352-1KG	1 kg

L-Cysteine hydrochloride

[52-89-1] $HSCH_2CH(NH_2)COOH \cdot HCl$ $C_3H_7NO_2S \cdot HCl$ FW 157.62

NMDA glutamatergic receptor agonist that is also an agonist at AMPA glutamatergic receptors at high concentrations.

▶ anhydrous, from non-animal source, BioReagent, suitable for cell culture, ≥98.0%

solubility
H_2O ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

C7477-25G	25 g
C7477-100G	100 g
C7477-1KG	1 kg

L-Cystine

(R,R)-3,3′-Dithiobis(2-aminopropionicacid)
[56-89-3] $[-SCH_2CH(NH_2)CO_2H]_2$ $C_6H_{12}N_2O_4S_2$ FW 240.30

▶ from non-animal source, meets EP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
1 M HCl ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

C7602-10MG	10 mg
C7602-25G	25 g
C7602-100G	100 g
C7602-1KG	1 kg

L-Cystine dihydrochloride

[30925-07-6] $C_6H_{12}N_2O_4S_2 \cdot 2HCl$ FW 313.22

▶ from non-animal source, BioReagent, suitable for cell culture, ≥98.0% dry basis

solubility
2 M HCl ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

C6727-25G	25 g
C6727-100G	100 g
C6727-1KG	1 kg

L-Glutamic acid

Glu; (*S*)-2-Aminopentanedioic acid
[56-86-0] $HO_2CCH_2CH_2CH(NH_2)CO_2H$ $C_5H_9NO_4$ FW 147.13

An excitatory amino acid neurotransmitter that is an agonist at all subtypes of glutamate receptors (metabotropic, kainate, NMDA, and AMPA).

▶ from non-animal source, meets EP testing specifications, suitable for cell culture, 98.5-100.5%

powder
solubility
1 M HCl ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

G8415-10MG	10 mg
G8415-100G	100 g
G8415-1KG	1 kg

DL-Glutamic acid monohydrate

(±)-2-Aminopentanedioic acid; (±)-2-Aminoglutaric acid
[19285-83-7] $HOOCCH_2CH_2CH(NH_2)COOH \cdot H_2O$ $C_5H_9NO_4 \cdot H_2O$ FW 165.14

Excitatory amino acid neurotransmitter that is an agonist at kainate, NMDA, AMPA, and metabotropic glutamatergic receptors.

BioReagent, suitable for cell culture

Qualified for use in cell culture applications where mixed isomers are indicated.

L-Glutamic acid is a proteinogenic amino acid incorporated into proteins as directed by the genetic code. This product is a mixture of D and L isomers of glutamic acid.

ship: ambient store at: room temp

G5513-10G	10 g
G5513-100G	100 g
G5513-1KG	1 kg

L-Glutamine

(*S*)-2,5-Diamino-5-oxopentanoic acid; L-Glutamic acid 5-amide
[56-85-9] $H_2NCOCH_2CH_2CH(NH_2)CO_2H$ $C_5H_{10}N_2O_3$ FW 146.14

L-Glutamine is an essential amino acid that is a crucial component of culture media that serves as a major energy source for cells in culture. L-Glutamine is very stable as a dry powder and as a frozen solution. In liquid media or stock solutions, however, L-glutamine degrades relatively rapidly. Optimal cell performance usually requires supplementation of the media with L-glutamine prior to use.

endotoxin ..... tested

meets USP testing specifications, cell culture tested, 99.0-101.0%, from non-animal source

powder

solubility
H_2O ..... 25 mg/mL

ship: ambient store at: room temp

G8540-10MG	10 mg
G8540-25G	25 g
G8540-100G	100 g
G8540-1KG	1 kg
G8540-25KG	25 kg

γ-irradiated, BioXtra, suitable for cell culture

vial = 0.292 g

50 mL amber serum vial with rubber stopper.

Prepares a 200 mM solution when reconstituted to 10 mL with sterile water.

ship: ambient store at: room temp

G6392-1VL	1 vial
G6392-10VL	10 vials

L-Glutamine solution

[56-85-9]

200 mM, solution, sterile-filtered, BioXtra, suitable for cell culture

Prepared in cell culture grade water.

endotoxin ..... tested

ship: dry ice store at: –20°C

G7513-20ML	20 mL
G7513-100ML	100 mL

Glutamine Medium Supplementation

Sigma's liquid media are formulated without antibiotics, serum supplement, and in many instances without L-glutamine. This prolongs shelf lives and gives additional flexibility to their applications. The desired amount of serum and antibiotics should be added, using aseptic techniques, immediately prior to use. If glutamine is to be added, we recommend using a 200 mM solution (Cat. No. G7513) or the crystalline form (Cat. No. G6392).

Please note that G7513 is a sterile-filtered solution and G6392 has been sterilized by exposure to 2.5-3.5 mRads of γ-irradiation. Cat. No. G8540 is not a sterile preparation, and should be solubilized and sterilized by filtration prior to use. Refer to the following table for appropriate amounts of L-glutamine in solid or liquid form to add to prepared media to effect the recommended concentration of the amino acid.

Liquid Media	Cat. No.	L-Glutamine 200 mM (mL/L)	L-Glutamine Powder (g/L)
BME EBSS	B1522	10.0	0.292
DME	D0422	20.0	0.584
DME HEPES	D6171	20.0	0.584
DME	D5546	20.0	0.584
DME	D5671	20.0	0.584
DME w/o phenol red	D5921	20.0	0.584
DME	D6546	20.0	0.584
DME/F-12	D6421	12.5	0.365
Glasgow MEM	G5154	10.0	0.292
IMDM	I3390	20.0	0.584
L-15	L5520	10.25	0.3
McCoy's 5A	M8403	7.5	0.2192
M-199 EBSS	M2154	3.4	0.1
M-199 HEPES	M7528	3.4	0.1
M-199 HBSS	M7653	3.4	0.1
MEM	M2414	10.0	0.292
MEM EBSS	M2279	10.0	0.292
MEM Alpha	M4526	10.0	0.292
MEM EBSS NEAA	M5650	10.0	0.292
MEM HBSS	M5775	10.0	0.292
MEM HEPES	M7278	10.0	0.292
MEM Joklik	M8028	10.0	0.292
NCTC 109	N1140	4.65	0.13573
Nut. Mix F-10	N2147	5.0	0.146
Nut. Mix F-12	N4888	5.0	0.146
Nut. Mix F-12 HEPES	N8641	5.0	0.146
Nut. Mix F-10	N6013	5.0	0.146
RPMI-1640	R0883	10.25	0.3
RPMI-1640 HEPES	R5886	10.25	0.3
RPMI-1640 mod.	R7513	10.25	0.3
RPMI-1640 w/o phenol red	R7509	10.25	0.3
RPMI-1640 Dutch mod.	R7638	10.25	0.3
Williams' Medium E	W4128	10.0	0.292

Glycine

Aminoethanoic acid; Aminoacetic acid; Glycocoll
[56-40-6] NH_2CH_2COOH $C_2H_5NO_2$ FW 75.07
pKa (25 °C) 2.35

from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, ≥98.5%

Amino acid for use in cell culture media development applications and existing media formulations.

powder
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

G8790-100G	100 g
G8790-1KG	1 kg
G8790-25KG	25 kg

Gly-Gln solution

200 mM, sterile-filtered, Biotechnology Performance Certified

Glutamine is an essential component of cell culture media but it is unstable in solution. To address this problem alternatives have been sought. Dipeptides such as gly-L-glutamine and L-ala-L-glutamine have proven to be effective stable forms of glutamine for use in cell culture.

Features and Benefits

Ready-to-use sterile filtered solution

Contains the dipeptide L-Glycyl-L-glutamine. 200 mM supplied in 0.85% NaCL.
endotoxin ..... tested
ship: ambient store at: 2-8°C

G8791-20ML	20 mL
G8791-100ML	100 mL

L-Histidine monohydrochloride monohydrate

L-α-Amino-β-(4-imidazolyl)propionic acid monohydrochloride
[5934-29-2] $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$ FW 209.63

Precursor of histamine by action of histidine decarboxylase.

from non-animal source, meets EP testing specifications, suitable for cell culture, 98.5-101.0%

solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

H5659-25G	25 g
H5659-100G	100 g
H5659-1KG	1 kg

trans-4-Hydroxy-L-proline

Hyp; (2*S*,4*R*)-4-Hydroxypyrrolidine-2-carboxylic acid
[51-35-4] $C_5H_9NO_3$ FW 131.13

Natural constituent of animal structural proteins such as collagen and elastin.

BioReagent, suitable for cell culture, ≥98.5%

solubility
H_2O ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

H5534-10MG	10 mg
H5534-25G	25 g
H5534-100G	100 g
H5534-1KG	1 kg

L-Isoleucine

(2*S*,3*S*)-2-Amino-3-methylpentanoic acid
[73-32-5] $C_2H_5CH(CH_3)CH(NH_2)CO_2H$ $C_6H_{13}NO_2$ FW 131.17

from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
1 M HCl ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

I7403-10MG	10 mg
I7403-25G	25 g
I7403-100G	100 g
I7403-1KG	1 kg

L-Leucine

(*S*)-2-Amino-4-methylpentanoic acid
[61-90-5] $(CH_3)_2CHCH_2CH(NH_2)CO_2H$ $C_6H_{13}NO_2$ FW 131.17

from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
1 M HCl ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

L8912-10MG	10 mg
L8912-25G	25 g
L8912-100G	100 g
L8912-1KG	1 kg
L8912-25KG	25 kg

L-Lysine monohydrochloride

(*S*)-2,6-Diaminohexanoic acid monohydrochloride
[657-27-2] $H_2N(CH_2)_4CH(NH_2)CO_2H{\cdot}HCl$ $C_6H_{14}N_2O_2 \cdot HCl$ FW 182.65

from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

L8662-25G	25 g
L8662-100G	100 g
L8662-1KG	1 kg
L8662-25KG	25 kg

MEM Amino Acids (50x) solution

Without L-glutamine, liquid, sterile-filtered, BioReagent, suitable for cell culture

Adding supplements of amino acids to media both stimulates growth and prolongs the viability of cells in culture. This concentrated supplement adds nutrients and reduces the biosynthetic burden on cell *in vitro*.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M5550-100ML	100 mL

MEM Amino Acids Solution 50X NEW

MEM Amino Acids (50x) solution

Without L-glutamine, liquid, sterile-filtered

Adding supplements of amino acids to media both stimulates growth and prolongs the viability of cells in culture. This concentrated supplement adds nutrients and reduces the biosynthetic burden on cell *in vitro*. Screened for cytotoxicity.

This is a fifty-fold concentrated stock of 12 MEM amino acids: L-Arg; L-Cys; L-His; L-Iso; L-Leu: L-Lys; L-Met; L-Phe; L-Thr; L-Typ; L-Tyr; L-Val.

For research or for further manufacturing use
ship: ambient store at: −20°C

58252C-100ML	100 mL

MEM Non-essential Amino Acid Solution (100×)

without L-glutamine, liquid, sterile-filtered, BioReagent, suitable for cell culture

Adding supplements of amino acids to media both stimulates growth and prolongs the viability of cells in culture. This concentrated supplement adds nutrients and reduces the biosynthetic burden on cell *in vitro*.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M7145-100ML	100 mL

L-Methionine

(*S*)-2-Amino-4-(methylmercapto)butyric acid; L-2-Amino-4-(methylthio)butanoic acid
[63-68-3] $CH_3SCH_2CH_2CH(NH_2)CO_2H$ $C_5H_{11}NO_2S$ FW 149.21

from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 99.0-101.0%

powder
solubility
H_2O ..... 25 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

M5308-10MG	10 mg
M5308-25G	25 g
M5308-100G	100 g
M5308-1KG	1 kg
M5308-25KG	25 kg

DL-Methionine

DL-2-Amino-4-(methylthio)butanoic acid; (±)-2-Amino-4-(methylmercapto)butyric acid
[59-51-8] $CH_3SCH_2CH_2CH(NH_2)COOH$ $C_5H_{11}NO_2S$ FW 149.21

BioReagent, suitable for cell culture, suitable for insect cell culture, ~99.5%

Use in mammalian and insect cell culture applications where mixed isomers are indicated.

ship: ambient store at: room temp

M2768-100G	100 g

L-Phenylalanine

(*S*)-2-Amino-3-phenylpropionic acid
[63-91-2] $C_6H_5CH_2CH(NH_2)CO_2H$ $C_9H_{11}NO_2$ FW 165.19

from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 98.5-101.0%

powder
solubility
1 M HCl ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

P5482-10MG	10 mg
P5482-25G	25 g
P5482-100G	100 g
P5482-1KG	1 kg

L-Proline

(*S*)-Pyrrolidine-2-carboxylic acid
[147-85-3] $C_5H_9NO_2$ FW 115.13

from non-animal source, meets EP, USP testing specifications, suitable for cell culture

powder
solubility
H_2O ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

P5607-25G	25 g
P5607-100G	100 g
P5607-1KG	1 kg
P5607-25KG	25 kg

RPMI 1640 Amino Acids Solution (50×)

Without L-glutamine, sterile-filtered, BioReagent, suitable for cell culture

Use as a concentrate to supplement RPMI-based mammalian cell culture media.

liquid
endotoxin ..... tested
ship: ambient store at: 2-8°C

R7131-100ML	100 mL

L-Serine

(*S*)-2-Amino-3-hydroxypropionic acid
[56-45-1] $HOCH_2CH(NH_2)CO_2H$ $C_3H_7NO_3$ FW 105.09

Precursor of glycine by serine hydroxymethyltransferase.

▸ **from non-animal source, meets EP, USP testing specifications, suitable for cell culture, 98.5-101.0%**

powder
solubility
H_2O ..... 50 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

S4311-25G	25 g
S4311-100G	100 g
S4311-1KG	1 kg

DL-Serine

(±)-2-Amino-3-hydroxypropionic acid
[302-84-1] $HOCH_2CH(NH_2)COOH$ $C_3H_7NO_3$ FW 105.09

▸ **BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98% (TLC)**

For use in insect and mammalian cell culture applications where the pure L-form is not required.

ship: ambient store at: room temp

S5386-25G	25 g
S5386-100G	100 g

L-Threonine

(2*S*,3*R*)-2-Amino-3-hydroxybutyric acid
[72-19-5] $CH_3CH(OH)CH(NH_2)CO_2H$ $C_4H_9NO_3$ FW 119.12

▸ **from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 99.0-101.0%**

powder
endotoxin ..... tested
ship: ambient store at: room temp

T8441-10MG	10 mg
T8441-25G	25 g
T8441-100G	100 g
T8441-1KG	1 kg

DL-Threonine

(±)-2-Amino-3-hydroxybutyric acid
[80-68-2] $CH_3CH(OH)CH(NH_2)COOH$ $C_4H_9NO_3$ FW 119.12

▸ **BioReagent, suitable for cell culture, suitable for insect cell culture**

For use in insect and mammalian cell culture applications where the pure L-form is not required.

ship: ambient store at: room temp

T1520-100G	100 g

L-Tryptophan

(*S*)-2-Amino-3-(3-indolyl)propionic acid; L-α-Amino-3-indolepropionic acid
[73-22-3] $C_{11}H_{12}N_2O_2$ FW 204.23

Amino acid precursor of serotonin and melatonin

▸ **from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, ≥99.0%**

powder
solubility
1 M HCl ..... 10 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

T8941-10MG	10 mg
T8941-25G	25 g
T8941-100G	100 g
T8941-1KG	1 kg

DL-Tryptophan

(±)-2-Amino-3-(3-indolyl)propionic acid; (±)-α-Amino-3-indolepropionic acid; DL-3β-Indolylalanine
[54-12-6] $C_{11}H_{12}N_2O_2$ FW 204.23

▸ **suitable for cell culture, ≥99% (TLC)**

For use in insect and mammalian cell culture applications where the pure L-form is not required.

Prepared from L-tryptophan produced by fermentation.

ship: ambient store at: room temp

T7425-25G	25 g

L-Tyrosine

(*S*)-2-Amino-3-(4-hydroxyphenyl)propionic acid; 3-(4-Hydroxyphenyl)-L-alanine
[60-18-4] $4\text{-}(HO)C_6H_4CH_2CH(NH_2)CO_2H$ $C_9H_{11}NO_3$ FW 181.19

Amino acid precursor of dopamine and other catecholamines

▸ **from non-animal source, meets EP, USP testing specifications, suitable for cell culture, ≥99.0%**

powder
solubility
1 M HCl ..... 25 mg/mL
endotoxin ..... tested
ship: ambient store at: room temp

T8566-10MG	10 mg
T8566-25G	25 g
T8566-100G	100 g
T8566-1KG	1 kg

L-Tyrosine disodium salt hydrate

L-3-(4-Hydroxyphenyl)alanine disodium salt hydrate
$C_9H_9NNa_2O_3 \cdot xH_2O$ FW 225.15 (Anh)

Amino acid precursor of dopamine and other catecholamines

▶ **BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98% (TLC)**

Extremely hygroscopic.

ship: ambient store at: 2-8°C

T1145-25G	25 g
T1145-100G	100 g
T1145-500G	500 g

L-Tyrosine hydrochloride

3-(4-Hydroxyphenyl)-L-alanine hydrochloride; 3-(4-Hydroxyphenyl)-L-alanine
[16870-43-2] $HOC_6H_4CH_2CH(NH_2)CO_2H{\cdot}HCl$ $C_9H_{11}NO_3 \cdot HCl$ FW 217.65

Amino acid precursor of dopamine and other catecholamines

▶ **BioReagent, suitable for cell culture**

ship: ambient store at: room temp

T2025-1G	1 g
T2025-5G	5 g

L-Valine

(*S*)-α-Aminoisovaleric acid; L-2-Amino-3-methylbutanoic acid
[72-18-4] $(CH_3)_2CHCH(NH_2)CO_2H$ $C_5H_{11}NO_2$ FW 117.15

▶ **from non-animal source, meets EP, JP, USP testing specifications, suitable for cell culture, 98.5-101.0%**

powder

solubility
H_2O ..... 25 mg/mL
endotoxin ..... tested

ship: ambient store at: room temp

V0513-10MG	10 mg
V0513-25G	25 g
V0513-100G	100 g
V0513-1KG	1 kg

L-Glutamine Stability Study

L-Glutamine, unlike most other amino acids, is labile in solution, and that concerns many cell culture scientists. This essential amino acid is required by virtually all mammalian cells in culture. Although the metabolic destiny remains a matter of conjecture, we conducted studies designed to confirm glutamine's lability and the conditions under which its deterioration could be minimized.

The first study series was designed to confirm the breakdown of L-glutamine as a function of time and temperature. Basal Medium Eagle (BME) with Earle's salts was prepared for cell culture use, i.e., dissolved in deionized water, supplemented with 2.2 g of sodium bicarbonate, and pH adjusted prior to sterile filtration. In addition, the medium contained 2 mM L-glutamine. Aliquots of the medium were stored at 4 °C, at room temperature (21 °C), and in an incubator at 35 °C. Samples were periodically harvested and derivatized with phenylisothiocyanate. Quantitative amino acid analysis was performed by reverse-phase HPLC and each sample analyzed in the presence of an internal standard. L-Glutamine peak heights were normalized and resulting data expressed as percent of glutamine remaining from the fresh BME preparation. Results of these studies are shown in **Figs. 1-3.**

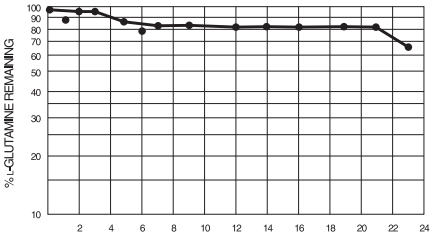


Fig. 1 DAYS IN STORAGE AT 4 °C

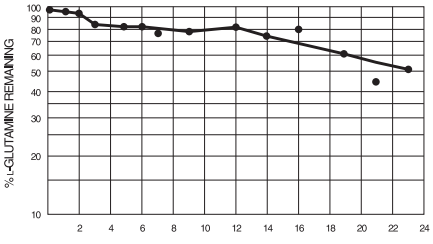


Fig. 2 DAYS IN STORAGE AT 21 °C

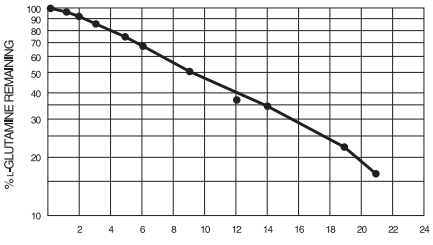


Fig. 3 DAYS IN STORAGE AT 35 °C

Glutamine was found to be most stable in medium stored at 4 °C. Little or no breakdown was detected until the end of the first week and virtually no further change was observed during the remainder of the test period. We recommend refrigerated storage for all media **(Fig. 1),** but do not recommend freezing liquid media because many components precipitate irreversibly when exposed to temperatures below 0 °C.

Breakdown occurred more rapidly at room temperature **(Fig. 2),** with approximately 20% of the glutamine deteriorating by day three. Deterioration occurred more rapidly when medium was stored at 35 °C **(Fig. 3).** Within nine days, glutamine degraded 50%, and less than 20% remained at the end of three weeks. These data underscore the need to replenish cell culture media every few days, and suggest that aliquots of media incubated to validate sterilization should be discarded.

The loss of bicarbonate as CO_2 with time from BME buffered with sodium bicarbonate, and the consequent rise in pH raised the question of glutamine stability. A second series of studies were conducted to assess the effect of pH on the stability of glutamine. Three 10 mM solutions of L-glutamine in water were prepared at pH 3.0, pH 7.3, and pH 9.0, and stored at room temperature. Samples were periodically harvested and analyzed as described above.

Results of these studies are shown in **Figs. 4-6.** Glutamine was most stable at neutral pH, and deteriorated at acidic and basic pH at similar rates. Data suggest that at least some of the breakdown of L-glutamine observed in media at room temperature **(Fig. 2)** resulted from a rise in pH.

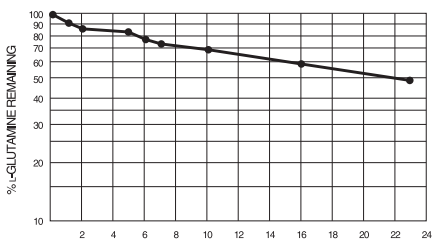


Fig. 4 DAYS IN STORAGE AT pH 3 AND 21 °C

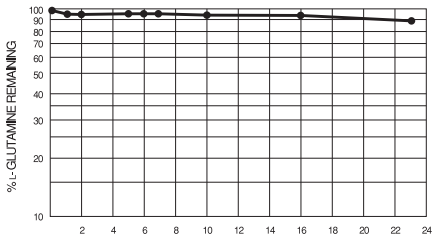


Fig. 5 DAYS IN STORAGE AT pH 7.3 AND 21 °C

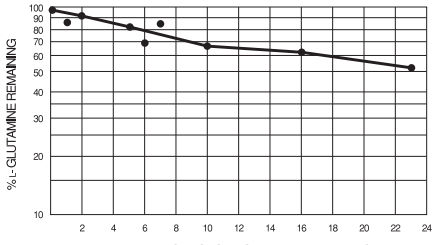


Fig. 6 DAYS IN STORAGE AT pH 9 AND 21 °C

Vitamins

BME Vitamins 100x solution

BioReagent, sterile-filtered, suitable for cell culture

ship: dry ice store at: –20°C

B6891-100ML	100 mL

Diamond Vitamin TWEEN® 80 Solution 40x NEW

BME Vitamins 100x solution

frozen liquid, suitable for cell culture

Adding supplements of vitamins to media both stimulates growth and prolongs the viability of cells in culture. This concentrated supplement adds nutrients and reduces the biosynthetic burden on cells *in vitro*.

for research or for further manufacturing use

ship: dry ice store at: –20°C

58980C-100ML	100 mL
58980C-500ML	500 mL

MEM Vitamin Solution (100×)

sterile-filtered, BioReagent, suitable for cell culture

Adding supplements of vitamins to media both stimulates growth and prolongs the viability of cells in culture. This concentrated supplement adds nutrients and reduces the biosynthetic burden on cell *in vitro*.

liquid

endotoxin ..... tested

ship: dry ice store at: –20°C

M6895-100ML	100 mL

Menadione sodium bisulfite

2-Methyl-1,4-naphthoquinone sodium bisulfite
[130-37-0] $C_{11}H_9NaO_5S$ FW 276.24

Water soluble vitamin K_3.

BioReagent, suitable for cell culture, ≥95% (TLC)

Use in cell culture to induce oxidative stress for mechanistic studies.

ship: ambient store at: –20°C

M2518-100G	100 g

Pyridoxamine dihydrochloride

4-(Aminomethyl)-5-hydroxy-6-methyl-3-pyridinemethanol dihydrochloride
[524-36-7] $C_8H_{12}N_2O_2 \cdot 2HCl$ FW 241.11

BioReagent, suitable for cell culture

Use in mammalian cell culture as an alternative to pyridoxine or pyridoxal. Vitamin B6 is a required component of cell culture media. Historically, media have been supplemented with vitamin B6 in the aldehyde form, pyridoxal. While this is a biologically active form of vitamin B6, it is unstable in liquid media. When cells are able to utilize either pyridoxine and/or pyridoxamine, they are preferred supplement versus pyridoxal. A number of classical media are now offered with a choice between pyridoxal and pyridoxine as the vitamin A source.

≥98%

powder

ship: ambient store at: –20°C

P9158-1G	1 g

Retinyl Acetate-Water Soluble

Vitamin A; Retinol Acetate
BioReagent, suitable for cell culture

With a minimum of 5 mg retinol acetate per gram; balance methyl-β-cyclodextrin.

Use to study retinoid regulation of cell transformation; cell differentiation and stem cell development.

Package size based on retinol acetate content

solubility

H_2O ..... 400 mg/mL

ship: ambient store at: –20°C

R0635-5MG	5 mg

RPMI 1640 Vitamins Solution (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

endotoxin ..... tested

ship: dry ice store at: –20°C

R7256-100ML	100 mL

(+)-Sodium L-ascorbate

L(+)-Ascorbic acid sodium salt; Vitamin C sodium salt
[134-03-2] $C_6H_7NaO_6$ FW 198.11

powder, BioReagent, suitable for cell culture

Antioxidant

Used for preparation of plant extracts; reductant for the isolation of chloroplasts.

≥98%

solubility

H_2O ..... 50 mg/mL

ship: ambient store at: room temp

A4034-100G	100 g
A4034-500G	500 g
A4034-1KG	1 kg

Antibiotics

The antibiotics described in this section have been specially prepared to control microbial contamination in cell culture or packaged especially for convenience of use in molecular biology applications. Please note that these products are intended for **research use only**. They are not for drug use.

Cell culture preparations have been γ-irradiated, sterile-filtered, aseptically-processed and/or lyophilized. The use of antibiotics in cell culture is not intended as a substitute for appropriate aseptic technique. In addition, many antibiotics have been found to be cytotoxic in some cases, even at concentrations approaching their effective levels. Cytotoxicity is cell dependent and care should be exercised when selecting the appropriate antibiotic concentration.

Molecular biology preparations are: (1) pre-weighed in quantities to give typical working concentrations when the entire package is added to 1 liter of agar preparation (50 plates at 20 ml per plate); (2) γ-irradiated for sterility and septum-capped for ease in injecting sterile diluent; (3) allow easy preparation of stock solutions directly in vials at any concentration in the recommended ranges and; (4) USP testing potency is carried out on control vials after γ-irradiation to assure full biological activity (except where noted).

References

1. Perlman, D., Use of antibiotics in cell culture media. *Methods in Enzymology, Vol. 58, Cell Culture*, W.B. Jakoby and I.H. Pastan, eds., Academic Press San Diego, CA., 110-116, 1979.
2. Sambrook, J., et al., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. 1989.
3. Ausbel, F.M., et al., Current Protocols in Molecular Biology. Green Publishing Associates and Wiley-Interscience, New York, 1987.
4. Adams, R.L.P., et al., The Biochemistry of Nucleic Acids. Chapman and Hall, New York, 1986.

Antibiotics Selection Guide

The following table is designed as a guide for selecting appropriate antibiotics and working concentrations. Care should be exercised when using 2 or more antibiotics in the same culture system. Combined antibiotics frequently exert cytotoxic effects at lower concentrations than those indicated as appropriate for the individual antibiotics. Refer to a comprehensive pharmacology guide for more informatioan on antibiotic incompatibilities, as well as other properties of antibiotics not included in this table.

Antibiotics and Antimycotics

Product	Cat. No.	Gram (+) Bacteria	Gram (-) Bacteria	Myco-bacteria	Yeasts	Molds	Myco-plasma	Working Concentration (µg/mL)	pcct/ mcct[1]
Amphotericin B	A2411				•	•		2.5 mg/L	mcct
Amphotericin B-Solubilized (Approx. 45%)	A9528				•	•		5.6 mg/L (of solid)	mcct
Amphotericin B- (250 mg/mL solution)	A2942				•	•		10 mL/L	mcct
Ampicillin	A0166	•	•					100 mg/L	mcct
Antibiotic Antimycotic Solution (100×) (10,000 units penicillin, 10 mg streptomycin, and 25 µg amphotericin B per mL) (Stabilized)	A5955	•	•		•	•		10 mL/L	mcct
Cephalothin	C3050	•	•					100 mg/L	mcct
Chloramphenicol	C3175	•	•				*	10-35 (TS)	mcct
Dihydrostreptomycin	D5155	•	•					100 mg/L	mcct
Erythromycin	E5389	•	•					100 mg/L	mcct
Gentamicin Sulfate	G1264	•	•				•	50 mg/L	mcct
Gentamicin Sulfate (10 mg/mL solution)	G1272	•	•				•	5 mL/L	mcct
Gentamicin Sulfate (50 mg/mL solution)	G1397	•	•				•	1 mL/L	mcct
Gentamicin Sulfate (50 mg/mL solution) (Hybri-Max™)	G1522	•	•				•	1 mL/L	mcct
Gentamicin-Glutamine Solution	G9654	•	•				•	10 mL/L	mcct
L-Glutamine-Penicillin-Streptomycin Solution (200 mM L-Glutamine, 10,000 units Penicillin, and 10 mg Streptomycin per mL)	G1146	•	•					10 mL/L	mcct
L-Glutamine-Penicillin-Streptomycin Solution (200 mM L-Glutamine, 10,000 units Penicillin, and 10 mg Streptomycin per mL) (Stabilized)	G6784	•	•					10 mL/L	mcct
Kanamycin Monosulfate	K1377	•	•				•	100 mg/L	mcct
Kanamycin Sulfate (10 mg/mL solution)	K0129	•	•				•	10 mL/L	pcct mcct
Kanamycin Sulfate (50 mg/mL solution)	K0254	•	•				•	2 mL/L	pcct mcct

Antibiotics and Antimycotics

Product	Cat. No.	Gram (+) Bacteria	Gram (-) Bacteria	Myco-bacteria	Yeasts	Molds	Myco-plasma	Working Concentration (µg/mL)	pcct/ mcct[1]
Lincomycin HCl	L2774	•						100 mg/L	mcct
Neomycin Sulfate	N6386	•	•					50 mg/L	mcct
Neomycin Sulfate (10 mg/mL solution)	N1142	•	•					5 mL/L	mcct
Nystatin (5000 units Nystatin per mg)	N6261				•	•		2.5×10^5 U/L (50 mg/L)	mcct
Nystatin γ-Irradiated	N4014				•	•		50 mg/L	mcct
Nystatin Suspension (10,000 units Nystatin per mL)	N1638				•	•		24 mL/L	mcct
Paromomycin Sulfate	P5057	•						100 mg/L	mcct
Penicillin-G (potassium salt)	P7794	•						100,000 U/L	mcct
Penicillin-G (sodium salt)	P3032	•						100,000 U/L	mcct
Penicillin-Streptomycin Solution (5000 units Penicillin-G and 5 mg Streptomycin per mL) (Stabilized)	P4458	•	•					20 m/L	mcct
Penicillin-Streptomycin Solution (10,000 units Penicillin-G and 10 mg Streptomycin per mL)	P0781	•	•					10 mL/L	mcct
Penicillin-Streptomycin Solution (10,000 units Penicillin-G and 10 mg Streptomycin per mL) (Hybri-Max™)	P7539	•	•					10 mL/L	mcct
Penicillin-Streptomycin Solution (10,000 units Penicillin-G and 10 mg Streptomycin per mL) (Stabilized)	P4333	•	•					10 mL/L	mcct
Penicillin-Streptomycin-Neomycin Solution (5000 units Penicillin-G, 5 mg Streptomycin and 10 mg Neomycin per mL)	P4083	•	•					10 mL/L	mcct
Phenoxymethylpenicillinic Acid (potassium salt) [Penicillin V]	P4807	•						100,000 U/L	mcct
Polymyxin B Sulfate	P4932		•					50 mg/L	mcct
Spectinomycin Dihydrochloride	S4014	•	•					7.5–20 mg/L	mcct
Spectromycin Sulfate	S9137	•	•					100 mg/L	mcct
Tetracycline Hydrochloride	T7660	•	•					10 mg/L	mcct
Tylosin Tartrate (8 mg/mL soln.)	T3397	•					•	1 mL/L	mcct
Tylosin Tartrate	T6271	•					•	8 mg/L	mcct

• Effective against most species
* Effective against certain species
[1] **pcct** = plant cell culture tested; **mcct** = mammalian cell culture tested

Antibiotic Selection Agents for Cell Culture

Product Name	Cat. No.	Mode of Action	Suggested Working Conc.
Actinomycin D	A9415	Complexes with DNA and interferes with RNA synthesis	1 µg/mL
Bleomycin Sulfate	B8416	Complexes with DNA, causing strand scissions	10-100 µg/mL
Chloramphenicol	C3175	Inhibits elongation at peptidyl transferase	5 µg/mL
G 418	A1720	Blocks polypeptide synthesis and inhibits chain elongation	100-800 µg/mL
G 418 (50 mg/mL solution)	G8168	Blocks polypeptide synthesis and inhibits chain elongation	100-800 µg/mL
Hygromycin B	H3274	Blocks polypeptide synthesis and inhibits chain elongation	
Mitomycin C	M4287	Inhibits nucleic acid synthesis	10-50 µg/mL
Mycophenolic Acid	M3536	Blocks inosine monophosphate dehydrogenase in guanosine mono-phosphate pathway	25 µg/mL
Puromycin • HCl	P8833	Inhibits protein synthesis	10-100 µg/mL

Cell Culture Antibiotics

Actinomycin D

Actinomycin IV; Actinomycin C_1; Dactinomycin
[50-76-0] $C_{62}H_{86}N_{12}O_{16}$ FW 1255.42

An antineoplastic antibiotic that inhibits cell proliferation by forming a stable complex with DNA and blocking the movement of RNA polymerase which interferes with DNA-dependent RNA synthesis. Induces apoptosis. Potent antitumor agent. For cell culture applications, actinomycin D is used as a selection agent and is used in banding techniques to differentiate between different regions of chromosomes.

color ..... red in powder

▸ from *Streptomyces* sp., suitable for cell culture, ≥95%

Mode of Action: Complexes with DNA and interferes with RNA synthesis.

Recommended for use in cell culture applications at 1 µg/ml.

powder
solubility
ethanol, DMSO ..... soluble (Stable in aqueous solutions at 2-8 °C.)
ship: ambient store at: 2-8°C

A9415-2MG	2 mg
A9415-5MG	5 mg
A9415-10MG	10 mg
A9415-25MG	25 mg

Amphotericin B solution

[1397-89-3] $C_{47}H_{73}NO_{17}$ FW 924.08

▸ 250 µg/mL in deionized water, sterile-filtered, BioReagent, suitable for cell culture

Mode of action: Interferes with fungal membrane permeability by forming channels in the membranes and causing small molecules to leak out.
Antimicrobial spectrum: Yeasts and molds.

Recommended for use in cell culture applications at 10 ml/L.

Prepared from A 9528.

Stable at 37 °C for 3 days.
ship: dry ice store at: –20°C

A2942-20ML	20 mL
A2942-50ML	50 mL
A2942-100ML	100 mL

Amphotericin B from *Streptomyces* sp.

Fungizone
[1397-89-3] $C_{47}H_{73}NO_{17}$ FW 924.08

Polyene antifungal antibiotic from *Streptomyces*. Affinity for sterols, primarily ergosterols, of fungal cell membranes. Forms channels in the membranes, causing small molecules to leak out.
Antimicrobial spectrum: fungi and yeast.

▸ BioReagent, suitable for cell culture, ~80% (HPLC)
solubility
H_2O ..... slightly soluble
ship: ambient store at: 2-8°C

A2411-250MG	250 mg
A2411-1G	1 g
A2411-5G	5 g

Amphotericin B solubilized

Fungizone; Amphotericin B from *Streptomyces* sp.
[1397-89-3] $C_{47}H_{73}NO_{17}$ FW 924.08

Polyene antifungal antibiotic from *Streptomyces*. Affinity for sterols, primarily ergosterols, of fungal cell membranes. Forms channels in the membranes, causing small molecules to leak out.
Antimicrobial spectrum: fungi and yeast.

▸ powder, γ-irradiated, BioXtra, suitable for cell culture

Mode of Action: Interferes with fungal membrane permeability by forming channels in the membranes and causing small molecules to leak out.

Recommended for use in cell culture applications at 5.6 mg (solid)/L.

Package size based on amphotericin B
solubility
sterile water .. 20 mg/mL (as a stock solution. Stock solutions should be stored at –20 °C. Stable at 37 °C for 3 days.)

Components

Amphotericin B ~45%
Sodium deoxycholate ~35%
Sodium phosphate balance
ship: ambient store at: 2-8°C

A9528-50MG	50 mg
A9528-100MG	100 mg
A9528-500MG	500 mg
A9528-1G	1 g
A9528-5G	5 g

Amphotericin B (Cat. No. A9528)

Amphotericin B is an anti-fungal agent produced by *Streptomyces*. Amphotericin B is offered as a gamma-irradiated, lyophilized powder containing 43% amphotericin B, 35% sodium deoxycholate and 22% sodium phosphate, pH 7.5. The quantity shown on the vial refers to the amount of Amphotericin B per vial.

Recommended usage:
For 50 mg of lyophilized powder, quickly add 10-20 ml of sterile deionized-distilled water. Swirl or gently pipette to dissolve powder. Further dilutions may be made as desired. For recommended concentrations, please see the antibiotic chart found at the beginning of the antibiotic listings.

Ampicillin sodium salt

D-(–)-α-Aminobenzylpenicillin sodium salt
[69-52-3] $C_{16}H_{18}N_3NaO_4S$ FW 371.39

▸ powder, BioReagent, suitable for cell culture

Recommended for antibacterial use in cell culture media at 100 mg/L.
Recommended for use in ampicillin-resistance studies at 20-125 µg/ml.
Stable at 37 °C for 3 days.

potency: ≥845 µg per mg
solubility
H_2O ..... 50 mg/mL (Stock solutions should be stored at –20 °C.)
color ..... white with slight yellow cast
ship: ambient store at: 2-8°C

A0166-5G	5 g
A0166-25G	25 g
A0166-100G	100 g

BioXtra, suitable for cell culture

endotoxin ..... tested
ship: ambient store at: 2-8°C

A8351-5G	5 g
A8351-25G	25 g

Antibiotic Antimycotic Solution (100×), Stabilized

with 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B per mL, sterile-filtered, BioReagent, suitable for cell culture

Mode of Action: Penicillin acts by inhibiting bacterial cell-wall synthesis. Streptomycin inhibits prokaryote protein synthesis by preventing the transition from initiation complex to chain-elongating ribosome and causes miscoding. Amphotericin B interferes with fungal membrane permeability by forming channels in the membranes and causing small molecules to leak out.
Antimicrobial spectrum: Gram-negative bacteria, Gram-positive bacteria, fungi and yeasts.

Solubilized in a proprietary citrate buffer. Formulated to contain 10,000 units/ml penicillin G, 10 mg/ml streptomycin sulfate and 25 µg/ml amphotericin B.

Recommended for use in cell culture applications at 10 ml/L.

suspension
endotoxin ..... tested
color ..... yellow
ship: dry ice store at: –20°C

A5955-20ML	20 mL
A5955-100ML	100 mL

Bleomycin sulfate from *Streptomyces verticillus*

Blenoxane; Bleo; Blexane
[9041-93-4]

An antineoplastic antibiotic isolated from *Streptomyces verticillus*. Binds to DNA, inhibits DNA synthesis and causes DNA scissions at specific base sequences. Needs to bind oxygen and a metal ion such as copper or iron to cleave DNA. Highly selective cleavage of RNA. Inducer and regulator of apoptosis in a variety of cells. Inhibits tumor angiogenesis.

Recommended for use as a selection agent at 10-100 µg/ml.

activity: 1.5-2.0 units/mg solid, BioReagent, suitable for cell culture

A mixture of bleomycin sulfate salts

crystalline
solubility
H_2O ..... 20 mg/mL, clear, colorless
color ..... white
Cu ..... <0.1%
ship: ambient store at: 2-8°C

B8416-15UN	15 units

Cephalothin sodium salt

Cephalotin sodium salt; 7-(2-Thienylacetamido)cephalosporanic acid sodium salt
[58-71-9] $C_{16}H_{15}N_2NaO_6S_2$ FW 418.42

Mode of Action: Inhibits cell wall synthesis.
Antimicrobial spectrum: Gram-positive cocci.
Mode of Resistance: Cephalosporinase production.

First generation cephalosporin antibiotic.

BioReagent, suitable for cell culture

Mode of Action: Inhibits cell wall synthesis.
Antimicrobial spectrum: Gram-positive cocci.
Mode of Resistance: Cephalosporinase production.

Cephalotin is a first generation cephalosporin antibiotic.

ship: ambient store at: 2-8°C

C3050-250MG	250 mg
C3050-1G	1 g

Chloramphenicol

D-(–)-*threo*-2-Dichloroacetamido-1-(4-nitrophenyl)-1,3-propanediol; Chloromycetin™; D-(–)-*threo*-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-β-(4-nitrophenyl)ethyl]acetamide; D-*threo*-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-4-nitrophenethyl]acetamide
[56-75-7] $Cl_2CHCONHCH(CH_2OH)CH(OH)C_6H_4NO_2$ $C_{11}H_{12}Cl_2N_2O_5$ FW 323.13

Mode of Action: Inhibits translation on the 50S ribosomal subunit at the peptidyltransferase step (elongation inhibition). Bacteriostatic.
Mode of Resistance: Acetylation by chloramphenicol acetyltransferase (*cat* gene).

Biotechnology Performance Certified, suitable for plant cell culture

endotoxin ..... tested
ship: ambient store at: room temp

C1863-25G	25 g
C1863-100G	100 g
C1863-500G	500 g

Chloramphenicol-Water Soluble

powder, BioReagent, suitable for cell culture

Mode of Action: Inhibits elongation of peptidyl transferase.

Formulated to contain approx. 100 mg chloramphenicol per gram of powder; balance 2-hydroxypropyl-β-cyclodextrin.

Recommended for use in cell culture applications at approximately 5 µg/ml of active component (chloramphenicol).

Package size based on chloramphenicol
solubility
other salt solutions ..... not recommended
H_2O ..... 50-500 mg/mL (stock solution)
PBS ..... 50-500 mg/mL (stock solution)
ship: ambient store at: room temp

C3175-100MG	100 mg

Dihydrostreptomycin sesquisulfate

[5490-27-7] $C_{21}H_{41}N_7O_{12} \cdot 3/2H_2SO_4$ $C_{21}H_{41}N_7O_{12}·1.5H_2SO_4$ FW 730.71

Mode of action: inhibits protein synthesis by interfering with translation.

BioReagent, suitable for cell culture, ≥98% (TLC)

Use to study bacterial protein synthesis, codon misreading, at the level of the 30S ribosomal subunit and the 16S rRNA.

crystalline
ship: ambient store at: 2-8°C

D5155-25G	25 g
D5155-100G	100 g

Erythromycin

[114-07-8] $C_{37}H_{67}NO_{13}$ FW 733.93

Mode of Action: Inhibits elongation at transpeptidation step.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.

Macrolide antibiotic.

Erythrocin; E-Mycin
BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 100 mg/L

powder

potency: ≥850 µg per mg

solubility
ethanol ..... (Stock solutions should be stored at 2-8 °C. Stable at 37 °C for 3 days.)
2 M HCl ..... 50 mg/mL (Stock solutions should be stored at 2-8 °C. Stable at 37 °C for 3 days.)

ship: ambient store at: room temp

E5389-1G	1 g
E5389-5G	5 g

G 418 disulfate salt

Antibiotic G418
[108321-42-2] $C_{20}H_{40}N_4O_{10} \cdot 2H_2SO_4$ FW 692.71

Mode of Action: Blocks polypeptide synthesis by inhibiting protein elongation. For use in the selection and maintenance of eukaryotic cells stably transfected with neomycin resistance genes.

Aminoglycoside antibiotic similar in structure to gentamicin, neomycin and kanamycin.

powder, BioReagent, suitable for cell culture

Recommended for use as a selection agent at 100-800 µg/ml.

solubility
H_2O ..... 50 mg/mL (As a stock solution. Stock solutions should be stored at 2-8°C. Stable at 37°C for 8 days.)

ship: ambient store at: room temp

A1720-1G	1 g
A1720-5G	5 g
A1720-25G	25 g

Biotechnology Performance Certified, suitable for cell culture, suitable for plant cell culture

endotoxin ..... tested

ship: ambient store at: 2-8°C

A8601-1G	1 g
A8601-5G	5 g

G 418 disulfate salt solution

Antibiotic G418
[108321-42-2] $C_{20}H_{40}N_4O_{10} \cdot 2H_2SO_4$ FW 692.71

50 mg/mL in H_2O, sterile-filtered, BioReagent, suitable for cell culture

Recommended for use as selection agent at 100-800 µg/ml.

50 mg/mL in tissue culture grade water

endotoxin ..... tested

ship: ambient store at: 2-8°C

G8168-10ML	10 mL
G8168-100ML	100 mL

G418 (Cat. Nos. A1720, A8601, and G8168)

Although it is related to Gentamicin, G418 is not normally used as a standard antibiotic. Its most common application is in molecular biology as a selection agent. G418 sulfate is toxic to bacteria, yeast, protozoa, helminths, and mammalian cells. Resistance is conferred by one of two dominant genes of bacterial origin, which can be expressed in eukaryotic cells.

Recommended usage:
G418 is water soluble and can be stored at room temperature for as long as 1 year. Aqueous solutions should be stored frozen. The amount of G418 required for selection will vary with each cell type and growth cycle. Although cells that are multiplying will be affected sooner than those that are not, cells that are in log phase will still require 3 to 7 days for selection. In general, the amount of G418 required for selection of mammalian cells is 400 µg/ml and 200 µg/ml for maintenance. G418 has also been reported to be useful in the elimination of fibroblasts from primary melanocyte cultures. Cells were cultured in medium containing 200 µg/ml of G418 for 2-3 days. (See **wistar.upenn.edu/herlyn/cellculture.htm** - Lab Web site of Drs. Dorothee and Meenhard Herlyn, Wistar Institute, University of Pennsylvania.)

Gentamicin solution

[1405-41-0]

Mode of Action: Inhibits protein synthesis by binding to L6 protein of 50S ribosomal subunit.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

endotoxin ..... tested

50 mg/mL in deionized water, liquid, sterile-filtered, BioReagent, suitable for cell culture

Formulated to contain 50 mg/ml gentamicin in deionized water.

Recommended for use in cell culture applications at 1 ml/L.

Stable at 37°C for 5 days.

ship: ambient store at: 2-8°C

G1397-10ML	10 mL
G1397-100ML	100 mL

Garamycin; Gentiomycin C
10 mg/mL in deionized water, liquid, sterile-filtered, BioReagent, suitable for cell culture

Formulated to contain 10 mg/ml gentamicin in deionized water.

Recommended for use in cell culture applications at 5 ml/L.

Stable at 37 °C for 5 days.

ship: ambient store at: 2-8°C

G1272-10ML	10 mL
G1272-10X10ML	10 × 10 mL
G1272-100ML	100 mL

Gentamicin sulfate salt

[1405-41-0]

Mode of action: Gentamicin causes codon misreading by binding to the 30S ribosomal subunit, blocking the translocation of peptidyl-tRNA from the acceptor site to the donor site.[1,2] The bactericidal effect of gentamicin on *Pseudomonas aeruginosa* is exerted by the binding of gentamicin to the outer membrane, where it displaces natural cations, destabilizes the membrane, and forms holes in the cell surface.[3]
Antimicrobial spectrum: Gram-negative bacteria, *Staphylococcus aureus* and other Gram-positive bacteria

Lit. cited: 1. Korzybski, T., et al., *Antibiotics: origin, nature, and properties* , American Society for Microbiology (Washington, DC: 1977), 712-723
2. Lorian, V. (ed.), *Antibiotics in Laboratory Medicine* 2nd ed., Williams and Wilkins (Baltimore, MD: 1986), 694-696
3. Kadurugamuwa, J., et al., Surface action of gentamicin on *Pseudomonas aeruginosa J. Bacteriol.* **175**, 5798-5805 (1993)

▸ Garamycin; Gentiomycin C powder, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 50 mg/L

potency: ~600 µg Gentamicin per mg

solubility
H_2O ..... 50 mg/mL (As stock solution, store at −20°C. Stable at 37°C for 5 days.)
ship: ambient store at: 2-8°C

G1264-50MG	50 mg
G1264-250MG	250 mg
G1264-1G	1 g
G1264-5G	5 g
G1264-100G	100 g

▸ Biotechnology Performance Certified, suitable for cell culture

Mode of Action: Inhibits protein synthesis by binding to L6 protein of 50S ribosomal subunit.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

endotoxin ..... tested
ship: ambient store at: 2-8°C

G4793-250MG	250 mg
G4793-1G	1 g
G4793-5G	5 g

Gentamicin-Glutamine solution

▸ with 200mM L-glutamine and 5mg gentamicin per mL in tissue culture grade water, liquid, sterile-filtered, BioReagent, suitable for cell culture

Mode of Action: Glutamine is essential as an energy source for most mammalian cells in culture because of its amino acid carbon skeletons. Inhibits protein synthesis by binding to L6 protein of 50S ribosomal subunit.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

Recommended for use in cell culture applications at 10 ml/L.

Formulated to contain 200 mM L-glutamine and 5 mg/ml gentamicin in 0.9% sodium chloride.

Stable at 37°C for 5 days.

endotoxin ..... tested
ship: dry ice store at: −20°C

G9654-10X5ML	10 × 5 mL

L-Glutamine–Penicillin–Streptomycin solution

Mode of Action: Glutamine is essential as an energy source for most mammalian cells in culture because of its amino acid carbon skeleton. Penicillin acts by inhibiting bacterial cell wall synthesis. Streptomycin inhibits prokaryote protein synthesis by preventing the transition from initiation complex to chain-elongating ribosome and causes miscoding.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.

Recommended for use in cell culture applications at 10 ml/L.

endotoxin ..... tested

▸ with 200mM L-glutamine, 10,000 U penicillin and 10mg steptomycin/mL in 0.9% NaCl, sterile-filtered, BioReagent, suitable for cell culture

liquid

Formulated with 200 mM L-glutamine, 10,000 units/ml penicillin, and 10 mg/ml streptomycin in 0.9% sodium chloride.

ship: dry ice store at: −20°C

G1146-10X5ML	10 × 5 mL
G1146-100ML	100 mL

▸ L-glutamine: 200 mM, streptomycin: 10 mg/mL, penicillin: 10,000 units, sterile-filtered, BioReagent, suitable for cell culture

Solubilized in a proprietary buffer.

stabilized

ship: ambient store at: −20°C

G6784-10X5ML	10 × 5 mL
G6784-100ML	100 mL

Hygromycin B from *Streptomyces hygroscopicus*

[31282-04-9] $C_{20}H_{37}N_3O_{13}$ FW 527.52

Mode of Action: Blocks polypeptide synthesis and inhibits elongation. For use in the selection and maintenance of prokaryotic and eukaryotic cells.

▸ powder, BioReagent, suitable for cell culture, suitable for insect cell culture

Recommended for use as a selection agent at 100-800 µg/mL.

≥60% (HPAE)

Purified by ion exchange chromatography

solubility
H_2O ..... 50 mg/mL (As a stock solution. Stock solutions should be stored at 2-8°C. Stable at 37°C for 30 days.)

ship: ambient store at: 2-8°C

H3274-50MG	50 mg
H3274-100MG	100 mg
H3274-5X100MG	5 × 100 mg
H3274-250MG	250 mg
H3274-1G	1 g

Hygromycin B (Cat. No. H3274)

Hygromycin B is an aminoglycoside antibiotic which is effective against prokaryotic and eukaryotic micro-organisms and cells. Similar to G418, its most common application is in molecular biology as a selection agent. Insect and mammalian cells transformed with the *hph* gene, which encodes for hygromycin-B phosphotransferase are resistant to hygromycin B.

Recommended usage:
Hygromycin B is provided as a powder. The recommended concentration is 100-800 mg/ml selection medium:
Prokaryotes: 100 µg/ml
Lower eukaryotes: 200 µg/ml
Higher eukaryotes: 150-400 µg/ml
Refer to the literature for more specific application information.

Kanamycin solution from *Streptomyces kanamyceticus*

[25389-94-0]

Mode of Action: Binds to 70S ribosomal subunit; inhibits translocation; elicits miscoding.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

endotoxin ..... tested

▶ 50 mg/mL in 0.9% NaCl, BioReagent, liquid, sterile-filtered, suitable for cell culture

Recommended for use in cell culture applications at 2 ml/L.

Stable at 37 °C for 5 days.

ship: ambient store at: 2-8°C

K0254-20ML	20 mL

▶ 10 mg/mL kanamycin in 0.9% NaCl, sterile-filtered, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 10 ml/L.

Stable at 37°C for 5 days.

ship: ambient store at: 2-8°C

K0129-20ML	20 mL

Kanamycin sulfate from *Streptomyces kanamyceticus*

Kanamycin A; Kanamycin sulfate salt
[25389-94-0] $C_{18}H_{36}N_4O_{11} \cdot H_2O_4S$ FW 582.58

Mode of Action: Binds to 70S ribosomal subunit; inhibits translocation; elicits miscoding. Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, and mycoplasma.

▶ powder, BioReagent, suitable for cell culture

Use in biotechnology applications to inhibit protein synthesis. Recommended for use in cell culture applications at 100 mg/L.

potency: ≥750 µg per mg
solubility
H_2O .... 10-50 mg/mL (As a stock solution. Stock solutions should be stored at 2-8°C. Stable at 37°C for 5 days.)
kanamycin B ..... <5%
ship: ambient store at: room temp

K1377-1G	1 g
K1377-5G	5 g
K1377-25G	25 g

Lincomycin hydrochloride

Methyl 6,8-dideoxy-6-(1-methyl-4-propyl-2-pyrrolidinecarboxamido)-1-thio-D-*erythro*-α-D-galactooctopyranoside hydrochloride; Lincocin hydrochloride
[859-18-7] $C_{18}H_{34}N_2O_6S \cdot HCl$ FW 443.00

Lincomycin is a lincosamide antibiotic that forms cross-links within the peptidyl transferase loop region of the 23S rRNA.[1]
Mode of action: inhibits bacterial protein synthesis
Antimicrobial spectrum: Gram-positive bacteria

Lit. cited: 1. Kirillov, S. V., et al., Peptidyl transferase antibiotics perturb the relative positioning of the 3′-terminal adenosine of P/P′-site-bound tRNA and 23S rRNA in the ribosome *RNA* **5**, 1003-1013 (1999)

▶ BioReagent, suitable for cell culture

potency: 800-900 units per mg
ship: ambient store at: 2-8°C

L2774-1MU	1000000 units
L2774-5MU	5000000 units

Mitomycin C from *Streptomyces caespitosus*

[50-07-7] $C_{15}H_{18}N_4O_5$ FW 334.33

Inhibitor of DNA synthesis, nuclear division, and cancer cells. Antibacterial to gram positive, gram negative, acid-fast bacilli.

▶ powder, BioReagent, suitable for cell culture

Vial contains 2 mg mitomycin C and 48 mg NaCl.

Mitomycin C is used to generate mitotically inactive feeder cells used in cell culture systems, such as mitotically inactive fibroblast used in embryonic stem cell (ESC) systems.

Mitomycin C is an anti-neoplastic antibiotic, DNA inter-strand, cross-linking, alkylating agent that targets guanine nucleoside in the sequence 5′CpG-3′. It produces oxygen radicals and is preferentially toxic to hypoxic cells.

solubility
H_2O ..... 4 mL/vial, clear to slightly hazy, blue to purple (Stock solutions should be filter sterilized and stored at 2-8 °C in the dark.)
color ..... blue-gray
ship: ambient store at: 2-8°C

M4287-2MG	2 mg
M4287-5X2MG	5 × 2 mg

Mitomycin C (Cat. No. M4287)

Mitomycin C is an antibiotic produced from *Streptomyces caespitosus*. It is an alkylating agent with antineoplastic properties. While mitomycin C is not cell cycle specific, it is most active in the late G1 and early S phase of the cell cycle. It acts by suppressing the synthesis of nucleic acids.

In cell culture, mitomycin C mitotically arrests cells. It is often employed to prepare cells used in feeder layers or preparation of stimulator lymphocytes for the mixed lymphocyte reaction. For example, mitomycin C is used to prepare STO feeder layers used in embryonic stem cell work.

Recommended usage:
Mitomycin C is supplied as a powder. Dissolve the contents of each vial in 2 ml of sterile water (Cat. Nos. W3500 or W1503) or sterile PBS to prepare a 100x stock. Store the reconstituted solution in the dark at 2-8 °C. This solution should not be used for longer than 1 week. Mitomycin C is typically used in inactivation medium at a final concentration of 10-50 µg/ml for the treatment of feeder layers. Please refer to the literature for more specific application information.

Mycophenolic acid

6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoic acid; 6-(1,3-Dihydro-7-hydroxy-5-methoxy-4-methyl-1-oxoisobenzofuran-6-yl)-4-methyl-4-hexanoic acid
[24280-93-1] $C_{17}H_{20}O_6$ FW 320.34

Immunosuppressive agent. Suppresses cytokine-induced nitric oxide production.

Mode of Action: Blocks inosine monophosphate dehydrogenase in the guanosine monophosphate pathway.

Used to select animal cells expressing the *Escherichia coli* gene for xanthine-guanine phosphosribosyl transferase.

▸ powder, BioReagent, suitable for cell culture, ≥98%

Recommended for use as a selection agent at 25 µg/ml.

solubility
methanol ..... 50 mg/mL (Stock solutions should be stored at 2-8°C.)
ship: ambient store at: 2-8°C

M3536-50MG	50 mg
M3536-250MG	250 mg

Mycophenolic Acid (Cat. No. M3536)

Mycophenolic acid is an antibiotic produced by *Penicillium brevicompactum*. It is an inhibitor of the enzyme inosinate dehydrogenase (IMP dehydrogenase) and therefore inhibits *de novo* nucleotide synthesis, i.e. it inhibits XMP and GMP formation.

Mycophenolic acid is used as a selection agent in mammalian protein expression systems where the *E. coli* gene *(Ecogpt)* encoding the enzyme xanthine guanine phosphoribosyltransferase (XGPRT) has been inserted. Mammalian cells transformed with *Ecogpt* can be grown in medium containing aminopterin and mycophenolic acid with xanthine as the sole precursor for purine synthesis.

Recommended usage:
Mycophenolic acid is not soluble in water. It is soluble in ether, chloroform, and alcohol. The use concentration is approximately 25 mg/ml. Please refer to the literature for more specific information.

Neomycin solution

[119-04-0] $C_{23}H_{46}N_6O_{13}$ FW 614.64

▸ with 10 mg/mL neomycin in 0.9% NaCl, sterile-filtered, BioReagent, suitable for cell culture

Mode of action: binds to the 30S and in some cases the 50S subunit causing miscoding; inhibits initiation and elongation during protein synthesis.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.

Formulated to contain 10 mg/ml neomycin in 0.9% sodium chloride.

Recommended for use in cell culture applications at 5 ml/L.

liquid

Stable at 37 °C for 5 days.

endotoxin ..... tested
ship: ambient store at: 2-8°C

N1142-20ML	20 mL

Neomycin trisulfate salt hydrate

[1405-10-3] $C_{23}H_{46}N_6O_{13} \cdot 3H_2SO_4 \cdot xH_2O$ FW 908.88 (Anh)

Mode of action: binds to the 30S and in some cases the 50S subunit causing miscoding; inhibits initiation and elongation during protein synthesis.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.

▸ powder, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 50 mg/L.

potency: ≥600 µg per mg
solubility
H_2O ..... 50 mg/mL (As a stock solution. Stock solutions should be filter sterilized and stored at 2-8°C. Stable at 37°C for 5 days.)
ship: ambient store at: room temp

N6386-5G	5 g
N6386-25G	25 g
N6386-100G	100 g

Nystatin

Fungicidin; Mycostatin
[1400-61-9]

Mode of Action: Increases the permeability of the cell membrane of sensitive fungi by binding to sterols.
Antimicrobial spectrum: Yeasts and molds.
solubility
H_2O ..... insoluble

▸ powder, BioReagent, suitable for cell culture

potency: ≥4,400 USP units per mg

Nystatin is effective as a suspension. Prepare 50 mg/ml stock suspensions in water and store at −20 °C. Stable at 37 °C for 3 days. Use in tissue culture at 50 µg/ml.

Note: Non-sterile powder. Not recommended for aseptic work. See N1638, Nystatin Suspension, Cell Culture Tested.
ship: ambient store at: −20°C

N6261-500KU	500000 units
N6261-5MU	5000000 units
N6261-25MU	25000000 units

▸ powder, γ-irradiated, BioXtra, suitable for cell culture

vial = 240,000 units

Prepared from Nystatin (N 6261).

Nystatin is effective as a suspension. Prepare 50 mg/ml stock suspensions in water and store at −20 °C. Stable at 37 °C for 3 days. Use in tissue culture at 50 µg/ml.
ship: ambient store at: −20°C

N4014-50MG	50 mg

Nystatin Suspension

[1400-61-9]

▸ **suspension, with 10,000 units/mL nystatin in DPBS, aseptically processed, BioReagent, suitable for cell culture**

Mode of Action: Increases the permeability of the cell membrane of sensitive fungi by binding to sterols.
Antimicrobial spectrum: Yeasts and molds.

Recommended for use in cell culture applications at 24 ml/L.

Stable at 37 °C for 3 days.

endotoxin ..... tested
color ..... yellow
ship: dry ice store at: –20°C

N1638-20ML	20 mL
N1638-100ML	100 mL

Paromomycin sulfate salt

[1263-89-4] $C_{23}H_{45}N_5O_{14} \cdot H_2SO_4$ FW 713.71

Antimicrobial spectrum: Gram-negative and Gram-positive bacteria, some protozoan species, and limited antihelminth.
Mode of Action: Inhibits initiation and elongation during protein synthesis.

▸ **powder, BioReagent, suitable for cell culture, ≥98%**

Recommended for use in cell culture applications at 100 mg/L.

potency: ≥675 µg per mg
solubility
H_2O ..... 50 mg/mL (store stock solution at 2-8°C. Stable at 37°C for 5 days.)
ship: ambient store at: room temp

P5057-1G	1 g
P5057-5G	5 g

Penicillin G potassium salt

Benzylpenicillin potassium salt
[113-98-4] $C_{16}H_{17}KN_2O_4S$ FW 372.48

Mode of Action: Inhibits bacterial cell wall synthesis.
Antimicrobial spectrum: Gram-positive bacteria

▸ **powder, BioReagent, suitable for cell culture**

Recommended for use in cell culture applications at 100,000 units/L. Solutions should be filter sterilized and stored at 2-8 °C for up to 1 week, –20 °C for extended periods. Solutions are stable at 37 °C for 3 days.

potency: 1440-1680 units per mg
solubility
H_2O ..... 100 mg/mL
ship: ambient store at: room temp

P7794-1MU	1000000 units
P7794-10MU	10000000 units
P7794-100MU	100000000 units

Penicillin G sodium salt

Benzylpenicillin sodium salt
[69-57-8] $C_{16}H_{17}N_2NaO_4S$ FW 356.37

Mode of Action: Inhibits bacterial cell wall synthesis.
Antimicrobial spectrum: Gram-positive bacteria.

▸ **powder, BioReagent, suitable for cell culture**

potency: ≥1477 units per mg

Recommended for use in cell culture media at 100,000 units/L. Solutions should be filter sterilized and stored at 2-8 °C for up to 1 week, –20 °C for extended periods. Solutions are stable at 37 °C for 3 days.

solubility
H_2O ..... 100 mg/mL (Solutions should be filter sterilized and stored at 2-8°C for 1 week or at -20°C for extended periods. Solutions are stable at 37°C for 3 days.)
ship: ambient store at: room temp

P3032-1MU	1000000 units
P3032-10MU	10000000 units
P3032-25MU	25000000 units
P3032-100MU	100000000 units

Penicillin V potassium salt

Phenoxymethylpenicillinic acid potassium salt; Penicillin V potassium salt
$C_{16}H_{17}N_2O_5SK$ FW 388.48

▸ **powder, BioReagent, suitable for cell culture**

Penicillin V inhibits the synthesis of bacterial cell walls by blocking cell wall peptidoglycan chain cross-linking. Used in cell culture in combination with streptomcyin and other antibiotics.

activity: ~1,500 units/mg
ship: ambient store at: room temp

P4807-10MU	10000000 units

Penicillin-Streptomycin

Pen-Strep

Penicillin-Streptomycin is used to supplement cell culture media to control bacterial contamination.

liquid
endotoxin ..... tested

▸ **with 10,000 units penicillin and 10 mg streptomycin per mL in 0.9% NaCl, sterile-filtered, BioReagent, suitable for cell culture**

Penicillin-Streptomycin is used to supplement cell culture media to control bacterial contamination. Recommended for use in cell culture applications at 10 ml/L.

concentration ..... 100 ×
ship: dry ice store at: –20°C

P0781-20ML	20 mL
P0781-100ML	100 mL

▸ **Solution Stabilized, with 5,000 units penicillin and 5mg streptomycin/mL, sterile-filtered, BioReagent, suitable for cell culture**

Solubilized in a proprietary citrate buffer.

Recommended for use in cell culture media at 20 ml/L.

concentration ..... 50 ×
ship: dry ice store at: –20°C

P4458-20ML	20 mL
P4458-100ML	100 mL

▸ **Solution stabilized, with 10,000 units penicillin and 10 mg streptomycin/mL, sterile-filtered, BioReagent, suitable for cell culture**

Solubilized in a proprietary citrate buffer.

Penicillin-Streptomycin is used to supplement cell culture media to control bacterial contamination. Recommended for use in cell culture applications at 10 ml/L.

concentration ..... 100 ×
ship: dry ice store at: –20°C

P4333-20ML	20 mL
P4333-100ML	100 mL

Penicillin – Streptomycin – Neomycin Solution Stabilized

Pen-Strep-Neo
formulated to contain ~5,000 units penicillin, 5 mg streptomycin and 10 mg neomycin/mL, sterile-filtered, BioReagent, suitable for cell culture

Penicillin-Streptomycin is used to supplement cell culture media to control bacterial contamination.

liquid

endotoxin ..... tested
ship: dry ice store at: –20°C

P4083-20ML	20 mL
P4083-100ML	100 mL

Polymyxin B sulfate salt

[1405-20-5] $C_{55}H_{96}N_{16}O_{13} \cdot 2H_2SO_4$ FW 1385.61

Antibiotic with bactericidal action on *E. coli*.[1,2] Binds to the lipid A portion of bacterial lipopolysaccharides.[3] Induces pore formation in the membranes of cortex cells from excised sorghum roots.[4]
Mode of Action: Binds to and interferes with the permeability of the cytoplasmic membrane.
Antimicrobial spectrum: Gram-negative bacteria.

Mixture of Polymyxin B_1 and B_2 sulfate.

Lit. cited: 1. Cornu, J., *Ann. Microbiol.* **131B**, 121 (1980)
2. Storm, D.R., et al., *Annu. Rev. Biochem.* **46**, 723 (1977)
3. Morrison, D.C. and Jacobs, D.M., *Immunochemistry* **13**, 813 (1976)
4. Lerner, H.R., et al., *Physiol. Plant.* **57**, 90 (1983)

powder, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 50 mg/L.

potency: ≥6,000 USP units per mg

Stock solutions should be sterile filtered and stored at 2-8 °C. Stable at 37 °C for 5 days.

solubility
H_2O ..... 50 mg/mL
ship: ambient store at: 2-8°C

P4932-1MU	1000000 units
P4932-5MU	5000000 units

Puromycin dihydrochloride from *Streptomyces alboniger*

3′-[α-Amino-*p*-methoxyhydrocinnamamido]-3′-deoxy-N,N-dimethyladenosine dihydrochloride
[58-58-2] $C_{22}H_{29}N_7O_5 \cdot 2HCl$ FW 544.43

Nucleoside antibiotic. Protein synthesis inhibitor that causes premature chain termination by acting as an analog of the 3′-terminal end of aminoacyl-tRNA. Prevents growth of bacteria, protozoa, algae, and mammalian cells. Acts very quickly and can kill 99% of cells within 2 days, the resistance gene (puromycin acetyltransferase) gives very effective protection.

powder, BioReagent, suitable for cell culture

May be used in vitro as a selection agent for cells transfected with puromycin N-acetyl transferase gene (pac).

≥98% (HPLC)

solubility
H_2O ..... 50 mg/mL
ship: ambient store at: –20°C

P8833-10MG	10 mg
P8833-25MG	25 mg
P8833-100MG	100 mg

Spectinomycin dihydrochloride pentahydrate

[22189-32-8] $C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$ FW 495.35

Mode of Action: Inhibits protein synthesis (elongation) by interfering with peptidyl tRNA translocation.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria (*Gonnococcus* only).
Mode of Resistance: Mutation in *rps*E (the gene for ribosomal protein S5) prevents binding of spectinomycin.

powder, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 7.5-20 mg/L.

potency: ≥603 µg per mg
ship: ambient store at: 2-8°C

S4014-5G	5 g
S4014-25G	25 g

Streptomycin sulfate salt

[3810-74-0] $C_{21}H_{39}N_7O_{12} \cdot 1.5H_2O_4S$ FW 728.69

Mode of Action: Inhibits prokaryote protein synthesis. Binds to S12 protein of 30S ribosomal subunit, preventing the transition from initiation complex to chain-elongating ribosome, causing miscoding or inhibiting initiation.
Mode of Resistance: Mutation in *rps*L (gene for S12 ribosomal protein) prevents binding of streptomycin to ribosome. Aminoglycoside phosphotransferase also inactivates.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.

potency: ≥720 I.U. per mg

powder, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 100 mg/L. Stock solutions should be sterile-filtered and stored at 2-8 °C for up to a month or at –20 °C for extended periods. Solutions are stable at 37 °C for 3 days.
ship: ambient store at: 2-8°C

S9137-25G	25 g
S9137-100G	100 g

powder, BioXtra, suitable for mouse embryo cell culture

Recommended for use in embryo culture at 50 mg/L. Stock solutions should be sterile-filtered and stored at 2-8 °C for up to a month or at -20 °C for extended periods. Solutions are stable at 37 °C for 3 days. Use to inhibit bacterial protein synthesis at the level of initiation. Use to study mechanisms of streptomycin resistance. Use together with penicillin and other agents to inhibit bacterial contamination in cell culture applications.
ship: ambient store at: 2-8°C

S1277-5G	5 g
S1277-50G	50 g

Tetracycline hydrochloride

[64-75-5] $C_{22}H_{24}N_2O_8 \cdot HCl$ FW 480.90

Mode of Action: Inhibits protein synthesis (elongation) by preventing binding of aminoacyl-tRNA to the 30S subunit.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.
Mode of Resistance: Loss of cell wall permeability.

powder, BioReagent, suitable for cell culture

Recommended for use in cell culture applications at 10 mg/L.

solubility
H_2O ..... 10 mg/mL (as a stock solution. Stock solutions should be filtered sterilized and stored at –20°C. Stable at 37°C for 4 days.)
ship: ambient store at: –20°C

T7660-5G	5 g

Tylosin solution

[74610-55-2]

▶ 8 mg/mL in 0.9% NaCl, sterile-filtered, BioReagent, suitable for cell culture

Interferes with bacterial protein synthesis by binding to the 50S subunit. Antimicrobial spectrum: Gram-positive bacteria and mycoplasma.

Formulated to contain 8 mg/ml tylosin in 0.9% sodium chloride.

Recommended for use in cell culture applications at 1 ml/L.

endotoxin ..... tested

ship: ambient store at: 2-8°C

T3397-20ML	20 mL

Tylosin tartrate

Mixture of tylosin A, B, C, and D tartrates.

▶ powder, BioReagent, suitable for cell culture

Interferes with bacterial protein synthesis by binding to the 50S subunit. Antimicrobial spectrum: Gram-positive bacteria and mycoplasma.

Recommended for use in cell culture applications at 8 mg/L.

potency: ~900 µg tylosin per mg

solubility

H_2O ..... ≤50 mg/mL

ship: ambient store at: 2-8°C

T6271-1G	1 g
T6271-5G	5 g
T6271-10G	10 g

Attachment Factors

Normal attachment, growth, and development of many cell types are dependent on attachment factors and extracellular matrix components. While some cells are able to synthesize these components, others require an exogenous source, particularly when grown in serum-free culture.

To help facilitate attachment, cell spreading, growth, morphology, differentiation, and motility of your cells, Sigma offers an extensive line of attachment and matrix factors. Each lot is cell culture tested to assess its ability to promote cell attachment and spreading.

Attachment Factors Table

Name	Source and	Storage Temp	Target Cells For Attachment	Concentration For Use	Cat. No.
Chondroitin sulfate A, lyophilized powder	Chondroitin sulfate A sodium salt from bovine trachea	2-8°C	Appears to play a regulatory role for chondrocytes, neural cells, and some tumor cells	20 - 2000 µg/cm^2	C9819-5G C9819-25G
Collagen	Collagen solution from bovine skin	2-8°C	A highly purified collagen solution suitable for 3-D matrix formation in cell culture.	6 - 10 µg/cm^2	C4243-20ML C4243-100ML
Collagen	Collagen solution from bovine skin	2-8°C	A highly purified collagen solution suitable for 3-D matrix formation in cell culture.	6 - 10 µg/cm^2	C2124-50ML
Collagen, solid	Collagen from calf skin	2-8°C	Muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, Schwann cells. Mediates the attachment of many cell types. Recommended for use as a cell culture substratum at 6-10 µg/cm^2.	6 - 10 µg/cm^2	C9791-10MG C9791-50MG C9791-100MG C9791-250MG
Collagen, (0.1% solution in 0.1 M acetic acid)	Collagen from calf skin	2-8°C	Recommended for use as a cell culture substratum at 6-10 µg/cm^2. Not suitable for 3D gel formation. Muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, Schwann cells. Mediates the attachment of many cell types.	6 - 10 µg/cm^2	C8919-20ML
Collagen, powder	Collagen from chicken sternal cartilage	2-8°C	Recommeded for use as a cell culture substratum at 6-10 µg/cm^2.	6 - 10 µg/cm^2	C9301-5MG C9301-25MG C9301-100MG
Collagen, lyophilized powder (from sterile-filtered solution)	Collagen from Engelbreth-Holm-Swarm murine sarcoma basement membrane	−20°C	Recommended for use as a cell culture substratum at 6-10 µg/cm^2. Epithelial cells, endothelial cells, muscle cells, nerve cells	6 - 10 µg/cm^2	C0543-1VL
Collagen, powder	Collagen from human placenta	−20°C	Epithelial cells, endothelial cells, muscle cells, nerve cells	6 - 10 µg/cm^2	C5533-5MG
Collagen, powder	Collagen from kangaroo tail	2-8°C	Muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, Schwann cells. Mediates the attachment of many cell types.	6 - 10 µg/cm^2	C1809-10MG
Collagen, powder	Collagen from rat tail	2-8°C	Recommended for use as a cell culture substratum at 6-10 µg/cm^2. This product may not be suitable for 3-D gel formation. Muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, Schwann cells. Mediates the attachment of many cell types.	6 - 10 µg/cm^2	C7661-5MG C7661-10MG C7661-25MG C7661-50MG C7661-100MG

Name	Source and	Storage Temp	Target Cells For Attachment	Concentration For Use	Cat. No.
Collagen Solution	Collagen Solution from human fibroblasts	2-8°C	Purified human collagen solution is produced from neonatal human fibroblasts that have secreted human extracellular matrix (hECM) using a propriety cell culture production system. The human fibroblast cells have undergone significant testing demonstrating safety from viruses and other adventitious agents.	6 - 10 µg/cm^2	C2249-20ML
Collagen Solution	Collagen Solution from rat tail	-	A Type I collagen formulated for use in 3D culture environments.	6 - 10 µg/cm^2	C7868
Collagen, Type I	Collagen, Type I solution from rat tail	2-8°C	Used as a coating material to support adherent cells growth and differentiation.	6 - 10 µg/cm^2	C3867-1VL
Collagen Type IV	Collagen Type IV from human cell culture	–20°C	-	6 - 10 µg/cm^2	C6745-1ML
ECM Gel, liquid	ECM Gel from Engelbreth-Holm-Swarm murine sarcoma	–20°C	Epithelial cells, endothelial cells, muscle cells, nerve cells, tumor cells Recommended for use as a cell culture substratum. For a 24-well plate, use 230-250 µl/well. For a 96-well plate, use 50-100 µl/well. Thaw gel overnight at 2-8 °C before use. The thawed gel may be diluted up to two-fold with cold (2-8 °C) Dulbecco's Modified Eagle's Medium. Gel dilutions should be made before it is added to the plate. ECM will gel within 5 minutes at 20 °C. For prolonged manipulations, work should be conducted below 10 °C. Dispense gel to wells of a multiwell plate using pipettes pre-cooled to 2-8 °C. A gel forms at 37 °C and maintains this form with culture medium for at least 14 days. Cells may be plated on top of a thin gel layer (0.5 mm) or cultured inside a 1 mm layer. When cultured inside, cells should be added to the gel prior to plating at a recommended density of 3-4 × 10^4 cells per mL. To dissociate cells from the gel, use protease (dispase) dissolved in PBS without calcium, magnesium, and EDTA at a working concentration of 0.6-2.4 units/ml.	6 - 10 µg/cm^2	E1270-1ML E1270-5ML E1270-10ML
ECM Gel, liquid	ECM Gel from Engelbreth-Holm-Swarm murine sarcoma	–20°C	Epithelial cells, endothelial cells, muscle cells, nerve cells, tumor cells Recommended for use as a cell culture substratum. For a 24-well plate, use 230-250 µl/well. For a 96-well plate, use 50-100 µl/well. Thaw gel overnight at 2-8 °C before use. The thawed gel may be diluted up to two-fold with cold (2-8 °C) Dulbecco's Modified Eagle's Medium. Gel dilutions should be made before it is added to the plate. ECM will gel within 5 minutes at 20 °C. For prolonged manipulations, work should be conducted below 10 °C. Dispense gel to wells of a multiwell plate using pipettes pre-cooled to 2-8 °C. A gel forms at 37 °C and maintains this form with culture medium for at least 14 days. Cells may be plated on top of a thin gel layer (0.5 mm) or cultured inside a 1 mm layer. When cultured inside, cells should be added to the gel prior to plating at a recommended density of 3-4 × 10^4 cells per mL. To dissociate cells from the gel, use protease (dispase) dissolved in PBS without calcium, magnesium, and EDTA at a working concentration of 0.6-2.4 units/ml.	6 - 10 µg/cm^2	E6909-5ML E6909-10ML
Fibronectin, solution	Fibronectin from bovine plasma	2-8°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F1141-1MG F1141-2MG F1141-5MG
Fibronectin, powder	Fibronectin from bovine plasma	–20°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F4759-1MG F4759-2MG F4759-5MG
Fibronectin	Fibronectin solution from human fibroblasts	–20°C	-	-	F0556-100UL
Fibronectin, lyophilized powder	Fibronectin from human foreskin fibroblasts	–20°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F2518-.5MG
Fibronectin, lyophilized powder	Fibronectin from human plasma	–20°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F2006-1MG F2006-2MG F2006-5MG
Fibronectin, liquid	Fibronectin from human plasma	2-8°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F0895-1MG F0895-2MG F0895-5MG
Fibronectin, lyophilized powder	Fibronectin from human plasma	–20°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F1056-1MG F1056-2MG F1056-5MG

Name	Source and	Storage Temp	Target Cells For Attachment	Concentration For Use	Cat. No.
Fibronectin, powder	Fibronectin from rat plasma	–20°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	1 - 5 µg/cm^2	F0635-.5MG F0635-1MG F0635-2MG
Superfibronectin, solution	Superfibronectin from human plasma and *Escherichia coli*	2-8°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells Recommended for use as a cell culture substratum at 1 µg/cm^2. Optimal conditions for attachment must be determined for each cell line and application.	1 µg/cm^2	S5171-.5MG
Fibronectin Fragment III_1-C, lyophilized powder	Fibronectin Fragment III_1-C human and *Escherichia coli*	–20°C	Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells	0.45 µg/cm^2	F3542-.5MG
Gelatin	Gelatin solution	2-8°C	Used for attachment of a variety of cell types	100 - 200 µg/cm^2	G1393-20ML G1393-100ML
Gelatin, powder	Gelatin from bovine skin	room temp	Used for attachment of a variety of cell types	100 - 200 µg/cm^2	G9391-100G G9391-500G
Gelatin, powder	Gelatin from porcine skin	room temp	Used for attachment of a variety of cell types	100 - 200 µg/cm^2	G1890-100G G1890-500G G1890-1KG
Gelatin, lyophilized powder	Gelatin from porcine skin	room temp	Used for attachment of a variety of cell types	100 - 200 µg/cm^2	G9136-10MG
HydroMatrix™ Peptide Cell Culture Scaffold, powder	HydroMatrix™ Peptide Cell Culture Scaffold	–20°C	-	-	A6982-1ML A6982-5ML A6982-10ML
Laminin, aqueous solution	Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane	–20°C	Laminin supports growth and differentiation of many cell types including epithelial, endothelial, neural, muscle, and liver cells.	1 - 2 µg/cm^2	L2020-1MG
Laminin, liquid	Laminin from human fibroblasts	–70°C	Laminin supports growth and differentiation of many cell types including epithelial, endothelial, neural, muscle, and liver cells.	1 - 2 µg/cm^2	L4544-100UL
Laminin, liquid	Laminin from human placenta	–70°C	Epithelial cells, endothelial cells, muscle cells, tumor cells, hepatocytes, Schwannoma	1 - 2 µg/cm^2	L6274-.5MG
MaxGel™ ECM, liquid	MaxGel™ ECM	–70°C	-	-	E0282-100UL E0282-1ML
SPARC, lyophilized powder	SPARC from murine parietal yolk sac (PYS-2) cells	–20°C	Expressed in a variety of tissues, it inhibits cell spreading and diminishes focal contacts *in vitro*.	4 - 40 µg/cm^2	S5174-25UG
Vitronectin, lyophilized powder	Vitronectin from bovine plasma	2-8°C	Cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma	0.1 µg/cm^2	V9881-50UG
Vitronectin, lyophilized powder	Vitronectin from human plasma	2-8°C	Cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma	0.1 µg/cm^2	V8379-50UG
Vitronectin, lyophilized powder	Vitronectin from rat plasma	2-8°C	Cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma	0.1 µg/cm^2	V0132-50UG

Poly-Lysine

Sigma offers both Poly-D-Lysine and Poly-L-Lysine in several molecular weight ranges. Poly-Lysine enhances electrostatic interaction between negatively-charged ions of the cell membrane and positively-charged surface ions of attachment factors on the culture surface. When adsorbed to the culture surface, it increases the number of positively-charged sites available for cell binding.

Name	Form	Molecular Weight	Conc.	Storage Temp	Application	Cat. No.
Poly-D-lysine	lyophilized powder, γ-irradiated	average mol wt 30,000-70,000	-	–20°C	Recommended as a cell culture substratum when using 0.5 - 1.0 mL of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P7280-5MG
Poly-D-lysine	lyophilized powder, γ-irradiated	70,000-150,000	-	–20°C	Recommended as a cell culture substratum when using 0.5 - 1.0 mL of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P6407-5MG
Poly-D-lysine	lyophilized powder, γ-irradiated	>300,000	-	–20°C	Recommended as a cell culture substratum when using 0.5 - 1.0 mL of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P7405-5MG

Name	Form	Molecular Weight	Conc.	Storage Temp	Application	Cat. No.
Poly-L-lysine	sterile-filtered	70,000-150,000	0.01%	2-8°C	Recommended as a cell culture substratum when using 0.5 mL of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P4707-50ML
Poly-L-lysine	sterile-filtered	150,000-300,000	0.01%	2-8°C	Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P4832-50ML
Poly-L-lysine	lyophilized powder, γ-irradiated	30,000-70,000	-	−20°C	Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P9155-5MG
Poly-L-lysine	lyophilized powder, γ-irradiated	70,000-150,000	-	−20°C	Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P6282-5MG
Poly-L-lysine	lyophilized powder, γ-irradiated	≥300,000	-	−20°C	Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.	P5899-5MG
Poly-L-ornithine	sterile-filtered	30,000-70,000	0.01%	2-8°C	Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2.	P4957-50ML

Fibronectin Proteolytic Fragments

Proteolytic fragments can be used for mapping regions, functions, and activities of fibronectin.

Name	Form	Source	Storage Temp	Binding Activity	Cat. No.
Fibronectin Proteolytic Fragment	lyophilized powder	Fibronectin Proteolytic Fragment from human plasma	−20°C	Gelatin binding fragment.	F0162-.5MG F0162-0.5MG
Fibronectin Proteolytic Fragment	lyophilized powder	Fibronectin Proteolytic Fragment from human plasma	−20°C	Heparin and gelatin binding fragment	F0287-.5MG
Fibronectin Proteolytic Fragment	lyophilized powder	Fibronectin Proteolytic Fragment from human plasma	−20°C	Heparin binding fragment	F9911-.5MG

Disintegrins

Disintegrins interfere with cell adhesion to the extracellular matrix, including adhesion of melanoma cells and fibroblasts to fibronectin. They are also potent inhibitors of platelet aggregation. To meet your research needs, Sigma offers several disintegrins isolated from venom sources. These peptides are of low molecular weight, cysteine-rich, and contain the arginine-glycine-aspartic acid (RGD) sequence. They are all integrin β_1 and β_3 inhibitors.

Name	Source	Form	Sterilization	Storage Temp	Cat. No.
Echistatin, >95%, SDS-PAGE	Echistatin from *Echis carinatus*	lyophilized powder	γ-irradiated	−20°C	E1518-50UG

Proteoglycans

Proteoglycans, which consist of a core protein with one or more glycosaminoglycan side chains, are a significant component of the extracellular matrix. Proteoglycans interact with a variety of molecules in the extracellular matrix, including various cell adhesion molecules and growth factors. To assist in you extracellular matrix research, Sigma offers several proteoglycans from different sources.

Name	Source	Sterilization	Form	Storage Temp	Cat. No.
Aggrecan	Aggrecan from bovine articular cartilage	sterile-filtered	lyophilized powder (from a sterile-filtered solution)	−20°C	A1960-1MG
Biglycan	Biglycan from bovine articular cartilage	sterile-filtered	essentially salt-free, lyophilized powder (from a sterile-filtered solution)	−20°C	B8041-.5MG
Heparan sulfate proteoglycan	Heparan sulfate proteoglycan	sterile-filtered	solution	−20°C	H4777-.1MG

Attachment Factor Products

Aggrecan from bovine articular cartilage

▶ lyophilized powder (from a sterile-filtered solution)

Major structural proteoglycan of cartilage extracellular matrix. Large proteoglycan with a molecular weight greater than 2,500 kDa. Approximately 100-150 glycosaminoglycan (GAG) chains are attached to the core protein (210-250 kDa). The majority of the GAG chains are chondroitin/dermatan sulfate with the remainder being keratan sulfate. This structural molecule produces a rigid, reversibly deformable gel that resists compression. It combines with hyaluronic acid to form very large macromolecular complexes. Addition of small amounts (0.1-2% w/w) of hyaluronic acid to an aggrecan solution (2mg/ml) results in the formation of a complex with an increased hydrodynamic volume and in a significant increase (30-40%) in the relative viscosity of the solution. Aggrecan is a critical component for cartilage structure and the function of joints. The synthesis and degradation of aggrecan are being investigated for their roles in cartilage deterioration during joint injury, disease, and aging. Contains three globular domains, G1, G2, and G3, that are involved in aggregation and hyaluronan binding, cell adhesion, and chondrocyte apoptosis.

Associated gene(s): AGC1 (280985)

sterile-filtered
solubility
H_2O ..... 2 mg/mL
salt ..... essentially free
color ..... white
ship: ambient store at: −20°C

A1960-1MG	1 mg

Biglycan from bovine articular cartilage

Interacts with collagen type I, as well as with fibronectin and TGF-β.

▶ essentially salt-free, lyophilized powder (from a sterile-filtered solution)

Biglycan is a leucine-rich repeat proteoglycan that interacts strongly with collagen types I and II. Biglycan is an extracellular component that binds TGF-β 1, facilitates bone mineralization and supports osteoblast differentiation thru BMP-4 binding.

Associated gene(s): BGN (280733)

sterile-filtered

mol wt 200-350 kDa (proteoglycan consisting of a 45 kDa core protein and two chrondroitin/dermatan sulfate glycosaminoglycan chains)
ship: ambient store at: −20°C

B8041-.5MG	0.5 mg

Chondroitin sulfate A sodium salt from bovine trachea

Alternating Copoly β-glucuronic acid-(1→3)-N-acetyl-β-galactosamine-4-sulfate-(1→4)
[39455-18-0]

▶ lyophilized powder, BioReagent, suitable for cell culture

Appears to play a regulatory role for chondrocytes, neural cells, and some tumor cells

Chondroitin sulfate A is composed of alternating N-acetylgalactosamine and glucuronic acid residues wherein carbon 4 of the N-acetygalactosamines is sulfated. Chondroitin sulfate is a structural component of cartilage.

≥60% (balance is chondroitin sulfate C)
ship: ambient store at: 2-8°C

C9819-5G	5 g
C9819-25G	25 g

Collagen solution from bovine skin NEW

▶ 6 mg/mL, sterile-filtered, BioReagent, suitable for cell culture

Purified using a GMP manufacturing process that included prion and/or viral inactivation steps.

A highly purified collagen solution suitable for 3-D matrix formation in cell culture.

The raw collagen used to prepare this product has been isolated from a closed herd and purified using a GMP manufacturing process that includes inactivation of any possible prion or viral contamination.

endotoxin ..... ≤0.5 EU/mg
ship: wet ice store at: 2-8°C

C2124-50ML	50 mL

Collagen from calf skin

[9007-34-5]

Muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, Schwann cells. Mediates the attachment of many cell types.

Collagen is classified into a number of structurally and genetically distinct types. We use the nomenclature proposed by Bornstein and Traub. Do not confuse Sigma type designations with recognized collagen classification types.

not available in Canada (at this time; for questions or status updates, please email us at antibody.canada@sial.com)

▶ Bornstein and Traub Type I, (0.1% solution in 0.1 M acetic acid), aseptically processed, BioReagent, suitable for cell culture

Recommended for use as a cell culture substratum at 6-10 µg/cm^2. Not suitable for 3D gel formation.

concentration ..... (Consult Certificate of Analysis for exact concentration)
ship: wet ice store at: 2-8°C

C8919-20ML	20 mL

▶ Bornstein and Traub Type I, solid, BioReagent, suitable for cell culture

Recommended for use as a cell culture substratum at 6-10 µg/cm^2.

Prepared by a modification of Gallop, P.M. and Seifter, S., *Meth. Enzymol.*, **VI**, 635 (1963).

solubility
0.1 M acetic acid ..... 1 mg/mL (Allow to stir at room temperature 1-3 hours until dissolved.)
ship: ambient store at: 2-8°C

C9791-10MG	10 mg
C9791-50MG	50 mg
C9791-100MG	100 mg
C9791-250MG	250 mg

Collagen from chicken sternal cartilage

[9007-34-5]

Collagen is classified into a number of structurally and genetically distinct types. We use the nomenclature proposed by Bornstein and Traub. Do not confuse Sigma type designations with recognized collagen classification types.

Type II (Miller), powder, BioReagent, suitable for cell culture

Major component of articular cartilage.

Recommeded for use as a cell culture substratum at 6-10 µg/cm^2.

Collagen terminology using the Miller designation originates from the reference; Miller, E.J., *Biochemistry*, 10,1652-1659 (1971)

Prepared by a modification of the method of Trentham, D.E., et al.

solubility
acetic acid .. 0.5-2.0 mg/mL (Dissolve for several hours at 2-8 °C, occasionally swirling.)

ship: ambient store at: 2-8°C

C9301-5MG	5 mg
C9301-25MG	25 mg
C9301-100MG	100 mg

Collagen from Engelbreth-Holm-Swarm murine sarcoma basement membrane

EHS-collagen
[9007-34-5]

Collagen is classified into a number of structurally and genetically distinct types. We use the nomenclature proposed by Bornstein and Traub. Do not confuse Sigma type designations with recognized collagen classification types.

Type IV (Miller), lyophilized powder (from sterile-filtered solution), BioReagent, suitable for cell culture

Native collagen

Epithelial cells, endothelial cells, muscle cells, nerve cells

Recommended for use as a cell culture substratum at 6-10 µg/cm^2.

Associated gene(s): Col4a1 (12826), Col4a2 (12827), Col4a3 (12828), Col4a5 (12830), Col4a6 (94216)

Chloroform treated.
Pepsin is not used in the preparation.

0.75 mg/vial

solubility
0.25% acetic acid ..... 0.5-2.0 mg/mL (Keep at 2-8 °C for several hours, occasionally swirling.)

ship: dry ice store at: –20°C

C0543-1VL	1 vial

Collagen from human placenta

[9007-34-5]

Collagen is classified into a number of structurally and genetically distinct types. We use the nomenclature proposed by Bornstein and Traub. Do not confuse Sigma type designations with recognized collagen classification types.

HIV and hepatitis B ..... none detected

Bornstein and Traub Type IV, powder, BioReagent, suitable for cell culture

Epithelial cells, endothelial cells, muscle cells, nerve cells

Associated gene(s): COL4A1 (1282), COL4A2 (1284), COL4A3 (1285), COL4A3BP (10087), COL4A4 (1286), COL4A5 (1287), COL4A6 (1288)

Prepared by a modification of the pepsin extraction method of Niyibizi, C. et. al

solubility
acetic acid ..... 0.5-2.0 mg/mL (Dissolve for several hours at 2-8 °C, occasionally swirling.)
HIV and hepatitis B ..... none detected (PCR)

ship: ambient store at: –20°C

C5533-5MG	5 mg

Collagen from rat tail

[9007-34-5]

Collagen is classified into a number of structurally and genetically distinct types. We use the nomenclature proposed by Bornstein and Traub. Do not confuse Sigma type designations with recognized collagen classification types.

Bornstein and Traub Type I, powder, BioReagent, suitable for cell culture

Muscle cells, hepatocytes, spinal ganglion, embryonic lung cells, Schwann cells. Mediates the attachment of many cell types.

Recommended for use as a cell culture substratum at 6-10 µg/cm^2. This product may not be suitable for 3-D gel formation.

Associated gene(s): Col1a1 (29393)

Prepared by a modification of the extraction method of Bornstein, M.B., *Lab. Invest.*, **7**, 134 (1958).

solubility
0.1 M acetic acid ..... 1 mg/mL (Allow to stir at room temperature 1-3 hours until dissolved.)

ship: ambient store at: 2-8°C

C7661-5MG	5 mg
C7661-10MG	10 mg
C7661-25MG	25 mg
C7661-50MG	50 mg
C7661-100MG	100 mg

Collagen Solution from human fibroblasts NEW

3 mg/mL, sterile-filtered, BioReagent, suitable for cell culture

Purified human collagen solution is produced from neo-natal human fibroblasts that have secreted human extracellular matrix (hECM) using a propriety cell culture production system. The human fibroblast cells have undergone significant testing demonstrating safety from viruses and other adventitious agents.

Collagen from human fibroblast (C2249) is approximately 97% Type I human collagen with the remainder being comprised of Type III collagen.

endotoxin ..... <0.5 EU/mg

ship: ambient store at: 2-8°C

C2249-20ML	20 mL

Collagen Type IV from human cell culture NEW

[9007-34-5]

Bornstein and Traub Type IV, 0.3 mg/mL, sterile-filtered, BioReagent, suitable for cell culture

Collagen IV, derived from human fibroblasts and epithelial cells in a co-culture system, creates an *in vitro* ECM (extracellular matrix) and serves as an excellent biological scaffold for three dimensional cell culture.

Collagen IV is a heterotrimeric molecules containing two α1-like and one α2-like chain. It is considered essential for completion of embryogenesis and is necessary for proper tissue organization and structural integrity. It is used *in vitro* as a substrate to enhance adherence and proliferation of many cell types. Produced by human fibroblasts and epithelial cells, Collagen IV is then purified biochemically and provided as a sterile solution at a concentration of 0.3mg/ml in 25% acetic acid.

Associated gene(s): COL4A1 (1282)

ship: dry ice store at: –20°C

C6745-1ML	1 mL

Echistatin from *Echis carinatus*

[129038-42-2]

Disintegrins represent a novel family of integrin β1 and β3 inhibitor proteins isolated from viper venoms. They are low molecular-weight, cysteine-rich peptides containing the Arg-Gly-Asp (RGD) sequence. They are the most potent known inhibitors of integrin function. Disintegrins interfere with cell adhesion to the extracellular matrix, including adhesion of melanoma cells and fibroblasts to fibronectin, and are potent inhibitors of platelet aggregation.

▸ lyophilized powder, γ-irradiated, BioXtra

>95% (SDS-PAGE)

ship: ambient store at: –20°C

E1518-50UG	50 µg

ECM Gel from Engelbreth-Holm-Swarm murine sarcoma

Epithelial cells, endothelial cells, muscle cells, nerve cells, tumor cells

Recommended for use as a cell culture substratum. For a 24-well plate, use 230-250 µl/well. For a 96-well plate, use 50-100 µl/well. Thaw gel overnight at 2-8 °C before use. The thawed gel may be diluted up to two-fold with cold (2-8 °C) Dulbecco's Modified Eagle's Medium. Gel dilutions should be made before it is added to the plate. ECM will gel within 5 minutes at 20 °C. For prolonged manipulations, work should be conducted below 10 °C. Dispense gel to wells of a multiwell plate using pipettes pre-cooled to 2-8 °C. A gel forms at 37 °C and maintains this form with culture medium for at least 14 days. Cells may be plated on top of a thin gel layer (0.5 mm) or cultured inside a 1 mm layer. When cultured inside, cells should be added to the gel prior to plating at a recommended density of 3-4 × 10^4 cells per mL. To dissociate cells from the gel, use protease (dispase) dissolved in PBS without calcium, magnesium, and EDTA at a working concentration of 0.6-2.4 units/ml.

dialyzed against chloroform

ECM gel may be stored up to 72 hours at 2-8 °C.

endotoxin ..... tested

▸ EHS matrix
liquid, BioReagent, suitable for cell culture

ECM gel is composed primarily of laminin, collagen type IV, heparan sulfate proteoglycan and entactin. Approximately 8-12 mg/ml basement membrane matrix protein in Dulbecco's modified Eagle's medium with 50 µg/ml gentamicin.

Associated gene(s): Lama1 (16772), Lama3 (16774), Lama5 (16776), Lamb1-1 (16777), Lamc1 (226519), Lamc2 (16782), Nid1 (18073), Sdc2 (15529)

concentration ..... 8 - 12 mg/mL

ship: dry ice store at: –20°C

E1270-1ML	1 mL
E1270-5ML	5 mL
E1270-10ML	10 mL

▸ growth-factor reduced, without phenol red, liquid, BioReagent, suitable for cell culture NEW

Associated gene(s): Hspg2 (15530), Lama1 (16772), Lama3 (16774), Lama5 (16776), Lamb1-1 (16777), Lamc1 (226519), Lamc2 (16782), Nid1 (18073), Sdc2 (15529)

concentration ..... 7-9 mg/mL

ship: dry ice store at: –20°C

E6909-5ML	5 mL
E6909-10ML	10 mL

Fibronectin from bovine plasma

Cold insoluble globulin; CIG
[86088-83-7]

Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells

Recommended for use as a cell culture substratum at 1-5 µg/cm^2 or 0.5-50 µg/ml. Optimal concentration depends on cell type as well as the application or research objectives.

Protein homogeneity is evaluated by immunoelectrophoresis.

Vortexing, excessive agitation, repeated freezing and thawing of reconstituted fibronectin are not recommended.

▸ solution, sterile-filtered, BioReagent, suitable for cell culture

Solution, 1mg/mL (in 0.5 M NaCl, 0.05 M Tris, pH 7.5)

suitable for

ship: wet ice store at: 2-8°C

F1141-1MG	1 mg
F1141-2MG	2 mg
F1141-5MG	5 mg

▸ powder, BioReagent, suitable for cell culture

Lyophilized from 0.05 M Tris buffered saline, pH 7.5

Store reconstituted solution in working aliquots at -20°C or lower.

solubility

H_2O .... 1 mg/mL 37 °C (Store reconstituted solution in working aliquots at -20°C or lower.)

ship: dry ice store at: –20°C

F4759-1MG	1 mg
F4759-2MG	2 mg
F4759-5MG	5 mg

Fibronectin solution from human fibroblasts NEW

▸ cell culture derived, ~0.5 mg/mL, sterile-filtered, BioReagent, suitable for cell culture

Fibronectin is a high molecular weight extracellular matrix glycoprotein that is involved in many cellular processes including tissue repair, embryogenesis, blood clotting, and cell migration/adhesion. It is also used *in vitro* as a substrate to enhance adherence and proliferation of many cell types. Fibronectin is produced by human fibroblasts, purified biochemically, and supplied as a sterile solution at a concentration of 0.5 mg/ml in CAPS saline buffer.

Associated gene(s): FN1 (2335)

ship: ambient store at: –20°C

F0556-100UL	100 µL

Fibronectin from human foreskin fibroblasts

CIG; Cold insoluble globulin
[86088-83-7]

Recommended for use as a cell culture substratum at 1-5 µg/cm^2 or 0.5-50 µg/ml. Optimal concentration depends on cell type as well as the application or research objectives.

▸ lyophilized powder, BioReagent, suitable for cell culture

Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells

Lyophilized in CAPS buffered saline

Associated gene(s): FN1 (2335)

dialyzed against chloroform

Store reconstituted solution in working aliquots at -20°C or lower.

Protein homogeneity is evaluated by immunoelectrophoresis.

Vortexing, excessive agitation, repeated freezing and thawing of reconstituted fibronectin are not recommended.

solubility
H_2O ..... 1 mg/mL 37 °C (Store reconstituted solution in working aliquots at -20°C or lower.)

ship: ambient store at: –20°C

F2518-.5MG	0.5 mg

Fibronectin from human plasma

Cold insoluble globulin; CIG
[86088-83-7]

Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells

Recommended for use as a cell culture substratum at 1-5 µg/cm^2 or 0.5-50 µg/ml. Optimal concentration depends on cell type as well as the application or research objectives.

Protein homogeneity is evaluated by immunoelectrophoresis.

Vortexing, excessive agitation, repeated freezing and thawing of reconstituted fibronectin are not recommended.

lyophilized powder, BioReagent, suitable for cell culture

Source material tested negative for HBsAg and HIV antibody.

Lyophilized from 0.05 M Tris buffered saline, pH 7.5

Associated gene(s): FN1 (2335)

Store reconstituted solution in working aliquots at -20°C or lower.

solubility
H_2O .... 1 mg/mL 37 °C (Store reconstituted solution in working aliquots at -20°C or lower.)

ship: dry ice store at: –20°C

F2006-1MG	1 mg
F2006-2MG	2 mg
F2006-5MG	5 mg

liquid, 0.1% (Solution), BioReagent, suitable for cell culture

Source material tested negative for HBsAg, HCV, and HIV antibody.

Associated gene(s): FN1 (2335)

ship: wet ice store at: 2-8°C

F0895-1MG	1 mg
F0895-2MG	2 mg
F0895-5MG	5 mg

lyophilized powder, BioReagent, suitable for cell culture, ≥95% (SDS-PAGE) NEW

Source material tested negative for HBsAg, HCV, and HIV antibody.

Lyophilized from 0.05 M Tris buffered saline, pH 7.5

Package size based on protein content

Store reconstituted solution in working aliquots at -20°C or lower.

Endotoxin tested

solubility
H_2O ..... 1 mg/mL 37 °C

ship: dry ice store at: –20°C

F1056-1MG	1 mg
F1056-2MG	2 mg
F1056-5MG	5 mg

Fibronectin from rat plasma

Cold insoluble globulin; CIG
[86088-83-7]

Recommended for use as a cell culture substratum at 1-5 µg/cm^2 or 0.5-50 µg/ml. Optimal concentration depends on cell type as well as the application or research objectives.

powder, BioReagent, suitable for cell culture

Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells

Lyophilized from 0.05 M Tris buffered saline, pH 7.5

Associated gene(s): Fn1 (25661)

Store reconstituted solution in working aliquots at -20°C or lower.

Single arc when reacted with anti-fibronectin by IEP (immunoelectrophoresis).

Vortexing, excessive agitation, repeated freezing and thawing of reconstituted fibronectin are not recommended.

solubility
H_2O ..... 1 mg/mL 37 °C, clear, colorless (some particles)

ship: dry ice store at: –20°C

F0635-.5MG	0.5 mg
F0635-1MG	1 mg
F0635-2MG	2 mg

Superfibronectin from human plasma

sFN
solution, sterile-filtered, BioReagent, suitable for cell culture

Complex of recombinant human fibronectin fragment III-C and human plasma fibronectin. Resembles *in vivo* matrix form of fibronectin.

Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells

Recommended for use as a cell culture substratum at 1 µg/cm^2. Optimal conditions for attachment must be determined for each cell line and application.

Solution in 0.05 M Tris buffered saline.

recombinant, expressed in *Escherichia coli*

Associated gene(s): FN1 (2335)

Source material tested for HBsAg and HIV antibody.

ship: ambient store at: 2-8°C

S5171-.5MG	0.5 mg

Fibronectin Adhesion-promoting Peptide

Trp-Gln-Pro-Pro-Arg-Ala-Arg-Ile [125720-21-0] $C_{47}H_{74}N_{16}O_{10}$ FW 1023.19

≥95% (HPLC)

Sequence found in the carboxy-terminal heparin-binding domain of fibronectin.

composition
Peptide content ~70%

ship: ambient store at: –20°C

F3667-1MG	1 mg
F3667-5MG	5 mg

Fibronectin Fragment III_1-C human

FF III_1-C

recombinant, expressed in *Escherichia coli*, lyophilized powder, >95% (SDS-PAGE)

Promotes cross-linking of fibronectin to form matrix fibril-like multimers.

Epithelial cells, mesenchymal cells, neuronal cells, fibroblasts, neural crest cells, endothelial cells

Associated gene(s): FN1 (2335)

essentially salt free

mol wt ~7 kDa

Package size based on protein content

ship: ambient store at: −20°C

F3542-.5MG	0.5 mg

Fibronectin-like Engineered Protein Polymer-plus genetically engineered

ProNectin® F Plus
powder, sterile; autoclaved, BioReagent, suitable for cell culture

Positively-charged, protein polymer which incorporates multiple copies of the RGD cell attachment epitope from human fibronectin between repeated structural peptide units.[1]

Recommended for use as a cell culture substratum at 2-10 µg/cm^2.

mol wt ~110 kDa, SDS-PAGE

Dilutions can be made to a working concentration with balanced salt solution; stable up to 2 days at RT. Coated labware is stable at least 4 months at RT.

Similar to F5022 but more highly charged polymer which encourages better adhesion in many cell lines.

solubility

H_2O ..... 1 mg/mL (stable for 12 mo. at RT)

Lit. cited: 1. Esty, A., Receptor-specific serum-free cell attachment using a highly stable engineered protein polymer. *Am. Biotechnol. Lab.* 44 (1991)

ship: ambient store at: room temp

F8141-1MG	1 mg

Fibronectin-like Protein Polymer genetically engineered

ProNectin® F
lyophilized powder, autoclaved, BioReagent, suitable for cell culture

Positively-charged, protein polymer which incorporates multiple copies of the RGD cell attachment epitope from human fibronectin between repeated structural peptide units.[1]

Recommended as a cell culture substratum at 2-10 µg/cm^2.

mol wt ~110 kDa, SDS-PAGE

mol wt 75 kDa, gene sequence (differences are due to the unusual amino acid composition of the protein)

Supplied with diluent in separate vial.

Dilutions can be made to a working concentration with balanced salt solution; stable up to 2 days at RT. Coated labware is stable at least 4 mos at RT.

solubility

4.5 M $LiClO_4$ ..... 1 mg/mL (stable for 12 mo. at RT.)

Lit. cited: 1. Esty, A., Receptor-specific serum-free cell attachment using a highly stable engineered protein polymer. *Am. Biotechnol. Lab.* 44 (1991)

ship: ambient store at: room temp

F5022-1MG	1 mg
F5022-5MG	5 mg

Fibronectin Proteolytic Fragment from human plasma

The proteolytic fragments can be used for mapping regions, functions, and activities of fibronectin.

Lyophilized from phosphate buffered saline with sucrose as a cryoprotectant

HIV and HBsAg ..... source material tested negative

Small proteolytic fragments ..... may contain traces

lyophilized powder, 45 kDa

Gelatin binding fragment.

≥90% (SDS-PAGE)

ship: ambient store at: −20°C

F0162-0.5MG	500 µg
F0162-.5MG	0.5 mg

lyophilized powder, 70 kDa

Heparin and gelatin binding fragment

Associated gene(s): FN1 (2335)

>90% (SDS-PAGE)

This N-terminal 70 kDa fragment is obtained by Cathepsin D digestion.

ship: ambient store at: −20°C

F0287-.5MG	0.5 mg

lyophilized powder, 30 kDa

Heparin binding fragment

Associated gene(s): FN1 (2335)

>90% (SDS-PAGE)

ship: ambient store at: −20°C

F9911-.5MG	0.5 mg

Gelatin solution

[9000-70-8]

Type B, 2% in H2O, tissue culture grade, sterile, BioReagent, suitable for cell culture

Applications include using gelatin coating cell culture plates to improve cell attachment for a variety of cell types, addition to PCR to help stabilize Taq DNA polymerase, and use as a blocking reagent in Western blotting, ELISA, and immunohistochemistry.

Gelatin is a heterogeneous mixture of water-soluble proteins of high average molecular matrices, present in collagen. The proteins are extracted by boiling skin, tendons, ligaments, bones, etc. in water. Type A gelatin is derived from acid-cured tissue and Type B gelatin is derived from lime-cured tissue.

Derived from lime-cured tissue bovine skin

endotoxin ..... tested

ship: ambient store at: 2-8°C

G1393-20ML	20 mL
G1393-100ML	100 mL

Gelatin from bovine skin

[9000-70-8]

Recommended for use as a cell culture substratum at 0.1-0.2 mg/cm^2 or 5-10 µl/cm^2. Optimal concentration depends on cell type as well as the application or research objectives.

Type B, powder, BioReagent, suitable for cell culture

Used for attachment of a variety of cell types

not available in Canada (at this time; for questions or status updates, please email us at antibody.canada@sial.com)

Derived from lime-cured tissue

gel strength ..... ~225 g Bloom

ship: ambient store at: room temp

G9391-100G	100 g
G9391-500G	500 g

Gelatin from porcine skin

[9000-70-8]

Used for attachment of a variety of cell types

Recommended for use as a cell culture substratum at 0.1-0.2 mg/cm^2 or 5-10 µl/cm^2. Optimal concentration depends on cell type as well as the application or research objectives.

Derived from acid-cured tissue

gel strength ..... ~300 g Bloom

Type A, powder, BioReagent, suitable for cell culture

ship: ambient store at: room temp

G1890-100G	100 g
G1890-500G	500 g
G1890-1KG	1 kg

Type A, lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture

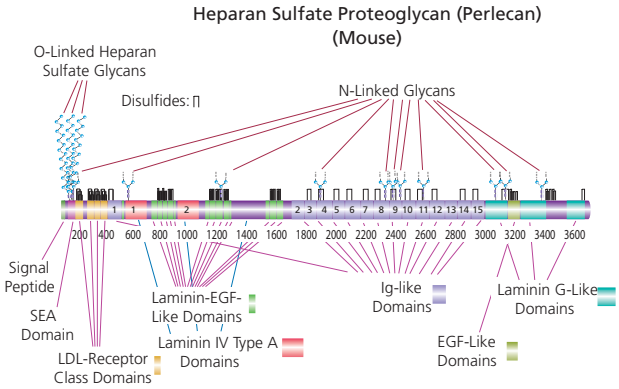
ship: ambient store at: room temp

G9136-10MG	10 mg

Heparan sulfate proteoglycan

HSPG

Extracellular matrix component that binds to fibroblast growth factors, vascular endothelial growth factor (VEGF) and VEGF receptors through its sugar moiety. Acts as a docking molecule for matrilysin (MMP-7) and other matrix metalloproteinases.



Heparan sulfate proteoglycan is an integral part of the basement membrane. It is a large biomolecule with a molecular mass >400 kDa, composed of a core protein covalently bound to heparan sulfate chains. The number of the polysaccharide chains and the size of the core protein may vary according to the source.

≥400 µg/mL glycosaminoglycan, sterile-filtered

Composed of a core protein covalently bound to heparan sulfate chains.

For cell culture use.

Isolated from basement membrane of Engelbreth-Holm-Swarm mouse sarcoma.

Solution in 50 mM Tris HCl, 150 mM NaCl, 1 mM EDTA, 0.1 mM PMSF, pH 7.4, containing ≥400 µg protein per ml.

solution

Uronic acid ..... ≥100 µg/mL

ship: dry ice store at: −20°C

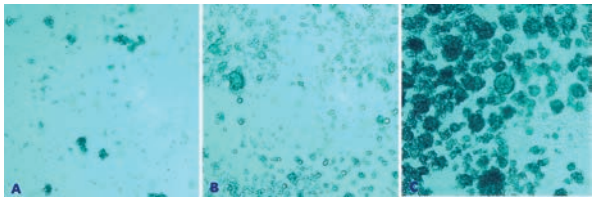
H4777-.1MG	0.1 mg

HydroMatrix™ Peptide Cell Culture Scaffold NEW

A synthetic peptide nanofiber scaffold, HydroMatrix offers the precision and control of a synthesized matrix with the natural three dimensional architecture of highly crosslinked peptide hydrogel. The HydroMatrix scaffold self-assembles from fluid precursors into a highly cross-linked peptide 3-dimensional hydrogel in response to changes in temperature or ionic strength. By adjusting the concentration of the HydroMatrix solution, researchers are able to control the flexibility of the 3-D architecture, and tailor the structure to meet their individual needs.

HydroMatrix promotes cell growth and migration and has been shown to support the proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, and keratinocytes.

from synthetic



HydroMatrix Peptide Hydrogel enables excellent cell growth. Rat neural stem cells (NSCs) were cultured on three surfaces. NSCs grew poorly on tissue culture plastic (A) and slightly better in poly-L-Lysine/laminin coated plates (B). NSCs demonstrated excellent growth on HydroMatrix Peptide Hydrogel 0.5% (w/v) (C).

mixture, powder

ship: ambient store at: –20°C

A6982-1ML	1 mL
A6982-5ML	5 mL
A6982-10ML	10 mL

96 well plate

ship: ambient store at: –20°C

H4165-1EA	1 ea

24 well plate

ship: ambient store at: –20°C

H4040-1EA	1 ea

6 well plate

ship: ambient store at: –20°C

H3915-1EA	1 ea

Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane

[114956-81-9]

Recommended for use as a cell culture substratum at 1-2 µg/cm^2. Optimal concentration depends on cell type as well as the application or research objectives.

1 mg/mL in Tris buffered NaCl, sterile-filtered, BioReagent, suitable for cell culture

Laminin supports growth and differentiation of many cell types including epithelial, endothelial, neural, muscle, and liver cells.

Associated gene(s): Lama1 (16772), Lama3 (16774), Lama5 (16776), Lamb1-1 (16777), Lamc1 (226519), Lamc2 (16782)

aqueous solution

ship: dry ice store at: –20°C

L2020-1MG	1 mg

Laminin from human fibroblasts NEW

cell culture derived, liquid, sterile-filtered

Laminin supports growth and differentiation of many cell types including epithelial, endothelial, neural, muscle, and liver cells.

Laminin is an epithelial cell adhesion glycoprotein, which is composed of 3 chains designated α1, β1, and γ1. Laminin promotes adhesion, differentiation, migration, and growth of many cells *in vitro*. Laminin is produced in human fibroblasts and epithelial cells in a co-culture system, which produces an extracellular matrix composed of many proteins, including laminin. Laminin is then purified biochemically and provided as a sterile solution at a concentration of 0.5 mg/ml in TBS (tris buffered saline), pH7.4.

Associated gene(s): LAMB1 (3912)

ship: ambient store at: –70°C

L4544-100UL	100 µL

Laminin from human placenta

[114956-81-9]

Recommended for use as a cell culture substratum at 1-2 µg/cm^2. Optimal concentration depends on cell type as well as the application or research objectives.

liquid, BioReagent, suitable for cell culture

Epithelial cells, endothelial cells, muscle cells, tumor cells, hepatocytes, Schwannoma

Associated gene(s): LAMB1 (3912)

sterile-filtered

Purified by immunoaffinity from pepsinized placenta.

concentration ..... 0.5 mg/mL in Tris buffered NaCl

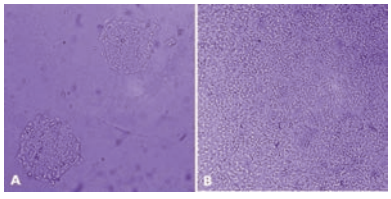
ship: dry ice store at: –70°C

L6274-.5MG	0.5 mg

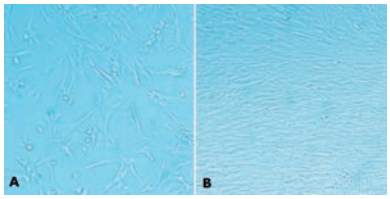
MaxGel™ ECM NEW

Produced *in vitro*, MaxGel human ECM Matrix provides a rich three dimensional environment to promote cellular proliferation. MaxGel ECM Matrix contains extracellular matrix components including collagens, laminin, fibronectin, tenascin, elastin, a number of proteoglycans and glycosamino-glycans. The cell-cultured derived BME effectively reproduces the cooperative interaction of epithelia and mesenchyme during development and in organotypic cell culture of skin.

The human MaxGel ECM Matrix promotes cell growth and migration and has been shown to support the proliferation of many cell types, including neural stem cells, neurons, glia, astrocytes, fibroblasts, hepatocytes and keratino-cytes.



MaxGel™ Human ECM enables improved expansion of Adult Keratinocytes. HaCaT cells (derived from human adult skin keratinocytes) were grown for 24 hours after plating on tissue culture plastic (A) and on 1% human ECM (B), which demonstrates that HaCaT cells proliferate better on MaxGel Human ECM.



MaxGel™ Human ECM enables improved expansion of Fetal Lung Fibroblasts. MRC-5 cells (derived from human fetal lung fibroblasts) were grown for 24 hours after plating on tissue culture plastic (A) and on 1% human ECM (B). As seem with other cells, MRC-5 cells propogate better on MaxGel Human ECM.

▸ mixture, liquid

ship: ambient store at: –70°C

E0282-100UL	100 µL
E0282-1ML	1 mL

▸ 96 well plate

Approximate volume per well is 75 μl

concentration ..... 0.8-1.2 ng/mL protein

ship: ambient store at: –70°C

M1073-1EA	1 ea

Poly-D-lysine hydrobromide

PDL

[27964-99-4] D-Lys-(D-Lys)$_n$-D-Lys · xHBr

Recommended as a cell culture substratum when using 0.5 - 1.0 mL of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.

Poly-lysine with mol. wt. >30,000 is useful in promoting cell adhesion to solid substrates.

Molecular weight based on viscosity.

For additional technical information on polyamino acids please visit the Polyamino acid FAQ resource.

▸ average mol wt 30,000-70,000, lyophilized powder, γ-irradiated, BioReagent, suitable for cell culture

This product has a lowerer binding site density, but is less viscous than P6407. It is a poly-D-lysine that can be used with cells that digest poly-L-lysine polymers.

ship: ambient store at: –20°C

P7280-5MG	5 mg

▸ mol wt 70,000-150,000, lyophilized powder, γ-irradiated, BioReagent, suitable for cell culture

This product has a higher binding site density, but is more viscous than P7280. It is a poly-D-lysine that can be used with cells that digest poly-L-lysine polymers.

ship: ambient store at: –20°C

P6407-5MG	5 mg

▸ mol wt >300,000, lyophilized powder, γ-irradiated, BioReagent, suitable for cell culture

ship: ambient store at: –20°C

P7405-5MG	5 mg

Poly-L-lysine solution

PDL Solution

[25988-63-0]

For additional technical information on polyamino acids please visit the Polyamino acid FAQ resource.

endotoxin ..... tested

▸ mol wt 70,000-150,000, 0.01%, sterile-filtered, BioReagent, suitable for cell culture

This poly-L-lysine is relatively low density which provides good handling characteristics. However, it may be degraded by some cell types. In that case a poly-D-lysine should be chosen.

Recommended as a cell culture substratum when using 0.5 mL of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.

ship: ambient store at: 2-8°C

P4707-50ML	50 mL

▸ mol wt 150,000-300,000, 0.01%, sterile-filtered, BioReagent, suitable for cell culture

This poly-L-lysine is relatively high density which provides good binding characteristics, but it is more viscous and difficult to handle. It may be degraded by some cell types. In that case a poly-D-lysine should be chosen.

Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.

ship: ambient store at: 2-8°C

P4832-50ML	50 mL

Poly-L-lysine hydrobromide

[25988-63-0]

Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm^2. Optimal conditions for attachment must be determined for each cell line and application.

Poly-lysine with mol. wt. >70,000 is useful in promoting cell adhesion to solid substrates.

Molecular weight based on viscosity. Also assayed by MALLS.

For additional technical information on polyamino acids please visit the Polyamino acid FAQ resource.

▸ PDL HBr
mol wt 30,000-70,000, lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture

ship: ambient store at: –20°C

P9155-5MG	5 mg

▸ PLL HBr
mol wt 70,000-150,000, lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture

ship: ambient store at: –20°C

P6282-5MG	5 mg

▸ PDL HBr
mol wt ≥300,000, lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture

ship: ambient store at: –20°C

P5899-5MG	5 mg

Poly-L-ornithine solution

[27378-49-0]

▸ mol wt 30,000-70,000, 0.01%, sterile-filtered, BioReagent, suitable for cell culture

Poly-L-ornithine has been used successfully for the attachement and study of neural cell differentiation and outgrowth. Recommended as a cell culture substratum when using 0.5 ml of a 0.1 mg/ml solution to coat 25 cm2.

Prepared in cell culture grade water.

For additional technical information on polyamino acids please visit the Polyamino acid FAQ resource.

endotoxin ..... tested

ship: ambient store at: 2-8°C

P4957-50ML	50 mL

SPARC from murine parietal yolk sac (PYS-2) cells

Secreted protein acidic and rich in cysteine; Osteonectin; BM-40

▸ ~80% (SDS-PAGE), lyophilized powder

A 43 kDa calcium binding glycoprotein. SPARC binds to albumin, collagen, and thrombospondin. It inhibits spreading of endothelial and smooth muscle cells and fibroblasts.

Expressed in a variety of tissues, it inhibits cell spreading and diminishes focal contacts *in vitro*.

Lyophilized from phosphate buffered saline

Associated gene(s): Sparc (20692)

ship: dry ice store at: –20°C

S5174-25UG	25 µg

Vitronectin from bovine plasma

Serum spreading factor
[83380-82-9]

Antigenically unrelated to fibronectin.

Recommended as a cell culture substratum at 0.1 µg/cm^2. Optimal conditions for attachment must be determined for each cell line and application.

▸ lyophilized powder, BioReagent, suitable for cell culture

Cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma

Lyophilized from buffered saline

Dissolve in water (1 ml/vial)

ship: ambient store at: 2-8°C

V9881-50UG	50 µg

Vitronectin from human plasma

Serum spreading factor
[83380-82-9]

Antigenically unrelated to fibronectin.

Recommended as a cell culture substratum at 0.1 µg/cm^2. Optimal conditions for attachment must be determined for each cell line and application.

▸ lyophilized powder, BioReagent, suitable for cell culture

Cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma

Associated gene(s): VTN (7448)

Dissolve in water (1 ml/vial)

Lyophilized from buffered saline

ship: ambient store at: 2-8°C

V8379-50UG	50 µg

Vitronectin from rat plasma

Serum spreading factor
[83380-82-9]

Antigenically unrelated to fibronectin.

Recommended as a cell culture substratum at 0.1 µg/cm^2. Optimal conditions for attachment must be determined for each cell line and application.

▸ lyophilized powder, BioReagent, suitable for cell culture

Cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma

Associated gene(s): Vtn (29169)

Dissolve in water (1 ml/vial)

Lyophilized from buffered saline

ship: ambient store at: 2-8°C

V0132-50UG	50 µg

Collagen Type I (Cat. Nos. C1809, C7661, C9791, C8919*)

Optimal conditions for attachment must be determined for each cell line and application.

1) Add collagen to 0.1 M acetic acid to obtain 0.1% (w/v) collagen solution. Allow to stir at room temperature 1-3 hours until dissolved. Collage solution (Catalog Number C8919) should be diluted 10-fold to obtain a working concentration of 0.01%.
2) We recommend transferring the collagen solution to a glass bottle with a screw cap and carefully layering chloroform at the bottom. The amount of chloroform to use should be approximately 10% of the volume of collagen solution. DO NOT SHAKE OR STIR. Allow to sit overnight in the cold. Aseptically remove the top layer containing your collagen solution. We do not recommend sterilizing the collagen solution by membrane filtration. We have found substantial protein loss by this method.
3) Coat dishes with 6-10 mg/cm^2 . Allow the protein to bind for several hours at room temperature, 37°C, or overnight at 2-8°C.
4) Remove excess fluid from the coated surface, and allow it to dry overnight. If the collagen solution is not sterile, the dried, coated surface can be sterilized by overnight exposure to UV light in a sterile tissue culture hood.
5) Rinse with sterile tissue culture grade water or a balanced salt solution before introducing cells and medium.
***Note:** Steps 1 and 2 are not necessary for the Collagen Solution, Cat. No. C8919.

Collagen, Type II and IV (Cat. Nos. C9301, C0543, C5533)

Optimal conditions for attachment must be determined for each cell line and application.

1) Collagen Types II and IV may be reconstituted to concentration of 0.5-2.0 mg/ml in 25% acetic acid. **Dissolve for several hours at 2-8°C, occasionally swirling.**
2) Coating of tissue culture plastic dishes may be performed by air-drying the above protein solution, or by preincubating the same solution overnight at 2-8°C (or several hours at 37°C) without air-drying.
3) Dried coated dishes can be sterilized by exposure to UV light in a sterile culture hood or by rinsing with 70% ethanol. Alternatively, the collagen solution may be sterilized by dialysis in a 0.25% acetic acid and 0.5% chloroform solution.

Fibronectin (Cat. Nos. F1141*, F0895*, F4759, F2006, F0635)

Optimal conditions for attachment must be determined for each cell line and application.

Cellular Fibronectin (Cat. No. F2518)

Optimal conditions for attachment must be determined for each cell line and application.

1) Reconstitute with 0.5 ml sterile water. Do not agitate. Allow the solution to stand 30 minutes to solubilize. A small amount of undissolved material may remain. This will not affect product performance.
2) Dilute fibronectin in sterile balanced salt solution and coat the culture surface with a minimal volume.
3) Allow to air dry for at least 45 minutes at room temperature. Excess fibronectin may be removed by aspiration, but is not necessary.

3-D Collagen Gel Prep

Type I collagen is often used as a 3-D matrix in cell culture applications. Sigma's collagens have not been use tested for 3-D gel formation, however the general protocol may be useful. Note that lot-to-lot variability exists with many collagen products due to extraction and purification methods. Because of this variability, formation of 3-D gels may not be suitable for some lots of collagen.

Material required

Collagen gel solution, 1.5-3 mg/ml concentration
10 × tissue culture medium containing phenol red
Sodium bicarbonate or HEPES buffer

Collagen Preparation

Collagen solution should be prepared at the desired concentration in 0.1 M acetic acid. This may require stirring for several hours at room temperature. Sterilization using chloroform is not recommended. The addition of antibiotics and antimycotics may be helpful (Cat. Nos. A5955 or A7292).

Procedure

1) Pipet 800 µl collagen solution into sterile tube
2) Add 100 µl 10× medium
3) Adjust pH to neutral with 1N sodium hydroxide (100µl or less, if required)
4) Mix contents well. Solution should maintain red color to indicate physiological pH.
5) Aseptically dispense solution into wells to a depth of 1-2 mm (approx. 15 µl/well in a 24 well plate).
6) Transfer to 37 °C incubator for 20-40 minutes.
7) Examine for gel formation.

NOTE: The above volumes represent quantities for use in 24-well plates. Volumes can be adjusted to accommodate other culture vessels.

Gelatin Solution, 2% (Cat. No. G1393)

Optimal conditions for attachment must be determined for each cell line and application.

1) Allow gelatin solution to completely liquefy at 37 °C.
2) Coat culture surface with 5-10 ml gelatin solution/cm^2 (i.e., 0.1-0.2 mg/cm^2 gelatin).
3) Allow to dry at least 2 hours before introducing cells and medium.

Gelatin (Cat. Nos. G9391, G1890, G9136)

Optimal conditions for attachment must be determined for each cell line and application.

1) Prepare a 2% (w/v) solution by dissolving gelatin in tissue culture grade water.
2) Sterilize by autoclaving at 121 °C, 15 psi for 30 minutes.
3) Coat culture surface with 5-10 ml gelatin solution/cm^2 (i.e., 0.1-0.2 mg/cm^2 gelatin).
4) Allow to dry at least 2 hours before introducing cells and medium.

Laminin (Cat. Nos. L2020, L6274)

Optimal conditions for attachment must be determined for each cell line and application.

1) Slowly thaw laminin at 2-8 °C to avoid the formation of a gel.
2) Dilute in a balanced salt solution and coat culture surface with a minimal volume.
3) Allow to air dry at least 45 minutes before introducing cells and medium.

Poly-Lysine (Cat. Nos. P7280, P6407, P7405, P9155, P6282, P5899, P4707*, P4832*)

Optimal conditions must be determined for each cell line and application.

1) Add 50 ml of sterile tissue culture grade water to 5 mg of poly-lysine.
2) Aseptically coat culture surface with 1.0 ml/25 cm^2 (only). Rock gently to ensure even coating of the culture surface.
3) After 5 minutes, remove solution by aspiration and thoroughly rinse surface with sterile tissue culture grade water.
4) Allow to dry at least 2 hours before introducing cells and medium.
***NOTE:** Step 1 is not necessary for Poly-lysine Solution, Cat. Nos. P4707 and P4832.

Vitronectin (Cat. Nos. V8379, V0132, V9881)

Optimal conditions must be determined for each cell line and application.

1) Resconstitute with tissue culture grade water and sterilize by filtration.
2) Material is reported to be active at a concentration of 0.1 mg/cm^2 of surface area. Optimal concentrations vary with each cell line.
3) Coat culture surface for 1-2 hours at 37 °C. Remove any remaining solution and wash with a balanced salt solution before introducing cells and medium.

Biological Buffers

For cell culture applications in which pH maintenance is critical and normal bicarbonate buffering is not adequate, the biological buffers listed here may be useful. Cells vary in their sensitivity to these buffers and may exhibit toxic reactions to them even at recommended working concentrations. We recommend that you consult the literature or test different concentrations to determine the most effective buffer system for a particular cell type and for individual culture conditions. The osmolality of some media may reach adverse levels when buffers are added. Should this occur, lowering the concentration of sodium chloride may correct this condition.

Bufferall 100x

sterile-filtered, BioReagent, suitable for cell culture

Contains EPPS, HEPES and MOPS.

Bufferall is a combination of three biological Goods buffers with pKa values of 7.2 (MOPS), 7.55 (HEPES) and 8.0 (EPPS). Bufferall is a general use buffer system effective in reducing pH fluctuations over the pH range 7.0 to 8.0. In comparison, the buffering capacity of Bufferall is markedly greater than that of carbonate. The addition of Bufferall to media with or without carbonate greatly enhances buffering capacity within the biological pH range. In our laboratories, Bufferall did not adversely affect the growth of any cell line tested.

Prepared in tissue culture grade water

endotoxin ..... tested
pH range ..... 6.9 - 8.0
ship: ambient store at: room temp

B8405-20ML	20 mL
B8405-100ML	100 mL

Ethanolamine

Monoethanolamine; 2-Aminoethyl alcohol; 2-Aminoethanol
[141-43-5] $NH_2CH_2CH_2OH$ C_2H_7NO FW 61.08

density ..... 1.012 g/mL, 25 °C
n_D^{20} 1.454
vd ..... 2.1 (vs air)
vp ..... 0.2 mmHg (20 °C)
ait ..... 1436 °F
lel 17%

liquid, BioReagent, suitable for cell culture, ≥98%

A medium supplement used as a precursor for phospholipid biosynthesis.

concentration ..... 16.6 M
ship: ambient store at: room temp

E0135-100ML	100 mL
E0135-500ML	500 mL

β-Glycerophosphate disodium salt hydrate NEW

Glycerol 2-phosphate disodium salt hydrate; BGP
[154804-51-0] $(HOCH_2)_2CHOP(O)(ONa)_2 \cdot xH_2O$ $C_3H_7Na_2O_6P \cdot xH_2O$
FW 216.04 (Anh)

BioUltra, suitable for cell culture, suitable for plant cell culture, ≥99.0% (titration)

Insoluble matter ..... ≤0.1%
L-α-isomer ..... ≤0.2 mol %

chloride (Cl^-)	≤0.05%	Fe	≤0.001%
sulfate (SO_4^{2-})	≤0.05%	K	≤0.005%
Al	≤0.0005%	Mg	≤0.0005%
Ca	≤0.001%	Pb	≤0.001%
Cu	≤0.0005%	Zn	≤0.0005%

ship: ambient store at: room temp

G9422-10G	10 g
G9422-50G	50 g
G9422-100G	100 g
G9422-500G	500 g

HEPES solution

N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)
[7365-45-9]

useful pH range ..... 6.8 - 8.2

1 M, pH 7.0-7.6, sterile-filtered, BioReagent, suitable for cell culture

HEPES is an organic zwitterionic buffering agent effective in the physiological pH range of 6.8 to 8.2 (pKa 7.55). It is typically used in cell culture at concentration between 5mM to 30 mM. This product has been qualified for use in cell culture.

liquid

Prepared from HEPES free acid and cell culture grade water.

Concentrations of HEPES over 40mM may be toxic to some cell types.

endotoxin ..... tested
pH range ..... 7.0 - 7.6
ship: ambient store at: 2-8°C

H0887-20ML	20 mL
H0887-100ML	100 mL

Sodium acetate

Acetic acid sodium salt
[127-09-3] CH_3COONa $C_2H_3NaO_2$ FW 82.03

solid

pKa ..... 4.76 (acetic acid)
ait ..... 1112 °F

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

S5636-500G	500 g

Sodium bicarbonate

Sodium hydrogen carbonate
[144-55-8] $NaHCO_3$ $CHNaO_3$ FW 84.01

pKa (25 °C) ..... (1) 6.37, (2) 10.25 (carbonic acid)

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, 99.5-100.5%

pH range ..... 6.8 - 7.4

ship: ambient store at: room temp

S5761-500G	500 g
S5761-1KG	1 kg
S5761-5KG	5 kg

Hybri-Max™, powder, suitable for hybridoma, ≥99.5%

endotoxin ..... tested

ship: ambient store at: room temp

S4019-500G	500 g
S4019-1KG	1 kg

Sodium bicarbonate solution

[144-55-8]

solution (7.5%), sterile-filtered, BioReagent, suitable for cell culture

Prepared in cell culture grade water.

endotoxin ..... tested
pH range ..... 6.8 - 7.4
pH range ..... 7.8 - 8.2

ship: ambient store at: 2-8°C

S8761-100ML	100 mL
S8761-500ML	500 mL

Sodium phosphate dibasic

Disodium hydrogen phosphate; *sec*-Sodium phosphate; Disodium phosphate; Sodium hydrogenphosphate
[7558-79-4] Na_2HPO_4 HNa_2O_4P FW 141.96

pKa (25 °C) ..... (1) 2.15, (2) 6.82, (3) 12.38 (phosphoric acid)

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

S5136-100G	100 g
S5136-500G	500 g
S5136-1KG	1 kg
S5136-5KG	5 kg

Sodium phosphate monobasic

Monosodium phosphate; Sodium dihydrogen phosphate
[7558-80-7] NaH_2PO_4 H_2NaO_4P FW 119.98

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.0% (titration)

Use in insect cell culture to adjust pH.

ship: ambient store at: room temp

S5011-100G	100 g
S5011-500G	500 g
S5011-1KG	1 kg

HEPES-Buffered Media

HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) Buffered Powdered Media are formulated by Sigma with 15-30 mM of HEPES buffer. HEPES supplemented media, in conjunction with the normal phosphate-bicarbonate* buffering system, provides more effective buffering in the optimal pH range (7.2-7.4 at 37 °C) than non-HEPES supplemented media. These media are designed for use in an open or closed system.

***NOTE:** Bicarbonate is added in appropriate concentrations during the liquid preparation of powdered media. See Sodium Bicarbonate Addition Table.

pH Control: HEPES Supplemented Cell Culture Media

Figure 1 shows the theoretical titration curves at 37 °C for both sodium bicarbonate and **HEPES** Buffers. The addition of **HEPES** to a sodium bicarbonate buffered system raises the theoretical buffering range closer to optimal physiological pH.

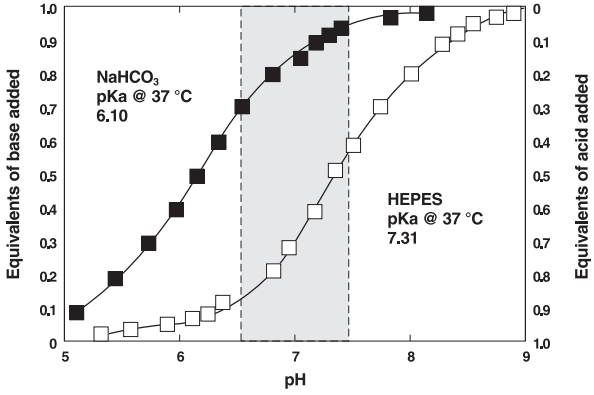


Figure 1. Titration curves at 37 °C for $NaHCO_3$ and HEPES buffers.

In our laboratories, we have formulated powdered media with appropriate concentrations of **HEPES** to provide both improved buffering conditions and minimal cytopathic effects. Figure 2 shows the titration curve of RPMI-1640 Medium (Catalog Number R6504) containing the normal phosphate-bicarbonate buffering system and RPMI-1640 Medium (Catalog Number R4130) containing phosphate-bicarbonate and 25 mM **HEPES** buffer.

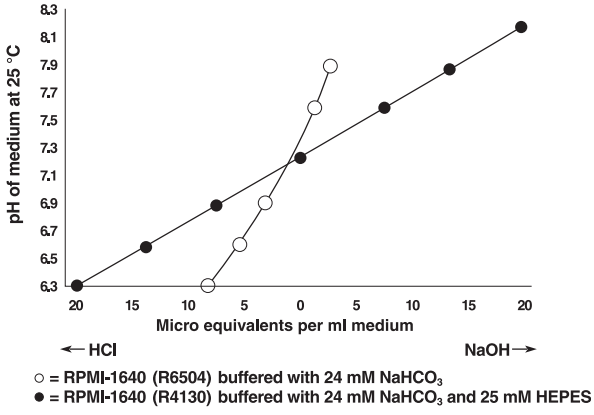


Figure 2. Titration curves of growth medium buffered with phosphate/$NaHCO_3$ and phosphate/$NaHCO_3$/HEPES.

Figure 3 shows the pH changes of RPMI-1640, without **HEPES** (Catalog Number R6504) and **HEPES** supplemented (Catalog Number R4130), during the growth and metabolism of HeLa cells over a 24-hour period. The **HEPES** buffered medium is resistant to rapid pH changes. As with all powdered tissue culture media from Sigma®, the **HEPES** buffered media are manufactured in accordance with their original formula except, where necessary, the sodium chloride concentration has been reduced to maintain the physiological osmolality. The sodium chloride concentrations are noted in the product insert accompanying each medium.

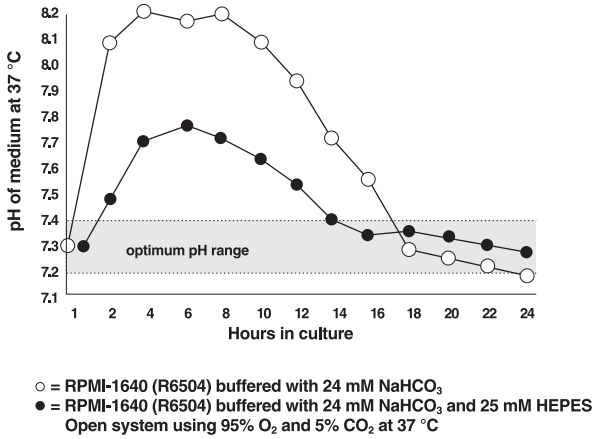


Figure 3. pH control in 24-hour culture of HeLa Cells.

In addition to sodium bicarbonate and **HEPES**, Sigma also offers a group of organic zwitterionic buffers that have been cell culture tested. The recommended concentrations are those reported in the references shown. Concentrations of organic buffers should be reduced if toxicity is apparent for a specific cell line as well as primary cultures. The inclusion of organic buffers into media may increase the osmolality of the media outside the normal physiological range (250-325 mOsm/Kg) and can be compensated for by reducing the sodium chloride concentration.

pH Control: Bufferall

Bufferall (Cat. No. B8405)

Changes in the pH of media affect mammalian cell growth, while the optimal pH varies with each cell line. In an effort to maintain the pH of media within the biological range of 6-8, phosphate and carbonate buffers have been used. Drawbacks to using these buffers are: [1] wide pH fluctuations occurring in media buffered in this manner; [2] phosphate, which is involved in metabolism, precipitates cations; and [3] carbonate buffering depends on carbon dioxide, which has limited solubility.

In 1966, Good and co-workers developed zwitterionic buffers which are chemically stable, with high solubility, and do not penetrate cells. Good's buffers, as well as other organic buffers, cover the biological pH range. Each individual buffer is most effective approximately two pH units centered on the pKa. Combinations of these biological buffers have been reported to reduce the wide pH fluctuations observed in cell culture.

Sigma's Bufferall is a combination of three biological buffers with pKa values of 7.2, 7.55, and 8.0. Bufferall is a general use buffer system effective in reducing pH fluctuations over the pH range 7.0 to 8.0. In comparison, the buffering capacity of Bufferall is markedly greater than that of carbonate (Fig. 1). The addition of Bufferall to media with or without carbonate greatly enhances buffering capacity within the biological pH range (Fig. 2). In our laboratories, Bufferall did not adversely affect the growth of any cell line tested.

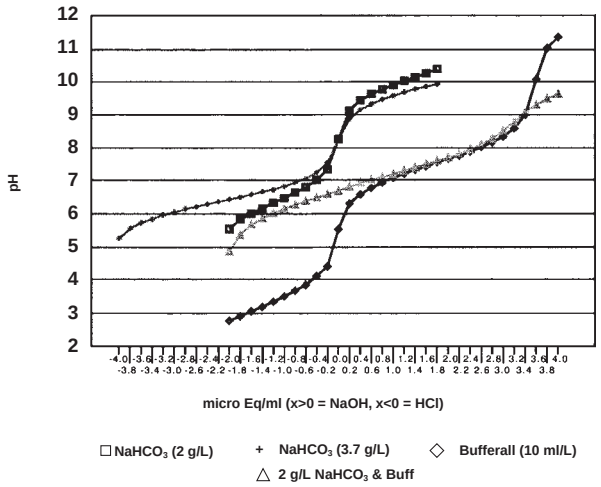


Figure 1. Titration curve of Bufferall at 25 °C and 0.03% CO_2

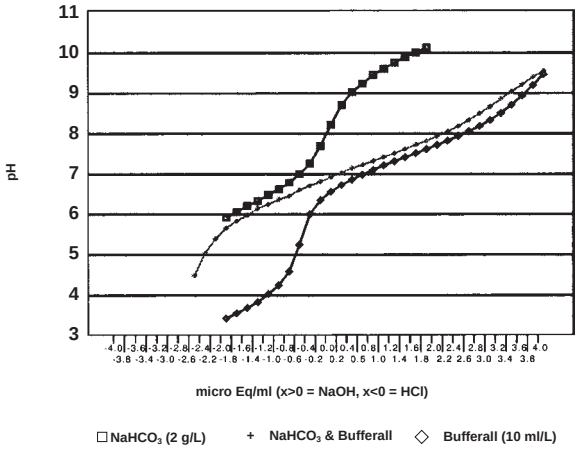


Figure 2. Titration curve of Bufferall in RPMI at 25 °C and 0.03% CO_2

References

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2. Taylor, A., *J. Cell Biol.*, **15,** 201-209, 1962.
3. Eagle, H., *Science,* **174,** 500-503, 1971.
4. Good, N., et al., *Biochem.*, **5,** 467, 1966.
5. Ceccarini, C. and Eagle, H., *Proc. Natl. Acad. Sci. USA*, **68,** 299, 1971.

Biological Detergents

IGEPAL® CA-630

(Octylphenoxy)polyethoxyethanol; Octylphenyl-polyethylene glycol
[9036-19-5] $(C_2H_4O)_nC_{14}H_{22}O$

Nonionic, non-denaturing detergent

Chemically indistinguishable from Nonidet™ P-40, which is no longer commercially available.

May develop turbidity or sediment during storage. A clear liquid can be obtained on heating to 40°C.

CMC ..... 0.08 mM (20-25°C)
HLB 13

viscous liquid

For solubilizing and isolation of membrane proteins.

non-ionic

mol wt ~603

viscosity 250-350 cP, neat (25 °C)(lit.)

density ..... 1.06 g/cm^3, 25 °C
ship: ambient store at: room temp

I3021-50ML	50 mL
I3021-100ML	100 mL
I3021-500ML	500 mL

Pluronic® F-68 solution

[106392-12-5]

solution, 10%, sterile-filtered, BioReagent, suitable for insect cell culture

Contains 100 g Pluronic F-68 per liter in cell culture grade water.

A nonionic copolymer surfactant

non-ionic

endotoxin ..... tested
ship: ambient store at: room temp

P5556-100ML	100 mL

Pluronic® F-127

[9003-11-6]

contains 100ppm BHT

powder, BioReagent, suitable for cell culture

A non-ionic copolymer surfactant qualified for use in insect cell culture applications as an antifoaming agent.

non-ionic
ship: ambient store at: room temp

P2443-250G	250 g
P2443-1KG	1 kg

TWEEN® 80

Polyethylene glycol sorbitan monooleate; Polyoxyethylenesorbitan monooleate; Polysorbate 80; POE (20) sorbitan monooleate
[9005-65-6]

Non-ionic detergent used for selective protein extraction and isolation of nuclei from mammalian cell lines.

non-ionic

composition
Oleic acid ~70% (balance primarily linoleic, palmitic, and stearic acids)

average mol wt 1310

TWEEN® 80KR
viscous liquid, Preservative Free, Low-peroxide; Low-carbonyls

micellar avg mol wt 79,000

cloud point ..... 65 °C
aggregation number 60
Carbonyls ..... ≤1.0 µmol/g
Peroxides ..... ≤0.5 µmol/g
water ..... ≤3%
CMC ..... 0.012 mM (20-25°C)
HLB 15
ship: ambient store at: −20°C

P6474-10ML	10 mL
P6474-5X10ML	5 × 10 mL
P6474-100ML	100 mL

viscous liquid, suitable for cell culture

mol wt ~1310
density ..... 1.07 g/cm^3, 25 °C CMC ..... 0.012 mM
density ..... 1.06 g/mL, 25 °C
ship: ambient store at: room temp

P4780-100ML	100 mL
P4780-500ML	500 mL
P4780-1GA	1 gal

viscous liquid, BioReagent, suitable for insect cell culture
ship: ambient store at: room temp

P4675-100ML	100 mL

TWEEN® 80 solution

Polyoxyethylenesorbitan Monooleate; Polyethylene glycol sorbitan monooleate solution
[9005-65-6]

Polysorbate 80
10%, low peroxide

Low Peroxide

Non-ionic detergent used for selective protein extraction and isolation of nuclei from mammalian cell lines

non-ionic

Contains BHT as an antioxidant.
ship: ambient store at: 2-8°C

P8192-5X10ML	5 × 10 mL

Cell Dissociation

Non-Enzymatic Dissociation Reagents

Cell Dissociation Solution Non-enzymatic 1x

Special non-enzymatic formulation for gently dislodging adherent cell types from plastic or glass surfaces.

Prepared in Hanks' balanced salt solution without calcium or magnesium, sterile-filtered, BioReagent, suitable for cell culture

ship: ambient store at: 2-8°C

C1419-100ML	100 mL

In PBS without Ca^{2+} and Mg^{2+}., sterile-filtered, BioReagent, suitable for insect cell culture

ship: ambient store at: 2-8°C

C1544-100ML	100 mL

Prepared in Hanks' Balanced Salt Solution without calcium and magnesium, sterile-filtered, BioReagent, suitable for cell culture

ship: ambient store at: 2-8°C

C5789-100ML	100 mL

Prepared in phosphate buffered saline without calcium and magnesium, sterile-filtered, BioReagent, suitable for cell culture

ship: ambient store at: 2-8°C

C5914-100ML	100 mL

NO-ZYME™

Cell Dissociation Solution Non-enzymatic 1x

Special non-enzymatic formulation for gently dislodging adherent cell types from plastic or glass surfaces.

liquid, sterile-filtered, suitable for cell culture

PBS Base, without calcium, without magnesium, without phenol red

for research or for further manufacturing use

ship: ambient store at: room temp

59226C-100ML	100 mL

Ethylenediaminetetraacetic acid

Edathamil; (Ethylenedinitrilo)tetraacetic acid; Ethylenedinitrilotetraacetic acid; EDTA

[60-00-4] $(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$ $C_{10}H_{16}N_2O_8$ FW 292.24

Calcium chelator used to eliminate inhibition of enzyme catalyzed reactions due to traces of heavy metals.

anhydrous, crystalline, BioReagent, suitable for cell culture

≥98.5%

solubility

3 M NaOH ..... 100 mg/mL

ship: ambient store at: room temp

E6758-100G	100 g
E6758-500G	500 g

Ethylenediaminetetraacetic acid solution

EDTA solution

[60-00-4] $C_{10}H_{16}N_2O_8$ FW 292.24

0.02% in DPBS (0.5 mM), sterile-filtered, BioReagent, suitable for cell culture

A chelator used to remove calcium from cell washing and suspension media. Reduces cell clumping.

Solution in Dulbecco's phosphate buffered saline without calcium and magnesium

endotoxin ..... tested

ship: ambient store at: 2-8°C

E8008-100ML	100 mL

Ethylenediaminetetraacetic acid tetrasodium salt dihydrate

Tetrasodium ethylenediaminetetraacetate dihydrate; EDTA tetrasodium salt; Edathamil

[10378-23-1] $(NaOOCCH_2)_2NCH_2CH_2N(CH_2COONa)_2 \cdot 2H_2O$ $C_{10}H_{12}N_2Na_4O_8 \cdot 2H_2O$ FW 416.20

Used to eliminate inhibition of enzyme catalyzed reactions due to traces of heavy metals

BioReagent, suitable for cell culture, 99.0-102.0%

solubility

H_2O ..... 160 mg/mL

ship: ambient store at: room temp

E6511-100G	100 g
E6511-500G	500 g

General Information About Sigma-Aldrich® Collagenase Products

Description of Sigma-Aldrich's Collagenase Products

The different Collagenase products developed by Sigma-Aldrich were selected because they digested some types of tissue (muscle, pancreas, heart, adipose) better than others. Besides meeting enzyme activity specifications every lot of many Sigma-Aldrich collagenase products must pass digestion tests with various tissues from rats. Some products that are also described as "cell culture tested" have undergone additional testing with mammalian cell lines to verify that they are not cytotoxic. Sigma-Aldrich's purified collagenase products have only trace amounts of caseinase (proteolytic) or clostripain activities. The purified Type VII Collagenase is also offered in Cell Culture Tested and sterile-filtered versions.

Collagenases, enzymes that break down the native collagen that holds animal tissues together, are made by a variety of microorganisms and by many different animal cells[1]. The most potent collagenase is the "crude" collagenase secreted by the anaerobic bacteria *Clostridium histolyticum*. The original 1953 fermentation and purification process described by MacLennan, Mandl and Howes[2] was first adopted by Sigma-Aldrich and eventually improved upon for higher activity products. "Crude" collagenase refers to the fact that the material is actually a mixture of several different enzymes besides collagenase that act together to break down tissue. It is now known that two forms of the collagenase enzyme are present[3, 4]. With a few exceptions different commercial collagenase are all made from *C. histolyticum*, or are recombinant versions where *Escherichia coli* expresses a gene cloned from *C. histolyticum*.

Collagenase Assays

The Type I and Type II forms of the purified collagenase enzymes differ in their specificities and relative activities on native collagen and synthetic substrates. These two collagenases can be mostly distinguished by their preference for one of the two different substrates used in Sigma-Aldrich® assays. The Collagenase Digestive Unit (CDU) assay[10, 11] measures predominantly the Collagenase I activity, which cleaves two of the three helical chains in the long, undenatured collagen protein. Collagenase II activity is measured by this enzyme's ability to cut a short synthetic peptide, N-[3-(2-Furyl)acryloyl)]-Leu-Gly-Pro-Ala (FALGPA, see Cat. No. F5135), in a second collagenase digestive assay[12, 13]. Purified preparations of either Collagenase I or II have been shown to be less effective at digesting various types of collagen or mammalian tissue when compared to a mixture of both forms of this enzyme. A purified collagenase containing only the Collagenase I and II forms of this enzyme is less effective at digesting tissue than the whole crude collagenase or combinations of the purified collagenase and various proteases. Obviously the combination of true collagenase and the different native proteases, Clostripain and aminopeptidases that have evolved in nature assist each other in digesting the collagen in different animal tissues. For tissue digestions the crude collagenase products have always been the most effective. Some researchers have tried mixtures of chromatographically purified collagenase with a protease such as trypsin or subtilisin to digest tissue.

In addition to the CDU and FALGPA assays for Collagenase activities Sigma-Aldrich tests each product lot for Caseinase[14, 15], Clostripain and Tryptic activities to look at the proteolytic enzymatic activities in the collagenase products. The Caseinase assay is the most important of the three for measuring the proteolytic activity that assists the digestion of animal tissue. Because the Clostripain present in crude collagenase must be reduced (e.g. by treatment with Dithiothreitol) in order to be active this enzyme probably contributes little to the tissue dissociation process in the laboratory. It is monitored because some researchers have reported that Clostripain may be damaging or toxic.

Many collagenase products that meet enzymatic specifications are also use-tested with various tissues obtained from rats. Type II (C6885, C1764) and Type VIII (C2139) collagenase lots are tested for the ability to release adipose (fat) cells from rat epididymal fat pads[5]. Fat cells are then screened for metabolic activity by measuring glucose oxidation rates with and without insulin addition. Type IV (C5138, C1889) and Type VIII (C2139) lots have been tested for the ability to release viable cells from rat liver[7]. Type V (C9263, C2104), Type XI (C7657, C4785, C9407, and C9697) and Type S (C6079) Collagenase lots must release intact islets of Langerhans from rat pancreas to pass their product test[8].

Factors that Affect Tissue Digestion-Dissociation by Collagenase

Based on our own R&D and from discussions with customers it is clear that the way a particular tissue is dissected and prepared has a significant effect on the speed and efficiency of any tissue digestion-dissociation with collagenase. Differences in the ages of the tissue donors can also be a major source of variation over time. Make sure that calcium ions are present in the digestion buffers at 5 mM. Chelating agents EGTA and EDTA can severely inhibit Collagenase activity by removing calcium ions required for enzyme stability and activity. β-mercaptoethanol[16], cysteine[16] and 8-hydroxyquinoline-5-sulfonate[16] are other inhibiting substances. A new lot of Collagenase with higher specific activity could cause excessive cell death at an established concentration. In that case use less collagenase and/or add BSA or serum (up to 0.5% and 5-10% respectively) to stabilize the cells to further digestion.

Notes:

- The separately prepared Collagenase and protease enzymes in the "Sigma® Blend" products (Cat. Nos. C7926, C8051 or C8176) give reproducible control of how much of each is used.
- DNAse will be inactivated by the shear of excessive stirring, and added enzymes may be digested by the neutral protease present in the Collagenase.
- Use EGTA (or EDTA) to remove Ca^{++} and flush away microorganisms, then wash tissue with buffer to remove the chelating agent. Do not add EGTA or EDTA to the enzyme solutions!

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Collagenase from *Clostridium histolyticum*

Clostridiopeptidase A
[9001-12-1]

One **collagen digestion unit** liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hr at pH 7.4 at 37 °C in the presence of calcium ions.
One **FALGPA hydrolysis unit** hydrolyzes 1.0 µmole of furylacryloyl-Leu-Gly-Pro-Ala per min at pH 7.5 at 25 °C in the presence of calcium ions.
One **neutral protease unit** hydrolyzes casein to produce color equivalent to 1.0 µmole tyrosine per 5 hr at pH 7.5 at 37 °C.
One **clostripain unit** hydrolyzes 1.0 µmole of BAEE per min at pH 7.6 at 25 °C in the presence of DTT.

▸ lyophilized powder, activity: ≥125 CDU/mg solid (CDU = collagen digestion units), activity: 0.5-5.0 FALGPA units/mg solid

Also contains clostripain, nonspecific neutral protease and tryptic activities.

Crude

This collagenase has been tested with cell lines to verify the product is not cytotoxic. Collagenase is typically used to digest the connective components in tissue samples to liberate individual cells. The concentration for cartilage dispersal is 1-2 mg/ml, but literature searches should be performed for species specific and/or tissue specific concentrations.

suitable for cell culture

Type I-A

This collagenase is obtained from the culture filtrate of Clostridium histolyticum. The culture filtrate is thought to contain at least 7 different proteases ranging in molecular weight from 68-130 kDa. This product is Type I-A.

ship: ambient store at: –20°C

C2674-100MG	100 mg
C2674-500MG	500 mg
C2674-1G	1 g

▸ sterile-filtered, release of physiologically active rat epididymal adipocytes tested, Type II-S, activity: 0.5-5.0 FALGPA units/mg solid

lyophilized powder

Prepared from Type II (C6885)

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

ship: ambient store at: –20°C

C1764-50MG	50 mg

▸ powder, Suitable for the digestion and isolation of physiologically active pancreatic islet cells, suitable for cell culture

Use for the digestion of collagen and the release of adherent cells from substrates.

Prepared from Type XI (C7657)

Also contains clostripain, nonspecific neutral protease and tryptic activities.

ship: ambient store at: –20°C

C9407-25MG	25 mg
C9407-100MG	100 mg
C9407-500MG	500 mg
C9407-1G	1 g
C9407-5G	5 g

▸ lyophilized powder (from sterile-filtered solution), Suitable for digestion and isolation of physiologically active pancreatic islet cells, suitable for cell culture

Prepared from Type XI (C9407).

This collagenase is obtained from the culture filtrate of Clostridium histolyticum. The culture filtrate is thought to contain at least 7 different proteases ranging in molecular weight from 68-130 kDa. This product is prepared from Type XI (C9407).

Also contains clostripain, nonspecific neutral protease and tryptic activities.

ship: ambient store at: –20°C

C9697-50MG	50 mg

▸ powder, suitable for cell culture, activity: 1000-3000 CDU/mg solid (CDU = collagen digestion units), activity: 4-12 FALGPA units/mg solid, high purity

Use for the digestion of collagen and the release of adherent cells from substrates.

purified by chromatography

Prepared from Type VII (C0773)

This collagenase is obtained from the culture filtrate of Clostridium histolyticum. The culture filtrate is thought to contain at least 7 different proteases ranging in molecular weight from 68-130 kDa. This product is prepared from Type VII (C0773)

neutral protease and clostripain ..... ≤1 unit/mg solid

ship: ambient store at: –20°C

C2799-7.5KU	7500 units
C2799-15KU	15000 units

▸ lyophilized powder (from sterile-filtered solution), suitable for cell culture, high purity

Prepared from Type VII (C2799)

This collagenase is obtained from the culture filtrate of Clostridium histolyticum. The culture filtrate is thought to contain at least 7 different proteases ranging in molecular weight from 68-130 kDa. Prepared from Type VII (C2799).

ship: ambient store at: –20°C

C9572-7.5KU	7500 units
C9572-15KU	15000 units

▸ Animal component-free, activity: ≥400 CDU/mg solid NEW

Also contains clostripain, nonspecific neutral protease, and tryptic activities.

suitable for cell culture

release of physiologically active rat pancreatic islets tested

essentially salt-free, lyophilized powder

activity: ≥1.0 FALGPA units/mg solid

Produced using a proprietary method utilizing animal-free micobial media

ship: ambient store at: –20°C

C0374-100MG	100 mg
C0374-500MG	500 mg
C0374-1G	1 g
C0374-5G	5 g

Enzymes

Accumax™ solution

Special formulation that gently and rapidly dissociates cell clumps to yield single cell suspensions for accurate and reproducible cell counts. This combines protease, collagenolytic and DNase activities which maximizes its versatility for cell aggregate dissociation.

Proven effective in dissociating clumped cells in suspension cultures of hybridomas, CHO, BHK, 293, COS, and Sf9 cells. Performs exceptionally well in dissociating cell counting, viral transfection assays, cell sorting, and flow cytometry as well as bioreactor scale-up. Does not contain mammalian or bacterial-derived products.

sterile-filtered, suitable for cell culture

Prepared in Dulbecco's PBS (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8 g/L NaCl, and 1.15 g/L Na_2HPO_4). Refer to Product Data Sheet on the website for product usage information.

Features and Benefits

Ready-to-use sterile liquid for *in vitro* cell applications

pH 6.8-7.8

ship: ambient store at: –20°C

A7089-100ML	100 mL

Accutase® solution

Special formulation that gently and rapidly dissociates tissues for cell isolation and propagation. This combines protease and collagenolytic activities which maximizes its versatility for cell detachment of adherent cells and tissue dissociation.

Proven effective in detaching primary fibroblasts, endothelial cells, neurons, tumor cell lines, and insect cells. Performs exceptionally well in detaching cells for analysis of cell surface markers, virus growth assay, and flow cytometry as well as bioreactor scale-up. Does not contain mammalian or bacterial-derived products.

sterile-filtered, suitable for cell culture

Prepared in Dulbecco's PBS (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8 g/L NaCl, and 1.15 g/L Na_2HPO_4) containing 0.5 mM EDTA•4Na and 3 mg/L Phenol Red. Refer to Product Data Sheet on the website for product usage information.

Features and Benefits

Ready-to-use liquid consisting of a proprietary mixture of proteolytic and collagenolytic enzymes for use in dissociation of cells from standard and adhesion-coated plasticware.

pH 6.8-7.8

ship: ambient store at: –20°C

A6964-100ML	100 mL

Catalase from bovine liver

H_2O_2:H_2O_2 oxidoreductase
[9001-05-2]

Activates the decomposition of hydrogen peroxide, Natural antioxidant used to study roles of reactive oxygen species in gene expression and apoptosis.

tetramer mol wt ~250 kDa

Catalase from bovine liver is a tetramer consisting of four equal subunits with a molecular weight of 60 kDa each. Each subunit contains iron bound to a protoheme IX group. The enzyme also strongly binds NADP, which is in close proximity to the heme group.

isoelectric point 5.4

powder, suitable for cell culture, activity: 2,000-5,000 units/mg protein

Associated gene(s): CAT (280743)

One unit will decompose 1.0 µmole of H_2O_2 per min at pH 7.0 at 25 °C, while the H_2O_2 conc. falls from 10.3 to 9.2 mM, measured by the rate of decrease of A_{240}.

Protein determined by biuret.

ship: ambient store at: –20°C

C1345-1G	1 g
C1345-10G	10 g
C1345-100G	100 g

Elastase from porcine pancreas

Elastase from hog pancreas; Pancreatopeptidase E
[39445-21-1]

One unit will hydrolyze 1.0 µmole of N-succinyl-L-Ala-Ala-Ala-p-nitroanilide per min, pH 8.0 at 25 °C.

lyophilized powder, BioReagent, suitable for cell culture

Enzyme that degrades elastin, a matrix component of tissues involved in stretching processes

activity: ≥4 units/mg protein

solubility
H_2O .....
H_2O .....

Trypsin ..... ≤50 BAEE units/mg protein

ship: ambient store at: –20°C

E7885-1MG	1 mg
E7885-5MG	5 mg
E7885-20MG	20 mg

Pancreatin from porcine pancreas

Pancreatin from hog pancreas
[8049-47-6]

Pancreatin will convert not less than 25 times its weight of potato starch into soluble carbohydrates in 5 minutes in water at 40 °C, will digest not less than 25 times its weight of casein in 60 minutes at pH7.5 at 40 °C and will release not less than 2 microequivalents of acid per min per mg pancreatin from olive oil at pH9.0 at 37 °C.

Contains many enzymes, including amylase, trypsin, lipase, ribonuclease and protease. The National Formulary (N.F.) and the U.S. Pharmacopeia (USP) specify amylase, protease and lipase only.

powder, suitable for cell culture, activity: 4 × USP specifications

contains lactose or sucrose as extender (The sucrose used might contain up to 3.25% starch.)

Sigma tests only for amylase activity.

ship: ambient store at: –20°C

P3292-25G	25 g
P3292-100G	100 g

Trypsin Solution 10X

NEW

Trypsin solution from porcine pancreas
[9002-07-7]

Used to release adherent cells from tissue culture plates for passaging.

for research or for further manufacturing use

Drug Master File available for SER-TAIN® γ-irradiated trypsin

ship: dry ice store at: –20°C

59427C-100ML	100 mL
59427C-500ML	500 mL

TrypZean® Solution, 1×

Trypsin
recombinant, expressed in corn, sterile-filtered

Used to release adherent cells from tissue culture plates for passaging. Eliminates the introduction of animal source contaminants found in traditional bovine and porcine trypsins.

aqueous solution

ship: ambient store at: –20°C

T3449-100ML	100 mL
T3449-500ML	500 mL

Trypsin

Using Trypsin to Remove Adherent Cells from a Culture Surface

Cells are most commonly removed from the culture substrate by treatment with trypsin, or trypsin-EDTA. If trypsin is being solubilized or diluted from a concentrated solution, it is important to use a buffered salt solution that contains no Ca^{2+} or Mg^{2+}, such as Hanks' Balanced Salt Solution, Modified (Catalog No. H8389). Adjust pH of trypsin solution to 7.4-7.6.

1) Remove medium from culture vessel by aspiration and wash the monolayer with Ca^{2+} and Mg^{2+} - free salt solution to remove all traces of serum. Remove salt solution by aspiration.
2) Dispense enough trypsin-EDTA solution into culture vessel(s) to completely cover the monolayer of cells and place in 37 °C incubator for approximately 2 minutes.
3) Remove the trypsin-EDTA solution by aspiration and return closed culture vessel(s) to incubator. The coated cells are allowed to incubate until cells detach from the surface. Progress can be checked by examination with an inverted microscope.
NOTE: The time required to remove cells from the culture surface is dependent on cell type, population density, serum concentration in the growth medium, potency of trypsin, and time since last subculture. Trypsin causes cellular damage and time of exposure should be kept to a minimum.
4) When trypsinization process is complete the cells will be in suspension and appear rounded.
5) It is advisable to add serum or medium containing serum to the cell suspension as soon as possible to inhibit further tryptic activity which may damage cells.
6) Cells can be resuspended by gently pipetting the cell suspension to break up the clumps. Further dilution can be made, if required, for cell counts and/or subculturing.

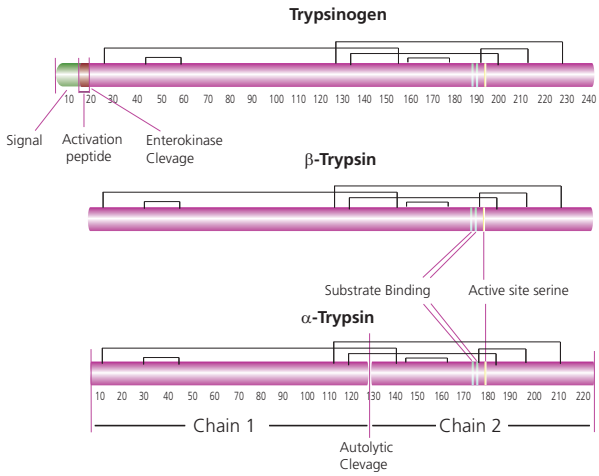
Trypsin from bovine pancreas

[9002-07-7]

mol wt 23.8 kDa

One BTEE unit = 320 ATEE units

Trypsin *in vivo* Processing
(Bovine Sequence)



Enterokinase activates pancreatic trypsinogen to trypsin by the hydrolysis of a hexapeptide(for bovine trypsin at the Lys6 - Ile7 peptide bond) from the NH2 terminus. Bovine trypsinogen consists of a single polypeptide chain of 229 amino acids and is cross linked by six disulfide bridges. Trypsin can autocatalytically activate more trypsinogen to trypsin. Trypsin consists of a single chain polypeptide of 223 amino acid residues. This native form of trypsin is refered to as β-trypsin. Autolysis of β-trypsin (which is cleaved at Lys131- Ser132 in the bovine sequence) results in α-trypsin which is held together by disulfide bridges.

essentially salt-free, lyophilized powder, activity: ≥9,000 BAEE units/mg protein, BioReagent, suitable for cell culture

Contains chymotrypsin activity.

Used to release adherent cells from tissue culture plates for passaging.

Derived from New Zealand-sourced pancreas

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25 °C using BAEE as substrate. Reaction volume = 3.2 ml (1 cm light path).

ship: ambient store at: –20°C

T9935-50MG	50 mg
T9935-100MG	100 mg

Trypsin Powder, Porcine 1:250

NEW

Trypsin from porcine pancreas
[9002-07-7]

mol wt 23.8 kDa

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25° C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

One BTEE unit = 320 ATEE units

Gamma irradiated by SER-TAIN® Process, dry powder

Used to release adherent cells from tissue culture plates for passaging.

for research or for further manufacturing use

Drug Master File available for SER-TAIN® γ-irradiation process

ship: ambient store at: –20°C

85450C-25G	25 g
85450C-100G	100 g
85450C-500G	500 g
85450C-1K	1 kg

Trypsin from porcine pancreas

[9002-07-7]

Contains chymotrypsin and elastase activities.

Lyophilized powder containing lactose

mol wt 23.8 kDa

One BAEE unit will produce a ΔA_{253} of 0.001 per min at pH 7.6 at 25° C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

One BTEE unit = 320 ATEE units

lyophilized powder, BioReagent, suitable for cell culture, activity: 1,000-2,000 BAEE units/mg solid

Used to release adherent cells from tissue culture plates for passaging.

ship: ambient store at: –20°C

T4799-5G	5 g
T4799-10X5G	10 × 5 g
T4799-10G	10 g
T4799-25G	25 g
T4799-100G	100 g
T4799-500G	500 g

lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture, activity: 1,000-1,500 BAEE units/mg solid

ship: ambient store at: 2-8°C

T5266-500MG	500 mg

Trypsin solution from porcine pancreas

[9002-07-7]

Used to release adherent cells from tissue culture plates for passaging.

10 ×, sterile-filtered, BioReagent, suitable for cell culture, 25 g porcine trypsin per liter in 0.9% sodium chloride

pH-range ..... 6.4 - 7.0

ship: dry ice store at: –20°C

T4549-20ML	20 mL
T4549-100ML	100 mL

10 ×, sterile-filtered, BioReagent, suitable for cell culture, 25 g porcine trypsin per liter in Hanks' Balanced Salt Solution with phenol red

Porcine parvovirus ..... none detected (9 CFR)

pH-range ..... 7.0 - 7.6

ship: dry ice store at: –20°C

T4674-100ML	100 mL

1 ×, sterile-filtered, BioReagent, suitable for cell culture, 2.5 g porcine trypsin per liter in Hanks' Balanced Salt Solution with phenol red

Porcine parvovirus ..... none detected (9 CFR)

pH-range ..... 7.0 - 7.6

ship: dry ice store at: –20°C

T4424-100ML	100 mL
T4424-500ML	500 mL

Trypsin Solution 10X NEW

Trypsin solution from porcine pancreas
[9002-07-7]

Used to release adherent cells from tissue culture plates for passaging.

for research or for further manufacturing use

Drug Master File available for SER-TAIN® γ-irradiated trypsin

ship: dry ice store at: –20°C

59427C-100ML	100 mL
59427C-500ML	500 mL

Trypsin-EDTA solution

Porcine parvovirus ..... none detected (9 CFR)

pH-range ..... 7.0 - 7.6

0.25%, sterile-filtered, BioReagent, suitable for cell culture, 2.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red

Used to release adherent cells from tissue culture plates for passaging.

ship: dry ice store at: –20°C

T4049-100ML	100 mL
T4049-500ML	500 mL

1 ×, sterile; sterile-filtered, BioReagent, suitable for cell culture, 0.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red

Used to release adherent cells from tissue culture plates for passaging.

ship: dry ice store at: –20°C

T3924-100ML	100 mL
T3924-500ML	500 mL

1 ×, sterile-filtered, BioReagent, suitable for cell culture, 500 BAEE units porcine trypsin and 180 µg EDTA • 4Na per ml in Dulbecco's Phosphate Buffered Saline without calcium and magnesium

For use with endothelial cell cultures.

ship: dry ice store at: –20°C

T4299-100ML	100 mL

10 ×, sterile-filtered, BioReagent, suitable for cell culture, 5.0 g porcine trypsin and 2 g EDTA • 4Na per liter of 0.9% sodium chloride

Used to release adherent cells from tissue culture plates for passaging.

ship: dry ice store at: –20°C

T4174-20ML	20 mL
T4174-100ML	100 mL

Trypsin-EDTA Solution 10X NEW

Trypsin solution from porcine pancreas
[9002-07-7]

0.5% trypsin, 0.2% EDTA, trypsin gamma irrdiated by SER-TAIN® Process, without phenol red, in saline

Used to release adherent cells from tissue culture plates for passaging.

for research or for further manufacturing use

Drug Master File available for SER-TAIN® process

ship: dry ice store at: –20°C

59418C-100ML	100 mL

Trypsin-EDTA Solution 1X

NEW

Trypsin-EDTA solution

0.25% trypsin, 0.02% EDTA, trypsin gamma irradiated by SER-TAIN® Process, in Hank's Balanced Salt Solution, sterile-filtered

for research or for further manufacturing use

Drug Master File available for SER-TAIN® γ-irradiation

ship: dry ice store at: –20°C

59428C-100ML	100 mL
59428C-500ML	500 mL

Trypsin-EDTA Solution 1X

NEW

Trypsin-EDTA solution

0.05% trypsin, 0.02% EDTA, trypin gamma irradiated by SER-TAIN® Process, in Hanks' Balanced Salt Solution

for research or for further manufacturing use

Drug Master File available for SER-TAIN® γ-irradiation

ship: dry ice store at: –20°C

59417C-100ML	100 mL
59417C-500ML	500 mL

Trypsin-EDTA Solution 1X

NEW

Trypsin-EDTA solution

Used to release adherent cells from tissue culture plates for passaging.

0.12% trypsin, 0.02% EDTA, trypsin gamma irradiated by SER-TAIN® Process, without phenol red, in Dulbecco's Phosphate Buffered Saline

Drug Master File available for SER-TAIN® γ-irradiation process

for research or for further manufacturing use

ship: dry ice store at: –20°C

59430C-100ML	100 mL
59430C-500ML	500 mL

Trypsin-EDTA Solution 1X

NEW

Trypsin-EDTA solution

0.25% trypsin, 0.1% EDTA, trypsin gamma irrdiated by SER-TAIN® Process, in Hank's Balanced Salt Solution, cell culture tested

1X

for research or for further manufacturing use

Drug Master File available for SER-TAIN® process

SAFC Biosciences has validated a process using γ radiation to significantly reduce the risks associated with adventitious agents such as Porcine Parvovirus (PPV), PorcineRespiratory and Reproductive Syndrome (PRRS) and Mycoplasma hyorhinis while maintaining product performance. The use of γ irradiated trypsin requires no change to the end user's methods or procedures while giving additional assurance against microbial contaminants associated with animal-derived products. All trypsin is obtained from the United States or other countries deemed free of Bovine Spongiform Encephalopathy (BSE).

ship: dry ice store at: –20°C

59429C-100ML	100 mL
59429C-500ML	500 mL

Protease Inhibitors

Proteolytic hydrolysis of peptide bonds was first studied as a function of digestion in higher animals. It is now recognized as an essential and ubiquitous mechanism for the regulation of a myriad of physiological processes.

Inhibition of proteolytic activity is generally employed for two purposes:

- For the prevention of unwanted degradation of proteins during their isolation and characterization.
- To study the regulatory aspects of specific proteolytic events as they relate to cellular processes. Some of the most studied proteolytic-related processes include the blood coagulation and complement cascades, hormonal regulation, apoptosis, extracellular matrix degradation, proteasome and lysosomal regulation, and disease states such as Alzheimer's and viral replication.

Four main classes of proteolytic enzymes have been routinely utilized to describe proteases. The serine proteases are probably the best characterized. This class of proteases includes trypsin, chymotrypsin, and elastase. The cysteine protease class includes papain, calpain, and lysosomal cathepsins. Aspartic proteases include pepsin and rennin. Metalloproteases include thermolysin and carboxypeptidase A.

During isolation and characterization one or all four classes of proteases may pose a threat to the fate of a protein. Broad spectrum protease inhibitors and mixtures (or cocktails) have been developed to help protect the integrity of isolated proteins. Sigma offers and manufactures the broadest range of protease inhibitors and inhibitor cocktails of any supplier. Sigma® Inhibitor Cocktails have been specifically formulated for particular applications as they relate to the biological source or method of expression. Protease inhibitors can be added during cell growth and protein expression or can be added at the time of extraction.

Aprotinin from bovine lung

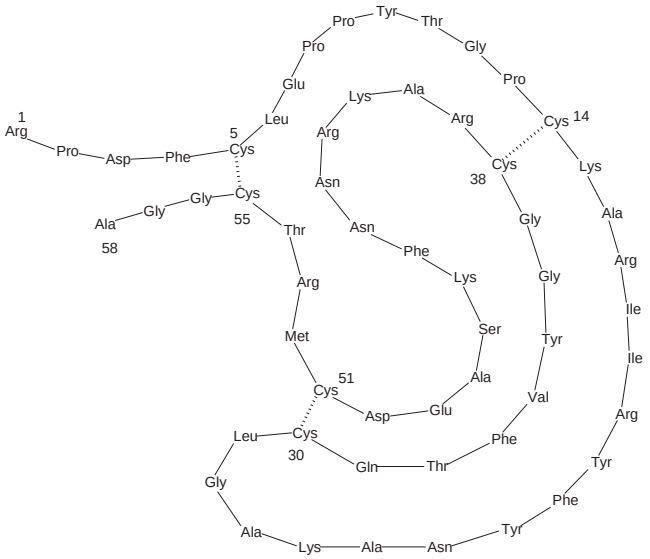
Trypsin inhibitor (basic)
[9087-70-1] $C_{284}H_{432}N_{84}O_{79}S_7$ FW 6511.44

Aprotinin is a competitive serine protease inhibitor that inhibits trypsin, chymotrypsin, kallikrein and plasmin. Aprotinin forms stable complexes with and blocks the active sites of enzymes. Binding is reversible with most aprotinin-protease complexes dissociating at pH >10 or <3. Effective concentration equimolar with protease.

Aprotinin is a protein consisting of 58 amino acids, arranged in a single polypeptide chain that is crosslinked by three disulfide bridges.

mol wt ~6,500

Another commonly used unit of activity is the KIU (Kallikrein Inhibitor Unit). 1 TIU ~ 1,300 KIU.



While aprotinin and bovine pancreatic trypsin inhibitor (BPTI) are the same protein sequence, the term aprotinin is typically used when describing the protein derived from bovine lung. Aprotinin is a single peptide chain with three disulfide bonds. Molecular Weight: ~6511

lyophilized powder, activity: 3-8 TIU/mg solid, BioReagent, suitable for cell culture

Note: When benzoyl-L-arginine ethyl ester (BAEE) is used as substrate one BAPNA unit is approx. 45 BAEE µmolar units at pH 8.0 at 25 °C or approx. 9,000 BAEE A_{253} units at pH 7.6 at 25 °C.

Associated gene(s): PTI (404172)

One trypsin inhibitor unit (TIU) will decrease the activity of 2 trypsin units by 50% where one trypsin unit will hydrolyze 1.0 µmole of Nα-benzoyl-DL-arginine p-nitroanilide (BAPNA) per min at pH 7.8 at 25°C.

ship: ambient store at: 2-8°C

A3428-10MG	10 mg
A3428-25MG	25 mg
A3428-100MG	100 mg
A3428-250MG	250 mg

Trypsin inhibitor from *Glycine max* (soybean)

SBTI
[9035-81-8]

Soybean trypsin inhibitor inhibits trypsin and to a lesser extent chymotrypsin and plasmin. It forms a 1:1 stoichiometric complex with trypsin. Upon formation of this complex, trypsin may cleave a single arginine-isoleucine bond in the inhibitor. Dissociation of this complex may yield the modified form or the native inhibitor. At the optimal pH for trypsin binding (pH 8.0), the association constant is ≥ $10x10^8$.

Trypsin inhibitor is soluble in water and phosphate buffers at concentrations of 10 mg/ml or higher. Solutions at higher concentrations may be hazy and have a yellow to amber color.

powder, BioReagent, suitable for cell culture

After trypsinizing cells, resuspend cells in 1 mL trypsin inhibitor solution (1mg/ml) for every mL of trypsin solution used for dissociation. Centrifuge the cell suspension at 1000 rpm for 5 minutes. A cell pellet should form. Remove as much of the trypsin inhibitor solution as possible and resuspend the pellet in serum-free medium. Culture cells as desired.

Type I-S

activity: (One mg will inhibit 1-3 mg of trypin with activity of approx. 10,000 BAEE units per mg protein.)

Chromatographically prepared.

One trypsin unit = ΔA_{253} of 0.001 per minute with N–α-benzoyl-L-arginine ethyl ester (BAEE) as substrate at pH 7.6 at 25 °C. Reaction volume = 3.2 mL (1 cm light path).

Protein determined by biuret.

solubility
serum-free medium .....
balanced salt solution ..... 1 mg/mL

ship: ambient store at: 2-8°C

T6522-25MG	25 mg
T6522-100MG	100 mg
T6522-5X100MG	5 × 100 mg
T6522-250MG	250 mg
T6522-500MG	500 mg
T6522-1G	1 g
T6522-5G	5 g

solution, 1 ×, sterile-filtered, BioReagent, suitable for cell culture

Contains chromatographically purified soybean trypsin inhibitor and iron supplemented calf serum.

Optimized for passage of endothelial cell cultures.

Prepared in Dulbecco's Phosphate Buffered Saline without calcium and magnesium.

endotoxin ..... tested

ship: dry ice store at: –20°C

T6414-100ML	100 mL
T6414-6X100ML	6 × 100 mL

Trypsin Inhibitor (Cat. No. T6522)

1) Prepare the trypsin inhibitor by dissolving it in serum-free medium at a concentration of 1 mg/ml.
2) Sterile filter the trypsin inhibitor solution through a 0.2 µm cellulose acetate membrane.
3) After trypsinizing cells, resuspend them in 1 ml trypsin inhibitor solution per ml of trypsin.
4) Centrifuge the cells at 1,000 rpm for 5 minutes.
5) Remove as much of the trypsin inhibitor solution as possible and resuspend the pellet in serum-free medium.
6) Culture as desired.

Phosphatase Inhibitor Cocktail 1

DMSO solution

A mixture of inhibitors that will inhibit the L-isozymes of alkaline phosphatase as well as serine/threonine protein phosphatases such as PP1 and PP2A. Contains microcystinLR, cantharidin, and (–)-p-bromotetramisole. This product has been replaced with P0044.

Recommended usage is a 100-fold dilution.

Tested on cell extracts from animal tissues such as bovine liver, human placenta, and rabbit muscle, as well as on extracts from A431 and Jurkat cell lines.

ship: wet ice store at: 2-8°C

P2850	

Phosphatase Inhibitor Cocktail 2

aqueous solution (gray coloration may develop upon storage, which does not affect the activity)

A mixture of inhibitors that will inhibit acid and alkaline phosphatase as well as tyrosine protein phosphatases. Contains sodium vanadate, sodium molybdate, sodium tartrate, and imidazole.

Recommended usage is a 100-fold dilution.

Tested on cell extracts from animal tissues such as bovine liver, human placenta, and rabbit muscle, as well as on extracts from A431 and Jurkat cell lines.

ship: wet ice store at: 2-8°C

P5726-1ML	1 mL
P5726-5ML	5 mL

Protease Inhibitor Cocktail

for use in tissue culture media, DMSO solution

Mixture of protease inhibitors with broad specificity for the inhibtion of serine, cysteine, aspartic and aminopeptidases. Contains aprotinin, bestatin, leupeptin, E-64, and pepstatin A. Contains no metal chelators

Use at a dilution of 1:200 or more in tissue culture media to prevent proteolytic degradation of secreted proteins.

Solution in DMSO (D 2650, Hybri-Max™).

After 48 hours exposure, the product is non-toxic to adherent cell lines A431, CHO, COS, HepG2 and HeLa; and to Jurkat and HL-60 cell lines grown in suspension.

ship: dry ice store at: –20°C

P1860-1ML	1 mL

for general use, lyophilized powder

Mixture of water-soluble protease inhibitors with broad specificity for the inhibition of serine, cysteine, and metalloproteases. Contains 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), E-64, bestatin, leupeptin, aprotinin, and sodium EDTA.

One bottle makes 100 mL of cocktail. One mL is recommended for the inhibition of proteases equivalent to 1 mg of USP pancreatin.

ship: ambient store at: –20°C

P2714-1BTL	1 bottle

for use with mammalian cell and tissue extracts, DMSO solution

A mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, aspartic proteases and aminopeptidases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pepstatinA, E-64, bestatin, leupeptin, and aprotinin. Contains no metal chelators.

One mL is recommended for the inhibition of proteases extracted from 20 g of bovine liver.

ship: ambient store at: –20°C

P8340-1ML	1 mL
P8340-5ML	5 mL

for use in purification of Histidine-tagged proteins, DMSO solution

Mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, aspartic, and aminopeptidases, and thermolysin-like activities. Formulated with no metal chelators that might inhibit binding of histidine-tagged proteins to metal affinity resins (IMAC). Contains 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), bestatin, pepstatin A, E-64, and phosphoramidon.

One mL is recommended for the inhibition of proteases extracted from 20 g of *Escherichia coli* or 10 g of baculovirus-infected *Spodoptera frugiperda* pupal ovary cells in a total volume of 100 ml.

ship: ambient store at: –20°C

P8849-1ML	1 mL
P8849-5ML	5 mL

SIGMA*FAST*™ Protease Inhibitor Tablets

For General Use

Tableted formulation containing water-soluble protease inhibitors with broad specificity for the inhibition of serine, cysteine, and metalloproteases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), E-64, bestatin, leupeptin, aprotinin, and EDTA (sodium salt).

One tablet makes 100 mL of cocktail. One mL is recommended for the inhibition of proteases equivalent to 1 mg of USP pancreatin. One tablet is recommended for the inhibition of proteases present in a maximum of 20 g of cell extract.

ship: ambient store at: 2-8°C

S8820-20TAB	20 tablets

Composition and Usage of Inhibitor Cocktails

	Protease Inhibitor Cocktails — Cat. No.					– Phosphatase Inhibitor Cocktails – Cat. No.	
	P1860	P8340	P8849	P2714	S8820	P5726	P2850
Target (Tissues or Enzymes)	Tissue Culture Media	Mammalian Tissues	Histidine-tagged proteins	General Use	General Use	Tyrosine protein phosphatases and acid and alkaline phosphatases	Ser/Thr protein phosphatases and L-isozymes of alkaline phosphatases
AEBSF*	•	•	•	•	•		
Aprotinin	•	•		•	•		
Bestatin	•	•	•	•	•		
EDTA				•	•		
E-64	•	•	•	•	•		
Leupeptin	•	•		•	•		
Pepstatin A	•	•	•				
Phosphoramidon			•				
(—)-p-Bromotetramisole							•
Canthardin							•
Imidazole						•	
Microcystin LR							•
Sodium molybdate						•	
Sodium orthovanadate						•	
Sodium tartrate						•	
Form of Cocktail	Solution in DMSO	Solution in DMSO	Solution in DMSO	Lyophilized powder (water soluble)	Tablet (water soluble)	Solution in water	Solution in DMSO
Usage: 1 mL of cocktail will inhibit:	Dilution of >1:200 in tissue culture media to prevent proteolytic degradation of secreted proteins	100 ml of cell lysate from 20 g (wet weight) of bovine liver or 10 mL of cell lysate obtained from CHO cells at a density of 107 cells per mL	100 mL of cell lysate from 20 g (wet weight) of *E. coli* cells or 10 g (wet weight) of baculovirus infected cells	1 mg of pancreatin	1 mg of USP pancreatin	100,000 x *g* supernatant from human placenta, bovine liver, rabbit muscle, A431 or Jurkat cell extracts at a protein concentration of approx. 5 mg/mL	100,000 x *g* supernatant from human placenta, bovine liver, rabbit muscle, A431 or Jurkat cell extracts at a protein concentration of approx. 5 mg/mL

AEBSF: A non-toxic alternative to PMSF and DFP

Equipment

Cell dissociation sieve - Tissue Grinder Kit

non-sterile; autoclavable

stainless steel

Kit contains one 85 mL cup, 5 each of 40, 50 and 60 mesh screens, 5 screen replacement keys and 2 glass pestles.

ship: ambient store at: room temp

CD1-1KT	1 kit

Key for screen replacement for CD-1™

ship: ambient store at: room temp

K3878-5EA	5 ea

Cell Dissociation Solutions (Cat. Nos. C1419, C1544, C5789, C5914)

Product Description

Sigma's Cell Dissociation Solutions are designed for the gentle removal of cells from the growing surface of the culture vessel. The unique formula contains no protein, and allows dislodging of cells without the use of enzymes. Cellular proteins are preserved without enzymatic modification or adsorption of foreign proteins. These products may be particularly useful for immunochemical studies that are dependent upon the recognition of cell surface proteins. Cell Dissociation Solutions are prepared in either Hanks' balanced salts or phosphate buffered saline. In addition, each contains EDTA, glycerol, and sodium citrate.

Recommended Use:
The following protocol is suggested for using the Cell Dissociation Solutions:
1) Prewarm all reagents.
2) Remove all medium from the culture vessel.
3) Rinse the cells with a balanced salt solution without calcium and magnesium. Gently rock the vessel for 30 seconds and remove the buffer.
4) Add the Cell Dissociation Solution (about 5 ml/75 cm^2 flask) and rock the vessel to bathe the cell monolayer.
5) Incubate 5-10 minutes.
6) Sharply tap the vessel against the palm of your hand to dislodge the cells. Strongly adherent cells may require additional time to become dislodged.
7) Add complete growth medium to the cells and pipette repeatedly to dissociate clumps.

Product Storage:
Cell Dissociation Solutions should be stored at refrigerator temperatures (2-8 °C). Freezing may cause irreversible precipitation and is not recommended.

Cell Freezing

Freezing living cells with the hope of full recovery began with the freezing of sperm in glycerol in 1949.[1] Later DMSO was shown to be a better preservative as it can better permeate cells like red blood cells.[2] Cryopreservation for all practical purposes is limited to individual cells or small clumps of cells. So preserving mammalian cells, plant cells, blood, sperm, and embryos is routine practice for clinicians and researchers. DMSO has proven to be the most robust cryopreservative agent and the most widely used. DMSO has some drawbacks in that it is cytotoxic and given that it permeates cells, it is difficult to completely eliminate. There are a number of non-permeating cryoprotectants that have also proven effective such as hydroxyethyl starch, PVP and CMC. Typically non-permeating cryoprotectants are not as efficient at preserving cell viability and are often used in concert with DMSO to enhance the efficiency of cryoprotection.

There are two common methods of cryopreservation, controlled rate freezing and vitrification. Controlled rate freezing typically involves placing the cells in a cryoprotectant media and cooling at a rate of –1 to –3 °C per minute, to about –80 °C and finally storing at –196 °C in liquid nitrogen. In this method, ice crystals form outside of cells first, and the higher concentrations of solutes that are excluded from ice crystals draw water from the cells and prevent or minimize freezing inside the cells. Controlled rate freezing is the most commonly practiced method, as it has proven to be robust and widely applicable. However, the osmotic changes and ice crystals formed using this technique can lead to reduced recovery and cryopreservation-induced delayed-onset cell death. Vitrification involves rapid freezing in higher concentration of cryoprotectants. The higher concentrations of cryoprotectants and rapid freezing prevent the formation of crystalline ice and promote the formation of an amorphous ice or glass. Unfortunately, vitrification is not without some problems as near toxic concentrations of DMSO are often required to promote the formation of a glass. Furthermore at this time the conditions required to achieve vitrification vary from application to application.

Sigma offers a wide selection of equipment and reagents for your cryopreservation needs that include DMSO, prepared freezing media and Mr. Frosty Freezers. In addition, we recenty introduced CryoStor® a completely optimizable serum free cryopreservation system, HypoThermosol®, a highly protective media for storage of cells and tissues at 2–8 °C and will soon offer sericin, a protein from silkworm that has proved to be a valuable FBS replacement in cryopreservation.

References

1. Revival of spermatozoa after vitrification and dehydration at low temperatures. C. Polge, et al., Nature 164 (1949) 666–667.
2. Prevention of Freezing Damage to Living Cells by Dimethyl Sulphoxide. Lovelock, J. E. and Bishop, M. W. H. Nature, 183: 1394–1395 (1959).

Cell Freezing Media and Reagents

Carboxymethylcellulose sodium

Carboxymethylcellulose sodium salt; Sodium carboxymethylcellulose [9004-32-4]

meets USP testing specifications, Medium viscosity

Useful for the study of attached cell and three-dimensional tissue culture models.

viscosity 400-800 cP, 2% in H_2O (25 °C)(lit.)

ship: ambient store at: room temp

C9481-500G	500 g
C9481-1KG	1 kg

Cell Freezing Medium-DMSO 1×

sterile-filtered, suitable for cell culture

Complete ready-to-use medium. Proprietary formulation with Minimum Essential Medium, dimethyl sulfoxide, calf serum and fetal bovine serum.

Recommended for the cryopreservation of cells.

endotoxin ..... tested

ship: dry ice store at: –20°C

C6164-50ML	50 mL
C6164-6X50ML	6 × 50 mL

Cell Freezing Medium-DMSO Serum free 1x

sterile-filtered, suitable for cell culture

Complete ready-to-use medium containing Minimum Essential Medium, dimethyl sulfoxide (8.7%) and methyl cellulose. Prepared according to the formulation of Waymouth[1].

Recommended for the cryopreservation of cells.

endotoxin ..... tested

Lit. cited: 1. Waymouth, C. and Varnum, D., Simple freezing procedure for storage in serum-free media of cultured and tumor cells of mouse. *TCA Manual* (1976), **2(1)**, 311-313

ship: dry ice store at: –20°C

C6295-50ML	50 mL

Cell Freezing Medium-Glycerol 1×

sterile-filtered, suitable for cell culture

Complete ready-to-use medium. Proprietary formulation containing Minimum Essential Medium, glycerol, calf serum and fetal bovine serum.

Recommended for the cryopreservation of cells.

endotoxin ..... tested

ship: dry ice store at: –20°C

C6039-50ML	50 mL

Cell Freezing Medium-Serum-free 1×

sterile-filtered, suitable for cell culture

Ready-to-use formula that does not contain dimethyl sulfoxide or serum.

For use with cells grown in serum-free conditions.

ship: ambient store at: 2-8°C

C2639-50ML	50 mL

Dimethyl sulfoxide

DMSO; Methyl sulfoxide

[67-68-5] $(CH_3)_2SO$ C_2H_6OS FW 78.13

Supercools easily and remelts slowly at room temperature. Solidified product can be re-liquified by warming to room temperature without detriment to the product.

density ..... 1.10 g/mL
n_D^{20} 1.479
vd ..... 2.7 (vs air)
vp ..... 0.42 mmHg (20 °C)
ait ..... 573 °F
lel(63 °F) 42%

Hybri-Max™, sterile-filtered, BioReagent, suitable for hybridoma, ≥99.7%

This product is a Hybri-Max product. It is hybridoma tested and is assessed for suitability in cell freezing. This product is sterile filtered (0.2 micron) and tested for endotoxin levels.

DMSO is a polar aprotic solvent used in chemical reactions, in polymerase chain reactions (PCR) and as a cryoprotectant vitrification agent for the preservation of cells, tissues and organs. DMSO is used in cell freezing media to protect cells from ice crystal induced mechanical injury. It is used for frozen storage of primary, sub-cultured, and recombinant heteroploid and hybridoma cell lines; embryonic stem cells (ESC), and hematopoietic stem cells. DMSO is frequently used in the combinations with BSA or fetal bovine serum (FBS).

5 mL and 10 mL in flame sealed ampules, 100 mL in amber bottle

endotoxin ..... tested

ship: ambient store at: room temp

D2650-5X5ML	5 × 5 mL
D2650-5X10ML	5 × 10 mL
D2650-100ML	100 mL

sterile-filtered, Biotechnology Performance Certified, meets EP, USP testing specifications, suitable for hybridoma

Human and animal cell lines grown in culture are generally stored frozen. Freezing protects the cell line from changes due to genetic drift and minimizes risk of contamination. Liquid nitrogen used in conjunction with a cryoprotective agent such as DMSO is a widely used method for preserving cells. Without the presence of a cryoprotective agent, freezing is lethal to most mammalian cells. Damage is caused by mechanical injury by ice crystals, concentration of electrolytes, pH changes, and denaturation of proteins. These lethal effects are minimized by adding a cryoprotective agent which lowers the freezing point and allows for a slower cooling rate. BPC-tested DMSO also meets the requirements of biotechnology and tissue engineering applications.

Features and Benefits

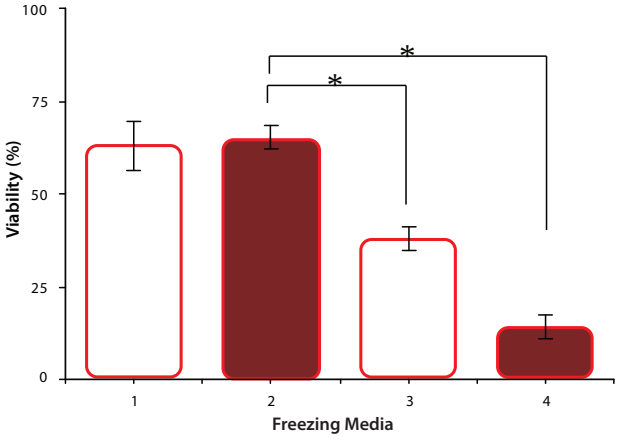
Ready-to-use sterile filtered product conveniently packaged septum screw-capped amber bottles. Meets USP and EP testing requirements (USP XXIV)

ship: ambient store at: room temp

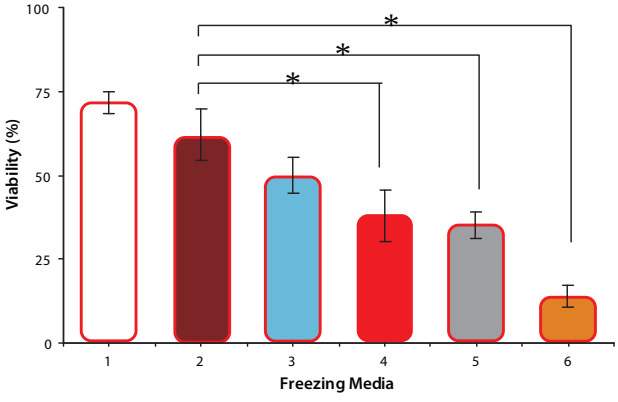
D2438-5X10ML	5 × 10 mL
D2438-50ML	50 mL

Sericin *Bombyx mori* (silkworm) NEW

Sericin is a protein derived from the silkworm cocoon and has a number of beneficial characteristics for culturing mammalian cells. It has cryoprotective properties that allow for replacement of FBS in cryopreservation media. 1% sericin (w/v) along with 0.5% (w/v) maltose, 0.3% (w/v) proline, 0.3% (w/v) glutamine and 10% DMSO is comparable to 90% FBS and 10% DMSO[1]. Furthermore, sericin can act as a FBS replacement in cell culture and stimulate cell growth[2,3] and in certain situations, inhibit cell death[4].

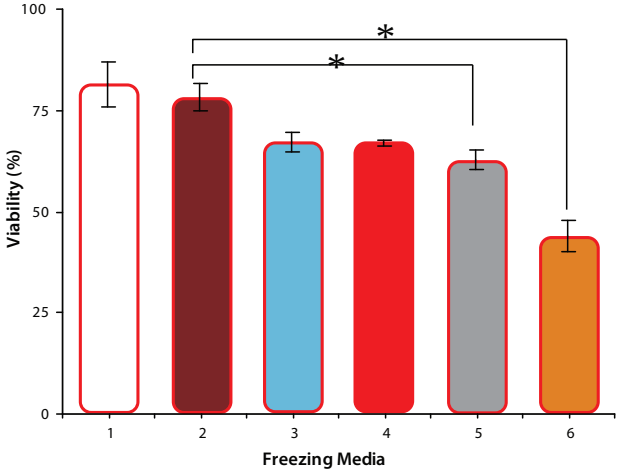


P3U1 myeloma cells (1×10^6) in 1 mL of freezing medium were frozen at -80 °C for 1 day. The cryovials were then thawed and the viability was determined. Freezing medium **1:** FBS supplemented with 10% DMSO; freezing medium **2:** freezing medium 3 supplemented with 1% sericin; freezing medium **3:** PBS supplemented with 0.5% maltose, 0.3% proline, 0.3% glutamine and 10% DMSO; freezing medium **4:** PBS supplemented with 10% DMSO. The error bars indicate S.D. (n=3). *P<0.05.

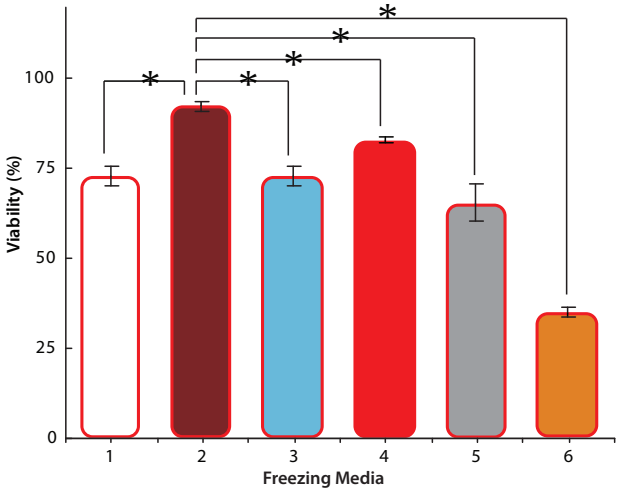


P3U1 myeloma cells 1×10^6 in 1 mL of freezing medium were frozen at -80 °C for 1 day. The vials were then thawed and the viability of the cells was determined immediately. Freezing medium **1:** FBS supplemented with 10% DMSO; freezing medium **2:** PBS supplemented with 1% sericin, 0.5% maltose, 0.3% proline, 0.3% glutamine and 10% DMSO; freezing medium **3:** Competitor A **4:** Competitor B **5:** Competitor C; **6:** PBS supplemented with 10% DMSO. The error bars indicate S.D. (n=3). *P<0.05.

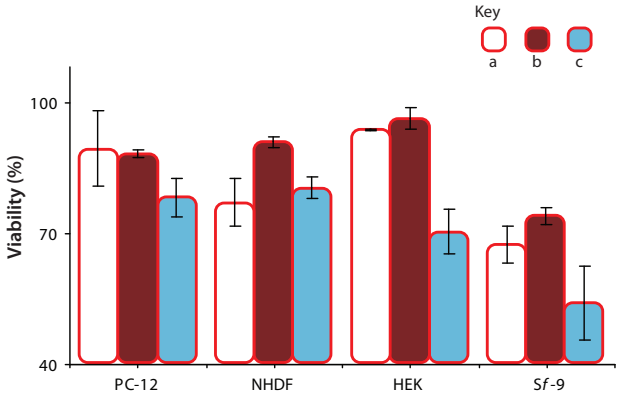
Sericin *Bombyx mori* (silkworm) *(continued)*



Hybridoma cells (1×10^6) in 1 mL of freezing medium were frozen at -80 °C for 1 day. The vials were then thawed and then the vials were frozen and thawed again before the viability was determined. Freezing medium **1:** FBS supplemented with 10% DMSO; freezing medium **2:** PBS supplemented with 1% sericin, 0.5% maltose, 0.3% proline, 0.3% glutamine and 10% DMSO; freezing medium **3:** Competitor A **4:** Competitor B **5:** Competitor C; **6:** PBS supplemented with 10% DMSO. The error bars indicate S.D. (n=3). *P<0.05.



CHO cells (1×10^6) in 1 mL of freezing medium were frozen at -80 °C for 1 day. The vials were then thawed and the viability of the cells was determined immediately. Freezing medium **1:** FBS supplemented with 10% DMSO; freezing medium **2:** PBS supplemented with 1% sericin, 0.5% maltose, 0.3% proline, 0.3% glutamine and 10% DMSO; freezing medium **3:** Competitor A **4:** Competitor B **5:** Competitor C; **6:** PBS supplemented with 10% DMSO. The error bars indicate S.D. (n=3). *P<0.05.



PC12, NHDF, HEK or insect *Sf*-9 cells (1×10^6) in 1 mL of freezing medium were frozen at -80° C for 1 day. The vials were then thawed and the viability of the cells was determined. Freezing media: FBS supplemented with 10% DMSO (**a**); PBS supplemented with 1% sericin, 0.5% maltose, 0.3% proline, 0.3% glutamine and 10% DMSO (**b**); and PBS supplemented with 10% DMSO (**c**). The error bars indicate S.D. (n=3). *P<0.05.

Lit. cited: 1. Sasaki, M., et al., Development of a novel serum-free freezing medium for mammalian cells using the silk protein sericin. *Biotechnol. Appl. Biochem.* **42**, 183-188 (2005)
2. Terada, S., et al, Sericin, a protein derived from silkworms, accelerates the proliferation of several mammalian cell lines including a hybridoma *Cytotechnology* **40**, 3-12 (2002)
3. Tsubouchi, K., et al, Sericin enhances attachment of cultured human skin fibroblasts *Biosci. Biotechnol. Biochem.* **69**, 403-5 (2005)
4. Takahashi, M, The silk protein, sericin, protects against cell death caused by acute serum deprivation in insect cell culture *Biotechnol. Lett.* **25**, 1805-9 (2003)
ship: ambient store at: room temp

S5201-1G	1 g
S5201-5G	5 g

CryoSOfree™ DMSO-free Cryopreservation Medium NEW

CryoSOfree is a chemically defined DMSO-free cryopreservation medium. It is animal component free and has been shown[1,2] to preserve the potential of stem cells for differentiation.

- Preserves pluripotency of stem cells
- Low cytotoxicity
- ≥90% recovery is typical
- Chemically defined, serum free, protein free
- Ready to use

The following cell lines have been recovered at ≥90 following cryopreservation with CryoSOfree:
L929, KB, MC3T3, B16F1, Colon26, CaCO2, HT1080, MG63

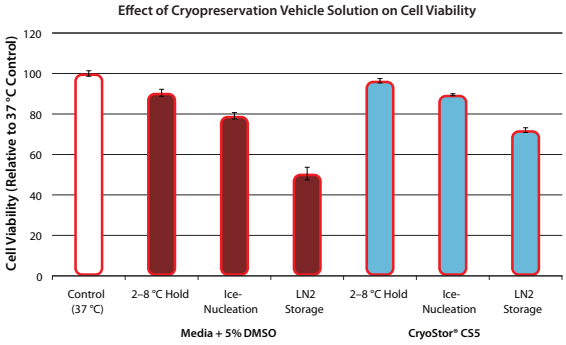
1 ml of CyroSOfree is sufficient for 5x10^5- 5x10^6 cells.

Lit. cited: 1. Matsumura K, Bae JY, Hyon SH, Polyampholytes as cryoprotective agents for mammalian cell cryopreservation. *Cell. Transplant.* **19**, 691-9 (2010)
2. Matsumura K, Hyon SH, Polyampholytes as low toxic efficient cryoprotective agents with antifreeze protein properties. *Biomaterials* **30**, 4842-9 (2009)
ship: ambient store at: 2-8°C

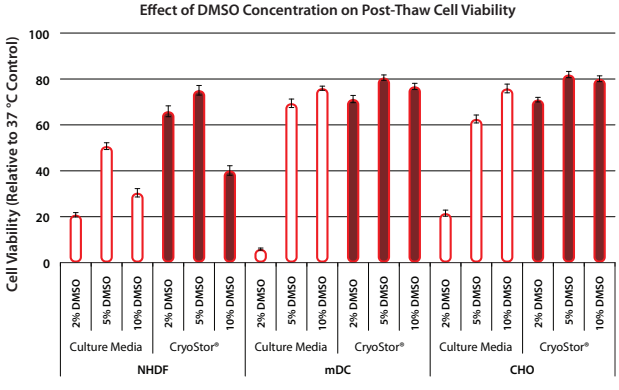
C9249-100ML	100 mL

The CryoStor® line of serum free cryoprotective products allow higher recovery rates than using media with 10% FBS and DMSO (Figure 1). CryoStor® allows researchers to optimize, and if necessary, minimize the amount of DMSO needed (Figure 2). The CryoStor® line is serum-free so concerns of viruses, BSE or other FBS/protein based contaminations are eliminated. In addition, CryoStor® is robust and has been tested with the following cell types.

- Liver Cells (Hepatocytes & C3A)
- Cardiomyocytes
- Stem Cells Hybridomas
- PBMC Pancreatic Islets
- Cord Blood Skeletal Muscle Cells (SKMC)
- T-Cells Smooth Muscle Cells (CASMC)
- Lymphocytes PBSC
- Coronary Artery Endothelial Cells (CAEC) Chondrocytes
- Cancer Cells (PC3, LNCaP) CHO
- Skeletal Myoblasts C2C12
- Neuronal Cells Ocular (BCE, HCE)
- Keratinocytes (A431) Engineered tissue
- Renal Cells (RPTEC, MDCK) Organ / Tissue
- Fibroblasts (NHDF, 3T3) Vascular Tissue
- Transfected Cells Hair Follicle
- HEK Dendritic Cells



Viability of NHDF cells following exposure to cell culture media + 5% DMSO or CryoStor® + 5% DMSO (CS5). Solution efficacy was investigated following a 10 min hold at 2–8 °C, following ice-nucleation, and after LN2 Storage. For each of the test conditions, samples were removed, thawed, diluted with media and plated. Relative cell viability was determined 1 day post-thaw and overall viability was compared to 37 °C non-cryopreserved control cultures.



Post-thaw viability of cells following cryopreservation with varying concentrations of DMSO in either cell culture media or CryoStor®. Relative cell viability was determined 1 day post-thaw as described in the methods and overall viability was compared to 37 °C non-cryopreserved control cultures. DMSO concentration and vehicle solution impact cell viability post-thaw.

CryoStor® cell cryopreservation media

NEW

CryoStor, a series of cell-specific, optimized preservation media, is uniquely formulated to address the molecularbiological aspects of cells during the cryopreservation process thereby directly reducing the level of Cryopreservation-Induced Delayed-Onset Cell Death and improving post-thaw cell viability and function.

store at: 2-8°C

CS2

Formulation contains 2% DMSO.

CryoStor CS2 is formulated to contain 2% dimethyl sulfoxide (DMSO). Suggested when reducing DMSO is of primary concern.

ship: ambient store at: room temp

C3124-100ML	100 mL

CS5

Formulation contains 5% DMSO.

Cryostor CS5 is formulated to contain 5% dimethyl sulfoxide (DMSO). Recommended for cryopreservation of most cell types.

ship: ambient store at: room temp

C2999-100ML	100 mL

CS10

Formulation contains 10% DMSO.

CryoStor® CS10 is a uniquely formulated cryopreservation medium containing 10% dimethyl sulfoxide (DMSO). Recommended for the preservation of hepatocytes, tissue samples and other extremely sensitive cell types.

ship: ambient store at: room temp

C2874-100ML	100 mL

HypoThermosol®-FRS Preservation Solution

NEW

HypoThermosol is an optimized hypothermic (2-8°C) preservation media that enables improved and extended preservation of cells, tissues and organs. HypoThermosol-FRS is uniquely formulated to address the molecular-biological response of cells during the hypothermic preservation process.

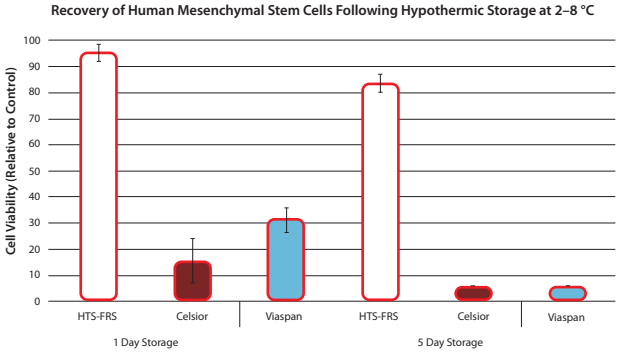
HypoThermosol-FRS includes key ions at concentrations that balance the intracellular state at hypothermic temperatures. Additional components include pH buffers, energy substrates, free radical scavengers, and osmotic/oncotic stabilizers.

ship: ambient store at: 2-8°C

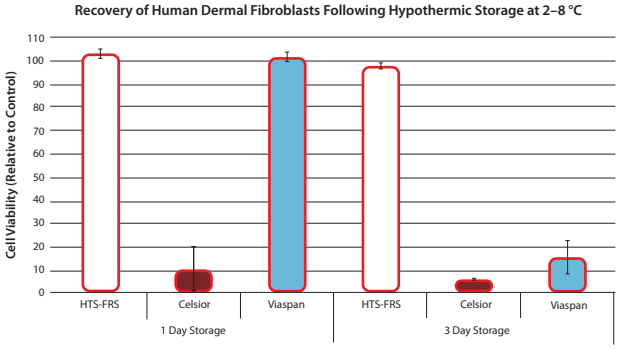
H4416-100ML	100 mL

Figures 1 and 2 clearly show that HypoThermosol® outperforms commonly used hypothermic preservation media on Human Mesenchymal Stem Cells (hMSC) and on Human Dermal Fibroblasts. In addition to these cell lines hypothermosol has been tested on the following cell lines, tissues and organs:

- Liver Cells (Hepatocytes & C3A)
- Cardiomyocytes
- Stem Cells Hybridomas
- PBMC Pancreatic Islets
- Cord Blood Skeletal Muscle Cells (SKMC)
- T-Cells Smooth Muscle Cells (CASMC)
- Lymphocytes PBSC
- Coronary Artery Endothelial Cells (CAEC) Chondrocytes
- Cancer Cells (PC3, LNCaP) CHO
- Skeletal Myoblasts C2C12
- Neuronal Cells Ocular (BCE, HCE)
- Keratinocytes (A431) Engineered tissue
- Renal Cells (RPTEC, MDCK) Organ / Tissue
- Fibroblasts (NHDF, 3T3) Vascular Tissue
- Transfected Cells Hair Follicle
- HEK Dendritic Cells



Recovery of human mesenchymal stem cells (hMSC) following hypothermic preservation at 2 –8 °C for either 1 day or 5 days. Cells were assayed for metabolic activity following 24 hours recovery post-preservation.



Recovery of human dermal fibroblasts (NHDF) following hypothermic preservation at 2–8 °C for either 1 day or 3 days. Cells were assayed for metabolic activity following 24 hours recovery post-preservation.

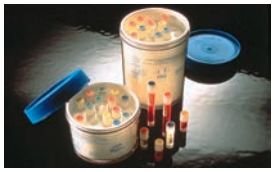
Equipment

Freezing container, Nalgene® Mr. Frosty

Polycarbonate container, blue high-density polyethylene closure, white high-density polyethylene vial holder, and foam insert.
Provides the critical, repeatable, 1 °C/min cooling rate required for successful cryopreservation of cells. Easy to use in any mechanical freezer. Container is imprinted with graphic instructions. Requires only isopropyl alcohol. Rigid vial holder keeps vials from contacting alcohol, preventing contamination by wicking. Vial holder floats to allow thawing in a water bath. Numbers molded for identification. All components withstand repeated freeze/thaw cycles. Stackable.

Holds 18 1.2 and 2.0 mL cryovials

H:86 mm × diam.:117 mm



ship: ambient store at: room temp

C1562-1EA	1 ea

Borrelia Culture Products

BSK-H Medium

With sodium bicarbonate, suitable for *Borrelia burgdorferi* (Qualified)

BSK-H medium is a standardized complex medium designed to support the growth of the Lyme disease spirochete, Borrelia burgdorferi. To standardize the procedure for isolating and culturing Lyme disease spirochetes, Pollack, RJ et. al. modified the composition of the medium generally used for this purpose (BSK-II) and developed a system for its distribution. This medium contains no gelatin or agarose, and various components are used in proportions that differ from those in BSK-II. Each of the major proteinacious components was screened by substitution in samples of the complete product. The final medium was evaluated for the capacity to grow related spirochetes including Borrelia burgdorferi N40, Guilford, and JD-1 as well as strains of Borrelia hermsii (HS-1) and of Borrelia coriaceae (CO53). Standardized medium, supplemented with prescreened rabbit serum, facilitates comparison of research results between laboratories and may eventually permit definitive clinical diagnosis of Lyme disease based on demonstration of the pathogen. The standardized medium is designated BSK-H.

For best results, serum used to supplement the medium should be prescreened for its ability to support the growth of Borrelia burgdorferi. Prescreened, sterile filtered Rabbit Serum [Product No. R7136] is available from Sigma.

ship: dry ice store at: –20°C

B3528-100ML	100 mL
B3528-500ML	500 mL
B3528-1L	1 L

BSK-H Medium, Complete

sterile-filtered, With 6% rabbit serum, suitable for *Borrelia burgdorferi*

BSK-H medium is a standardized complex medium designed to support the growth of the Lyme disease spirochete, Borrelia burgdorferi. To standardize the procedure for isolating and culturing Lyme disease spirochetes, Pollack, RJ et. al. modified the composition of the medium generally used for this purpose (BSK-II) and developed a system for its distribution. This medium contains no gelatin or agarose, and various components are used in proportions that differ from those in BSK-II. Each of the major proteinacious components was screened by substitution in samples of the complete product. The final medium was evaluated for the capacity to grow related spirochetes including Borrelia burgdorferi N40, Guilford, and JD-1 as well as strains of Borrelia hermsii (HS-1) and of Borrelia coriaceae (CO53). Standardized medium, supplemented with prescreened rabbit serum, facilitates comparison of research results between laboratories and may eventually permit definitive clinical diagnosis of Lyme disease based on demonstration of the pathogen. The standardized medium is designated BSK-H.

BSK-H Medium is supplied as a sterile-filtered liquid. After thawing, mix well by inverting the bottle prior to use. If the thawed medium will not be used within a few days it is recommended that the medium be refrozen in working aliquots to avoid repeated free-thaw cycles. Other supplements may be added aseptically as desired. The nature of the supplement may affect storage conditions and shelf-life of the medium.

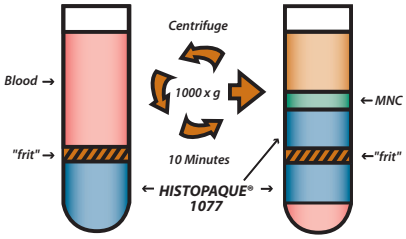
ship: dry ice store at: –20°C

B8291-100ML	100 mL
B8291-500ML	500 mL
B8291-1L	1 L

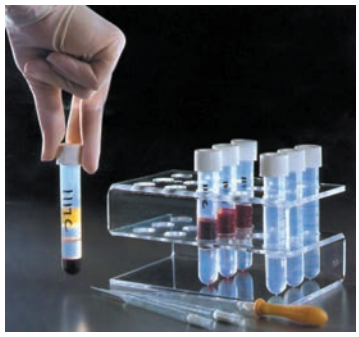
Cell Separation

Accuspin™

Sigma-Aldrich offers a complete line of products for the separation or extraction of leukocytes, viruses, DNA, RNA, organelles, as well as many other applications. Featured in the product line is Accuspin™, a sterile, 2-chamber tube separated by a porous frit. Whole blood can be added directly to the Accuspin™ tube without the risk of mixing with the HISTOPAQUE®-1077 contained in the lower chamber. For application assistance, contact our technical team at (800) 325-0250 or by e-mail at clintech@sial.com.



Accuspin™ SYSTEM-HISTOPAQUE®-1077



The Accuspin™ System Histopaque-1077
Method: Density Gradient Centrifugation on Sodium Diatrizoate-Polysucrose Gradients
Principle: Accuspin™ System Histopaque-1077 employs centrifuge tubes specially designed with two chambers separated by a porous high-density polyethylene barrier("frit"). The lower chamber contains Histopaque-1077 which allows the addition of anticoagulated whole blood without risk of mixing with the separation medium. On centrifugation, erythrocytes & granulocytes descend through the frit to pellet below the Histopaque-1077. The erythrocytes aggregate, increasing their sedimentation rate, resulting in pelleting at the bottom of the Accuspin™ tube. Lymphocytes and other mononuclear cells, i.e., monocytes, remain above the frit at the plasma-Histopaque-1077 interface.

Accuspin™ System-Histopaque®-1077

Accuspin™ System-HISTOPAQUE-1077 employs centrifuge tubes specially designed with two chambers separated by a porous high-density polyethylene barrier ("frit"). The lower chamber contains HISTOPAQUE-1077 which allows the addition of anticoagulated whole blood without risk of mixing with the separation medium. On centrifugation, the whole blood descends through the frit to contact with the HISTOPAQUE-1077 below the frit, giving a clear separation of the blood components. The erythrocytes aggregate and the granulocytes become slightly hypertonic, increasing their sedimentation rate, resulting in pelleting at the bottom of the Accuspin™ tube. Lymphocytes and other mononuclear cells, i.e., monocytes, remain at the plasma-HISTOPAQUE-1077 interface.

for *in vitro* diagnostic use

store at: 2-8°C

Accuspin™ System-Histopaque®-1077 *(continued)*

Polypropylene radiation sterilized tube fitted with a high density polyethylene barrier and sterile filled with HISTOPAQUE®-1077.

Each tube contains 3 mL Histopaque 1077-1 and will separate 3-6 mL of anticoagulated blood.

ship: ambient store at: room temp

A6929-40X3ML	40 × 3 mL

Histopaque®-1077 Hybri-Max™ (Cat. No. H8889)

Product Description

Histopaque®-1077 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 +/- 0.001 g/ml. This medium facilitates rapid recovery of viable lymphocytes from small volumes of whole blood. This technique is suitable for use in cell-mediated lympholysis and for human lymphocyte antigen (HLA) typing. It may also be employed as the initial isolation step prior to enumeration of T-, B-, and 'null' lymphocytes.

Performance Characteristics

Anticoagulated venous blood is layered onto HISTOPAQUE®-1077. During centrifugation, erythrocytes and granulocytes are aggregated by polysucrose and rapidly sediment. Lymphocytes and other mononuclear cells remain at the plasma-HISTOPAQUE®-1077 interface. Erythrocyte contamination is negligible. Most extraneous platelets are removed by low speed centrifugation during the washing steps.

Product Information

Density: 1.076-1.078 g/ml
Endotoxin: <0.3 ng/ml
Solution pH: 8.7-9.1
Splenocyte separation: Clear interface with 90% cell viability
Sterility: Sterile by USP XXIII

Reconstitution and Use

1. To 3 ml whole blood, collected in heparin or EDTA, add 5 ml PBS without calcium and magnesium and mix well by inversion.
2. To a 15 ml conical centrifuge tube, add 3 ml of HISTOPAQUE-1077 and bring to room temperature.
3. Carefully layer 8 ml of the blood-saline mixture onto the HISTOPAQUE-1077. Centrifuge at 400 x g for EXACTLY30 MINUTES at room temperature. Lower temps may cause clumping and poor recovery.
4. After centrifugation, use a Pasteur pipette to aspirate the upper layer to within 0.5 cm of the opaque interface containing the mononuclear cells. Discard the upper layer.
5. With a Pasteur pipette, carefully transfer the opaque interface to a clean, conical centrifuge tube.
6. Add to this tube 10 ml PBS and mix by inversion.
7. Centrifuge at 250 x g for EXACTLY 10 MINUTES.
8. Aspirate the supernatant and discard.
9. Resuspend lymphocyte pellet with 5 ml PBS and mix by gentle trituration with Pasteur pipette.
10. Centrifuge at 250 x g for EXACTLY 10 MINUTES.
11. Repeat steps 8, 9, and 10, discard supernatant and resuspend pellet in 0.5 ml PBS.

Histopaque®-1077 Hybri-Max™

liquid, sterile-filtered, BioReagent, suitable for hybridoma

Used to create a density medium for the purification of lymphocytes and other mononuclear cells. Histopaque®-1077 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 +/- 0.001 g/ml. This medium facilitates rapid recovery of viable lymphocytes from small volumes of whole blood. This technique is suitable for use in cell mediated lympholysis and for human lymphocyte antigen (HLA)2 typing. It may also be employed as the initial isolation step prior to enumeration of T-, B-, and 'null' lymphocytes.

endotoxin ..... tested
density ..... 1.077 g/mL, 25 °C
ship: ambient store at: 2-8°C

H8889-100ML	100 mL
H8889-500ML	500 mL

Ficoll®

Ficoll® 400
[26873-85-8]

A nonionic synthetic polymer of sucrose.

Used for cell separation and organelle isolation.

BioXtra, for molecular biology, lyophilized powder

Dialyzed and lyophilized

DNase, RNase, NICKase and protease ..... none detected
ship: ambient store at: room temp

F2637-5G	5 g
F2637-10G	10 g
F2637-25G	25 g
F2637-100G	100 g
F2637-500G	500 g

Ficoll® 400

[26873-85-8]

Dialyzed

A nonionic synthetic polymer of sucrose.

Used for cell separation and organelle isolation.

lyophilized powder, Type 400-DL, BioReagent, suitable for cell culture

ship: ambient store at: room temp

F8016-5G	5 g
F8016-100G	100 g
F8016-500G	500 g

lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture

Ficoll is a hydrophilic neutral highly branched polysaccharide use to establish density gradients to separate blood component cells and organelles. It is part of the separation medium called Ficoll-Paque.

ship: ambient store at: room temp

F8636-25G	25 g

Cell Viability and Proliferation

Assays to measure proliferation, viability, and cytotoxicity are commonly used to monitor the response and health of cells in culture after treatment with various stimuli. The proper choice of an assay method depends on the number and type of cells used as well as the expected outcome. Assays for cell proliferation may monitor the number of cells over time, the number of cellular divisions, metabolic activity, or DNA synthesis. Cell counting using viability dyes such as trypan blue or calcein-AM can provide both the rate of proliferation as well as the percentage of viable cells.

5(6)-Carboxyfluorescein diacetate N-succinimidyl ester (CFSE) is a popular choice for measuring the number of cellular divisions a population has undergone. Upon entering the cell, CFSE is cleaved by intracellular esterases to form the fluorescent compound and the succinimidyl ester group covalently reacts with primary amines on intracellular proteins. Upon division, the fluorescence intensity of each daughter cell is halved which allows for the simple detection of the number of cell divisions by flow cytometry.

Assays that measure metabolic activity are suitable for analyzing proliferation, viability, and cytotoxicity. The reduction of tetrazolium salts such as MTT and XTT to colored formazan compounds or the bioreduction of resazurin only occurs in metabolically active cells. Actively proliferating cells increase their metabolic activity while cells exposed to toxins will have decreased activity.

Kits

Cell Counting Kit

sufficient for 500 tests

The Cell Counting Kit (CCK) is utilized for the fluorometric detection of living cell numbers. The amount of a fluorescent dye, calcein, produced from calcein-AM (3′,6′-Di(O-acetyl)-2′,7′-bis[*N*,*N*-bis-(carboxymethyl) aminomethyl]-fluorescein, tetraacetoxymethyl ester) by esterases in cells is directly proportional to the number of viable cells in a culture medium. Since calcein-AM is highly lypohilic because of the acetoxymethy groups in the molecule, it can rapidly permeate into the cytoplasm through the cell membrane. The CCK assay does not require any radioisotopes (such as in the [^{3}H]- thymidine incorporated assay) or a solubilization procedure (such as in the MTT assay). Therefore, it allows the users to obtain highly reproducible and accurate cell proliferation assay results.

Kit contents: Calcein-AM and Dulbecco's phosphate-buffered solution, protocol for the fluorometric detection of the living cell numbers (48927F and 19361F)

ship: ambient store at: –20°C

03285-1KT-F	1 kit

Cell Counting Kit - 8

Cell Counting Kit-8 (CCK-8) allows convenient assays using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS. CCK-8 solution is added directly to the cells, no pre-mixing of components is required. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. Since the CCK-8 solution is very stable and it has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible.
Cell Counting Kit-8 allows sensitive colorimetric assays for the determination of the number of viable cells in the proliferation and cytotoxicity assays. The detection sensitivity is higher than any other tetrazolium salts such as MTT, XTT or MTS.

ship: dry ice store at: –20°C

96992-500TESTS-F	500 test
96992-3000TESTS-F	3000 test

In Vitro Toxicology Assay Kit, Lactic Dehydrogenase based

LDH reduces NAD^+, which then converts a tetrazolium dye to a soluble, colored formazan derivative.

Tox7 is a member of Sigma's CytoToxicity Kit series. It is based on the measurement of lactate dehydrogenase (LDH) release from cells as a measure of cell membrane damage and cytotoxicity. This technique has been utilized as an alternative to 51Cr release for cell mediated cytotoxicity assays, as well as conventional cytotoxicity resulting from interaction of a test material with the cell.

1 kit sufficient for 500 tests

ship: ambient store at: –20°C

TOX7-1KT	1 kit

In Vitro Toxicology Assay Kit, MTT based

Conversion of MTT to a water-insoluble colored formazan derivative which is then solubilized in acidic isopropanol.

For spectrophotometric measurement of cell viability by mitochodrial dehydrogenase. Absorbance of converted dye is measured at a wavelength of 570 nm.

1 kit sufficient for 1,000 tests

ship: ambient store at: 2-8°C

TOX1-1KT	1 kit

In Vitro Toxicology Assay Kit, Neutral Red based

Neutral red is taken up by viable cells and stored in the lysosomes. The dye is extracted and the uptake is quantitated by spectroscopy.

For spectrophotometric measurement of viable cells. Absorbance of converted dye is measured at a wavelength of 540 nm.

1 kit sufficient for 1,000 tests

ship: ambient store at: 2-8°C

TOX4-1KT	1 kit

In Vitro Toxicology Assay Kit, Resazurin based

Bioreduction of the dye reduces the amount of its oxidized form (blue) and concomitantly increases the fluorescent intermediate (red).

For spectrophotometric measurement of metabolic activity of living cells. Absorbance of converted dye is measured at a wavelength of 600 nm.

1 kit sufficient for 2,000 tests
ship: ambient store at: 2-8°C

TOX8-1KT	1 kit

In Vitro Toxicology Assay Kit, Sulforhodamine B based

Dye binds to cellular protein and is then solubilized in base.

For spectrophotometric measurement of biomass (viable and non-viable cells) by total protein. Absorbance of dye is measured at a wavelength of 565 nm.

1 kit sufficient for 1,000 tests
ship: ambient store at: room temp

TOX6-1KT	1 kit

In Vitro Toxicology Assay Kit, XTT based

Conversion of XTT to a water-soluble colored formazan derivative.

For spectrophotometric measurement of cell viability by mitochodrial dehydrogenase. Absorbance of converted dye is measured at a wavelength of 450 nm.

1 kit sufficient for 1,000 tests
ship: ambient store at: –20°C

TOX2-1KT	1 kit

Live/Dead Cell Double Staining Kit

Staining kit for live/dead cells

The Live/Dead Cell Double Staining Kit is utilized for simultaneous fluorescence staining of viable and dead cells. This kit contains calcein-AM and propidium iodide (PI) solutions, which stain viable and dead cells, respectively. Calcein-AM, acetoxymethyl ester of calcein, is highly lipophilic and cell membrane permeable. Though calcein-AM itself is not a fluorescent molecule, the calcein generated from Calcein-AM by esterase in a viable cell emits a strong green fluorescence (λ_{ex} 490 nm, λ_{em} 515 nm). Therefore, calcein-AM only stains viable cells. Alternatively, the nuclei staining dye PI cannot pass through a viable cell membrane. It reaches the nucleus by passing through disordered areas of dead cell membrane, and intercalates with the DNA double helix of the cell to emit red fluorescence (λ_{ex} 535 nm, λ_{em} 617 nm). Since both calcein and PI-DNA can be excited with 490 nm light, simultaneous monitoring of viable and dead cells is possible with a fluorescence microscope. Using λ_{ex} 545 nm, only dead cells can be observed.

passes test for fluorescence

Components

Solution A (Calcein AM solution) 4 × 50 µl
Solution B (propidium iodide solution) 300 µl
ship: ambient store at: –20°C

04511-1KT-F	1 kit

Senescence Cells Histochemical Staining Kit

▸ **sufficient for 100 tests**

Replicative senescence is a growth-arrest state associated with loss of division potential, changes in cell morphology, shape and physical appearance, and the pattern of gene expression in cells.

Histochemical staining of β-galactosidase activity is performed at pH 6.0. Under these conditions, β-galactosidase is a biomarker specific for senescent cells, but is not found in quiescent, immortal, or tumor cells.

Manufactured under license to US Patent Nos. 5,491,069 and 5,795,728.
ship: wet ice store at: –20°C

CS0030-1KT	1 kit

Bright-Line™ Hemacytometer

H-shaped moat forms two cell-counting areas. The surface features enhanced Neubauer rulings. Replacement cover slips sold separately.

Supplied with two cover slips.

ship: ambient store at: room temp

Z359629-1EA	1 ea

Stains, Dyes and Indicators

Giemsa stain

Azure mixture sicc. Giemsa stain
[51811-82-6] $C_{14}H_{14}ClN_3S$ FW 291.80

▸ **powder, BioReagent, suitable for cell culture**

Used as a chromosome stain and to differentiate nuclear morphology of platelets, RBCs, WBCs, and other cell types.

solubility
ethanol ..... 1 mg/mL
ship: ambient store at: room temp

G9641-5G	5 g
G9641-25G	25 g

Hoechst Stain solution

Bisbenzimide H 33258
[23491-45-4]

A fluorescent DNA stain qualified for use in Mycoplama staining.

Contains 0.5µg/ml Hoechst bisbenzimide 33258 fluorochrome stain and thimerosal.
ship: ambient store at: 2-8°C

H6024-10ML	10 mL

Neutral Red

3-Amino-7-dimethylamino-2-methylphenazine hydrochloride; Toluylene red; Basic Red 5
[553-24-2] $C_{15}H_{17}ClN_4$ FW 288.78

Useful as an indicator for preparing neutral red paper, and as a biological stain.

pH range ..... 6.8 - 8.0, red to yellow

▶ powder, BioReagent, suitable for cell culture

composition
Dye content ≥90%

Purified

solubility
H_2O ..... 10 mg/mL

ship: ambient store at: 2-8°C

N4638-1G	1 g
N4638-5G	5 g

Nigrosin water soluble

Acid black 2
[8005-03-6]

▶ powder, BioReagent, suitable for cell culture

Used as a viable cell staining agent especially in stem cell biology.

solubility
H_2O ..... 10 mg/mL

ship: ambient store at: room temp

N4763-25G	25 g

Phenol Red

Phenolsulfonphthalein
[143-74-8] $C_{19}H_{14}O_5S$ FW 354.38

Suitable for use as a pH indicator.

visual transition interval ..... 6.8 - 8.2, yellow to red

▶ powder, BioReagent, suitable for cell culture

solubility
1 M NaOH ..... 1 mg/mL

ship: ambient store at: room temp

P3532-5G	5 g
P3532-25G	25 g

Phenol red solution

[143-74-8]

▶ 0.5%, liquid, sterile-filtered, BioReagent, suitable for cell culture

For use as a pH indicator in cell culture applications.

Prepared in Dulbecco's Phosphate Buffered Saline.

endotoxin ..... tested

ship: ambient store at: room temp

P0290-100ML	100 mL

Phenol Red sodium salt

Phenolsulfonephthalein sodium salt
[34487-61-1] $C_{19}H_{13}NaO_5S$ FW 376.36

Suitable for use as a pH indicator.

visual transition interval ..... 6.8 - 8.2, yellow to red

▶ powder, BioReagent, suitable for cell culture, suitable for insect cell culture

solubility
H_2O ..... 1 mg/mL

ship: ambient store at: room temp

P5530-5G	5 g
P5530-25G	25 g
P5530-50G	50 g

Resazurin sodium salt

7-Hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt
[62758-13-8] $C_{12}H_6NNaO_4$ FW 251.17

▶ powder, BioReagent, suitable for cell culture

For the measurement of metabolic activity of living cells. The bioreduction of the dye reduces the amount of the oxidized form (blue) and concomitantly increases the fluorescent intermediate (red).

composition
Dye content ~80%

solubility
H_2O .....

λ ..... 600 nm

pH range ..... 3.8 - 6.5, orange to violet

ship: ambient store at: room temp

R7017-1G	1 g
R7017-5G	5 g

Sulforhodamine B sodium salt

[3520-42-1] $C_{27}H_{29}N_2NaO_7S_2$ FW 580.65

▶ powder, BioReagent, suitable for cell culture

Use as a fluorescent dye to quantify cellular proteins in cultured cells.

composition
Dye content ~75%

solubility
methanol ..... 1 mg/mL

ship: ambient store at: room temp

S1402-1G	1 g
S1402-5G	5 g
S1402-25G	25 g

Thiazolyl Blue Tetrazolium Bromide

MTT; 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; Methyl-thiazolyldiphenyl-tetrazolium bromide
[298-93-1] $C_{18}H_{16}BrN_5S$ FW 414.32

▶ powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥97.5% (TLC)

Use as a colorimetric metabolic activity indicator in cell viability assays.

solubility
H_2O ..... 5 mg/mL

ship: ambient store at: 2-8°C

M5655-100MG	100 mg
M5655-500MG	500 mg
M5655-1G	1 g

Trypan Blue

Direct blue 14
[72-57-1] $C_{34}H_{24}N_6O_{14}S_4Na_4$ $C_{34}H_{24}N_6Na_4O_{14}S_4$ FW 960.81

▸ powder, BioReagent, suitable for cell culture

Use to detect dead and dying cells in cytotoxicity assays and for routine assessment of cell viability.

composition
Dye content ~40%
solubility
H_2O ..... 10 mg/mL
ship: ambient store at: room temp

T6146-5G	5 g
T6146-25G	25 g
T6146-100G	100 g

Trypan Blue solution

[72-57-1] $C_{34}H_{24}N_6Na_4O_{14}S_4$ FW 960.81

▸ 0.4%, liquid, sterile-filtered, suitable for cell culture

Trypan blue solution may be used in trypan blue based cytotoxitiy and proliferation assays. It is a vital stain that is not absorbed by healthy viable cells. When cells are damaged or dead, trypan blue can enter the cell allowing dead cells to be counted. The method is sometimes referred to as the dye exclusion method.

Prepared in 0.81% sodium chloride and 0.06% potassium phosphate, dibasic.
ship: ambient store at: room temp

T8154-20ML	20 mL
T8154-100ML	100 mL

XTT sodium salt

2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt
[111072-31-2] $C_{22}H_{16}N_7NaO_{13}S_2$ FW 673.52

In living cells, XTT is metabolically reduced to produce a colorimetric, water-soluble formazan product.

XTT has been used in conjunction with phenazine methosulfate (PMS) to screen human cancer cell lines.[1] The tetrazolium dye has also been used to study fungal cell damage,[2] in testing antimicrobial susceptibility of staphylococci,[3] and in *Candida* biofilm research.[4] Limitations to the XTT assay have been reported.[5]

Lit. cited: 1. Scudiero, D.A., et al., Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res.* **48**, 4827-33 (1998)
2. Meshulam, T., et al., A simplified new assay for assessment of fungal cell damage with the tetrazolium dye, (2,3)-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT). *J. Infect. Dis.* **172**, 1153-6 (1995)
3. Brady, A.J., et al., *J. Microbiol. Methods* **71**, 305-11 (2007)
4. da Silva, W.J., et al., Improvement of XTT assay performance for studies involving *Candida albicans* biofilms. *Braz. Dent. J.* **19**, 264-9 (2008)
5. Kuhn, D.M., et al., Uses and limitations of the XTT assay in studies of *Candida* growth and metabolism. *J. Clin. Microbiol.* **41**, 506-8 (2003)

▸ powder, BioReagent, suitable for cell culture

≥90%
solubility
H_2O ..... 2.5 mg/mL (hot)
ship: ambient store at: 2-8°C

X4626-100MG	100 mg
X4626-500MG	500 mg

Neutral Red Solution (0.33%)

Neutral Red solution; Toluylene red; 3-Amino-7-dimethylamino-2-methylphenazine hydrochloride
[553-24-2] $C_{15}H_{17}ClN_4$ FW 288.78

▸ 3.3 g/L in DPBS, sterile-filtered, BioReagent, suitable for cell culture

Can be used as a vital stain, to stain living cells.

endotoxin ..... tested
ship: ambient store at: 2-8°C

N2889-20ML	20 mL
N2889-100ML	100 mL

MTT (Cat. No. M5655)

Applications

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes.[1] This water insoluble formazan can be solubilized using isopropanol or other solvents and the dissolved material is measured spectrophotometrically yielding absorbance as a function of concentration of converted dye.

The cleavage and conversion of the soluble yellow dye to the insoluble purple formazan has been used to develop an assay system alternative to the conventional 3H-thymidine uptake and other assays for measurement of cell proliferation. Active mitochondrial dehydrogenases of living cells will cause this conversion. Dead cells do not cause this change. This has been applied in measurement of interleukin-2 activity in a multiwell assay.[2] Modification has improved the sensitivity.[3] Other uses such as measurement of cytotoxicity[4] and cell number have also been developed.

In our testing we dissolve MTT, (Cat. No. M5655), 5 mg/ml in RPMI-1640 without phenol red. This is available as a powder (Cat. No. R8755) or liquid (Cat. No. R7509). The solution is filtered through a 0.2 µm filter and stored at 2-8 °C for frequent use or frozen for extended periods.

Routinely, MTT stock solution (5 mg/ml) is added to each culture being assayed to equal one tenth the original culture volume and incubated for 3 to 4 hr. At the end of the incubation period the medium can be removed if working with attached cells and the converted dye may be solubilized with acidic isopropanol (0.04-0.1 N HCl in absolute isopropanol). When working with suspension cells the dye is added directly and dissolution is accomplished by trituration. Absorbance of converted dye is measured at a wavelength of 570 nm with background subtraction at 630-690 nm.

MTT may also be used to score hybridoma development or clonal development. Clones will convert the dye and become readily visible without magnification. It should be noted that MTT is a mutagen and the resultant cells may be affected. The concentration used may be reduced by dilution of the stock solution (5 mg/ml) to 0.1 mg/ml and adding a tenth volume to each well. Incubation should be monitored by observing for stained clones. Cells can be recovered by gently washing the cells and adding growth medium.

Summary

MTT stock solution: 5 mg/mL
Typical use: Add 1/10th of culture volume
Solvent: 0.04–0.1 N HCl in isopropanol
Can also use: 1:1, DMSO:Isopropyl
Spectrophotometric reading: 570 nm
Background wavelength: 630–690 nm

References

1. Slater, T.F., et al., *Biochim. Biophys. Acta.,* **77,** 383, 1963.
2. Mossman, T., *J. Immunol. Methods,* **65,** 55, 1983.
3. Denizot, F. and Lang, R., *J. Immunol. Methods,* **89,** 271, 1986.
4. Carmichael, J., et al., *Cancer Research,* **47,** 936, 1987.

Trypan Blue (Cat. Nos. T8154, T6146, and Z359629)

Use of Trypan Blue Stain and the Hemocytometer to Determine Total Cell Counts and Viable Cell Number

Trypan Blue is one of several stains recommended for use in dye exclusion procedures for viable cell counting. This method is based on the principle that live (viable) cells do not take up certain dyes, whereas dead (non-viable) cells do. Staining facilitates the visualization of cell morphology.

NOTE: Trypan Blue has a greater affinity for serum proteins than for cellular protein. If the background is too dark, cells should be pelleted and resuspended in protein-free medium or salt solution prior to counting.

1) Prepare a cell suspension in a balanced salt solution (e.g., Hanks' Balanced Salts [HBSS], Catalog Number H9269).

2) Transfer 0.5 ml of 0.4% Trypan Blue solution (w/v) to a test tube. Add 0.3 ml of HBSS and 0.2 ml of the cell suspension (dilution factor = 5) and mix thoroughly. Allow to stand for 5 to 15 minutes.
NOTE: If cells are exposed to Trypan Blue for extended periods of time, viable cells, as well as non-viable cells, may begin to take up dye.

3) With the cover-slip in place, use a Pasteur pipette or other suitable device to transfer a small amount of Trypan Blue-cell suspension mixture to both chambers of the hemacytometer. Carefully touch the edge of the cover-slip with the pipette tip and allow each chamber to fill by capillary action. Do not overfill or underfill the chambers.

4) Starting with chamber 1 of the hemacytometer, count all the cells in the 1 mm center square and four 1 mm corner squares (see **Diagram I**). Non-viable cells will stain blue. Keep a separate count of viable and non-viable cells.
NOTE: Count cells on top and left touching middle line of the perimeter of each square. Do not count cells touching the middle line at bottom and right sides (see **Diagram II**).

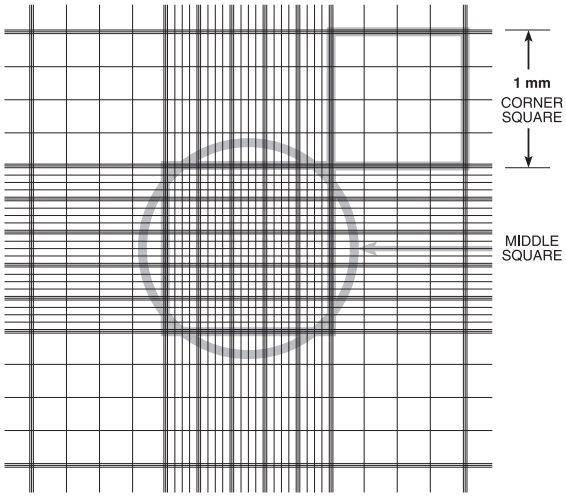
5) Repeat this procedure for chamber 2.
NOTE: If greater than 10% of the cells appear clustered, repeat entire procedure making sure the cells are dispersed by vigorous pipetting in the original cell suspension as well as the Trypan Blue-cell suspension mixture. If less than 200 or greater than 500 cells (i.e., 20-50 cells/square) are observed in the 10 squares, repeat the procedure adjusting to an appropriate dilution factor.

6) Withdraw a second sample and repeat count procedure to ensure accuracy.

7) **CELL COUNTS** - Each square of the hemacytometer, with cover-slip in place, represents a total volume of 0.1 mm^3 or 10^{-4} cm^3. Since 1 cm^3 is equivalent to approximately 1 ml, the subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations:
CELLS PER ml = the average count per square × dilution factor × 10^4 (count 10 squares)
Ex: If the average count per square is 45 cells × 5 × 10^4 = 2.25 × 10^6 cells/ml.
TOTAL CELLS = cells per ml x the original volume of fluid from which cell sample was removed.
Ex: 2.25 × 10^6 (cells/ml) × 10 ml (original volume) = 2.25 × 10^7 total cells.

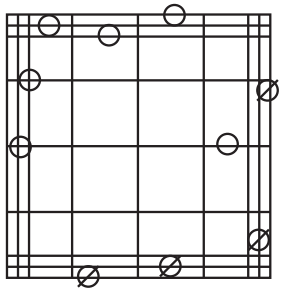
8) **CELL VIABILITY** (%) = total viable cells (unstained) ÷ total cells (stained and unstained) × 100.
Ex: If the average count per square of unstained (viable) cells is 37.5, the total viable cells = [37.5 × 5 × 10^4] viable cells/ml × 10 ml (original volume) = 1.875 × 10^7 viable cells. Cell viability (%) = 1.875 × 10^7 (viable cells) ÷ 2.25 × 10^7 (total cells) × 100 = 83% viability.

DIAGRAM I
STANDARD HEMOCYTOMETER CHAMBER



The circle indicates the approximate area covered at 100x microscope magnification (10× ocular and 10x objective). Include cells on top and left touching middle line (O). Do not count cells touching middle line at bottom and right (Φ). Count 4 corner squares and middle square in both chambers (one chamber represented here).

DIAGRAM II
CORNER SQUARE (ENLARGEMENT)



Count cells on top and left touching middle line (O). Do not count cells touching middle line at bottom and right (Φ).

Media Analysis Kit

Glutamine and Glutamate Determination Kit

For enzymatic determination of L-glutamine and L-glutamate via reduction of NAD to NADH, measured spectrophotometrically at 339 nm.
The detection ranges for this kit are: 0.056 - 0.56 mM L-glutamate; 0.14 - 1.4 mM L-glutamine.

1 kit sufficient for 50 tests

ship: ambient store at: 2-8°C

GLN1-1KT	1 kit

Endothelial Cell Culture Products

Human Umbilical Vein Endothelial Cell (HUVEC) Culture Media

CS-C Medium Without Serum for Endothelial Cell Lines

With L-glutamine, sodium bicarbonate, and HEPES, without phenol red, liquid, sterile-filtered, suitable for cell culture

Optimized for endothelial cell growth (HUVEC).

Packaged in PETG plastic bottles.

Should be supplemented with 10% serum, Endothelial Cell Growth Factor (E 9640) and Endothelial Cell Attachment Factor (E 9765) for best results.

endotoxin ..... tested

ship: ambient store at: 2-8°C

C1556-100ML	100 mL

CS-C Medium With Serum for Endothelial Cell Lines

With 10% iron supplemented bovine calf serum, L-glutamine, sodium bicarbonate, and HEPES, without phenol red, liquid, sterile-filtered, suitable for cell culture

Optimized for endothelial cell growth (HUVEC).

Should be supplemented with Endothelial Cell Growth Factor (E 9640) and Endothelial Cell Attachment Factor (E 9765) for best results.

endotoxin ..... tested

ship: ambient store at: 2-8°C

C1431-100ML	100 mL

Human Umbilical Vein Endothelial Cell (HUVEC) Culture Reagents

Endothelial Cell Attachment Factor (ECAF)

liquid, sterile-filtered, suitable for cell culture

With gelatin, factors from iron-supplemented calf serum associated with endothelial cell attachment and spreading, sodium bicarbonate and HEPES buffers, and phenol red.

ECAF is optimized to promote the attachment, spreading and polarity of endothelial cells. It contains gelatin (in HEPES buffered medium) to which factors separated from serum by chromatography and dialysis have been added. Use in combination with C1431, C1556, E9640 and T4299.

endotoxin ..... tested

ship: dry ice store at: −70°C

E9765-5ML	5 mL
E9765-10ML	10 mL

Endothelial cell growth factor

100 ×, sterile-filtered, suitable for cell culture

Optimized for endothelial cell growth. Drives mitogenic proliferation when supplemented to CS-C medium.

5 mg/ml each porcine heparin and HBGF-1 in DPBS without calcium and magnesium

endotoxin ..... tested

ship: dry ice store at: −70°C

E9640-1ML	1 mL
E9640-5ML	5 mL
E9640-10ML	10 mL

Trypsin-EDTA Solution 1X NEW

Trypsin-EDTA solution

for research or for further manufacturing use

0.25% trypsin, 0.1% EDTA, trypsin gamma irrdiated by SER-TAIN® Process, in Hank's Balanced Salt Solution, cell culture tested

1X

Drug Master File available for SER-TAIN® process

SAFC Biosciences has validated a process using γ radiation to significantly reduce the risks associated with adventitious agents such as Porcine Parvovirus (PPV), PorcineRespiratory and Reproductive Syndrome (PRRS) and Mycoplasma hyorhinis while maintaining product performance. The use of γ irradiated trypsin requires no change to the end user's methods or procedures while giving additional assurance against microbial contaminants associated with animal-derived products. All trypsin is obtained from the United States or other countries deemed free of Bovine Spongiform Encephalopathy (BSE).

ship: dry ice store at: −20°C

59429C-100ML	100 mL
59429C-500ML	500 mL

0.25% trypsin, 0.02% EDTA, trypsin gamma irradiated by SER-TAIN® Process, in Hank's Balanced Salt Solution, sterile-filtered

Drug Master File available for SER-TAIN® γ-irradiation

ship: dry ice store at: −20°C

59428C-100ML	100 mL
59428C-500ML	500 mL

0.05% trypsin, 0.02% EDTA, trypin gamma irradiated by SER-TAIN® Process, in Hanks' Balanced Salt Solution

Drug Master File available for SER-TAIN® γ-irradiation

ship: dry ice store at: –20°C

59417C-100ML	100 mL
59417C-500ML	500 mL

Endothelial Cell Attachment Factor (ECAF) (Cat. No. E9765)

ECAF is optimized to promote the attachment, spreading and polarity of endothelial cells. It contains gelatin (in HEPES buffered medium) to which factors separated from serum by chromatography and dialysis have been added.

Product Use

1. ECAF should be thawed and warmed to 37 °C before use. Cover the tissue culture surface to be plated for 1 minute.
2. Aspirate coating solution. Cells can be plated immediately.
3. ECAF can (if desired) be moved from one well of a multiwell plate to another, or from one flask to another without reducing its effectiveness.
4. Store ECAF at -70 °C. Shelf life at 2-8 °C is two weeks.

Endothelial Cell Attachment Factor (ECAF)

liquid, sterile-filtered, suitable for cell culture

With gelatin, factors from iron-supplemented calf serum associated with endothelial cell attachment and spreading, sodium bicarbonate and HEPES buffers, and phenol red.

ECAF is optimized to promote the attachment, spreading and polarity of endothelial cells. It contains gelatin (in HEPES buffered medium) to which factors separated from serum by chromatography and dialysis have been added. Use in combination with C1431, C1556, E9640 and T4299.

endotoxin ..... tested

ship: dry ice store at: –70°C

E9765-5ML	5 mL
E9765-10ML	10 mL

Directions for Thawing Human Endothelial Cells

1. Frozen cells should be stored under liquid nitrogen.
2. Rapidly thaw the cells by immersion in a 37 °C water bath.
3. Immerse in 70% ethanol. Dry with sterile gauze.
4. Transfer contents to a 15 ml centrifuge tube. Add 1.0 ml CS-C medium (C1431/C1556) and mix gently.
5. Wait one minute, add 2.0 ml CS-C medium and mix gently.
6. Wait one minute, add 4.0 ml CS-C medium and mix gently.
7. Pellet cells by gentle centrifugation. Aspirate supernatant, leaving a minimal volume to cover cells. Loosen pellet by flicking sharply with fingers.
8. Add Endothelial Cell Growth Factor (E9640) to loosened cell pellet at 1% of the final culture medium volume. Mix gently.
9. Add CS-C medium to final volume. Mix gently.
10. Plate cells on Endothelial Cell Attachment Factor (E9765) coated tissue culture surface.

Passage of Endothelial Cell Cultures

1. Remove medium from endothelial cells and replace with sufficient Trypsin-EDTA solution (T4299) to cover the cell layer. Washing the cell layer with PBS or EDTA before adding the protease is optional.
2. Incubate at 37 °C until the cells are well rounded and begin to detach. Some cells may detach more quickly than others as a function of where they are in the cell cycle and the amount of extracellular matrix present.
3. Cells may be harvested after rounding by sharply rapping the culture flask with the hand or against a hard surface.
4. Add an equal volume (or a minimum of 5 ml) of Trypsin Inhibitor solution (T0800) to the cell suspension and transfer to a sterile centrifuge tube.
5. Rinse culture flask with CS-C medium (C1431/C1556) to remove remaining cells. Add cells to the centrifuge tube. Pellet cells by gentle centrifugation. Aspirate supernatant, leaving a minimal volume to cover cells.
6. Loosen the cell pellet by flicking the centrifuge tube with fingers. Do not vortex.
7. Add Endothelial Cell Growth Factor (E9640) to the loosened pellet at 1% of the final culture medium volume. Mix gently.
8. Adjust cell concentration for plating or counting.
9. Inoculate cells on an Endothelial Cell Attachment Factor (E9765) coated tissue culture surface. Incubate at 37 °C, 5% CO2, and 100% humidity.
10. Feed cultures CS-C medium every two days when actively proliferating.
11. Add Endothelial Cell Growth Factor directly to refed cultures (1% v/v) or to CS-C medium (1% v/v) just prior to feeding cells. Adding the growth factor to an entire bottle of CS-C medium is **not recommended**.

Growth Factors and Cytokines

Cytokines and Growth Factors

The importance of cytokines and growth factors in cell culture has been demonstrated time and time again since the inception of cell culture. Beginning with the first successful efforts of growing cells in culture, there have been so called 'secret ingredients' added to culture medium that have, over time, given rise to a the belief that cell culture is as much of an art as a technique. Serum and other undefined extracts and the painstaking, continued attempts to characterize them have greatly contributed to this situation.

Studies published as early as 1921 acknowledged the fact that there were critical unknowns that were essential for normal growth, metabolism, and development of cells in culture. Zilva, Goldblatt, Sanford and a number of other cell biology pioneers were the first to admit that there were unknown factors influencing the general health of both cellular and animal models.

Over time, we have learned that many of these unknown factors are nutrients such as vitamins, amino acids, sugars, albumins and transferrins. However, even when all the necessary nutrients are present certain cells especially primary cells do not proliferate. Serum seemed to provide the unknown factors that encouraged cell proliferation. Today we know the agents that are responsible for cell proliferation and differentiation are growth factors and cytokines.

Growth factors can be described as proteins that bind to receptors on the cell surface of non-hematopoietic cells and result in proliferation or differentiation of the affected cells. Each family of growth factors affect specific cell types. For example, epidermal growth factors (EGF), affect epithelial cell types, similarly platelet derived growth factors (PDGF), affect only fibroblasts commonly found in connective tissues.

Cytokines, often compared with growth factors, are a class of signaling molecules (proteins, peptides and glycoproteins) that affect primarily the cells of the immune system but can affect other diverse cell types outside of the immune system as well. Cytokines are generally thought of as part of the signaling mechanism that orchestrates the immune response to bacterial infection. The effects of cytokines on cells are varied, some like growth factors cause cell proliferation, others may cause chemotaxis between different cell types, and others can even cause apoptosis.

Cytokines and growth factors are somewhat similar in their structure and mechanism of action. Both bind to specific cell surface receptors that initiate signaling pathways and well as having receptors that share distinct structural homologies. Many growth factors and cytokines also share several intracellular signaling components through which the activated cell surface receptor transmits its message to the cell nucleus.

In both the research and pharmaceutical community, there is a growing need for defined serum-free media that eliminates the variability and the potential virus and prion contamination as well as facilitates the purification of recombinant proteins. The development of serum-free media will often necessitate the use of certain growth factors, and cytokines. This is always true for primary cell lines but often necessary for transformed cell lines and hybridomas as well.

Selecting the appropriate growth factors and cytokines for your application is an important task and can be often based on existing protocols or by chance. Growth factors and cytokines are critical to successful cell differentiation and proliferation. Within this section, you will find s listing of cell culture tested cytokines and growth factors along with helpful information that will make choosing the products you need easier.

Epidermal Growth Factors (EGF) and NRG Family

The epidermal growth factor (EGF) family of growth factors is comprised of the normal mammalian gene products of **EGF, amphiregulin, betacellulin, heparin-binding EGF (HB-EGF), and transforming growth factor-α (TGF-α)**, plus pox virus EGF-like protein (PVGF), lin-3 from *Caenorhabditis elegans*, and *spitz* from *Drosophila*. The neuregulin (NRG) or neu differentiation factor (NDF) family of growth factors includes the **heregulins (HRG-α and HRG-β)**, acetylcholine receptor inducing activity (ARIA) and glial growth factor (GGF). The EGF and NRG families are similar in that all members contain one or more EGF-like motifs in the extracellular domain that interacts with a cell surface receptor. Many members of both families also undergo proteolytic cleavages in the region between the EGF-like motif and transmembrane domain and also further toward the N-terminus to release molecules that bind and activate one or more members of the EGF receptor (EGFR) family. Among the members of both families there is a great deal of variations in other extracellular and intracellular structures of the precursor forms. EGF family members all bind and activate EGFR. Members of the NRG family bind to one or more of the EGFR-related receptors designated as erbB-2, erbB-3 or erbB-4. Emphasizing the homologies among the two families, some authors designate the EGFR as HER1 (human EGF receptor-1) and erbB-2-4 as HER2-4.

References

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5. Earp, H.S., et al., Heterodimerization and functional interaction between EGF receptor family members: a new signaling paradigm with implications for breast cancer researc. *Breast Canc. Res. Treat.*, **35**, 115-132 (1995).

Amphiregulin human

AR
[117147-70-3]

AREG; CRDGF; SDGF
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

Also known as keratinocyte autocrine factor (KAF); expression of amphiregulin mRNA has been found in numerous carcinoma cell lines and epithelial cells of several human tissues including colon, stomach, breast, ovary, and kidney.

Lyophilized from 0.2 µm filtered solution in phosphate buffered saline containing 5 mg bovine serum albumin.

Associated gene(s): AREG (374)

ED_{50}/EC_{50}: 10-100 ng/mL

mol wt ~11 kDa

The proliferative activity is tested in culture using a mouse fibroblast cell line BALB/3T3.

endotoxin ..... tested
ship: ambient store at: –20°C

A7080-.1MG	0.1 mg

Betacellulin human

BTC
[163150-12-7]

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

Betacellulin, a heparin-binding member of the EGF family, appears to play a role in the growth and differentiation of islet cells in human pancreas. BTC binds with high affinity to both EGFR and to erbB-4. Overexpression of BTC with erbB-3 and erbB-4 are linked to human endometrial adenocarcinoma. Human and mouse precursor forms of BTC show 79% amino acid sequence identity.

Used to study the differentiation of pancreatic acinar cell line (AR42J) into insulin-secreting cells.

Lyophilized from 0.2 µm filtered solution in phosphate buffered saline containing bovine serum albumin.

Associated gene(s): BTC (685)

protein mol wt 9.5 kDa

The bioactivity is measured in a cell proliferation assay using BALB/3T3 fibroblasts.

endotoxin ..... tested
ship: ambient store at: –20°C

B3670-10UG	10 µg

Epidermal Growth Factor human

EGF
[62253-63-8]

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Qualified for use as a mitogen for a variety of epidermal and epithelial cells, including fibroblasts, glial cells, mammary epithelial cells, vascular and corneal endothelial cells, bovine granulosa, rabbit chondrocytes, HeLa cells, and SV40-3T3 cells.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline

Associated gene(s): EGF (1950)

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.08-0.8 ng/mL

≥98% SDS-PAGE

The biological activity of recombinant, human EGF is measured by its ability to stimulate the mouse fibroblast cell line, BALB/3T3. The EC50 is defined as the effective concentration of growth factor that elicits a 50% increase in cell growth in a cell based bioassay.

endotoxin ..... ≤1 EU/µg (EGF)
ship: ambient store at: −20°C

E9644-.2MG	0.2 mg

Epidermal Growth Factor from murine submaxillary gland

Epidermal cell growth factor; EGF
[62229-50-9]

Lyophilized from 0.2 µm filtered solution in ammonium acetate buffer.

Associated gene(s): Egf (13645)

lyophilized powder

The biological activity is measured in a proliferation assay using BALB/MK cells.

endotoxin ..... tested

suitable for cell culture

ED_{50}/EC_{50}: 0.1-10 ng/mL

Purified by gel filtration.
ship: ambient store at: 2-8°C

E4127-.1MG	0.1 mg
E4127-5X.1MG	5 × 0.1 mg

lyophilized powder, BioReagent, suitable for cell culture

EC_{50}: 0.02-2.0 ng/mL

Purified by gel filtration and ion-exchange chromatography.
ship: ambient store at: 2-8°C

E1257-.1MG	0.1 mg

Epiregulin from mouse

Binds and activates the tyrosine kinase ErbB family receptors (ErbB1-ErbB4). Inhibits the growth of several epithelial tumor cells and stimulates the growth of fibroblasts and other cell types. Its expression is upregulated in a number of carcinoma cell lines.

EREG
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.5 mg bovine serum albumin.

Associated gene(s): Ereg (13874)

≥97% (SDS-PAGE)

predicted mol wt ~5.5 kDa

The biological activity is measured by its ability to stimulate the ^{3}H-thymidine incorporation in a mouse fibroblast cell line, Balb/3T3.

endotoxin ..... tested
ship: ambient store at: −20°C

E8780-50UG	50 µg

Heparin-Binding EGF-Like Growth Factor human

HB-EGF; Heparin-Binding Epidermal Growth Factor-Like Growth Factor

hHB-EGF
recombinant, expressed in *Sf*21 cells, lyophilized powder, suitable for cell culture

Heparin-binding epidermal growth factor (HB-EGF), like BTC, is a heparin-binding EGF family member. Natural soluble HB-EGF is a 19 kDa glycoprotein of multiple forms due to heterogeneous truncations. Soluble HB-EGF is mitogenic and chemotactic for fibroblasts, keratinocytes, and smooth muscle cells but not capillary endothelial cells. Production of HB-EGF is detected in monocytes, macrophages, and vascular and aortic smooth muscle cells (SMC). HB-EGF may play a role in the pathogenesis of atherosclerosis. It may also be an effector of Jun-induced oncogenic transformation. HB-EGF binds to EGFR with high affinity and to ErbB with moderate affinity.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 2.5 mg bovine serum albumin.

≥97% (SDS-PAGE and N-terminal analysis)

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation in the EGF-responsive BALB/3T3 cells.

endotoxin ..... tested
ship: ambient store at: −20°C

E4643-50UG	50 µg

Heregulin-α, EGF Domain human

HRG-α

Heregulin (HRG) is the human homologue to the neu differentiation factor (NDF) in rat. The heregulin family members contain one EGF-like motif and an IgD-like motif in the extracellular domain. They bind to ErbB-2, ErbB-3, and ErbB-4 (receptors closely related to EGFR). HRG-α and HRG-β isoforms differ slightly in the EGF domain due to alternate splicing. HRG-β isoforms are further subdivided into β1, β2, and β3 isoforms, which show identical binding and activation characteristics. Both α and β HRG isoforms bind to ErbB-3 and ErbB-4 homodimers, but not directly to ErbB-2. HRG-α binding to ErbB-3 and ErbB-4 is reported to be approximately 100-fold weaker than that of HRG-β. When ErbB-2 is combined into a heterodimer with ErbB-3 or ErbB-4, the binding affinities of both α and β isoforms are substantially improved. HRGs are mitogenic for Schwann cells in culture and weakly to moderately mitogenic for a variety of epithelial cells, including mammary, ovarian, lung, and gastric cells. HRGs inhibit proliferation and induce differentation in some tumor cell lines, such as certain mammary tumor cells, which are arrested at the G2M phase. HRGs also induce expression of acetylcholine receptors and possibly other molecules in muscle cells at newly formed neuromuscular synapses, suggesting they may play a role in neuromuscular synapse maturation and maintenance.

Heregulin-α, EGF Domain *(continued)*

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 2.5 mg bovine serum albumin.

Associated gene(s): NRG1 (3084)

protein mol wt ~7 kDa

The bioactivity is tested in culture using a proliferation assay with the human cell line MCF-7.

endotoxin ..... tested

ship: ambient store at: −20°C

H5529-50UG	50 µg

LONG® EGF human

NEW

EGF; Epidermal Growth Factor
[62229-50-9]

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

LONG®EGF is a recombinant analog of human epidermal growth factor (EGF) that provides a potent and cost-effective mitogen for cell culture applications. LONG®EGF is ideal for both research and large-scale culture systems utilizing serum-free or low-level serum applications. It is effective in a variety of fibroblast and endothelial cell types and also acts synergistically with LONG®R^{3}IGF-I (Catalog No. 85580C) to produce extremely potent results in cell culture. LONG®EGF is produced in an *E. coli* expression system free of animal-derived components.

for research or for futher manufacturing use

media grade

not available in Japan

The biological activity is measured by the stimulation of protein synthesis in L6 myoblasts

Available in USA, Canada, and Europe. Please check with your local supplier regarding availability and restrictions in other countries.

ship: ambient store at: 2-8°C

85570C-1MG	1 mg
85570C-5MG	5 mg
85570C-10MG	10 mg

LONG® EGF human

Epidermal Growth Factor human; EGF
[62253-63-8]

powder, recombinant, expressed in *Escherichia coli*, suitable for cell culture

A genetically engineered recombinant human analog of EGF

Associated gene(s): EGF (1950)

Lyophilized from 0.1 M acetic acid.

ship: ambient store at: 2-8°C

E4269-.1MG	0.1 mg
E4269-1MG	1 mg

Fibroblast Growth Factors (FGF)

Fibroblast Growth Factors (FGFs) are potent regulators of cell proliferation, differentiation and function and are critically important in normal development, tissue maintenance and wound repair. FGFs are also linked with several pathological conditions.[1,2,3] There are at least 20 FGF members, designated FGF-1 through FGF-20, but acidic FGF and basic FGF are names commonly used for FGF-1 and FGF-2, and keratinocyte growth factor (KGF) for FGF-7. Although FGF was originally named after its fibroblast mitogenicity,[4] some FGFs do not induce fibroblast growth at all. Members of the FGF family generally share 30-50% amino acid sequence homology, have two conserved cysteine residues, and bind with high affinity to heparin. Several FGF members are oncogene products, e.g., FGF-3 (int-2), FGF-4 (hst-1, K-FGF), FGF-5 and FGF-6 (hst-2). FGFs interact with four distinct FGF receptors on cells of mesodermal, ectodermal and endodermal origin, eliciting changes in migration, morphology, function or proliferation. FGFs play several roles, including angiogenesis, wound healing, tissue regeneration, embryonic development, endocrine modulation and neurotrophic support.[3]

Acidic FGF (aFGF) and basic FGF (bFGF) are the prototypic FGF members named because of their different isoelectric points. They share a 55% homology in amino acid sequence and similar size, depending on translation extensions and truncations (15-18 kDa for aFGF and 16-24 kDa for bFGF). Neither aFGF nor bFGF genes include a secretory signal sequence and the prinicple mechanism of their release into extracellular fluid has not yet been resolved. Acidic FGF has high expression levels in brain, retina, bone matrix and osteosarcomas. Basic FGF is found in a variety of tissues, including pituitary gland, neural tissue, adrenal cortex, corpus luteum, and placenta. Acidic and basic FGFs stimulate proliferation in all cells of mesodermal origin, and many cells of neuroectodermal, ectodermal, and endodermal origin. They are chemotactic and mitogenic for endothelial cells and induce the release of agents that break down basement membranes. These two FGFs appear to play significant roles in modulating such normal processes as angiogenesis, tissue repair, embryonic development, and neural function. They also appear to participate in some pathological conditions that involve excessive cell proliferation or angiogenesis, such as tumor production.

References

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β-Endothelial Cell Growth Factor human

β-ECGF
[105843-51-4]

ECGS
recombinant, expressed in *Escherichia coli*, suitable for cell culture

β-ECGF is a potent endothelial cell mitogen and its mitogenic activity is potentiated by heparin. β-ECGF stimulates the growth of quiescent HUVEC cells in culture.

14 amino acid, N-terminal extended form of human FGF-acidic.

Lyophilized from a 0.2 µm filtered solution in 10 mM MOPS, 50mM Sodium Sulfate, 0.2 mM DTT, and 0.2 mM EDTA, pH 7.2, containing 50 µg bovine serum albumin per 1 µg cytokine.

Associated gene(s): VEGFB (7423)

≥97% (SDS-PAGE)

lyophilized powder

ED_{50}/EC_{50}: 0.03-0.3 ng/mL

The proliferative activity is tested in culture by using quiescent NR6R-3T3 fibroblasts.

endotoxin ..... tested

ship: ambient store at: −20°C

E1388-25UG	25 µg

Endothelial cell growth supplement from bovine neural tissue

suitable for cell culture

An extract of bovine neural tissue containing growth promoting factors for vascular endothelial cells of mammalian origin. ECGS has also been reported to be beneficial as a media supplement for the fusion and growth of hybridoma cells in monoclonal antibody production.

ED_{50}/EC_{50}: 2-200 µg/mL

Endothelial cell growth supplement (ECGS) is an extract of bovine neural tissue containing growth promoting factors for vascular endothelial cells of mammalian origin. Endothelial cell growth supplement is prepared using a modification of the method of Maciag, et al., and is lyophilized from a sterile solution containing NaCl and streptomycin sulfate.

The proliferative activity of ECGS is tested in culture using fetal bovine heart endothelial cells (ATCC® CRL 1395) seeded at low density. The EC_{50} is defined as the effective concentration of growth factor that elicits a 50% increase in cell growth in a cell based bioassay.

endotoxin ..... tested

ship: ambient store at: 2-8°C

E2759-15MG	15 mg
E2759-5X15MG	5 × 15 mg

Fibroblast Growth Factor-4 human

FGF-4; K-FGF

hFGF-4
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

FGF-4 (hst-1, hst/KS53) is a 19 kDa protein identified in NIH-3T3 cell assays as a transforming gene from DNA derived from human tumors, including stomach (*hst*) and colon cancers, Kaposi's sarcoma, and hepatocellular carcinoma. FGF-4 includes a secretory signal sequence and shares 42% sequence identity with bFGF at the amino acid level. Both FGF-4 and bFGF bind to the same receptors. Mouse and human FGF-4 shares 82% homology with species cross-reactivity. FGF-4 does not appear to be expressed in normal adult tissues, but during embryogenesis, the gene is spatially and temporally regulated. FGF-4 is a mitogen for fibroblasts and endothelial cells and a potent promoter of angiogenesis. It is believed to be critical in embryonic limb development.

Lyophilized from a 0.2 µm filtered buffer solution containing a carrier protein.

≥95% (SDS-PAGE and HPLC)

The biological activity is measured by the dose-dependent stimulation of thymidine uptake using BaF3 cells expressing FGF receptors.

endotoxin ..... tested

ship: ambient store at: −20°C

F8424-25UG	25 µg

Fibroblast Growth Factor-5 human

FGF-5

hFGF-5
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE and N-terminal analysis)

FGF-5, a 27 kDa protein, has a secretory signal sequence and shares 45% sequence identity with bFGF. Expression of FGF-5 is limited to neonatal brain and certain human tumor lines.[2]

Lyophilized from a 0.2 µm filtered solution in 10mM MOPS, 50 mM $NaSO_4$, 0.5 mM EDTA, 0.5 mM DTT and 5% trehalose, pH 7.0 containing 50 µg BSA per 1 µg cytokine.

Associated gene(s): FGF5 (2250)

The biological activity is tested in culture with quiescent NR6R-3T3 fibroblasts.[1]

endotoxin ..... tested

Lit. cited: 1. Rizzino, A., et al., Regulatory effects of cell density on the binding of transforming growth factor beta, epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor. *Cancer Res.* **48**, 4266-4271 (1988)
2. Vainikka, S., et al., Nicola, N., ed., Fibroblast growth factors (FGFs). *Guidebook to Cytokines and Their Receptors* , Oxford Press (New York, NY: 1994), 214

ship: ambient store at: −20°C

F4537-50UG	50 µg

Fibroblast Growth Factor-6 human

FGF-6

hFGF-6
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE and N-terminal analysis)

FGF-6 (*hst*-2), a 14 kDa protein, shares a 70% amino acid sequence identity with FGF-4. FGF-6 is a potent mitogen for fibroblasts but is only slightly mitogenic for endothelial cells or melanocytes that normally require bFGF.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.05% CHAPS and 2.5 mg bovine serum albumin.

Associated gene(s): FGF6 (2251)

The bioactivity is determined by measuring the FGF-6 dependent ^{3}H-thymidine incorporation in quiescent NR6R-3T3 fibroblasts.

endotoxin ..... tested

ship: ambient store at: −20°C

F4662-25UG	25 µg

Fibroblast Growth Factor-9 human

FGF-9; Glial Activating Factor; GAF

hGFG-9
recombinant, expressed in *Sf*21 cells, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

FGF-9 (23 kDa protein) is mitogenic for glial cells, fibroblasts, and oligodendrocyte type 2 astrocyte progenitor cells. It is not mitogenic for endothelial cells. Like aFGF and bFGF, FGF-9 has no secretory signal sequence. FGF-9 shares ~30% amino acid sequence identity with other FGF members.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 1.25 mg bovine serum albumin.

Fibroblast Growth Factor-9 *(continued)*

Associated gene(s): FGF9 (2254)

mol wt ~25 kDa

The biological activity is measured in a bioassay using BALB/3T3 cells.

endotoxin ..... tested

ship: ambient store at: –20°C

F1168-25UG	25 µg

Fibroblast Growth Factor from bovine pituitary

FGF
[62031-54-3]

suitable for cell culture

Although the major mitogenic component of this product is FGF2, the activity per unit weight of protein is less than that of purified FGF2.

A natural mitogenic growth factor used in cell culture applications to study cell signaling and to promote fibroblast and endothelial cell growth and survival.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.1 mg/ml BSA.

lyophilized powder

Purified glands by a modification of the method of Gospodarowicz.

The biological activity is measured using a 3-day MTT assay.

endotoxin ..... tested

ship: ambient store at: –20°C

F3133-10UG	10 µg
F3133-5X10UG	5 × 10 µg

Fibroblast Growth Factor-Acidic human

aFGF; FGF-1

recombinant, expressed in *Escherichia coli*, suitable for cell culture

A potent mitogenic agent for a wide variety of mesoderm-derived cells including BALB/c 3T3 fibroblasts, capillary and endocardial endothelial cells, myoblasts, vascular smooth muscle cells, mesothelial cells, glial and astroglial cells, and adrenal cortex

The product is lyophilized from a 0.2 µm filtered solution of 20 mM Tris, 1 M NaCl, 5 mM DTT containing 50 µg of bovine serum albumin per 1 µg of cytokine.

Associated gene(s): FGF1 (2246)

≥97% (SDS-PAGE)

lyophilized powder

The proliferative activity is tested in culture using quiescent NR6R-3T3 fibroblasts.

endotoxin ..... tested

ship: ambient store at: –20°C

F5542-25UG	25 µg

Fibroblast Growth Factor-Basic from bovine pituitary

bFGF; FGF2
[106096-93-9]

suitable for cell culture

A natural mitogenic growth factor used in cell culture applications to study cell signaling and to promote fibroblast and endothelial cell growth and survival.

Lyophilized from a 0.2 µm filtered solution in sodium phosphate and sodium chloride containing 0.1 mg bovine serum albumin

~90% (SDS-PAGE and N-terminal analysis)

lyophilized powder

ED_{50}/EC_{50}: 0.03-3.0 ng/mL

Purified glands by a modification of the method of Gospodarowicz, et al.[1]

The proliferative activity is tested in culture using fetal bovine heart endothelial cells.

endotoxin ..... tested

Lit. cited: 1. Gospodarowicz, D., et al., Isolation of brain fibroblast growth factor by heparin-Sepharose affinity chromatography: identity with pituitary fibroblast growth factor. *Proc. Natl. Acad. Sci. U. S. A.* **81**, 6963-6967 (1984)

ship: ambient store at: –20°C

F5392-1UG	1 µg

Fibroblast Growth Factor-Basic human

bFGF; FGF2
[106096-93-9]

recombinant, expressed in *Escherichia coli*, suitable for cell culture

FGF-2 is required for the maintenance of human embryonic stem cells in culture, and various other stem cell lines such as mesenchymal stem (stroma) cells (MSC).

FGF2 is found in basement membranes and sub-endothelial extracellular matrix. FGF2 is induced early in the development of the embryo where it is necessary (but not sufficient) for pluripotency and self-renewal of embryonic stem cells (ESC) and lineage defined stem cells; wherein it functions to support proliferation and inhibit differentiation. Self-renewal of hESC depends upon activation of the Activin/Nodal/Smad2,3 branch and suppression of the BMP/GDF/Smad5 branch of the TGFβ-family cell signaling program. FGF2 is a competence factor for Nodal support of pluripotency. Activin-A is necessary and sufficient to maintain stemness. It induces the expression of both Nodal and FGF2 in human ES cells.

Lyophilized from 0.2 µm filtered solution in 20 mM Tris and 1 M NaCl, pH 7.0, containing 50 µg bovine serum albumin per 1 µg BFGF.

Associated gene(s): FGF12 (2257)

≥97% (SDS-PAGE)

lyophilized powder

The bioactivity of bFGF was measured in a fluorometric assay using the redox sensitive dye, Resazurin.

ship: ambient store at: –20°C

F0291-25UG	25 µg

Fibroblast Growth Factor-Basic Heparin Stabilized human

rhbFGF
[106096-93-9]

recombinant, expressed in *Escherichia coli*, sterile-filtered, aqueous solution, suitable for cell culture

A heparin stabilized form of a natural mitogenic growth factor used in cell culture applications to study cell signaling and to promote fibroblast and endothelial cell growth and survival.

Associated gene(s): FGF2 (2247)

ED_{50}/EC_{50}: 0.03-3.0 ng/mL

potency: ~675 ng/mL

endotoxin ..... tested

ship: ambient store at: 2-8°C

F9786-1ML	1 mL
F9786-5ML	5 mL

Keratinocyte Growth Factor human

FGF-7; KGF
[148348-15-6]

hKGF; hFGF-7
recombinant, expressed in *Escherichia coli*, powder, suitable for cell culture

Keratinocyte growth factor (18.9 kDa, 163 amino acids) includes a secretory signal sequence and shares 39% identity with bFGF. It is expressed in a number of stromal fibroblast cell lines but absent from normal glial cells and epithelial cell lines. KGF stimulates proliferation and differentiation of keratinocytes and other cells in the epithelium and induces chemotaxis of microvascular endothelial cells and neovascularization of rat cornea.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.5 mg bovine serum albumin.

≥95% (SDS-PAGE and HPLC)

ED_{50}/EC_{50}: 1.0-10 ng/mL

The proliferative activity is tested in culture using the murine hematopoietic cell line BaF3.

endotoxin ..... tested

ship: ambient store at: −20°C

K1757-10UG	10 µg

Hematopoietic Cytokines

Numerous cytokines are involved in the regulation of hematopoiesis within a complex network of positive and negative regulators. Some cytokines have very narrow lineage specificities of their actions, while many others have rather broad and overlapping specificity ranges. Listed within this section include the cytokines whose predominant action appears to be the stimulation or regulation of hematopoietic cells. The term "colony stimulating factor" (CSF) was a designation originally given to agents discovered to stimulate growth of colonies containing differentiated myeloid cells from single bone marrow-derived precursor cells plated in semisolid medium. The glycoproteins considered to be CSFs include **granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), interleukin-3 (multi-CSF or IL-3), interleukin-5 (IL-5), erythropoietin (EPO),**[1] **and thrombopoietin (TPO).**[2]

A number of other cytokines exert profound effects on the formation and maturation of hematopoietic cells, with most of these belonging to the structural class known as the "4-α-helical bundle" family of cytokines,[3] which include **stem cell factor (SCF), flt-3/flk-2 Ligand (FL) and leukemia inhibitory factor (LIF)**. Other cytokines or ligands, such as jagged-1, transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α) also play significant roles in modulating hematopoiesis. Descriptions and listings of IL-3, IL-5, TGF-β and TNF-α products are listed in other sections by name.

References

1. Brugger, W., et al., Clinical role of colony stimulating factors. *Acta Haematol.*, **86**, 138-147 (1991).
2. Lok, S., and Foster, D., The structure, biology and potential therapeutic applications of recombinant thrombopoietin. *Stem Cells*, **12**, 586-598 (1994).
3. Nicola, N., An introduction to the cytokines, in Guidebook to Cytokines and Their Receptors, Nicola, N., ed., Oxford Press (New York, N.Y.: 1994), pp. 1-7.

Erythropoietin human

EPO
[11096-26-7]

Erythropoietin is a glycoprotein that is the principal regulator of red blood cell growth and differentiation.

recombinant, expressed in CHO cells, lyophilized powder, cell culture tested, activity: ~100,000 units/mg protein

Lyophilized as 10 units EPO, 10 mg trisodium citrate and 10 mg NaCl at pH ≅7.0.

Associated gene(s): EPO (2056)

The biological activity is assayed in tissue culture using mouse fetal liver cells.

ship: ambient store at: −20°C

E5627-10UN	10 units

Erythropoietin from rat

EPO

Erythropoietin is a glycoprotein that is the principal regulator of red blood cell growth and differentiation.

recombinant, expressed in insect cells, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.5 mg bovine serum albumin.

calculated mol wt 18.5 kDa

mol wt 22-26 kDa, SDS-PAGE

The biological acitivity is measured by its ability to stimulate cell proliferation using TF-1 cell line.

endotoxin ..... tested

ship: ambient store at: −20°C

E8905-10UG	10 µg

Flt-3/Flk-2 ligand human

FL
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥98% (SDS-PAGE and HPLC)

Flt-3/Flk-2 Ligand (FL, or Flt-3L) is a transmembrane glycoprotein that binds and activates the Flt-3 receptor. Expression of Flt-3 is primarily restricted among hematopoietic cells to the most primitive progenitor cells.

Mouse and human Flt-3 ligand share ~72% homology and both demonstrate cross-species activity.

Lyophilized, 0.2 µm sterile filtered, with with 0.5x PBS.

Associated gene(s): FLT3 (2322)

The biological activity is measured by its ability to stimulate the proliferation of human AML5 cells.

endotoxin ..... tested
ship: ambient store at: −20°C

F3422-5UG	5 µg

Granulocyte colony-stimulating factor human

recombinant, expressed in *Escherichia coli*, suitable for cell culture

Lyophilized from 10 mM acetic acid containing 250 µg bovine serum albumin

from human

≥95% (SDS-PAGE)

lyophilized powder

mol wt 18.8 kDa (175 amino acids including N-terminal methionine)

The biological activity is measured in a cell proliferation assay using a murine myeloblastic cell line, NFS-60.

endotoxin ..... tested
ship: ambient store at: −20°C

G0407-5UG	5 µg

Granulocyte Colony-Stimulating Factor from mouse

G-CSF

recombinant, expressed in *Escherichia coli*, suitable for cell culture

G-CSF is a lineage-restricted hematopoietic growth factor, stimulating final mitotic divisions and the terminal cellular maturation of the partially differentiated hematopoietic progenitors. G-CSF[3,4] is produced by monocytes and fibroblasts. It stimulates granulocyte colony formation, activates neutrophils and mature granulocytes, and promotes differentiation of certain myeloid leukemic cells. Natural G-CSF is a glycoprotein of 177 amino acids and a molecular weight of 19 kDa.[5] Human and murine G-CSF have about 75% homology and show biological cross-reactivity.

Lyophilized from 20 mM acetic acid containing 250 µg bovine serum albumin

from mouse

Associated gene(s): Csf3r (12986)

>97% (SDS-PAGE)

lyophilized powder

ED_{50}/EC_{50}: 0.005-0.05 ng/mL

mol wt 19 kDa (179 amino acids including N-terminal methionine)

The biological activity is measured in a cell proliferation assay using a murine myeloblastic cell line, NFS-60.

endotoxin ..... tested
ship: ambient store at: −20°C

G8160-5UG	5 µg

Granulocyte-Macrophage Colony-Stimulating Factor human

GM-CSF human

lyophilized powder

endotoxin ..... tested

from human, recombinant, expressed in *Escherichia coli*, suitable for cell culture

Package size based on protein content

Lyophilized from a 0.2 µm filtered, 10 mM sodium citrate, pH 3.5 solution.

≥98% (SDS-PAGE and HPLC)

EC_{50}: ≤0.1 ng/mL

ED_{50}/EC_{50}: 0.01-0.1 ng/mL

mol wt 14.6 kDa

The proliferative activity of human GM-CSF is tested in culture using human TF-1 cells.[1]

Lit. cited: 1. Kitamura, T., et al., Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. *J. Cell Physiol.* **140**, 323-334 (1989)

ship: ambient store at: −20°C

G5035-5UG	5 µg

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

≥95% (SDS-PAGE)

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 15-36 kDa (glycosylated)

HumanKine® GM-CSF is expressed as a 15-36 kDa glycosylated monomer in human HEK 293 cells. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications. Although human and mouse GM-CSF share 54% amino acid sequence homology, their biological actions are species-specific and exhibit no cross-species reactivity.

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

Although human and mouse GM-CSF share 54% amino acid sequence homology, their biological actions are species-specific and exhibit no cross-species reactivity.

ship: ambient store at: room temp

H5666-5UG	5 µg

Granulocyte-Macrophage Colony-Stimulating Factor from mouse

GM-CSF
from mouse, recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from 10 mM acetic acid plus 250 µg BSA.

≥97% (SDS-PAGE)

EC_{50}: ≤0.2 ng/mL (corresponds to ≥5 × 10^6 units/mg)

mol wt 14.8 kDa (125 amino acids including N-terminal methionine)

The EC_{50} activity of mouse GM-CSF is tested in culture using murine FDP-1 cells.

endotoxin ..... tested
ship: ambient store at: −20°C

G0282-5UG	5 µg

Granulocyte-Macrophage Colony-Stimulating Factor from rat

GM-CSF

from rat, recombinant, expressed in *Escherichia coli*

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.25 mg bovine serum albumin.

>97% (SDS-PAGE)

lyophilized powder

Recombinant Rat Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is produced from a DNA sequence encoding mature rat GM-CSF. This recombinant protein is a mixture of two rat GM-CSFs, the 127 amino acid form and the 128 amino acid methionyl form. Mature rat GM-CSF has a calculated mass of 14.7 kDa. GM-CSF, an acidic glycoprotein, is species-specific.

The biological activity is measured by its ability to induce proliferation of the factor-dependent mouse cell line, DA3.

endotoxin ..... tested
ship: ambient store at: −20°C

G0792-5UG	5 µg

Leukemia Inhibitory Factor human

LIF

Leukemia Inhibitory Factor (LIF) is a pleiotropic glycoprotein originally described to inhibit the proliferation of the murine myeloid leukemic cell line M1, while inducing differentiation into macrophages. Other activities were later identified to LIF, which is known by a variety of synonyms, including DIF, D-factor, DIA, DRF, CNDF, HILDA, HSF-III, and MLPLI. Human LIF exerts its actions through a receptor comprising a 190 kDa LIF-binding α-chain (130 kDa, mouse) and a 130 kDa signal-transducing β-chain (gp130), which is shared with CNTF, OSM, L-6 and IL-11. As such, it is a member of the gp130 family of the cytokine receptor superfamily. LIF receptors have been identified on several cells, including monocytes, liver, placenta and embryonic stem cells. Natural LIF is heavily glycosylated, showing an apparent molecular weight of 32 kDa to 62 kDa, depending on the source, but absence of glycosylation appears not to affect its biological activity. A single gene encodes LIF, which is secreted as a single chain glycoprotein containing 180 amino acids for human or mouse with a conserved disulfide bond. Human and mouse LIF share 78% sequence homology. Human LIF can activate mouse cells, but mouse LIF cannot activate human cells.

recombinant, expressed in *Escherichia coli*, 10 µg/ml, buffered aqueous solution (pH 7.4), suitable for cell culture

Solution, 0.2 µm filtered, in phosphate buffered saline containing 0.02% TWEEN® 20.

≥95% (SDS-PAGE)

ED_{50}/EC_{50}: 0.1-1.5 ng/mL

The proliferative activity of human LIF is measured in culture using the human leukemic cell line, TF-1.

Manufactured by Chemicon International, Inc. LIF is protected under US Patent 5,443,825 and 5,427,925 and is not available for resale.

endotoxin ..... tested
ship: ambient store at: 2-8°C

L5283-10UG	10 µg

Leukemia Inhibitory Factor from mouse

LIF

Leukemia Inhibitory Factor (LIF) is a pleiotropic glycoprotein originally described to inhibit the proliferation of the murine myeloid leukemic cell line M1, while inducing differentiation into macrophages. Other activities were later identified to LIF, which is known by a variety of synonyms, including DIF, D-factor, DIA, DRF, CNDF, HILDA, HSF-III, and MLPLI. Human LIF exerts its actions through a receptor comprising a 190 kDa LIF-binding α-chain (130 kDa, mouse) and a 130 kDa signal-transducing β-chain (gp130), which is shared with CNTF, OSM, L-6 and IL-11. As such, it is a member of the gp130 family of the cytokine receptor superfamily. LIF receptors have been identified on several cells, including monocytes, liver, placenta and embryonic stem cells. Natural LIF is heavily glycosylated, showing an apparent molecular weight of 32 kDa to 62 kDa, depending on the source, but absence of glycosylation appears not to affect its biological activity. A single gene encodes LIF, which is secreted as a single chain glycoprotein containing 180 amino acids for human or mouse with a conserved disulfide bond. Human and mouse LIF share 78% sequence homology. Human LIF can activate mouse cells, but mouse LIF cannot activate human cells.

recombinant, expressed in *Escherichia coli*, 10 µg/ml, buffered aqueous solution, suitable for cell culture

Solution, 0.2 µm filtered, in phosphate buffered saline containing 0.02% Tween 20.

≥95% (SDS-PAGE)

The proliferative activity of mouse LIF is measured in culture using the M1 murine cell line.[1]

Manufactured by Chemicon International, Inc. LIF is protected under US Patent 5,750,654 and is not available for resale.

endotoxin ..... tested
Lit. cited: 1. Gearing, D.P., et al., Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). *EMBO J.* **6**, 3995-4002 (1987)
ship: ambient store at: 2-8°C

L5158-5UG	5 µg

Macrophage Colony-Stimulating Factor human

MCSF

▸ recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

M-CSF augments many of the physiological functions of macrophages, and support osteoclast differentiation.

159 amino acids including N-terminal methionine (each monomer).

Lyophilized from a sterile filtered buffered solution.

≥98% (SDS-PAGE and HPLC)

dimer mol wt 37 kDa

The proliferative activity is tested in culture using the cytokine-dependent murine monocytic cell line M-NFS-60 cells.

endotoxin ..... tested

ship: ambient store at: −20°C

M6518-5UG	5 µg

Macrophage Colony-Stimulating Factor from mouse

M-CSF; CSF-1
[81627-83-0]

▸ mM-CSF; mCSF-1
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a sterile filtered buffered aqueous solution.

≥97% (SDS-PAGE)

dimer mol wt 18.2 kDa (containing 156 amino acid residues)

The proliferative activity is tested by the dose-dependent stimulation of the murine monocytic cell line, M-NFS-60.

endotoxin ..... tested

ship: ambient store at: −20°C

M9170-10UG	10 µg

Stem Cell Factor human

c-*Kit* ligand; SCF

Stem Cell Factor (SCF), also known as c-*Kit* ligand (KL), steel factor (SLF) and mast cell growth factor (MGF), is a 30 kDa glycoprotein with broad activities on various tissues, including hematopoietic cells, pigment cells, and primordial germ cells. SCF is secreted by endothelial cells, fibroblasts, and bone marrow stromal cells as a membrane-bound form which may be cleaved to release the soluble form. Both forms are active in promoting colony formation from murine bone marrow cells, but membrane-bound SCF is more effective in promoting hematopoieses *in vivo*, suggesting a role in cellular interactions between hematopoietic and stromal cells. The soluble is thought to exist in solution as a noncovalently linked dimer. SCF is structurally related to M-CSF (CSF-1) and Flt-3/Flk-2 Ligand (FL) with all three sharing a similar size, existence of transmembrane and soluble forms, four conserved cysteines, and alternative splicing exon locations, but they share little sequence homology. SCF alone is a modest colony stimulating factor. However, in the presence of other cytokines such as EPO, TPO, GM-CSF, G-CSF, M-CSF, IL-3, and IL-7, SCF is a potent costimulant that works synergistically to increase the size of myeloid, erythroid or lymphoid lineage colonies without influencing the lineage differentiation of the progenitors.

endotoxin ..... tested

▸ recombinant, expressed in *Escherichia coli*, powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in PBS containg 50 µg BSA /µg cytokine.

>95% (SDS-PAGE)

ED_{50}/EC_{50}: 2.5-10 ng/mL

protein mol wt 18.5 kDa

The proliferative activity is tested in culture using TF-1 cells or by the dose dependent proliferation of human MO-7e cells.

ship: ambient store at: −20°C

S7901-10UG	10 µg

▸ recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 25-100 ng/mL

dimer mol wt 35-45 kDa (glycosylated)

HumanKine® SCF is expressed in human HEK 293 cells as a glycosylated monomer with an apparent molecular mass of 35-45 kDa due to glycosylation. Production in human 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

ship: ambient store at: room temp

H8416-10UG	10 µg

Thrombopoietin human

TPO; Megakaryocyte colony-stimulating factor; c-MPL ligand; MGDF

Thrombopoeitin is a primary regulatory factor for megakaryocytopoiesis and thrombopoiesis.[1,2,3,4] The mature form of TPO is a highly conserved glycoprotein, showing homology among various mammals. It is produced by liver and kidney cells. TPO stimulates growth and maturation of mega-karyocytes and megakaryocytic colonies from bone marrow cultures. TPO binds and activates an 68-78 kDa glycoprotein receptor belonging to the GH family of cytokine receptors, a family that includes receptors to growth hormone (GH), erythropoietin (EPO), and prolactin (PRL).[5,6] Like GH and EPO, TPO may bind to its receptor at two distinct sites, initiating receptor dimerization and activation.[7] Analysis of mRNA indicates also the existence of a novel truncated and potentially soluble form of TPO receptor. The viral oncogene v-*mlp* of the myeloproliferative leukemia virus (MPLV) contains the gene sequence for the entire cytoplasmic and transmembrane domains and a portion of the extracellular domain of c-*mlp* (TPO receptor).

The biological activity is measured in a cell proliferation assay using MO7e cells.

Lit. cited: 1. Lok, S., and Foster, D., The structure, biology and potential therapeutic applications of recombinant thrombopoietin. *Stem Cells* **12**, 586-598 (1994)
2. Kato, T., et al., Native thrombopoietin: structure and function. *Stem Cells* **16**, 11-19 (1998)
3. Kuter, D., et al., eds., *Thrombopoiesis and Thrombopoietins* , Humana Press (Totowa, N.J: 1997)
4. Callard, R., and Gearing, A., *The Cytokine Facts Book* , Academic Press (New York: 1994)
5. Heim, M.H., The Jak-STAT pathway: cytokine signalling from the receptor to the nucleus. *Receptor Signal Transduction Res.* **19**, 75-120 (1999)
6. Skoda, R., Specificity of signaling by hematopoietic cytokine receptors: instructive versus permissive effects. *J. Recept. Signal Transduct. Res.* **19**, 741-772 (1999)
7. Hou, J., Zhan, H., Expression of active thrombopoietin and identification of its key residues responsible for receptor binding. *Cytokine* **10**, 319 (1998)

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥98% (SDS-PAGE and HPLC)

Lyophilized from a 0.1 % TFA solution containing 250 µg bovine serum albumin

Associated gene(s): THPO (7066)

mol wt 18.6 kDa

endotoxin ..... tested

ship: ambient store at: –20°C

T1568-5UG	5 µg

Thrombopoietin from mouse

TPO

Thrombopoeitin (TPO), also known as the ligand for the *c-mpl* proto-oncogene (Mpl-L) and as megakaryocyte colony stimulating factor (Meg-CSF), is a primary regulatory factor for megakaryocytopoiesis and thrombopoiesis.[1,2,3,4] Produced mainly by hepatocytes but also by kidney cells, TPO is a humoral glycoprotein that stimulates growth and maturation of megakaryocytes and megakaryocytic colonies from bone marrow cultures. TPO binds and activates an 68-78 kDa glycoprotein receptor belonging to the GH family of cytokine receptors, a family that includes receptors to growth hormone (GH), erythropoietin (EPO) and prolactin (PRL).[5,6] Like GH and EPO, TPO may bind to its receptor at two distinct sites, initiating receptor dimerization and activation.[7] Analysis of mRNA indicates also the existence of a novel truncated and potentially soluble form of TPO receptor. The viral oncogene v-*mlp* of the myeloproliferative leukemia virus (MPLV) contains the gene sequence for the entire cytoplasmic and transmembrane domains and a portion of the extracellular domain of c-*mlp* (TPO receptor). TPO receptors are found on megakaryocytes and their precursors and on platelets. The mature form of TPO is a highly conserved glycoprotein, showing approximately 70% sequence homology among various mammals. Human TPO contains 332 amino acids (mouse 335) including two internal disulfide bonds and 6 N-linked glycosylation sites (mouse 7). The N-terminal 153 amino acid region of TPO shows structural and amino acid sequence homology to erythropoietin and a pair of arginines (potential cleavage site) separating it from the highly glycosylated carboxyterminal domain.

Lit. cited: 1. Lok, S., and Foster, D., The structure, biology and potential therapeutic applications of recombinant thrombopoietin. *Stem Cells* **12**, 586-598 (1994)
2. Kato, T., et al., Native thrombopoietin: structure and function. *Stem Cells* **16**, 11-19 (1998)
3. Kuter, D., et al., eds., *Thrombopoiesis and Thrombopoietins* , Humana Press (Totowa, N.J: 1997)
4. Callard, R., and Gearing, A., *The Cytokine Facts Book* , Academic Press (New York: 1994)
5. Heim, M.H., The Jak-STAT pathway: cytokine signalling from the receptor to the nucleus. *Receptor Signal Transduction Res.* **19**, 75-120 (1999)
6. Skoda, R., Specificity of signaling by hematopoietic cytokine receptors: instructive versus permissive effects. *J. Recept. Signal Transduct. Res.* **19**, 741-772 (1999)
7. Hou, J., Zhan, H., Expression of active thrombopoietin and identification of its key residues responsible for receptor binding. *Cytokine* **10**, 319 (1998)

recombinant, expressed in mouse NSO cells, lyophilized powder, suitable for cell culture, >97% (SDS-PAGE)

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.25 mg bovine serum albumin

Associated gene(s): Thpo (21832)

predicted mol wt 35 kDa

mol wt ~75 kDa, SDS-PAGE (as a result of glycosylation)

The biological activity was measured in a cell proliferation assay using MO7e cells.

endotoxin ..... tested

ship: ambient store at: –20°C

T4184-5UG	5 µg

Hepatocyte Growth Factor/Scatter Factor

Hepatocyte Growth Factor human

HGF; Hepatopoietin A; Scatter Factor

The NSO-expressed recombinant HGF is greater than 95% heterodimeric HGF. The insect cell line-expressed recombinant HGF is a mixture of single chain HGF (predominant) with some heterodimeric HGF. Single chain HGF and heterodimeric HGF are equally active in *in vitro* assays.

lyophilized powder

endotoxin ..... tested

recombinant, expressed in *Baculovirus* infected High-5 cells, suitable for cell culture

Lyophilized from a sterile filtered solution in 0.1 M L-Arginine hydrochloride and 10 mM Tris, pH 8.0, with 200 mM NaCl

Associated gene(s): HGF (3082)

≥98% (SDS-PAGE)

mol wt ~80 kDa

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation in the HGF responsive monkey epithelial cell line, 4MBr-5.

ship: ambient store at: –20°C

H1404-5UG	5 µg

recombinant, expressed in mouse NSO cells, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 50 µg of bovine serum albumin per 1µg of cytokine.

Associated gene(s): HGF (3082)

≥95% (SDS-PAGE)

mol wt 70-80 kDa, SDS-PAGE (non-reducing)

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation in the HGF responsive monkey epithelial cell line, 4MBr-5.

ship: ambient store at: –20°C

H9661-5UG	5 µg

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

The hepatocyte growth factor gene spans ~70 kb and consists of 18 exons interrupted by 17 introns. The organization of the human HGF gene is highly homologous to that of human plasminogen. HGF maps to the long arm of human chromosome 7, 7q21.1.

≥95% (SDS-PAGE)

ED_{50}: 10-50 ng/mL

dimer mol wt 70 kDa (glycosylated)

HumanKine® HGF is expressed as a glycosylated 70 kDa single chain monomer in human HEK 293 cells. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of the monkey epithelial cell line 4MBr-5.

ship: ambient store at: room temp

H5791-5UG	5 µg

HumanKine® Growth Factors and Cytokines

Sigma-Aldrich® is proud to bring you HumanKine® growth factors and cytokines. These highly purified reagents are developed from an efficient human cell-based technology, and are excellent choices for your critical inflammation, cancer, stem cell, and antibody development research applications.

HumanKine® Growth Factors are:

- Produced in HEK 293 cells
- Recombinant, animal component-free
- Have authentic human glycosylation and post-translational modification patterns
- Highly pure, low endotoxin proteins

Activin A human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.5-5 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

The specific activity was determined by the dose-dependent inhibition of proliferation of the MPC-11 cell line (mouse plasmocytoma cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H4666-5UG	5 µg

Bone Morphogenetic Protein 4 human NEW

BMP-4

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

Bone Morphogenetic Proteins (BMP) are members of the TGF-β superfamily of cytokines that affect bone and cartilage formation. Similar to other TGF-β family proteins, BMPs are highly conserved across animal species. Mature BMPs are 30-38 kDa proteins that assume a TGF-β-like cysteine knot configuration. Unlike TGF-β, BMPs do not form latent complexes with their propeptide counterparts. Most BMPs are homodimers, but bioactive natural heterodimers have been reported.

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 2-10 ng/mL

dimer mol wt 34 kDa (glycosylated)

This BMP-4 is expressed in human HEK 293 cells as a glycosylated 34 kDa homodimer. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications. Mature human and mouse BMP-4 are 98% and 100% identical, respectively, to mature rat BMP-4 in their amino acid sequence.

The specific activity was determined by its ability to induce alkaline phosphatase production in a dose response to BMP-4 in the ATDC-5 cell line (mouse chondrogenic cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H4916-10UG	10 µg

Bone Morphogenetic Protein 2 human NEW

BMP-2

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

Bone Morphogenetic Protein 2 is a member of the TGF-β superfamily of cytokines that affect bone and cartilage formation. It is important for skeletal development during embryogenesis. BMP-2 induces chondrocyte formation, osteoblast differentiation, and is involved in embryo dorsal-ventral patterning and organogenesis.

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 1-5 ng/mL

dimer mol wt 30-38 kDa (glycosylated)

This Bone Morphogenetic Protein 2 is produced from a DNA sequence encoding the human BMP-2 protein, expressed in HEK 293 cells. It is a glycosylated homodimer linked by a single disulfide bond with an apparent molecular mass of 30-38 kDa.

The specific activity was determined by its ability to induce alkaline phosphatase production in a dose response to BMP-2 in the ATDC-5 cell line (mouse chondrogenic cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H4791-10UG	10 µg

Cystatin C human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

IC_{50}: 0.3-1.5 µM

dimer mol wt 12-13 kDa (non-glycosylated)

This product is lyophilized from a PBS solution. IC50: 0.3-1.5 microM.

The inhibitory function of cystatin c on papain's protease activity was measured by a colorimetric assay using L-BAPA as substrate. IC_{50} value was measured at 5 to 20 µg/mL (0.3 to 1.5 µM) with a range of 1.56 µg/mL to 50 µg/mL cystatin C in presence of 0.55 µM papain and 0.44 µM L-BAPA.

endotoxin ..... tested

ship: ambient store at: room temp

H5041-10UG	10 µg

Erythropoietin human NEW

EPO
[11096-26-7]

Erythropoietin is a glycoprotein that is the principal regulator of red blood cell growth and differentiation.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

Erythropoietin has been cloned from various species including human, murine, canine, and others. The mature proteins from the various species are highly conserved and exhibit greater than 80% amino acid sequence identity. EPO contains three N-linked glycosylation sites. The glycosylation of erythropoietin is required for the biological activities of erythropoietin in vivo.

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.5-2.5 ng/mL

dimer mol wt 36 kDa (glycosylated)

HumanKine® EPO is expressed as a glycosylated 36 kDa monomer in human HEK 293 cells. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested
ship: ambient store at: room temp

H5166-5UG	5 µg

Fibroblast Growth Factor-8b human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 15-60 ng/mL

dimer mol wt 30-45 kDa (glycosylated)

HumanKine® Fibroblast Growth Factor-8b, expressed in human HEK 293 cells is a glycosylated monomer with an apparent molecular mass of 30-45 kDa due to glycosylation. Production in human HEK 293 cells offers authentic glycosylation, which is absent when this cytokine is expressed in E. coli. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of the BALB/3T3 cell line.

endotoxin ..... tested
ship: ambient store at: room temp

H5291-10UG	10 µg

FLT3 Ligand human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.2-0.8 ng/mL

monomer mol wt 24-30 kDa (glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of the human acute myeloid leukemia cell line OCI-AML5.

endotoxin ..... tested
ship: ambient store at: room temp

H5416-10UG	10 µg

Granulocyte-Macrophage Colony-Stimulating Factor human NEW

GM-CSF human

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 15-36 kDa (glycosylated)

HumanKine® GM-CSF is expressed as a 15-36 kDa glycosylated monomer in human HEK 293 cells. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications. Although human and mouse GM-CSF share 54% amino acid sequence homology, their biological actions are species-specific and exhibit no cross-species reactivity.

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

Although human and mouse GM-CSF share 54% amino acid sequence homology, their biological actions are species-specific and exhibit no cross-species reactivity.

endotoxin ..... tested
ship: ambient store at: room temp

H5666-5UG	5 µg

Granulocyte colony-stimulating factor human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.02-0.08 ng/mL

dimer mol wt 21-25 kDa (glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of murine M-NFS-60 cells (mouse myeloid leukemia indicator cell line).

endotoxin ..... tested
ship: ambient store at: room temp

H5541-5UG	5 µg

Hepatocyte Growth Factor human NEW

HGF; Hepatopoietin A; Scatter Factor

The NSO-expressed recombinant HGF is greater than 95% heterodimeric HGF. The insect cell line-expressed recombinant HGF is a mixture of single chain HGF (predominant) with some heterodimeric HGF. Single chain HGF and heterodimeric HGF are equally active in *in vitro* assays.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

The hepatocyte growth factor gene spans ?70 kb and consists of 18 exons interrupted by 17 introns. The organization of the human HGF gene is highly homologous to that of human plasminogen. HGF maps to the long arm of human chromosome 7, 7q21.1.

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 10-50 ng/mL

dimer mol wt 70 kDa (glycosylated)

HumanKine® HGF is expressed as a glycosylated 70 kDa single chain monomer in human HEK 293 cells. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of the monkey epithelial cell line 4MBr-5.

endotoxin ..... tested
ship: ambient store at: room temp

H5791-5UG	5 µg

Human Growth Hormone human

NEW

HumanKine®, recombinant, expressed in HEK 293 cells, cell culture tested, endotoxin tested

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.4-2 ng/mL

monomer mol wt 22 kDa (non-glycosylated)

HumanKine®, Human Growth Hormone (HGH) is expressed as a non-glycosylated monomer in human HEK 293 cells and has an apparent molecular mass of 22 kDa.

The activity was determined by the dose-dependent stimulation of the proliferation of rat lymphoma line Nb2-11 cells (prolactin indicator cell line).

ship: ambient store at: room temp

H5916-10UG	10 µg

Interferon α 2A human

NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.4 ng/mL

dimer mol wt 16 kDa (glycosylated)

HumanKine® IFNα-2A, expressed in human HEK 293 cells, is a glycosylated monomer with an apparent molecular mass of 16 kDa. Production in human HEK 93 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent proliferation of WISH (heteroploid human amnion cell line) in presence of VSV (vesicular stomatitis virus).

endotoxin ..... tested

ship: ambient store at: room temp

H6041-10UG	10 µg

Interferon α 2B human

NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.02-0.08 ng/mL

dimer mol wt 16 kDa (glycosylated)

HumanKine® IFNα-2B is a glycosylated monomer expressed in HEK 293 cells with an apparent molecular mass of 16 kDa. Production in human 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent proliferation of WISH (heteroploid human amnion cell line) in presence of VSV (vesicular stomatitis virus).

endotoxin ..... tested

ship: ambient store at: room temp

H6166-10UG	10 µg

Interleukin-1β human

NEW

IL-1β; Lymphocyte activating factor

Interleukin-1, originally known as Lymphocyte Activating Factor (LAF), activates T cell lymphocytes, which then proliferate and secrete interleukin-2 (IL-2). IL-1 is released primarily from stimulated macrophages and monocytes, but has also been shown to be released from several other types and is thought to play a key role in inflammatory and immune responses. Other synonyms for IL-1 include: endogenous pyrogen (EP), mitogenic protein (MP), Helper Peak-1 (HP-1), T Cell Replacing Factor III (TRF III or TRFM), B Cell Activating Factor (BAF) and B Cell Differentiation Factor (BDF) The two closely related agents Interleukin-1α (IL-1α) and Interleukin-1β (IL-1β) share 62% homology in amino acid sequence and elicit nearly identical biological responses. IL-1α and IL-1β are both approximately 17 kDa with some heterogeneity in the amount of glycosylation.

HumanKine®, recombinant, expressed in HEK 293 cells, cell culture tested, endotoxin tested

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.02-0.08 ng/mL

dimer mol wt 18-25 kDa (glycosylated)

HumanKine® Recombinant Human IL-1β, expressed in human HEK 293 cells, is a glycosylated monomer with a molecular mass of 18-25 kDa. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of mouse D10S cells.

ship: ambient store at: room temp

H6291-5UG	5 µg

Interleukin-3 human

NEW

IL-3

Interleukin-3 is a multifunctional protein produced by activated T lymphocytes. IL-3 supports the formation of multilineage colonies in the early development of multipotent hematopoietic progenitor cells. IL-3 also interacts with IL-2 to stimulate growth of T lymphocytes and induce IgG secretion from activated B cells.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥90% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.5-2 ng/mL

dimer mol wt 17-45 kDa (glycosylated)

HumanKine® IL-3, expressed in human HEK 293 cells, is a glycosylated monomer with an apparent molecular mass of 17-45 kDa due to glycosylation, which is absent when this cytokine is expressed in E. coli. Production in human HEK 293 cells offers authentic glycosylation which contributes to stability in cell growth media and other applications.

The activity was determined by the dosedependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H7166-10UG	10 µg

Interleukin-4 human

NEW

- **recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture**

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 14-19 kDa (glycosylated)

HumanKine® IL-4, expressed in human HEK 293 cells, is a glycosylated monomer with an apparent molecular mass of 14-19 kDa due to glycosylation, which is absent when this cytokine is expressed in E. coli. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent simulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H7291-10UG	10 µg

Interleukin-6 human

NEW

IL-6

Interleukin-6 is a multifunctional protein originally discovered in the media of cells stimulated with double stranded RNA. IL-6 appears to be directly involved in the responses that occur after infection and injury and may prove to be as important as IL-1 and TNF-α in regulating the acute phase response. IL-6 is reported to be produced by fibroblasts, activated T cells, activated monocytes or macrophages, and endothelial cells. It acts upon a variety of cells, including fibroblasts, myeloid progenitor cells, T cells, B cells and hepatocytes. IL-6 induces multiple effects, as indicated by its numerous synonyms: plasmacytoma growth factor (PCT-GF), interferon-β-2 (IFN-β_2), monocyte derived human B cell growth factor, B cell stimulating factor (BSF-2), hepatocyte stimulating factor (HSF), Interleukin Hybridoma/Plasmacytoma-1 (IL-HP1). In addition, IL-6 appears to interact with IL-2 in the proliferation of T lymphocytes. IL-6 also potentiates the proliferative effect of IL-3 on multipotential hematopoietic progenitors.

- **recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture**

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.2-1 ng/mL

dimer mol wt 26-30 kDa (glycosylated)

HumanKine® IL-6, expressed in human 293 cells, is a glycosylated monomer with an apparent molecular mass of 26-30 kDa due to glycosylation, which is absent when this cytokine is expressed in E. coli. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H7416-10UG	10 µg

Interleukin-10 human

NEW

IL-10

Interleukin-10 regulates lymphoid and myeloid cell functions. It blocks the activation of cytokine synthesis and several accessory functions of macrophages. IL-10 enhances cytotoxic T cell development and co-stimulates B cell differentiation and Ig secretion. IL-10 regulates angiogenesis by inducing cell-type dependent expression of either angiogenic or angiostatic factors.

Interleukin-10 is an important regulator of the functions of lymphoid and myeloid cells. IL-10 can block the activation of cytokine synthesis and several accessory functions of macrophages. Human and mouse IL-10 share a 73% sequence homology. However, human IL-10 acts on both human and mouse target cells, while mouse IL-10 has species-specific activity. In mouse, the cellular sources of IL-10 consist of Th2 cells, activated fetal thymocytes, macrophages, keratinocytes, LY-1^+ ($CD5^{+)}$, and normal B cells. In human, the cellular sources of IL-10 consist of $CD4^+$ T cells and T cell clones, thymocytes, B cells, B cell lymphomas, macrophages, mast cell lines and keratinocytes. IL-10 stimulates the growth of stem cells, mast cells and thymocytes. IL-10 enhances cytotoxic T cell development, and co-stimulates B cell differentiation and Ig secretion. IL-10 regulates angiogenesis by inducing the cell-type dependent expression of either angiogenic or angiostatic factors.

- **recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture**

endotoxin tested

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.3-1.5 ng/mL

dimer mol wt 17 kDa (glycosylated)

HumanKine® IL-10 is expressed in human HEK 293 cells as a glycosylated, non-disulfide linked homodimer with an apparent molecular mass of 17 kDa due to glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of MC/9 cells (mouse master cell line) in presence of 200 pg/mL IL-4.

ship: ambient store at: room temp

H7541-10UG	10 µg

Interleukin-12 human

NEW

IL-12

Interleukin-12 (IL-12) was identified as a factor secreted by human Epstein-Barr (EBV)-transformed B cell lines. IL-12, or Natural Killer Cell Stimulatory Factor (NKSF), is a 75 kDa disulfide-linked heterodimer of a 35 kDa subunit and 40 kDa subunit. IL-12 is produced predominantly by monocytes and NK cells and induces T cells and NK cells to produce IFN-γ. Human IL-12 is not active on mouse cells, but murine IL-12 is active on both murine and human lymphocytes. The biological activity of recombinant human IL-12 was measured by its ability to stimulate the proliferation of PHA-activated human T lymphoblasts. IL-12 has anti-angiogenic properties.

Interleukin-12 *(continued)*

HumanKine®, recombinant, expressed in HEK 293 cells, cell culture tested, endotoxin tested

≥95% (SDS-PAGE)

aqueous solution

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 57 kDa (glycosylated)

The specific activity was determined by the dose-dependent release of IFN-γ from the human NK92 cell line in presence of 20 ng/mL rIL-2.

ship: ambient store at: room temp

H7666

Interleukin-17F human NEW

HumanKine®, recombinant, expressed in HEK 293 cells, cell culture tested, endotoxin tested

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 100-500 ng/mL

dimer mol wt 38 kDa (glycosylated)

The specific activity was determined by the dose-dependent induction of IL-6 secretion from NDHF adult fibroblasts.

ship: ambient store at: room temp

H7916-10UG 10 µg

Interleukin-23 human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

aqueous solution

ED_{50}: 2-20 ng/mL

dimer mol wt 55 kDa (glycosylated)

The specific activity was determined by the dose-dependent secretion of IL-17 from mouse splenocytes activated with 10 ng/mL PMA.

endotoxin ..... tested

ship: ambient store at: room temp

H8041-5UG 5 µg

Keratinocyte Growth Factor human NEW

FGF-7; KGF
[148348-15-6]

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 10-40 ng/mL

dimer mol wt 17-30 kDa (glycosylated)

HumanKine® Keratinocyte Growth Factor (KGF) is expressed as glycosylated 17 and 30 kDa monomers in human HEK 293 cells. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of 4MBr-5 cells (monkey epithelial cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H6666-10UG 10 µg

LEFTY-B human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥90% (SDS-PAGE)

lyophilized powder

ED_{50}: 10-40 ng/mL

monomer mol wt 38 kDa (glycosylated)

The specific activity was determined by dose dependent ability to inhibit BMP-4 (6.5 ng/mL) induction of alkaline phosphatase production in the MC-3T3-E1 cell line (mouse chondrogenic cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H6791-10UG 10 µg

Macrophage Colony-Stimulating Factor human NEW

MCSF

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.5-4 ng/mL

dimer mol wt 35-40 kDa (glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of murine M-NFS-60 cells (mouse myeloid Leukemia indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H6916-10UG 10 µg

Nerve Growth Factor β Human NEW

NGF-β human

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: ≤16 ng/mL

mol wt 13 kDa (non-glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of the human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H9666-10UG 10 µg

Noggin human

NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 20-100 ng/mL

dimer mol wt 65 kDa (glycosylated)

HumanKine® Noggin is expressed in human HEK 293 cells using a scaleable suspension cell culture system. The protein is a highly stable, authentically glycosylated,disulfide linked 65 kDa homodimer. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose dependent inhibition of rhBMP4 induced alkaline phosphate production by ATDC5 cells.

endotoxin ..... tested

ship: ambient store at: room temp

H6416-5UG	5 µg

Oncostatin M human

NEW

OSM

[106956-32-5]

Oncostatin M, LIF, G-CSF, IL-6, and ciliary neurotrophic factor (CNTF) are structurally related members of the same cytokine family sharing similarities in their primary amino acid sequences, predicted secondary structure, and receptor components. Oncostatin M is a growth-regulating cytokine, affecting a number of tumor and normal cells. This material was first identified by its ability to inhibit the growth of A375 melanoma cells and other human tumor cells, but not inhibit the growth of normal human fibroblasts. It acts synergistically with TGF β1 to inhibit the proliferation of tumor cells like A375 melanoma cells. It induces an increase in LDL receptor expression and LDL uptake by hepatoma cells. OSM activates synovial fibroblast-like cells to produce urokinase type plasminogen activator. Oncostatin M is secreted by macrophages and activated T lymphocytes.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 2-10 ng/mL

dimer mol wt 30 kDa (glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H6541-5UG	5 µg

Platelet-Derived Growth Factor-AA human

NEW

PDGF–AA

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 200-1000 ng/mL

dimer mol wt 35-45 kDa (glycosylated)

HumanKine® recombinant human PDGF-AA, expressed in human HEK 293 cells, is a mixture of pro-form, pro-mature, and mature dimers with apparent molecular masses of 35, 40, and 45 kDa, respectively. They are disulfide linked glycosylated homodimers. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of 3T3 cells.

endotoxin ..... tested

ship: ambient store at: room temp

H8291-10UG	10 µg

pro Insulin-like Growth Factor-II human

NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 2-8 ng/mL

monomer mol wt 25 kDa (glycosylated)

The specific activity was determined by the dose-dependent proliferation of MCF-7 cells (human breast cancer cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H8166-10UG	10 µg

Stem Cell Factor human

NEW

c-*Kit* ligand; SCF

Stem Cell Factor (SCF), also known as c-*Kit* ligand (KL), steel factor (SLF) and mast cell growth factor (MGF), is a 30 kDa glycoprotein with broad activities on various tissues, including hematopoietic cells, pigment cells, and primordial germ cells. SCF is secreted by endothelial cells, fibroblasts, and bone marrow stromal cells as a membrane-bound form which may be cleaved to release the soluble form. Both forms are active in promoting colony formation from murine bone marrow cells, but membrane-bound SCF is more effective in promoting hematopoieses *in vivo*, suggesting a role in cellular interactions between hematopoietic and stromal cells. The soluble is thought to exist in solution as a noncovalently linked dimer. SCF is structurally related to M-CSF (CSF-1) and Flt-3/Flk-2 Ligand (FL) with all three sharing a similar size, existence of transmembrane and soluble forms, four conserved cysteines, and alternative splicing exon locations, but they share little sequence homology. SCF alone is a modest colony stimulating factor. However, in the presence of other cytokines such as EPO, TPO, GM-CSF, G-CSF, M-CSF, IL-3, and IL-7, SCF is a potent costimulant that works synergistically to increase the size of myeloid, erythroid or lymphoid lineage colonies without influencing the lineage differentiation of the progenitors.

Stem Cell Factor *(continued)*

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 25-100 ng/mL

dimer mol wt 35-45 kDa (glycosylated)

HumanKine® SCF is expressed in human HEK 293 cells as a glycosylated monomer with an apparent molecular mass of 35-45 kDa due to glycosylation. Production in human 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells (human erythroleukemic indicator cell line).

endotoxin ..... tested

ship: ambient store at: room temp

H8416-10UG	10 µg

Transforming Growth Factor-β1 human NEW

TGF-β1

TGF-β1 is produced by many cell types, but is reported to be most concentrated in mammalian platelets, where it is present at approximately four times the level of TGF-β2.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

HumanKine® TGF-β1 is expressed in human HEK 293 cells as a mature, disulfide linked, non- glycosylated, homodimer with a predicted molecular mass of 25 kDa.

The specific activity was determined by the dose dependent inhibition of IL-4 induced proliferation of mouse HT-2 cells (BALB/c spleen activated by sheep erythrocytes in the presence of IL-2).

endotoxin ..... tested

ship: ambient store at: room temp

H8541-5UG	5 µg

Transforming Growth Factor-β2 human NEW

TGF-β2

TGF-β2, like TGF-β1, is produced by many cell types and reported to be most concentrated in mammalian platelets.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

HumanKine® TGF-β2 is expressed in human HEK 293 cells as a mature, non-glycosylated, disulfide-linked homodimer with a predicted molecular mass of approx. 25 kDa.

The specific activity was determined by the dose-dependent inhibition of IL-4 induced proliferation of mouse HT-2 (BALB/c spleen activated by sheep erythrocytes in the presence of IL-2).

endotoxin ..... tested

ship: ambient store at: room temp

H8666-5UG	5 µg

Transforming Growth Factor-β3 human NEW

TGF-β3

TGF-β3 is less prevalent in natural expression than either TGF-β1 or TGF-β2, but it is the most abundant mRNA expressed in chick embryos. It is also expressed in human umbilical cord, in a variety of mesenchymal cells of human and rodent origin, and in several human carcinoma cells.

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: ≤1.0 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

HumanKine® TGF-β3 is expressed in human HEK 293 cells as a mature, disulfide linked, non- glycosylated homodimer with a predicted molecular mass of 25 kDa.

The specific activity was determined by the dose-dependent inhibition of IL-4 induced proliferation of mouse HT-2 cells (BALB/c spleen activated by sheep erythrocytes in the presence of IL-2).

endotoxin ..... tested

ship: ambient store at: room temp

H8791-5UG	5 µg

Tumor Necrosis Factor-α human NEW

TNF-α
[94948-59-1]

Tumor necrosis factor-α, also known as cachectin, is expressed as a 26 kDa membrane bound protein and is then cleaved by TNF-α converting enzyme (TACE) to release the soluble 17 kDa monomer, which forms homotrimers in circulation. TNF-α plays roles in antitumor activity, immune modulation, inflammation, anorexia, cachexia, septic shock, viral replication and hematopoiesis. TNF-α is expressed by a great variety of cells, with numerous inductive and suppressive agents. Primarily, TNF-α is produced by macrophages in response to immunological challenges such as bacteria (lipopolysaccharides), viruses, parasites, mitogens and other cytokines. TNF-α is cytotoxic for many transformed cells (its namesake activity) but in normal diploid cells, it can stimulate proliferation (fibroblasts), differentiation (myeloid cells) or activation (neutrophils). TNF-α also shows antiviral effects against both DNA and RNA viruses and it induces production of several other cytokines. Although TNF-α is used in clinical trials as an antitumor agent, Sigma's cytokine, growth factor and hormone products are for research only. TNF-α and the related molecule TNF-β (LT-α) share close structural homology with 28% amino acid sequence identity and both activate the same TNF receptors, TNFR1 and TNFR2. Mouse and human TNF-α share 79% amino acid sequence identity. Unlike human TNF-α, the mouse form is glycosylated.

mol wt ~17.4 kDa

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: ≤1.00 ng/mL

mol wt 17 kDa (glycosylated)

HumanKine® TNF-α is expressed in human HEK 293 cells and has been shown to be predominantly a glycosylated, non-covalently linked homotrimer with a molecular mass of 51 kDa (gel filtration). Production in human 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent cytotoxity of the TNF alpha sensitive cell line L-929 in the presence of actinomycin D.

endotoxin ..... tested

ship: ambient store at: room temp

H8916-10UG	10 µg

Vascular Endothelial Growth Factor 121 human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

HumanPro VEGF-121 is expressed in human HEK 293 cells as a disulfide linked, glycosylated cytokine with an apparent molecular mass of 37 kDa as a homodimer and 50 kDa as a homotrimer. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 2-8 ng/mL

dimer mol wt 37 kDa (glycosylated)

dimer mol wt 50 kDa (glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of HUVEC cells (human umbilical vein endothelial cells).

endotoxin ..... tested

ship: ambient store at: room temp

H9041-5UG	5 µg

Vascular Endothelial Growth Factor 165 human NEW

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture

HumanKine® VEGF165 is expressed in human HEK 293 cells as a glycosylated homodimer with an apparent molecular mass of 45 kDa. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 2-10 ng/mL

dimer mol wt 45 kDa (glycosylated)

The specific activity was determined by the dose-dependent stimulation of the proliferation of HUVEC cells (human umbilical vein endothelial cells).

endotoxin ..... tested

ship: ambient store at: room temp

H9166-10UG	10 µg

Insulin-like Growth Factors (IGF)

Insulin-like growth factors (IGF-I and IGF-II) are mitogenic and anabolic peptides structurally homologous to insulin. IGF-I and -II are single polypeptide chains of approximately 7.5 kDa comprised of 70 and 67 amino acid residues, respectively. IGF-I and -II share 70% homology in amino acid sequence, while IGF-I and proinsulin share 48% homology. Both IGFs are highly conserved between species, with 100% identity among human, bovine and porcine IGFs. Unlike insulin, IGF-I and -II are primarily involved in normal growth and development. Circulating IGF is mainly secreted from the liver and acts as an endocrine to distant cells.

Many other tissues also make IGFs, where they act with autocrine and paracrine functions to regulate a number of different cellular functions.IGF-I receptor (IGF-IR) is homologous to the insulin receptor (IR) and is comprised of two 130-kDa ligand-binding a-subunits and two 95-kDa transmembrane b-subunits. IGF-IR binds IGF-I with highest affinity, IGF-II with somewhat lower affinity, and insulin with rather weak affinity. IGF-IR is a tyrosine kinase receptor with signal transduction pathways that include substrates IRS-1, IRS-2, Shc, and Grb10. IGF-IIR has anabolic functions (like IR) but also shows three distinguishing qualities concerned with growth: 1) It signals mitosis in a variety of cells. 2) It is a necessary factor in establishing and maintaining cells in a transformed phenotype. 3) It protects cells from apoptosis, both *in vitro* and *in vivo*. This last quality is the subject of considerable interest, as it was found that IGF-I administration to cells stimulates the formation of bcl-2, a prominent anti-apoptotic intracellular messenger. While other known anti-apoptosis treatments inhibit apoptotic pathways without actually preventing cell death, IGF-I stimulation may actually decrease the probability of apoptosis initiation.

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Insulin-like Growth Factor-I human

IGF; IGF-I; somatomedin c

IGF-I also known as somatomedin C, is secreted from the liver into circulation in a process regulated by pituitary growth hormone (GH) and so it mediates the growth-promoting activity of GH. In the developing embryo IGF-I is expressed primarily by mesenchymal-derived cells. After birth IGF-I expression in most extrahepatic tissues declines and hepatic expression of IGF-I becomes GH-regulated. Expression of IGF-I outside the liver is regulated differently, depending on the specific tissues. For example, gonadotropins and sex steroids regulate IGF-I expression within the reproductive system, while parathyroid hormone and sex steroids regulate IGF-I expression in bone. IGF-I is produced in several human tumors. IGF-I is mitogenic for a variety of cells including fibroblasts, osteoblasts, smooth muscle cells, fetal brain cells, neuroglial cells, and erythroid progenitor cells. IGF-I exerts its actions exclusively through the IGF-I receptor (IGF-IR). IGF-I induces endothelial cell migration and is involved in the regulation of angiogenesis.

Insulin-like Growth Factor-I *(continued)*

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from 0.2 µm filtered solution in 50 mM acetic acid.

Associated gene(s): Igf1 (16000)

≥97% (SDS-PAGE or HPLC)

ED_{50}/EC_{50}: 0.1-10 ng/mL

Reconstitute in water

The biological activiy is calculated by the dose-dependent proliferation of murine BALB/C3T3 cells. ED_{50} corresponds to a specific activity of at least 1 x 10^7 units/mg.

endotoxin ..... tested
ship: ambient store at: –20°C

I3769-50UG	50 µg

Insulin-like Growth Factor-II human

IGF-II

IGF-II also known as multiplication stimulating activity (MSA), shows virtually identical bioactivities as IGF-I. Expression of IGF-II is highest in fetal tissues, where it is believed to play a major role in overall growth and development. After birth IGF-II expression decreases to much lower levels in a variety of tissues. IGF-II is produced also in several tumors. IGF-II binds to IGF-II receptor (IGF-IIR), to IGF-IR and weakly to the insulin receptor (IR). All IGF-II bioactivities in adult tissues are generally attributed to its interaction with IGF-IR. However, during embryogenesis the roles of both IGF-IIR and IR may be significant in promoting normal growth.

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution containing 100 mM acetic acid.

≥97% (SDS-PAGE or HPLC)

ED_{50}/EC_{50}: 5.0-10.0 ng/mL

The biological activity is measured in a serum-free cell proliferation assay using the human cell line MCF-7.

endotoxin ..... tested
ship: ambient store at: –20°C

I2526-50UG	50 µg

DES (1-6) IGF II

[141909-47-9]

lyophilized powder, recombinant, expressed in *Escherichia coli*, suitable for cell culture

Recombinant analog of insulin-like growth factor II.

Used for studies of IGF-II function in cell culture applications. Use in combination with IGF-I, insulin and other forms of IGF-II to study receptor functions and cell signaling.

DES (1-6) IGF-II is a 61 amino acid analog of human insulin-like growth factor II, lacking the first six N-terminal amimo acids. DES (1-6) IGF-II exhibits reduced affinity for IGF binding proteins (IGFBPs), which are known to inhibit the actions of IGF's.

≥95% (HPLC)

endotoxin ..... tested
ship: ambient store at: 2-8°C

I1521-20UG	20 µg

Insulin-Like Growth Factor Binding Protein-2 human

IGFBP-2

During development, insulin-like growth factor binding protein-2 is expressed in a number of tissues with the highest expression level found in the central nervous system. IGFBP-2 exhibits a 2-10 fold higher affinity for IGF II than for IGF I. Mouse and human IGFBP-2 share approximately 82% homology.

recombinant, expressed in mouse NSO cells, lyophilized powder, suitable for cell culture, ≥95% (SDS-PAGE)

Lyophilized from a 0.2 µm filtered solution of 20% acetonitrile with 0.1% TFA

Associated gene(s): IGFBP2 (3485)

calculated mol wt ~31 kDa

mol wt ~36 kDa, SDS-PAGE

The biological activity is measured by its ability to inhibit recombinant human IGF-II on MCF-7 cells.

endotoxin ..... tested
ship: ambient store at: –20°C

I5403-25UG	25 µg

Insulin-Like Growth Factor Binding Protein-3 human

IGFBP-3; IGF Binding Protein-3

recombinant, expressed in mouse NSO cells, lyophilized powder, suitable for cell culture, ≥95% (SDS-PAGE)

Human IGFBP-3 is the major insulin-like growth factor (IGF) binding protein in plasma, where it exists in a ternary complex with IGF-I or IGF-II and the acid-labile subunit (ALS).

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% TFA.

Associated gene(s): IGFBP3 (3486)

The biological acitivity is measured as the ability to inhibit the biological activity of 14 ng/ml recombinant human IGF-I on MCF-7 cells.

endotoxin ..... tested
ship: ambient store at: –20°C

I5278-25UG	25 µg

R^3 IGF-1 human

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Product stimulates protein synthesis in L6 myoblasts.

Recombinant analog of insulin-like growth factor-I with the substitution of Arg for Glu at position 3.

R3 IGF-1 is qualified for use in cell culture applications where recombinant protein is preferred.

≥85% (HPLC)

Prepared from 0.1 M acetic acid

endotoxin ..... tested

ship: ambient store at: 2-8°C

I1146-.1MG	0.1 mg

Insulin-like Growth Factor-II from mouse

IGF-II

lyophilized powder, recombinant, expressed in *Escherichia coli*

Lyophilized from a 0.2 µm filtered solution of 30% acetonitrile and 0.1% of TFA.

>97% (SDS-PAGE)

The biological activity is measured in a serum-free cell proliferation assay using the human breast carcinoma cell line MCF-7.

endotoxin ..... tested

ship: ambient store at: –20°C

I8904-50UG	50 µg

LONG® R^3 IGF-I human

LONG R^3 IGF-I is a recombinant analog of human insulin-like growth factor-I (IGF-I) that has been specifically engineered for the enhancement of cell culture performance. It is more biologically potent *in vitro* than either insulin or native IGF-I and has been shown to significantly increase recombinant protein production. It is ideal for both research and large-scale culture systems utilizing serum-free or low-level serum applications. All cells that have a growth response to insulin in cell culture have the potential to respond to LONG R^3 IGF-I. LONG R^3 IGF-I is effective in commercially relevant cell types including CHO, PER.C6® and HEK 293. Hybridomas and fibroblasts have also been shown to respond to LONG R^3 IGF-I.

LONG R^3 IGF-I is produced in an *Escherichia coli* expression system without the use of animal-derived components.

recombinant, expressed in *Escherichia coli*, Recombinant Analog, suitable for cell culture

A genetically engineered recombinant human analog of IGF-1.

≥95% (HPLC)

powder

Lyophilized from 0.1M acetic acid

endotoxin ..... tested

ship: ambient store at: 2-8°C

I1271-.1MG	0.1 mg
I1271-1MG	1 mg

Interleukins

Interleukin-1α

Interleukin-1α human

Interleukin-1α human; Lymphocyte activating factor

IL-1α; hIL-1α.
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Interleukin-1, originally known as Lymphocyte Activating Factor (LA F), activates T cells and lymphocytes, which then proliferate and secrete Interleukin-2 (IL-2). The proliferative activity of human IL-1α is tested in culture using the murine cell line D10S.

Lyophilized from 0.2 µm filtered 10 mM tris, pH 8.0, plus 50 mm NaCl and 100 µg bovine serum albumin.

Associated gene(s): IL1A (3552)

≥97% (SDS-PAGE)

The proliferative activity of human IL-1α is tested in culture using the murine cell line D10S.

endotoxin ..... tested

ship: ambient store at: –20°C

I2778-2UG	2 µg

Interleukin-1α from mouse

IL-1α

mIL-1α
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Interleukin-1, originally known as Lymphocyte Activating Factor (LA F), activates T cells and lymphocytes, which then proliferate and secrete Interleukin-2 (IL-2). The proliferative activity of human IL-1α is tested in culture using the murine cell line D10S.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.25 mg bovine serum albumin.

Associated gene(s): IL1A (3552)

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.001-0.01 ng/mL

The biological activity is measured in a cel proliferation assay using the murine helper T cell line, D10.G4.1.

endotoxin ..... tested

ship: ambient store at: –20°C

I5396-5UG	5 µg

Interleukin-1α from rat

Lymphocyte activating factor; IL-1α

rIL-1α
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Interleukin-1, originally known as Lymphocyte Activating Factor (LA F), activates T cells and lymphocytes, which then proliferate and secrete Interleukin-2 (IL-2). The proliferative activity of human IL-1α is tested in culture using the murine cell line D10S.

Lyophilized from 0.2 µm filtered phosphate buffered saline containing 0.25 mg of bovine serum albumin.

Associated gene(s): Il1a (24493)

≥97% (SDS-PAGE)

The biological activity of recombinant rat IL-1α was measured by its ability to stimulate cell proliferation using a mouse helper T cell line, D10.G4.1.

endotoxin ..... tested

ship: ambient store at: –20°C

I3901-5UG	5 µg

Interleukin-1β

Interleukin-1β from rat

Lymphocyte activating factor; Interleukin-1β from rat

IL-1β
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered buffered solution.

Associated gene(s): Il1b (24494)

≥98% (SDS-PAGE and HPLC)

ED_{50}/EC_{50}: 0.01-0.1 ng/mL

The biological activity is measured in a cell proliferation assay using murine D10S cells.

The two closely related agents, Interleukin-1α (IL-1α) and Interleukin-1B (IL-1B) bind to the same cell surface receptor, elicit nearly identical biological responses and yet share 25% homology in their amino acid sequence.

ship: ambient store at: –20°C

I2393-10UG	10 µg

Interleukin-1β from mouse

Lymphocyte activating factor; IL-1β

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from 0.2 µm filtered solution in 10mM Sodium Citrate, pH 4.0

Associated gene(s): Il1b (16176)

≥98% (SDS-PAGE and HPLC)

mol wt 17.5 kDa (containing 153 amino acid residues)

The biological activity is measured by the dose-dependent stimulation of murine D10S cells.

The two closely related agents, Interleukin-1α (IL-1α) and Interleukin-1B (IL-1B) bind to the same cell surface receptor, elicit nearly identical biological responses and yet share 25% homology in their amino acid sequence.

endotoxin ..... tested

ship: ambient store at: –20°C

I5271-5UG	5 µg

Interleukin-2

Interleukin-2 human

Interleukin-2; T-cell growth factor

Interleukin-2 is an immunomodulatory factor produced by certain subsets of T lymphocytes. This lymphokine promotes long term growth of activated T cells and related cell types. Interleukin-2 plays a role in the activation and proliferation of NK cells, induces γ-interferon and B cell growth factor secretion, and modulates the expression of the IL-2 receptor.

endotoxin ..... tested

IL-2; hIL-2
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized, 0.2 µm filtered, no additives.

from human

mol wt 15.5 kDa

The biological activity is measured by the dose-dependent stimulation of the proliferation of murine CTLL-2 cells.

ship: ambient store at: –20°C

I2644-10UG	10 µg

IL-2
recombinant, expressed in *Pichia pastoris*, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in 50 mM sodium phosphate buffer, pH 7.0, containing 0.1% human serum albumin per 0.1 mL of cytokine.

≥98% (SDS-PAGE and HPLC)

predicted mol wt ~15 kDa

The biological activity is tested in a cell proliferation assay using an IL-2 dependent murine cytotoxic T cell line, CTLL-2.

ship: dry ice store at: –20°C

I7908-10KU	10000 units

HumanKine®, recombinant, expressed in HEK 293 cells, cell culture tested, endotoxin tested NEW

≥95% (SDS-PAGE)

ED_{50}: 0.2-2 ng/mL

dimer mol wt 15 kDa (glycosylated)

HumanKine® IL-2 expressed in human HEK 293 cells is a glycosylated monomer with a molecular mass of 15 kDa. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of mouse CTLL-2 cells (mouse cytotoxic T cell line).

ship: ambient store at: room temp

H7041-10UG	10 µg

Interleukin-2 from mouse

IL-2; T-cell growth factor

mIL-2
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Interleukin-2 is an immunomodulatory factor produced by certain subsets of T lymphocytes. This lymphokine promotes long term growth of activated T cells and related cell types. Interleukin-2 plays a role in the activation and proliferation of NK cells, induces γ-interferon and B cell growth factor secretion, and modulates the expression of the IL-2 receptor.

Lyophilized from a 0.2 µm filtered solution in 10 mM sodium citrate, pH 4.0.

≥97% (SDS-PAGE)

Activity was determined by dose-dependent stimulation of murine CTLL-2 cells. ED_{50} : ≤ 0.2 ng/ml (corresponds to a specific activity of 5×10^6 units/mg)

endotoxin ..... tested

ship: ambient store at: −20°C

I0523-20UG	20 µg

Interleukin-3

Interleukin-3 from mouse

Interleukin-3 from mouse

Interleukin-3 is a multifunctional protein, originally called colony forming unit-stimulating activity (CFU-SA), and is produced by activated T lymphocytes. IL-3 supports the formation of multilineage colonies in the early development of multipotent hematopoietic progenitor cells. IL-3 has been shown to induce colony formation of macrophages, neutrophils, mast cells, and megakaryocytes from agar-suspended bone marrow cells. IL-3 also interacts with IL-2 to stimulate growth of T lymphocytes and to induce IgG secretion from activated B cells. Other synonyms for IL-3 include pan-specific hemopoietin, multicolony stimulating factor, mast cell growth factor, burst promoting activity, histamine-producing cell stimulating factor, P cell stimulating factor, and WEHI-3 factor.

IL-3; mIL-3
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized with 0.5 mg of bovine serum albumin. Sterile filtered through a 0.2 µm filter.

≥98% (SDS-PAGE and HPLC)

ED_{50}/EC_{50}: 0.01-0.1 ng/mL

mol wt 15.1 kDa

The biological activity is measured by the dose-dependent stimulation of the proliferation of mouse M-NFS-60 cells.

endotoxin ..... tested

ship: ambient store at: −20°C

I4144-10UG	10 µg

Interleukin-3 human

hIL-3; IL-3

Interleukin-3 is a multifunctional protein produced by activated T lymphocytes. IL-3 supports the formation of multilineage colonies in the early development of multipotent hematopoietic progenitor cells. IL-3 also interacts with IL-2 to stimulate growth of T lymphocytes and induce IgG secretion from activated B cells.

endotoxin ..... tested

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized with no additives. Sterile filtered through a 0.2 µm filter.

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: ≤1.0 ng/mL

mol wt 15 kDa

The biological activity is measured by the dose-dependent stimulation of the proliferation of human TF-1 cells.

ship: ambient store at: −20°C

I1646-10UG	10 µg

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution.

≥95% (SDS-PAGE)

One unit is the amount of IL-3 required to induce half-maximal incorportion of ^3H thymidine. Activity also is expressed in Reference units (NIBSC reference preparation for IL-3 code 88/87).

ship: dry ice store at: −20°C

I7389-4UG	4 µg

Interleukin-4

Interleukin-4 human

IL-4; hIL-4
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lymphokine with profound effects on the growth and differentiation of immunologically competent cells, inhibits VEGF-induced and βFGF-induced angiogenesis.

Lyophilized from a sterile filtered solution.

Associated gene(s): IL4 (3565)

≥98% (SDS-PAGE and HPLC)

mol wt 14.9 kDa (containing 129 amino acid residues)

The biological activity is measured by the dose-dependent stimulation of the proliferation of human TF-1 cells.

endotoxin ..... tested

ship: ambient store at: −20°C

I4269-5UG	5 µg

Interleukin-4 from mouse

Interleukin-4 human

Interleukin-4, a lymphokine with profound effects on the growth and differentiation of immunologically competent cells, inhibits VEGF-induced and bFGF-induced angiogenesis. Human and mouse IL-4 share 50% amino acid sequence identity, but their biological actions are species-specific.

mIL-4
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized, 0.2 µm filtered, with no additives.

≥98% (SDS-PAGE)

ED_{50}/EC_{50}: 0.1-2.0 ng/mL

mol wt ~13.5 kDa

The proliferative activity is tested in culture using mouse HT-2 cells.[1]

endotoxin ..... tested

Lit. cited: 1. Fernandez-Botran, R., et al., B cell-stimulatory factor 1 (BSF-1) promotes growth of helper T cell lines. *J. Exp. Med.* **164**, 580-593 (1986)

ship: ambient store at: –20°C

I1020-5UG	5 µg

Interleukin-4 from rat

IL-4

Interleukin-4, a lymphokine with profound effects on the growth and differentiation of immunologically competent cells, inhibits VEGF-induced and bFGF-induced angiogenesis. Human and mouse IL-4 share 50% amino acid sequence identity, but their biological actions are species-specific.

Interleukin-4 is a lymphokine with profound effects on the growth and differentiation of immunologically competent cells. IL-4 is also known as B cell stimulatory factor-1 (BSF-1), T cell growth factor-2 (TCGF-2) and mast-cell growth factor-2 (MCGF-2). Inhibits VEGF-induced and bFGF-induced angiogenesis. IL-4 is a complex glycoprotein released by a subset of activated T cells. Human and mouse IL-4 share 50% amino acid sequence homology, but their biological actions are species-specific.

rIL-4
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.25 mg bovine serum albumin.

Associated gene(s): Il4 (287287)

The biological activity of recombinant rat IL-4 was measured by its ability to stimulate proliferation of rat splenocytes.

endotoxin ..... tested

ship: ambient store at: –20°C

I3650-5UG	5 µg

Interleukin-6

Interleukin-6 human

hIL-6; IL-6

Interleukin-6 is a multifunctional protein originally discovered in the media of cells stimulated with double stranded RNA. IL-6 appears to be directly involved in the responses that occur after infection and injury and may prove to be as important as IL-1 and TNF-α in regulating the acute phase response. IL-6 is reported to be produced by fibroblasts, activated T cells, activated monocytes or macrophages, and endothelial cells. It acts upon a variety of cells, including fibroblasts, myeloid progenitor cells, T cells, B cells and hepatocytes. IL-6 induces multiple effects, as indicated by its numerous synonyms: plasmacytoma growth factor (PCT-GF), interferon-β-2 (IFN-β_2), monocyte derived human B cell growth factor, B cell stimulating factor (BSF-2), hepatocyte stimulating factor (HSF), Interleukin Hybridoma/Plasmacytoma-1 (IL-HP1). In addition, IL-6 appears to interact with IL-2 in the proliferation of T lymphocytes. IL-6 also potentiates the proliferative effect of IL-3 on multipotential hematopoietic progenitors.

endotoxin ..... tested

from human (osteosarcoma cells), solution, suitable for cell culture

Solution in 0.2 µm filtered phosphate buffered saline containing 0.1% human serum albumin.

≥90% (SDS-PAGE)

Produced in MG-63 osteosarcoma cells induced with human IL-1β.[1]

One unit equals the amount of IL-6 required to induce half maximal proliferation of T-1165 cell *in vitro*.

Lit. cited: 1. Van Damme, J., et al., Interleukin 1 and poly(rI).poly(rC) induce production of a hybridoma growth factor by human fibroblasts. *Eur. J. Immunol.* **17**, 1-7 (1987)

ship: dry ice store at: –20°C

I3268-10KU	10000 units

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution of phosphate buffer saline (PBS), pH 7.4, containing 500 µg bovine serum albumin.

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.4-2.0 ng/mL

ship: ambient store at: –20°C

I1395-10UG	10 µg

Interleukin-6 from mouse

IL-6

Interleukin-6 is a multifunctional protein originally discovered in the media of cells stimulated with double stranded RNA. IL-6 appears to be directly involved in the responses that occur after infection and injury and may prove to be as important as IL-1 and TNF-α in regulating the acute phase response. IL-6 is reported to be produced by fibroblasts, activated T cells, activated monocytes or macrophages, and endothelial cells. It acts upon a variety of cells, including fibroblasts, myeloid progenitor cells, T cells, B cells and hepatocytes. IL-6 induces multiple effects, as indicated by its numerous synonyms: plasmacytoma growth factor (PCT-GF), interferon-β-2 (IFN-β_2), monocyte derived human B cell growth factor, B cell stimulating factor (BSF-2), hepatocyte stimulating factor (HSF), Interleukin Hybridoma/Plasmacytoma-1 (IL-HP1). In addition, IL-6 appears to interact with IL-2 in the proliferation of T lymphocytes. IL-6 also potentiates the proliferative effect of IL-3 on multipotential hematopoietic progenitors.

mIL-6
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a sterile filtered solution with no additives.

≥97% (SDS-GE)

EC_{50}: 0.002-0.02 ng/mL

mol wt ~21.7 kDa

The biological activity of recombinant mouse IL-6 was measured in a cell proliferation assay using the mouse 7TD1 cells.

endotoxin ..... tested
ship: ambient store at: –20°C

I9646-5UG	5 µg

Macrophage Inflammatory Proteins (MIPs)

Macrophage Inflammatory Protein-1β from mouse

MIP-1β

mMIP-1β
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

MIP-1β has endogenous pyrogenic activity when it is injected intravenously into rabbits. MIP-1 can synergize with the hematopoietic growth factors granulocytemacrophage CSF (GM-CSF) or macrophage CSF (M-CSF) to enhance colony formation.

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% trifluoroacetic acid containing 0.5 mg bovine serum albumin

Associated gene(s): Ccl4 (20303)

≥97% (SDS-PAGE and N-terminal analysis)

ED_{50}/EC_{50}: 40-100 ng/mL

The biological activity of MIP-1β was tested in culture by measuring its ability to inhibit hematopoietic stem cell proliferation in an in vitro colony assay.

endotoxin ..... tested
ship: ambient store at: –20°C

M6542-10UG	10 µg

Macrophage Inflammatory Protein-3α human

MIP-3α; LARC; Exodus

hMIP-3α
suitable for, lyophilized powder (from PBS solution), suitable for cell culture

Expression of the chemokine is detected in the lymphoid tissues, fetal lung and fetal liver. Recombinant and native MIP-3α are known chemoattractants of lymphocytes *in vitro*. Additionally, a growing body of work has identified MIP-3α as a functional ligand of GPR-CY4 orphan receptor or CCR6 and given useful information about *in vivo* function.

≥95% (SDS-PAGE)

The biological activity is measured by its ability to induce calcium flux in HEK293 cells stably expressing CCR-6 and for chemotaxis of mouse BaF/3 cells transfected with hCCR6.

endotoxin tested ..... contains
ship: ambient store at: –20°C

M249-25UG	25 µg

Neurotrophic Factors

Neurotrophic factors are agents that are important for survival, growth, or differentiation of discrete neuronal populations. Based on amino acid sequence homologies of cytokines and receptors and based on similarities in cytokine-receptor binding characteristics, neurotrophic factors can be divided into three general families.[1,2] The "neurotrophin family" includes **nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrohin-3 (NT-3) and neurotrohin-4 (NT-4)**.[3] The "CNTF family" includes **ciliary neurotrophic factor (CNTF)**, leukemia inhibitory factor (LIF) and interleukin-6 (IL-6).[2] Due to their broad biological actions, LIF and IL-6 are listed in separate sections. The "GDNF family" includes glial cell linederived neurotrophic factor (GDNF), neurturin (NTN), artemin(ART) and persephin (PSP).[4]

References

1. Henderson, C.E., Role of neurotrophic factors in neuronal development. *Curr. Opinion Neurobiol.*, **6**, 64-70 (1996).
2. Frostick, S.P., et al., Schwann cells, neurothrophic factors, and peripheral nerve regeneration. *Microsurgery*, **18**, 397-405 (1998).
3. Barde, Y.-A., The nerve growth factor family-neurotrophins, in Guidebook to Cytokines and Their Receptors, Nicola, N., ed., Oxford Press (New York, N.Y.: 1994), pp. 140-143.
4. Airaksinen, M.S., et al., GDNF family neurotrophic factor signaling: four masters, one servant? *Molec. Cell. Neurosci.*, **13**, 313-325 (1999).
5. Sieber-Blum, M., Growth factor synergism and antagonism in early neural crest development. *Biochem. Cell Biol.*, **76**, 1039-1050 (1998).

NGF-β human

NGFB; hNGFβ; HSAN5
from human, recombinant, expressed in mouse NSO cells, lyophilized powder, suitable for cell culture

NGF-β promotes the survival and cholinergic phenotype of basal forebrain cholinergic neurons and stimulates neurite outgrowth of embryonic trigeminal motor neurons *in vitro*.

Lyophilized from 0.2% acetic acid containing 5 mg bovine serum albumin.

Associated gene(s): NGF (4803)

>97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.15-3.0 ng/mL

The biological activity is measured in a cell proliferation assay using a factor-dependent human erythroleukemic cell line, TF-1.

endotoxin ..... tested
ship: ambient store at: –20°C

N1408-.1MG	0.1 mg

NGF-β from rat

Nerve Growth Factor-β from rat
[86923-98-0]

recombinant, expressed in *Sf*21 cells, lyophilized powder, suitable for cell culture

NGF-β promotes the survival and cholinergic phenotype of basal forebrain cholinergic neurons and stimulates neurite outgrowth of embryonic trigeminal motor neurons *in vitro*.

Lyophilized from a 0.2 µm filtered solution in PBS and 1 M NaCl containing 50 µg of bovine serum albumin per 1 µg of cytokine

NGF-β *(continued)*

Associated gene(s): NGF (4803)

≥97% (SDS-PAGE)

The biological activity is measured in a cell proliferation assay using the factor-dependent human erythroleukemic cell line, TF-1.

endotoxin ..... tested
ship: ambient store at: –20°C

N2513-.1MG	0.1 mg

Brain-derived neurotrophic factor human

BDNF

Abrineurin
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Brain-derived neurotrophic factor (BDNF) is a 13.6 kDa (or 27.2 kDa dimer) member of the neurotrophin family. BDNF has identical amino acid sequence in human, mouse, and pig with full cross-reactivities. BDNF is important in development and maintenance of neuronal populations within the central nervous system or cells directly associated with it. BDNF has been shown to enhance the survival and differentiation of several classes of neurons *in vitro*, including neural crest and placode-derived sensory neurons, dopaminergic neurons in the substantia nigra, basal forebrain cholinergic neurons, hippocampal neurons, and retinal ganglial cells.

The product is lyophilized from a sterile 0.2 µm filtered solution containing 250 µg BSA

≥98% (SDS-PAGE and HPLC)

The bioactivity is determined by its ability to stimulate the proliferation of rat C6 cells.

endotoxin ..... tested
ship: ambient store at: –20°C

B3795-5UG	5 µg

Ciliary Neurotrophic Factor human

CNTF

Ciliary neurotrophic factor was first identified as a survival factor for neurons from the ciliary ganglion of chicken embryos. Most of its known actions are restricted to cells of the nervous system, including motor neurons, sympathetic ganglion neurons, sensory neurons, hippocampal neurons, and medial septal neurons. CNTF also prevents degeneration of motor axons after axotomy and promotes astrocyte differentiation and oligodendrocyte survival and maturation. Outside the nervous system, CNTF maintains embryonic stem cells in an undifferentiated, pluripotent state. CNTF is structurally related to leukemia inhibitory factor (LIF), interleukin-6 (IL-6), interleukin-11 (IL-11) and oncostatin M (OSM). CNTF exerts its actions through the activation of the high-affinity CTNF receptor complex, which contains the ligand-binding α-subunit (CNTF Rα) and two signal transducing β-subunits (LIF Rβ and gp130). The LIF Rβ subunit is also shared by receptors for LIF and OSM. The gp130 subunit is also shared by receptors for LIF, OSM, IL-6, and IL-11. CNTF is localized in the cell nucleus subsequent to receptor binding. Human and rat CNTF share ~83% sequence homology and show cross-reactivity in bioactivity.

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 0.5 mg bovine serum albumin.

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 20-250 ng/mL

mol wt 22.8 kDa

The proliferative activity is tested in a cell proliferation assay using the cytokine-dependent human erythroleukemic cell line, TF-1.

endotoxin ..... tested
ship: ambient store at: –20°C

C3710-10UG	10 µg

Ciliary Neurotrophic Factor from rat

CNTF

Ciliary neurotrophic factor was first identified as a survival factor for neurons from the ciliary ganglion of chicken embryos. Most of its known actions are restricted to cells of the nervous system, including motor neurons, sympathetic ganglion neurons, sensory neurons, hippocampal neurons, and medial septal neurons. CNTF also prevents degeneration of motor axons after axotomy and promotes astrocyte differentiation and oligodendrocyte survival and maturation. Outside the nervous system, CNTF maintains embryonic stem cells in an undifferentiated, pluripotent state. CNTF is structurally related to leukemia inhibitory factor (LIF), interleukin-6 (IL-6), interleukin-11 (IL-11) and oncostatin M (OSM). CNTF exerts its actions through the activation of the high-affinity CTNF receptor complex, which contains the ligand-binding α-subunit (CNTF Rα) and two signal transducing β-subunits (LIF Rβ and gp130). CNTF is localized in the cell nucleus subsequent to receptor binding. Human and rat ciliary neurotrophic factor share ~83% sequence homology and show cross-reactivity in bioactivity.

The proliferative activity is tested in a cell proliferation assay using the cytokine-dependent human erythroleukemic cell line, TF-1.

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥98% (SDS-PAGE and HPLC)

Lyophilized from a 0.2 µm filtered solution of 5 mM sodium acetate, pH 6.5.

Associated gene(s): Cntf (25707)

mol wt 22.7 kDa

endotoxin ..... tested
ship: ambient store at: –20°C

C3835-10UG	10 µg

Glial Cell Line-derived Neurotrophic Factor human

GDNF

Glial Cell Line-Derived Neurotrophic Factor is a member of the cysteine-knot superfamily of growth factors that assume stable dimeric protein structures. GDNF is founding member of the GDNF family of ligands, which to date include GDNF, neurturin (NTN), persephin (PSP) and artemin (ART). GDNF is a glycosylated disulfide-linked homodimeric protein of ~15 kDa. Mature rat and human GDNF share ~93% sequence homology, with strong species cross-reactivity. GDNF signals through a multicomponent receptor system, composed of a RET and one of the four GFRα (α1-α4) receptors. GDNF specifically promotes dopamine uptake and survival and morphological differentiation of midbrain neurons. Using the Parkinson's disease mouse model, GDNF has been shown to improve conditions such as bradykinesia, rigidity, and postural instability. GDNF promotes survival of various neuronal cells in central and peripheral nervous systems and different stages of development, including motoneurons, midbrain dopaminergic neurons, Purkinje cells and sympathetic neurons. Cells known to express GDNF include Sertoli cells, type 1 astrocytes, Schwann cells, neurons, pinealocytes and skeletal muscle cells. In addition, exogenously applied GDNF has been shown to rescue damaged facial motor neurons *in vivo*.

ATF
recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture, ≥98% (SDS-PAGE and HPLC)

Lyophilized from a 0.2 µm filtered solution of 10 mM sodium citrate and 150 mM sodium chloride containing 0.5 mg bovine serum albumin.

Associated gene(s): GDNF (2668)

The biological activity of GDNF is determined by the dose-dependent dopamine uptake by rat ventral mesencephalic cultures.

endotoxin ..... tested
ship: ambient store at: −20°C

G1777-10UG	10 µg

Glial Cell Line-derived Neurotrophic Factor from rat

GDNF

Glial cell-derived neurotrophic factor (GDNF) is a neurotrophic factor that is a member of the TGF-β superfamily. GDNF is founding member of the GDNF family of ligands, which to date include GDNF, neurturin (NTN), persephin (PSP) and artemin (ART). GDNF is a glycosylated disulfide-linked homodimeric protein of ~15 kDa. Mature rat and human GDNF share 93% sequence homology with strong species cross-reactivity. GDNF promotes survival of various neuronal cells in central and peripheral nervous systems and different stages of development including motoneurons, midbrain dopaminergic neurons, Purkinje cells, and sympathetic neurons. Cells known to express GDNF include Sertoli cells, type 1 astrocytes, Schwann cells, neurons, pinealocytes, and skeletal muscle cells. In addition, exogenously applied GDNF has been shown to rescue damaged facial motor neurons *in vivo*.

Astrocyte-derived trophic factor; ATF
recombinant, expressed in *Sf*21 cells, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% trifluoroacetic acid (TFA) containing 50 µg bovine serum albumin (BSA) per 1 µg of cytokine.

Associated gene(s): Gdnf (25453)

The biological activity of GDNF is measured by its ability to bind to immobilized rrGFRa1/Fc in a functional ELISA.

endotoxin ..... tested
ship: ambient store at: −20°C

G1401-10UG	10 µg

NGF-β human

NGFB; hNGFβ; HSAN5
from human, recombinant, expressed in mouse NSO cells, lyophilized powder, suitable for cell culture

NGF-β promotes the survival and cholinergic phenotype of basal forebrain cholinergic neurons and stimulates neurite outgrowth of embryonic trigeminal motor neurons *in vitro*.

Lyophilized from 0.2% acetic acid containing 5 mg bovine serum albumin.

Associated gene(s): NGF (4803)

>97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.15-3.0 ng/mL

The biological activity is measured in a cell proliferation assay using a factor-dependent human erythroleukemic cell line, TF-1.

endotoxin ..... tested
ship: ambient store at: −20°C

N1408-.1MG	0.1 mg

Nerve Growth Factor from *Vipera lebetina* venom

[9061-61-4]

lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in sodium acetate buffer.

ED_{50}/EC_{50}: 0.2-20 µg/mL

protein mol wt 32.5 kDa

Isolated from the snake venom of *V. lebetina*.

The biological activity of Nerve Growth Factor from *Vipera lebetina* venom is measured in a cell proliferation assay using PC-12 cells.

endotoxin ..... tested
ship: ambient store at: −20°C

N8133-.1MG	0.1 mg

Nerve Growth Factor-7S from murine submaxillary gland

[93928-24-6]

NGF-7S
lyophilized powder, suitable for cell culture

Lyophilized from 0.2 µm filtered solution in 25 mM sodium phosphate, pH 7.0.

Associated gene(s): Ngf (18049)

ED_{50}/EC_{50}: 2-250 ng/mL

protein mol wt 130 kDa

NGF-7S is isolated from male mouse submaxillary glands using a modification of the method of Varon, et al.[1]

The biological activity is measured using a 3-day MTT assay.

endotoxin ..... tested
Lit. cited: 1. Varon, S., et al., The isolation of the mouse nerve growth factor protein in a high molecular weight form. *Biochemistry* **6**, 2202-2209 (1967)
ship: ambient store at: −20°C

N0513-.1MG	0.1 mg

Nerve Growth Factor-2.5S from murine submaxillary gland

[93928-24-6]

NGF-2.5S
lyophilized powder, suitable for cell culture

2.5S subunit of NGF-7S is essentially the β-subunit when isolated from male mouse submaxillary glands under initially dissociative conditions by a modification of the method of Bocchini and Angeletti.

Lyophilized from a 0.2 µm filtered solution in sodium acetate buffer.

Associated gene(s): Ngf (18049)

EC_{50}: 0.1-30 ng/mL (using PC-12 cells)

The biological activity is measured using a 3-day MTT assay.

endotoxin ..... tested
ship: ambient store at: −20°C

N6009-10UG	10 µg
N6009-100UG	100 µg

Neurotrophin-3 human

NT-3

NTF3; HDNF
recombinant, expressed in *Sf*21 cells, lyophilized powder, suitable for cell culture, >97% (SDS-PAGE)

Neurotrophin-3 (13.6 kDa or 27.2 kDa dimer) is a member of the neurotrophin family. It has identical amino acid sequence in human, mouse, and pig with full cross-reactivities. NT-3 is important in development and maintenance of neuronal populations and promotes differentiation of neural crest derived sensory and sympathetic neurons. It is critical for proprioceptive 1a afferent neurons, which relay information from peripheral muscle spindles to motoneurons, sending projections to spinocerebellar neurons. NT-3 is also critical in the superior cervical and nodose ganglia.

Lyophilized from a 0.2 m filtered solution in phosphate buffered saline (PBS) containing 50 µg of bovine serum albumin (BSA) per 1 µg of cytokine.

Associated gene(s): NTF3 (4908)

The biological activity is measured in a cell proliferation assay using a TrkB-transfected cell line BaF-TrkB-BD.

endotoxin ..... tested
ship: ambient store at: –20°C

N1905-5UG	5 µg

Neurotrophin-4 human

NT-4

NTF4
recombinant, expressed in *Sf*21 cells, lyophilized powder, suitable for cell culture, ≥97% (SDS-PAGE)

Neurotrophin-4 (also called NT-4/5 or NT-5) is a 14 kDa member of the neurotrophin family. NT-4 shares 95% sequence homology between human and rat. NT-4 from human, rat, and *Xenopus* are active on chick dorsal root ganglia. NT-4 is expressed in many tissues and major brain regions. NT-4 promotes survival and differentiation of various cells in culture including spinal neuons, basal forebrain cholinergic neurons, hippocampal neurons, cerebellar granule cells, embryonic dopaminergic neruons of mesencephalon, noradrenergic neurons of the locus coeruleus, dopaminergic, GABAergic, and serotoninergic neurons of the substantia nigra, and embryonic trigeminal and jugular neurons.

Lyophilized from a 0.2 µm filtered solution in phosphate buffered saline, pH 7.4, containing 50 µg bovine serum albumin per 1 µg of cytokine.

Associated gene(s): NTF4 (4909)

mol wt 14 kDa

The biological activity is measured in a cell proliferation assay using a TrkB-transfected cell line BaF-TrkB-BD.

endotoxin ..... tested
ship: ambient store at: –20°C

N1780-5UG	5 µg

Platelet Derived Growth Factors (PDGF)

Platelet-Derived Growth Factor is the principal mitogen found in mammalian serum and is released from platelets during clot formation.[1] PDGF elicits multifunctional actions with a variety of cells, including mitogenesis of mesoderm-derived cells, increased extracellular matrix synthesis, and chemotaxis and activation of neutrophils, monocytes and fibroblasts. PDGF is mitogenic for dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells and chondrocytes. PDGF appears to interact with Transforming Growth Factor-1 in acceleratting wound healing.[2] However, PDGF may also be pathogenic in arteriosclerosis and neoplasia.[3]

The mitogenic activities of all PDGF products are tested in culture using Swiss 3T3 cells or NR6-3T3 fibroblasts.[4]

References

1. Ross, R., et al., A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells *in vitro*. *Proc. Natl. Acad. Sci. USA*, **71**, 1207-1210 (1974).
2. Pierce, G., et al., Platelet-derived growth factor and transforming growth factor-b enhance tissue repair activities by unique mechanisms. *J. Cell. Biol.*, **109**, 429-440 (1989).
3. Ross, R., Atherosclerosis: a problem of the biology of arterial wall cells and their interactions with blood components. *Arteriosclerosis*, **1**, 293-311 (1981)
4. Raines, E., et al., Purification of human platelet-derived growth factor. *Meth. Enzymol.*, **109**, 749-773 (1985).

Platelet-Derived Growth Factor from human platelets

PDGF

The principal mitogen found in mammalian serum, released from platelets during clot formation. Elicits mitogenesis of mesoderm-derived cells, increased extracellular matrix synthesis, and chemotaxis and activation of neutrophils, monocytes and fibroblasts. Mitogenic for dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells and chondrocytes. Appears to interact with transforming growth factor-β1 in accelerating wound healing. However, PDGF may also be pathogenic in arteriosclerosis and neoplasia.

250 ng/vial, lyophilized powder, suitable for cell culture

Isolated from human platelets, predominantly containing two covalently linked subunits (A and B).

Lyophilized from a 0.2-µm filtered solution in 40% acetonitrile plus 0.1% TFA

≥95% (SDS-PAGE)

EC_{50}: 0.5-10.0 ng/mL

glycoprotein mol wt 28-31 kDa

The mitogenic activity is tested in culture using Swiss 3T3 cells or NR6-3T3 fibroblasts.

endotoxin ..... tested
ship: ambient store at: –20°C

P8147-1VL	1 vial

Platelet-Derived Growth Factor from porcine platelets

PDGF

The principal mitogen found in mammalian serum, released from platelets during clot formation. Elicits mitogenesis of mesoderm-derived cells, increased extracellular matrix synthesis, and chemotaxis and activation of neutrophils, monocytes and fibroblasts. Mitogenic for dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells and chondrocytes. Appears to interact with transforming growth factor-β1 in accelerating wound healing. However, PDGF may also be pathogenic in arteriosclerosis and neoplasia.

lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in 40% acetonitrile and 0.1% trifluoroacetic acid, pH 2.0.

≥97% (SDS-PAGE and N-terminal analysis)

glycoprotein mol wt 38 kDa

The mitogenic activity is tested in culture using Swiss 3T3 cells or NR6-3T3 fibroblasts.

endotoxin ..... tested

ship: ambient store at: −20°C

P8953-5UG	5 µg

Platelet-Derived Growth Factor-AA human

PDGF−AA

endotoxin ..... tested

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

PDGF is believed to play an essential role in the cellular response to tissue injury, both as a stimulant of mesodermal cell growth and activity and as a chemoattractant to other cells involved in the repair process.

Homodimer of the A chain of human PDGF.

Lyophilized from a sterile filtered buffered solution.

Associated gene(s): PDGFRA (5156)

≥98% (SDS-PAGE and HPLC)

mol wt 28.5 kDa (containing 250 amino acid residues)

The biological activity is measured by the dose-dependent stimulation of thymidine uptake by BALB/c 3T3 cells.

ship: ambient store at: −20°C

P3076-10UG	10 µg

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

≥95% (SDS-PAGE)

ED_{50}: 200-1000 ng/mL

dimer mol wt 35-45 kDa (glycosylated)

HumanKine® recombinant human PDGF-AA, expressed in human HEK 293 cells, is a mixture of pro-form, pro-mature, and mature dimers with apparent molecular masses of 35, 40, and 45 kDa, respectively. They are disulfide linked glycosylated homodimers. Production in human HEK 293 cells offers authentic glycosylation. Glycosylation contributes to stability in cell growth media and other applications.

The specific activity was determined by the dose-dependent stimulation of the proliferation of 3T3 cells.

ship: ambient store at: room temp

H8291-10UG	10 µg

Platelet-Derived Growth Factor-AB human

PDGF-AB

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Cellular actions of PDGF include chemoattraction and activation of neutrophils, monocytes, and fibroblasts. PDGF appears to play a crucial role in the cellular response to tissue injury and is critically involved in the pathological progression of atherosclerosis.

Heterodimer of the A and B chains of PDGF disulfide linked.

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% trifluoroacetic acid.

Associated gene(s): PDGFA (5154), PDGFB (5155)

≥97% (SDS-PAGE and N-terminal analysis)

ED_{50}/EC_{50}: 10-100 ng/mL

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation in quiescent NR6R-3T3 fibroblasts.

endotoxin ..... tested

ship: ambient store at: −20°C

P3326-10UG	10 µg

Platelet-Derived Growth Factor-BB human

PDGF-BB

PDGF elicits multifunctional actions with a variety of cells. It is mitogenic to mesoderm-derived cells, such as dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells, and chondrocytes. PDGF is a potent chemoattractant and activator of neutrophils, monocytes, and fibroblasts. It increases the synthesis of phospholipids, cholesterol esters, glycogen and prostaglandins, and modulates LDL receptor binding.

Dimer of the B chain of human PDGF.

Associated gene(s): PDGFB (5155)

≥97% (SDS-PAGE)

endotoxin ..... tested

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a buffered aqueous solution

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation in quiescent NR6R-3T3 fibroblasts.

ship: ambient store at: −20°C

P3201-10UG	10 µg

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution.

The biological activity is measured by the dose-dependent stimulation of thymidine uptake in BALB/c 3T3 cells.

ship: ambient store at: −20°C

P4306-10UG	10 µg

Platelet-Derived Growth Factor-BB from rat

PDGF-BB

recombinant, expressed in *Escherichia coli*, lyophilized powder, suitable for cell culture

PDGF elicits multifunctional actions with a variety of cells. It is mitogenic to mesoderm-derived cells, such as dermal and tendon fibroblasts, vascular smooth muscle cells, glial cells, and chondrocytes. PDGF is a potent chemoattractant and activator of neutrophils, monocytes, and fibroblasts. It increases the synthesis of phospholipids, cholesterol esters, glycogen and prostaglandins, and modulates LDL receptor binding.

Dimer of the B chain of rat PDGF.

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% trifluoroacetic acid containing 2.5 mg bovine serum albumin.

Associated gene(s): Pdgfb (24628)

≥97% (SDS-PAGE)

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation in quiescent NR6R-3T3 fibroblasts.

endotoxin ..... tested
ship: ambient store at: −20°C

P4056-50UG	50 µg

Transforming Growth Factors

The transforming growth factor-β (TGF-β) superfamily of cytokines include the structurally related subfamily of **TGF-β s**, the subfamily of **bone morphogenic proteins (BMP)**, decapentaplegic (dpp) and Vg1, the subfamily of Mullerian inhibitory substances (MIS), and the subfamily of activins and inhibins.[1,2] In general, individual members of this superfamily were originally purified and characterized with a specific functional assay, but most of these have broader biological activities that are particularly relevant in development. TGF-β superfamily members have a conserved set of six cysteine residues that form a rigid "cysteine knot" in the carboxyterminal region. They are all secreted as large propeptide molecules which then form homodimers (or sometimes heterodimers with certain other superfamily members). Because TGF-β s and BMPs receptors share close similarities, these two subfamilies are grouped together in this section.

References

1. Gitelman, S., and Derynck, R., Transforming growth factor β (TGF-β), in Guidebook to Cytokines and Their Receptors, Nicola, N., ed., Oxford Press (New York, NY: 1994), pp. 223-226.
2. O'Kane, S., and Ferguson, M., Transforming growth factor-βs and wound healing. *Int. J. Biochem. Cell Biol.*, **29**, 63-78 (1997).

Activin B human

recombinant, expressed in CHO cells, suitable for cell culture

Activins have a wide range of biological activities including mesoderm induction, neural cell differentiation, bone remodeling, hematopoiesis, and reproductive physiology. Activins influence erythropoiesis and the potentiation of erythroid colony formation, oxytocin secretion, paracrine, and autocrine regulation.

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% TFA containing 0.25 mg bovine serum albumin.

≥90% (SDS-PAGE)

calculated mol wt ~14.5 kDa

The biological activity is measured by its ability to induce hemoglobin expression in K562 cells.

endotoxin ..... tested
ship: ambient store at: −20°C

A1729-5UG	5 µg

Activin AB human

recombinant, expressed in CHO cells, suitable for cell culture

Activins have a wide range of biological activities including mesoderm induction, neural cell differentiation, bone remodeling, hematopoiesis, and reproductive physiology. Activins influence erythropoiesis and the potentiation of erythroid colony formation, oxytocin secretion, paracrine, and autocrine regulation.

Lyophilized from a 0.2 µm filtered solution in 35% acetonitrile and 0.1% TFA containing 0.25 mg bovine serum albumin.

≥90% (SDS-PAGE)

apparent mol wt ~14 kDa, SDS-PAGE (reducing) (Both A and B monomers)

The biological activity is measured by its ability to induce hemoglobin expression in K562 cells.

endotoxin ..... tested
ship: ambient store at: −20°C

A1604-5UG	5 µg

Bone Morphogenetic Protein 5 human

BMP-5

recombinant, expressed in mouse NSO cells, lyophilized powder, suitable for cell culture, >95% (SDS-PAGE)

Cellular responses to BMP-5 is mediated by the formation of hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors.

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile, 0.1% TFA, with 5% trehalose and 50 µg of BSA per 1 µg of cytokine

Associated gene(s): BMP5 (653)

The biological activity is measured by its ability to induce alkaline phosphatase production in ATDC5 chondrogenic cells.

endotoxin ..... tested
ship: dry ice store at: −20°C

B9803-50UG	50 µg

Transforming Growth Factor-α human

TGF-α
Val-Val-Ser-His-Phe-Asn-Asp-Cys-Pro-Asp-Ser-His-Thr-Gln-Phe-Cys-Phe-His-Gly-Thr-Cys-Arg-Phe-Leu-Val-Gln-Glu-Asp-Lys-Pro-Ala-Cys-Val-Cys-His-Ser-Gly-Tyr-Val-Gly-Ala-Arg-Cys-Glu-His-Ala-Asp-Leu-Leu-Ala [Disulfide bridges: 8-21; 16-32; 34-43] [105186-99-0] $C_{239}H_{348}N_{70}O_{72}S_6$ FW 5546.14

Transforming growth factor-α (TGF-α), is a 5.5 kDa polypeptide containing 50 amino acids in its mature form. It was originally identified as an agent that reversibly confers a transformed phenotype upon normal non-neoplastic cells, such as normal rat kidney fibroblasts. This activity requires the presence of transforming growth factor-β (TGF-β), which potentiates the action of TGF-α via a separate receptor. TGF-α is synthesized by monocytes, keratinocytes and many tissues and tumors. Mice genetically engineered to be lacking TGF-α expression showed no abnormalities except for the detection of a "wavy" hair coat. TGF-α exerts its action through the EGF receptor.

hTGF-α
recombinant, expressed in *Escherichia coli*, powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered buffered solution.

≥98% (SDS-PAGE and HPLC)

The biological activity is measured by its ability to stimulate ^{3}H-thymidine incorporation into BALB/c 3T3 cells.

endotoxin ..... tested

ship: ambient store at: –20°C

T7924-.1MG	0.1 mg

Transforming Growth Factor-β1 human

TGF-β1

TGF-β1 is produced by many cell types, but is reported to be most concentrated in mammalian platelets, where it is present at approximately four times the level of TGF-β2.

endotoxin ..... tested

hTGF-β1
recombinant, expressed in CHO cells, powder, suitable for cell culture

TGF-β1 is produced by many cell types, but is reported to be most concentrated in mammalian platelets, where it is present at approximately four times the level of TGF-β2.

Transforming Growth Factor-β1 is a 25 kDa multifunctional peptide capable of influencing cell proliferation, differentiation, and other functions in a wide range of cell types. Transformed as well as non-neoplastic tissues release transforming growth factors and essentially all cells possess a specific TGF-β1 receptor. The multi-modal nature of TGF-β1 is seen in its ability to stimulate or inhibit cellular proliferation. In general, cells of mesenchymal origin appear to be stimulated by TGF-β1; whereas, hepatocytes, T and B lymphocytes, keratinocytes, and many epithelial cells are inhibited by the peptide. TGF-β1 interacts with Epidermal Growth Factor, Platelet Derived Growth Factor, Fibroblast Growth Factor, and T Cell Growth Factor either by enhancing or antagonizing their characteristic actions. TGF-β1 plays a fundamental role in tissue growth and differentiation by involvement in adipogenesis, myogenesis, chondrogenesis, osteogenesis, epithelial cell differentiation, and immune cell function.

Lyophilized from a 0.2 µm filtered buffered solution.

≥98% (SDS-PAGE)

protein mol wt 25 kDa

The biological activity of TGF-β1 is measured in culture by inhibition of mouse IL-4-dependent proliferation of mouse HT-2 cells.

ship: ambient store at: –20°C

T7039-2UG	2 µg

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

HumanKine® TGF-β1 is expressed in human HEK 293 cells as a mature, disulfide linked, non- glycosylated, homodimer with a predicted molecular mass of 25 kDa.

The specific activity was determined by the dose dependent inhibition of IL-4 induced proliferation of mouse HT-2 cells (BALB/c spleen activated by sheep erythrocytes in the presence of IL-2).

ship: ambient store at: room temp

H8541-5UG	5 µg

Transforming Growth Factor-β1 from human platelets

TGF-β1

TGF-β1 is produced by many cell types, but is reported to be most concentrated in mammalian platelets, where it is present at approximately four times the level of TGF-β2.

lyophilized powder, suitable for cell culture, activity: 1 × 10^{6} units/mg

Lyophilized from a 0.2 µm filtered solution in 35% acetonitrile, 0.1% TFA, containing 50 ug of BSA per 1 ug cytokine

≥97% (SDS-PAGE and N-terminal analysis)

ED_{50}/EC_{50}: 0.04-0.3 ng/mL

protein mol wt 25 kDa

The biological activity is measured by its ability to inhibit the IL-4-dependent proliferation of mouse HT-2 cells.

endotoxin ..... tested

ship: ambient store at: –20°C

T1654-1UG	1 µg

Transforming Growth Factor-β1 from porcine platelets

TGF-β1

TGF-β1 is produced by many cell types, but is reported to be most concentrated in mammalian platelets, where it is present at approximately four times the level of TGF-β2.

powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in 35% acetonitrile and 0.1% trifluoroacetic acid containing 0.05 mg bovine serum albumin per µg cytokine.

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.01-0.1 ng/mL

protein mol wt 25 kDa

The biological activity is measured by its ability to inhibit the IL-4-dependent proliferation of mouse HT-2 cells.

endotoxin ..... tested

ship: ambient store at: –20°C

T5050-1UG	1 µg

Transforming Growth Factor-β2 human

TGF-β2

TGF-β2, like TGF-β1, is produced by many cell types and reported to be most concentrated in mammalian platelets.

endotoxin ..... tested

hTGF-β2
recombinant, expressed in mouse NSO cells, powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in 30% acetonitrile and 0.1% trifluoroacetic acid containing 0.1 mg bovine serum albumin

Associated gene(s): TGFBR2 (7048)

≥97% (SDS-PAGE)

The biological activity is measured by its ability to inhibit the IL-4-dependent proliferation of mouse HT-2 cells.

ship: ambient store at: –20°C

T2815-2UG	2 µg

Transforming Growth Factor-β2 *(continued)*

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: 0.1-0.5 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

HumanKine® TGF-β2 is expressed in human HEK 293 cells as a mature, non-glycosylated, disulfide-linked homodimer with a predicted molecular mass of approx. 25 kDa.

The specific activity was determined by the dose-dependent inhibition of IL-4 induced proliferation of mouse HT-2 (BALB/c spleen activated by sheep erythrocytes in the presence of IL-2).

ship: ambient store at: room temp

H8666-5UG	5 µg

Transforming Growth Factor-β2 from porcine platelets

TGF-β2

TGF-β2, like TGF-β1, is produced by many cell types and reported to be most concentrated in mammalian platelets.

powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in 25% acetonitrile and 0.1% trifluoroacetic acid containing 0.05 mg bovine serum albumin.

≥97% (SDS-PAGE and N-terminal analysis)

ED_{50}/EC_{50}: 0.05-0.5 ng/mL

protein mol wt 25 kDa

The biological activity is measured by its ability to inhibit the IL-4-dependent proliferation of mouse HT-2 cells.

The biological activity of porcine TGF-β2 was tested in culture by measuring its ability to inhibit thymidine incorporation in the IL-4 dependent mouse T-helper cell line HT-2. The EC50 is defined as the effective concentration of growth factor that elicits 50% inhibition of cell growth in a cell based bioassay.

endotoxin ..... tested

ship: ambient store at: −20°C

T5300-1UG	1 µg

Transforming Growth Factor-β3 human

TGF-β3

TGF-β3 is less prevalent in natural expression than either TGF-β1 or TGF-β2, but it is the most abundant mRNA expressed in chick embryos. It is also expressed in human umbilical cord, in a variety of mesenchymal cells of human and rodent origin, and in several human carcinoma cells.

endotoxin ..... tested

hTGF-β3
recombinant, expressed in *Sf*21 cells, powder, suitable for cell culture

Lyophilized from a 0.2 µm filtered solution in 35% acetonitrile and 0.1% trifluoroacetic acid containing 50 µg bovine serum albumin per 1 µg of cytokine.

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.01-0.09 ng/mL

predicted mol wt ~25 kDa

The biological activity is measured by its ability to inhibit the IL-4-dependent proliferation of mouse HT-2 cells.

ship: ambient store at: −20°C

T5425-2UG	2 µg

recombinant, expressed in HEK 293 cells, HumanKine®, suitable for cell culture NEW

≥95% (SDS-PAGE)

lyophilized powder

ED_{50}: ≤1.0 ng/mL

dimer mol wt 25 kDa (non-glycosylated)

HumanKine® TGF-β3 is expressed in human HEK 293 cells as a mature, disulfide linked, non- glycosylated homodimer with a predicted molecular mass of 25 kDa.

The specific activity was determined by the dose-dependent inhibition of IL-4 induced proliferation of mouse HT-2 cells (BALB/c spleen activated by sheep erythrocytes in the presence of IL-2).

ship: ambient store at: room temp

H8791-5UG	5 µg

Tumor Necrosis Factor (TNF) Superfamily

The tumor necrosis factor (TNF) superfamily comprise an increasing number of structurally related ligand-receptor pairs, including TNF-α, TNF-β (lymphotoxin, LT), TNFR1, TNFR2, LT-βR, osteoprotegrin (OPG), RANK, RANKL (TRANCE), TRAIL (APO-2L), DR4 (TRAIL-R1), DR5 (TRAIL-R2), DcR1 (decoy receptor 1, TRAIL-R3), and DcR2 (decoy receptor 2, TRAIL-R4). Except for one member, all receptors to the TNF superfamily bind TNF-related ligands and act mainly on the immune system. The exception is $p75^{NGF}$, distinctly homologous to TNF receptors, but which binds neurotrophins (NGF, BDNF, NT-3 and NT-4) and exerts its actions on the nervous system. (See Neurotrophic Factors section for this receptor description.)

Although a variety of actions are reported for the TNF superfamily, a common theme is their ability to regulate cell viability. They play important roles in lymphoid development and in T and B cell responses. Several TNF superfamily receptors induce apoptosis, but many TNF members may also induce lymphocyte proliferation and differentiation through costimulation with antigen receptors. TNF superfamily ligands (except neurotrophins) share several common features. Synthesized as a type II transmembrane protein (extracellular C-terminus) without secretion signal sequence, these ligands are usually released from the outer cell membrane by proteolytic cleavage, except for TNF-β (lymphotoxin, LT or LT-α) which has a nonfunctional transmembrane section and so is secreted from the cell directly. TNF superfamily members are usually long-chain β-sheet "jellyroll" cytokines that form cone-shaped homotrimers in solution. The surface form of lymphotoxin is unusual, as it is a heterotrimer containing one or two molecules of the membrane-attached LT-β with one or two LT-α (TNF-β) chains, with LT-α1LT-β2 as the predominant form.

References

1. Ware, C., et al., Tumor necrosis factor-related ligands and receptors, in The Cytokine Handbook, 3rd Edition, Thomson, A.W., ed., Academic Press (San Diego, CA: 1998), pp. 549-592.
2. Gravestein, L., and Borst, J., T., Tumor necrosis factor family membersin the immune system. *Sem. Immunol.*, **10**, 423-434 (1998).

Tumor Necrosis Factor-α human

hTNF-α; TNF-α
[94948-59-1]

Tumor necrosis factor-α, also known as cachectin, is expressed as a 26 kDa membrane bound protein and is then cleaved by TNF-α converting enzyme (TACE) to release the soluble 17 kDa monomer, which forms homotrimers in circulation. TNF-α plays roles in antitumor activity, immune modulation, inflammation, anorexia, cachexia, septic shock, viral replication and hematopoiesis. TNF-α is expressed by a great variety of cells, with numerous inductive and suppressive agents. Primarily, TNF-α is produced by macrophages in response to immunological challenges such as bacteria (lipopolysaccharides), viruses, parasites, mitogens and other cytokines. TNF-α is cytotoxic for many transformed cells (its namesake activity) but in normal diploid cells, it can stimulate proliferation (fibroblasts), differentiation (myeloid cells) or activation (neutrophils). TNF-α also shows antiviral effects against both DNA and RNA viruses and it induces production of several other cytokines. Although TNF-α is used in clinical trials as an antitumor agent, Sigma's cytokine, growth factor and hormone products are for research only. TNF-α and the related molecule TNF-β (LT-α) share close structural homology with 28% amino acid sequence identity and both activate the same TNF receptors, TNFR1 and TNFR2. Mouse and human TNF-α share 79% amino acid sequence identity. Unlike human TNF-α, the mouse form is glycosylated.

mol wt ~17.4 kDa

endotoxin ..... tested

buffered aqueous solution, recombinant, expressed in yeast, suitable for cell culture

Solution in phosphate buffered saline containing 1 mg/ml bovine serum albumin.

≥95% (SDS-PAGE)

activity: ≥2 × 10^7 units/mg

The cytolytic activity of TNF-α against WEHI 164 cells has been measured in culture using an MTT toxicity assay. The ED50 is defined as the concentration of TNF-alpha that mediates half-maximal cytotoxicity in the presence of 1 micro-g/ml actinomycin D.

ship: dry ice store at: –20°C

T0157-10UG	10 µg

powder, recombinant, expressed in *Escherichia coli*, suitable for cell culture

Lyophilized from a 0.2 µm-filtered buffer solution containing 3mM Tris-HCl and 5% trehalose.

Associated gene(s): TNF (7124)

≥97% (SDS-PAGE)

ED_{50}/EC_{50}: 0.02-0.3 ng/mL

The biological activity of TNF-α is measured by the cytolysis of murine L929 cells.

ship: ambient store at: –20°C

T6674-10UG	10 µg

Tumor Necrosis Factor-α from mouse

TNF-α

Tumor necrosis factor-α, also known as cachectin, is expressed as a 26 kDa membrane bound protein and is then cleaved by TNF-α converting enzyme (TACE) to release the soluble 17 kDa monomer, which forms homotrimers in circulation. TNF-α plays roles in antitumor activity, immune modulation, inflammation, anorexia, cachexia, septic shock, viral replication and hematopoiesis. TNF-α is expressed by a great variety of cells, with numerous inductive and suppressive agents. Primarily, TNF-α is produced by macrophages in response to immunological challenges such as bacteria (lipopolysaccharides), viruses, parasites, mitogens and other cytokines. TNF-α is cytotoxic for many transformed cells (its namesake activity) but in normal diploid cells, it can stimulate proliferation (fibroblasts), differentiation (myeloid cells) or activation (neutrophils). TNF-α also shows antiviral effects against both DNA and RNA viruses and it induces production of several other cytokines. Although TNF-α is used in clinical trials as an antitumor agent, Sigma's cytokine, growth factor and hormone products are for research only. TNF-α and the related molecule TNF-β (LT-α) share close structural homology with 28% amino acid sequence identity and both activate the same TNF receptors, TNFR1 and TNFR2. Mouse and human TNF-α share 79% amino acid sequence identity. Unlike human TNF-α, the mouse form is glycosylated.

mol wt 17.5 kDa

mTNF-α
recombinant, expressed in *Escherichia coli*, powder, suitable for cell culture

Lyophilized from 5 mM Tris, pH 8.0

ED_{50}/EC_{50}: 0.01-0.5 ng/mL

One unit is the amount required to induce half-maximal cytolysis of L929 cells.

endotoxin ..... tested

ship: ambient store at: –20°C

T7539-10UG	10 µg

Vascular Endothelial Growth Factors

Vascular endothelial growth factor (VEGF) is a family of closely related growth factors having a conserved pattern of eight cysteine residues and sharing common VEGF receptors. Originally known simply as VEGF, vasculotropin (VAS) or vascular permeability factor (VPF), this factor is now sometimes called VEGF-A. Four additional family members (placental growth factor, PlGF; VEGF-B; VEGF-C; and VEGF-D) have been identified to date.

VEGF-A (VEGF) is a potent growth factor for blood vessel endothelial cells, showing pleiotropic responses that facilitate cell migration, proliferation, tube formation, and survival. It is also one of the most potent permeability factors, so that VEGF-A is a common link of inflammation, permeability and angiogenesis. VEGF-A mRNA expression patterns are closely related to proliferation of blood vessels during the developing embryo and wound healing or in the ovary. Local hypoxia is a potent inducer of VEGF-A expression from adjacent cells but it is not synthesized in endothelial cells, indicating a paracrine regulation of vessel formation. In the developing embryo VEGF-A mRNA is expressed by cells within tissues undergoing capillarization. In most adult tissues the level of VEGF-A expression is low except in the kidney (Bowman's capsule podocytes). Expression of VEGF-A can be induced in macrophages, T cells, astrocytes, osteoblasts, smooth muscle cells, fibroblasts, endothelial cells, cardiomyocytes, skeletal muscle cells and keratinocytes. It is also expressed in a variety of human tumors. Due to alternative splicing of a single gene, VEGF-A may exist in four isoforms, designated by their expected final amino acid length (VEGF121, VEGF165, VEGF189 and VEGF206). These isoforms show similar biological activities but bind with different affinities to the heparin and result in different secretion patterns. The smallest isoform (VEGF121) is secreted and completely diffusible, the largest (VEGF206) is almost completely attached to the extracellular matrix, and the other two show intermediate heparinbinding affinities. VEGF-A exerts its actions through two receptors (VEGFR-1 and VEGFR-2).

Tumor Necrosis Factor-α *(continued)*

PlGF is expressed in the placenta and somewhat less in the heart, lung and thyroid gland. Placentally expressed PlGF may act as an autocrine on trophoblasts, which express both PlGF and its receptor (VEGFR-1). Since these cells also make VEGF-A, natural heterodimers (PlGF/VEGF-A) have also been detected. Two alternatively spliced isoforms of PlGF have been identified. Hypoxia does not induce PlGF synthesis, but the formation of heterodimers would be affected due to hypoxic control over VEGF-A expression.VEGF-B is largely cell-associated and expressed mostly in the heart, skeletal muscle, brain and kidney. It is often co-expressed with VEGF-A and heterodimers of A/B have been detected. VEGF-B expression is not regulated by hypoxia. The long half-life of its mRNA (>8 hours) suggests a chronic rather than acute regulation. VEGF-B exerts its actions through one receptor (VEGFR-1).VEGF-C, also called VEGF-related factor (VRP) or VEGF-2, in the adult is expressed primarily in the heart, placenta, lung, kidney, muscle, ovary and small intestine. During embryo development it is expressed in the cephalic mesenchyme, tail region and allantois and along the somites. VEGF-C may play roles in the development of the veinous and lymphatic vasculature systems. VEGF-C exerts its actions through two receptors (VEGFR-2 and VEGFR-3). VEGF-D, also called c-fos induced growth factor (FIGF), is a VEGF homologue induced by c-fos. It is expressed in adult lung, heart and small intestine and in fetal lung. It is reported mildly mitogenic for endothelial cells. VEGF-D and VEGF-C share 23% amino acid sequence homology. VEGF-D exerts its actions through two receptors (VEGFR-2 and VEGFR-3).

References

1. Ferrara, N., and Park, J.E., Vascular endothelial growth factor (VEGF), in Guidebook to Cytokines and Their Receptors, Nicola, N., ed., Oxford Press (New York, NY: 1994), pp. 232-234.
2. Achen, M.G., et al., Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc. Nat. Acad. Sci. (USA)*, **95**, 548-553 (1998).
3. Carmeliet, P., and Collen, D., Role of vascular endothelial growth factor and vascular endothelial growth factor receptors in vascular development. *Curr. Top. Microbiol. Immunol.*, **237**, 133-158 (1999).
4. Neufeld, G., et al., Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.*, **13**, 9-22 (1999).

Placenta Growth Factor human

PlGF; PGF

recombinant, expressed in *Escherichia coli*, powder, suitable for cell culture

PlGF is expressed in the placenta and somewhat less in the heart, lung and thyroid gland. Placentally expressed PlGF may act as an autocrine on trophoblasts, which express both PlGF and its receptor (VEGFR-1). Since these cells also make VEGF-A, natural heterodimers (PlGF/VEGF-A) have also been detected. Two alternatively spliced isoforms of PlGF have been identified.

Lyophilized from 81.7 µL of a 0.2 µm filtered solution in 30% acetonitrile and 0.1% trifluoroacetic acid (pH 7.4) containing 50 µg bovine serum albumin per 1 µg of cytokine.

≥97% (SDS-PAGE)

mol wt 29 kDa

Recombinant human Placenta Growth Factor is lyophilized from a 0.2 micron filtered solution of 30% CH3CN and 0.1% TFA (pH 7.4) containing 50 microg of bovine serum albumin per 1 microg of cytokine.

Reconstitute with sterile PBS containing at least 0.1% HSA or BSA. The rhP/GF concentration should be no less than 10 µg/mL.

The biological activity is measured by the ability to bind rhFlt-1/Fc in a functional ELISA. Immobilized rhFlt-1/Fc at 2 µg/mL (100 µL/well) can bind rhP/GF with a linear range at 0.1 - 5 ng/mL.

endotoxin ..... tested

ship: ambient store at: −20°C

P1588-10UG	10 µg

Vascular Endothelial Growth Factor human

VEGF

[127464-60-2]

powder, recombinant, expressed in *Escherichia coli*, suitable for cell culture

Vascular Endothelial Growth Factor (VEGF), also known as vasculotropin, is an angiogenic growth factor,which is heat and acid stable. VEGFs stimulate endothelial cell growth, angiogenisis, and capillary permeability. VEGF is a secreted homodimeric, heparin-binding glycoprotein,1 which has an iso-electric point of 8.5. VEGF promotes the growth of endothelial cells isolated from bovine adrenal cortex, cerebral cortex, fetal and adult aorta, and human umbilical vein. The target cell specificity of VEGF is restricted to vascular endothelial cells. VEGF has no mitogenic effect on cultured corneal endothelial cells, vascular smooth muscle cells, BHK-12 fibroblasts, keratino-cytes, human sarcoma cells, or lens epithelial cells. A variety of human tumor cell lines including sarcoma and carcinoma cells show a 3.7 kb RNA transcript that hybridizes with the VEGF probe in a Northern blot. Mouse sarcoma 180 cells express the VEGF mRNA and secrete a VEGF-like mitogen.

165 amino acids residue of human VEGF.

Lyophilized from a sterile filtered buffered solution with no additives.

Associated gene(s): VEGFA (7422)

≥98% (SDS-PAGE and HPLC)

ED_{50}/EC_{50}: 1-10 ng/mL

protein mol wt 38.2 kDa

endotoxin ..... tested

ship: ambient store at: −20°C

V7259-10UG	10 µg

Vascular Endothelial Growth Factor from mouse

VEGF

mVEGF
recombinant, expressed in *Escherichia coli*, powder, suitable for cell culture

Homodimeric protein consisting of two 165 amino acid polypeptide chains of mouse VEGF.

VEGF supports development of new blood vessels during embryonic development and after vascular injury.

Lyophilized from a 0.2 µm filtered buffered solution.

Associated gene(s): Vegfc (22341)

≥98% (SDS-PAGE and HPLC)

protein mol wt 39 kDa

The biological activity is measured by the ability to stimulate ^{3}H-thymidine incorporation in human umbilical vein endothelial cells (HUVEC) and bovine aortic endothelial cells.

endotoxin ..... tested

ship: ambient store at: −20°C

V4512-5UG	5 µg

Hormones

Hormone Quick Reference Table

Name	Reconstitution	Working Range	Storage	Cat. No.
Dexamethasone	To prepare 20 µg/ml stock solution, add 1ml absolute ethanol per mg product; gently swirl to dissolve. Add 49 ml sterile medium per ml of ethanol added, while mixing, to acheive final concentration of 20 µg/ml.	potency 4-500 ng/mL	powder 2-8 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw	D4902-25MG D4902-100MG D4902-500MG D4902-1G
Dexamethasone	To prepare 20 µg/ml stock solution: add 1 ml absolute ethanol; gently swirl to dissolve; add 49 ml sterile medium while mixing.	potency 4-500 ng/mL	powder 2-8 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw	D8893-1MG
Estradiol	-	-	-	E1024-1G E1024-25G E1024-100G
β-Estradiol	To prepare a 20 µg/ml stock solution, add 1 ml absolute ethanol to 1 mg β-estradiol; gently swirl to dissolve; add 49 ml sterile medium while mixing.	potency 0.2-10 ng/mL	powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw	E2758-250MG E2758-1G E2758-5G
β-Estradiol	To prepare 20 µg/ml stock solution: add 1 ml absolute ethanol; gently swirl to dissolve; add 49 ml sterile medium while mixing.	potency 0.2-10 ng/mL	powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw	E2257-1MG
Hydrocortisone	To prepare 50 µg/mL stock solution; add 1 mL absolute ethanol, gently swirl to dissolve, add 19 mL sterile medium while mixing.	potency 0.004-5 µg/mL	powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw	H0135-1MG
Hydrocortisone	To prepare 50 µg/mL stock solution, add 1.0 mL absolute ethanol to 1.0 mg of product, gently swirl to dissolve, add 19 mL sterile medium.	-	powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw	H0888-1G H0888-5G H0888-10G
Hydrocortisone solution	-	-	frozen in working aliquots, avoid repeated freeze/thaw	H6909-10ML
Insulin from bovine pancreas	Insulin has low solubility at neutral pH. It can be solubilized at 2 mg/ml in dilute acetic or hydrochloric acid, pH 2-3.	potency 0.001-20 µg/mL potency ≥27 USP units per mg	powder-0 °C; solution stable 2-8 °C, 1 year	I6634-50MG I6634-100MG I6634-250MG I6634-500MG I6634-1G I6634-5G
Insulin from bovine pancreas	To prepare 10 mg/ml stock solution, add 10 ml of acidified H_2O (pH ≤2) - prepared by addition of glacial acetic acid (approx. 0.1 ml).	potency 0.001-20 µg/mL	powder-0 °C; solution stable 2-8 °C, 1 year	I1882-100MG
Insulin solution from bovine pancreas	-	-	stable 2-8 °C	I0516-5ML
Insulin solution human	-	potency 0.001-20 µg/mL	stable 2-8 °C	I9278-5ML I9278-10ML
Progesterone	To prepare 20 µg/ml stock solution, add 1 ml absolute ethanol, gently swirl to dissolve, add 49 ml sterile medium.	potency 0.1-20 ng/mL	powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw	P6149-1MG
Progesterone	To prepare 20µg/ml stock solution, add 1ml absolute ethanol per mg progesterone, gently swirl to dissolve, add 49ml sterile medium per ml ethanol.	potency 0.1-20 ng/mL	powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw	P8783-1G P8783-5G P8783-25G
Somatostatin	To prepare 2 µg/ml stock solution, dissolve in 5 ml sterile culture medium per µg somatostatin.	-	powder-0 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw	S1763-.1MG S1763-1MG
Somatostatin	To prepare 2 µg/ml stock solution, add 10 ml sterile culture medium.	potency 0.3-50 ng/mL	powder-0 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw	S0885-20UG
L-Thyroxine	To prepare 20 µg/ml stock solution, dissolve in 50 ml sterile culture medium per mg thyroxine.	potency 5-50 ng/mL	powder-0 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw, solution stable 2-8 °C, 30 days	T1775-100MG T1775-500MG T1775-1G
L-Thyroxine sodium salt pentahydrate	To prepare 20 µg/ml stock solution, add 50 ml sterile culture medium.	potency 5-50 ng/mL	powder-0 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw, solution stable 2-8 °C, 30 days	T0397-1MG
3,3',5-Triiodo-L-thyronine sodium salt	To prepare 20 µg/ml stock solution: add 1ml 1N NaOH per mg 3,3', 5-triiodo-L-thyronine; gently swirl to dissolve. To this, add 49 ml sterile medium per ml 1N NaOH added.	potency 0.02-50 µg/mL	powder-0 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw, solution stable 2-8 °C, 30 days	T6397-100MG T6397-250MG T6397-1G
3,3',5-Triiodo-L-thyronine sodium salt	To prepare 20 µg/ml stock solution: add 1 ml 1 N NaOH; gently swirl to dissolve; add 49 ml sterile medium.	potency 0.02-50 µg/mL	powder-0 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw, solution stable 2-8 °C, 30 days	T5516-1MG

Dexamethasone

Prednisolone F; 9α-Fluoro-16α-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione; (11β,16α)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione; 9α-Fluoro-16α-methylprednisolone
[50-02-2] $C_{22}H_{29}FO_5$ FW 392.46

Glucocorticoid anti-inflammatory agent. Regulates T cell survival, growth, and differentiation. Inhibits the induction of nitric oxide synthase.

An anti-inflammatory glucocorticoid with a range of effects on cell survival, cell signaling and gene expression. Use to study apoptosis, cell signaling pathways and gene expression.

potency: 4-500 ng/mL

powder, BioReagent, suitable for cell culture, ≥97%

To prepare 20 µg/ml stock solution, add 1ml absolute ethanol per mg product; gently swirl to dissolve. Add 49 ml sterile medium per ml of ethanol added, while mixing, to acheive final concentration of 20 µg/ml.

ship: ambient store at: 2-8°C

D4902-25MG	25 mg
D4902-100MG	100 mg
D4902-500MG	500 mg
D4902-1G	1 g

powder, γ-irradiated, BioXtra, suitable for cell culture, ≥80% (HPLC)

To prepare 20 µg/ml stock solution: add 1 ml absolute ethanol; gently swirl to dissolve; add 49 ml sterile medium while mixing.

ship: ambient store at: 2-8°C

D8893-1MG	1 mg

Dexamethasone-Water Soluble

Dexamethasone – Cyclodextrin complex
[50-02-2]

BioReagent, suitable for cell culture

Contains approx. 65 mg dexamethasone per gram; balance 2-hydroxypropyl-β-cyclodextrin.

An anti-inflammatory glucocorticoid with a range of effects on cell survival, cell signaling and gene expression. Use to study apoptosis, cell signaling pathways and gene expression.

Package size based on dexamethasone

solubility
H_2O ..... 25 mg/mL, clear to slightly hazy

ship: ambient store at: room temp

D2915-100MG	100 mg

β-Estradiol

3,17β-Dihydroxy-1,3,5(10)-estratriene; 1,3,5-Estratriene-3,17β-diol; Dihydrofolliculin; 17β-Estradiol
[50-28-2] $C_{18}H_{24}O_2$ FW 272.38

The major estrogen secreted by the premenopausal ovary. Estrogens direct the development of the female phenotype in embryogenesis and during puberty by regulating gene transcription and, thus, protein synthesis. It also induces the production of gonadotropins which, in turn, induce ovulation. Exposure to estradiol increases breast cancer incidence and proliferation.

β-Estradiol is used to study cell differentiation and transformations (tumorigenicity).

powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw

potency: 0.2-10 ng/mL

BioReagent, powder, suitable for cell culture

≥98%

To prepare a 20 µg/ml stock solution, add 1 ml absolute ethanol to 1 mg β-estradiol; gently swirl to dissolve; add 49 ml sterile medium while mixing.

ship: ambient store at: room temp

E2758-250MG	250 mg
E2758-1G	1 g
E2758-5G	5 g

powder, γ-irradiated, BioXtra, suitable for cell culture

To prepare 20 µg/ml stock solution: add 1 ml absolute ethanol; gently swirl to dissolve; add 49 ml sterile medium while mixing.

ship: ambient store at: room temp

E2257-1MG	1 mg

β-Estradiol-Water Soluble

Cyclodextrin-encapsulated 17β-estradiol

BioReagent, suitable for cell culture

Contains 40-55 mg estradiol per gram of solid; balance 2-hydroxypropyl-β-cyclodextrin.

β-Estradiol is used to study cell differentiation and transformations (tumorigenicity). This product is not recommended for use in binding assays.

Package size based on estradiol

solubility
H_2O ..... 25 mg/mL, clear to slightly hazy

ship: ambient store at: room temp

E4389-100MG	100 mg

Hydrocortisone

4-Pregnene-11β,17α,21-triol-3,20-dione; 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione; Kendall's compound F; Cortisol; Reichstein's substance M; 17-Hydroxycorticosterone
[50-23-7] $C_{21}H_{30}O_5$ FW 362.46

Primary glucocorticoid secreted by the adrenal cortex. It has three times the anti-inflammatory potency of corticosterone but much lower Na^{2+} retention potency.

γ-irradiated, powder, BioXtra, suitable for cell culture

Hydrocortisone for use in epithelial and endothelial adherent cell culture applications.

potency: 0.004-5 µg/mL

To prepare 50 µg/mL stock solution; add 1 mL absolute ethanol, gently swirl to dissolve, add 19 mL sterile medium while mixing.

ship: ambient store at: room temp

H0135-1MG	1 mg

BioReagent, suitable for cell culture

≥98%

To prepare 50 µg/mL stock solution, add 1.0 mL absolute ethanol to 1.0 mg of product, gently swirl to dissolve, add 19 mL sterile medium.

ship: ambient store at: room temp

H0888-1G	1 g
H0888-5G	5 g
H0888-10G	10 g

Hydrocortisone solution

Cortisol; 17-Hydroxycorticosterone
[50-23-7]

▶ 50 µM, sterile-filtered, BioXtra, suitable for cell culture

Animal-component free

Hydrocortisone for use in epithelial and endothelial adherent cell culture applications.

frozen in working aliquots, avoid repeated freeze/thaw

endotoxin ..... tested

ship: ambient store at: −20°C

H6909-10ML	10 mL

Hydrocortisone 21-hemisuccinate sodium salt

Cortisol 21-hemisuccinate sodium salt; 11β,17α,21-Trihydroxy-4-pregnene-3,20-dione 21-hemisuccinate sodium salt
[125-04-2] $C_{25}H_{33}NaO_8$ FW 484.51

▶ powder, BioReagent, suitable for cell culture

Hydrocortisone for use in epithelial and endothelial adherent cell culture applications.

solubility
H_2O ..... 50 mg/mL

ship: ambient store at: −20°C

H2270-100MG	100 mg

Hydrocortisone-Water Soluble

Cyclodextrin-encapsulated hydrocortisone

▶ BioReagent, suitable for cell culture

Hydrocortisone for use in epithelial and endothelial adherent cell culture applications.

Package size based on hydrocortisone

solubility
H_2O ..... 100 mg/mL

ship: ambient store at: room temp

H0396-100MG	100 mg

Insulin from bovine pancreas

[11070-73-8] $C_{254}H_{377}N_{65}O_{75}S_6$ FW 5733.49

Two-chain polypeptide hormone produced by the β-cells of pancreatic islets. Its molecular weight is ~5800 Da. The α and β chains are joined by two interchain disulfide bonds. The α chain contains an intrachain disulfide bond. Insulin regulates the cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids and inhibits the breakdown of glycogen, protein, and fat.

Insulin is used as a growth factor in many mammalian cell culture systems.

▶ powder, BioReagent, suitable for cell culture

Associated gene(s): INS (280829)

potency: ≥27 USP units per mg

Insulin has low solubility at neutral pH. It can be solubilized at 2 mg/ml in dilute acetic or hydrochloric acid, pH 2-3.

ship: ambient store at: −20°C

I6634-50MG	50 mg
I6634-100MG	100 mg
I6634-250MG	250 mg
I6634-500MG	500 mg
I6634-1G	1 g
I6634-5G	5 g

▶ γ-irradiated, lyophilized powder, suitable for cell culture, activity: ≥25 USP units/mg

BioXtra

To prepare 10 mg/ml stock solution, add 10 ml of acidified H_2O (pH ≤2) - prepared by addition of glacial acetic acid (approx. 0.1 ml).

ship: ambient store at: −20°C

I1882-100MG	100 mg

Insulin solution from bovine pancreas

Two-chain polypeptide hormone produced by the β-cells of pancreatic islets. Its molecular weight is ~5800 Da. The α and β chains are joined by two interchain disulfide bonds. The α chain contains an intrachain disulfide bond. Insulin regulates the cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids and inhibits the breakdown of glycogen, protein, and fat.

▶ 10 mg/mL insulin in 25 mM HEPES, pH 8.2, BioReagent, sterile-filtered, suitable for cell culture

Recommended for use in cell culture applications at 0.5 to 1 mL per liter of medium.

solution

endotoxin ..... tested

ship: ambient store at: 2-8°C

I0516-5ML	5 mL

Insulin solution human

[11061-68-0]

Two-chain polypeptide hormone produced by the β-cells of pancreatic islets. Its molecular weight is ~5800 Da. The α and β chains are joined by two interchain disulfide bonds. The α chain contains an intrachain disulfide bond. Insulin regulates the cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids and inhibits the breakdown of glycogen, protein, and fat.

▶ Chemically defined, recombinant from *Saccharomyces cerevisiae*, sterile-filtered, BioXtra, suitable for cell culture

Recommended for use in cell culture applications at 0.5 to 1 mL per liter of medium.

solution

concentration ..... 9.5-10.5 mg/mL

ship: ambient store at: 2-8°C

I9278-5ML	5 mL
I9278-10ML	10 mL

Progesterone

4-Pregnene-3,20-dione
[57-83-0] $C_{21}H_{30}O_2$ FW 314.46

Induces maturation and secretory activity of the uterine endothelium; suppresses ovulation. Progesterone is implicated in the etiology of breast cancer.

Steroid hormone produced by the corpus luteum.

Use in cell culture applications to study sterol regulation of cell signaling and gene expression.

powder-RT; stock-frozen in working aliquots, avoid repeated freeze/thaw

synthesized from materials derived from plant source

powder

potency: 0.1-20 ng/mL

Progesterone *(continued)*

γ-irradiated, BioXtra, suitable for cell culture

To prepare 20 µg/ml stock solution, add 1 ml absolute ethanol, gently swirl to dissolve, add 49 ml sterile medium.

ship: ambient store at: room temp

P6149-1MG	1 mg

powder, BioReagent, suitable for cell culture

suitable for

To prepare 20µg/ml stock solution, add 1ml absolute ethanol per mg progesterone, gently swirl to dissolve, add 49ml sterile medium per ml ethanol.

solubility
absolute ethanol ..... 1 mg/mL

ship: ambient store at: room temp

P8783-1G	1 g
P8783-5G	5 g
P8783-25G	25 g

Progesterone-Water Soluble

Progesterone: HBC complex; Progesterone: 2-hydroxypropyl-β-cyclodextrin complex

powder, BioReagent, suitable for cell culture

Contains approx. 70 mg progesterone per gram; balance 2-hydroxypropyl-β-cyclodextrin.

This product is not recommended for use in binding assays.

Package size based on progesterone content

solubility
H_2O ..... 25 mg/mL, clear to slightly hazy

ship: ambient store at: room temp

P7556-100MG	100 mg

Prolactin human

Lactogenic Hormone; LTH; Luteotropic Hormone; PRL
[9002-62-4]

Prolactin is a neuroendocrine hormone. The prolactin receptor is a transmembrane glycoprotein that belongs to the cytokine hematopoietic receptor family. A large number of cells and organs express the receptor, including B cells, T cells, macrophages, monocytes, and neutrophils. Prolactin signal transduction involves the JAK/STAT families and the src kinase family. Induces lactation; inhibits secretion of gonadotropins; release is inhibited by dopamine.

hPRL
recombinant, expressed in *Escherichia coli*, lyophilized powder, BioReagent, suitable for cell culture, ≥97% (SDS-PAGE)

Contains an N-terminal methionine not present in the natural product.

Use in cell culture applications to study prolactin regulation of cell signaling and gene expression.

Lyophilized in 30% acetonitrile and 0.1% TFA.

Associated gene(s): PRL (5617)

predicted mol wt ~24 kDa

The biological activity is assayed by proliferation of the rat lymphoma cell line, Nb2-11.

endotoxin ..... tested

ship: ambient store at: −20°C

L4021-50UG	50 µg

Somatostatin

Somatotropin release inhibiting factor; Somatostatin-14; SRIF; Growth hormone release inhibiting factor
[38916-34-6] $C_{76}H_{104}N_{18}O_{19}S_2$ FW 1637.88

powder, BioReagent, suitable for cell culture

Somatostatin regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G protein-coupled somatostatin receptors and inhibition of the release of numerous secondary hormones.

≥97% (HPLC)

To prepare 2 µg/ml stock solution, dissolve in 5 ml sterile culture medium per µg somatostatin.

solubility
H_2O ..... 1 mg/mL

ship: ambient store at: −20°C

S1763-.1MG	0.1 mg
S1763-1MG	1 mg

γ-irradiated, powder, BioXtra, suitable for cell culture

Somatostatin regulates endocrine function and cell proliferation via interaction with G-protein-coupled somatostatin receptors. It exists in two active forms produced by alternative cleavage of a single preproprotein (14 aa and 28 aa forms). Somatosatin inhibits the release of growth hormone; thyroid-stimulating hormone and a range of gastrointestinal hormones: Cholecystokinin (CCK); Enteroglucagon; Gastrin; Gastric inhibitory polypeptide (GIP); Motilin; Secretin; Vasoactive intestinal peptide (VIP).

potency: 0.3-50 ng/mL

To prepare 2 µg/ml stock solution, add 10 ml sterile culture medium.

solubility
cell culture medium ..... 10 mL/vial

ship: ambient store at: −20°C

S0885-20UG	20 µg

L-Thyroxine

T_4; 3-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-L-alanine; 3,3′,5,5″-Tetraiodo-L-thyronine
[51-48-9] $HOC_6H_2(I)_2OC_6H_2(I)_2CH_2CH(NH_2)CO_2H$ $C_{15}H_{11}I_4NO_4$ FW 776.87

L-Thyroxine (T_4) and triiodo-L-thyronine (T_3) are iodine-containing hormones produced from thyroglobulin in the thyroid follicular cells. The stimulation of metabolic rate and regulation of growth and development by these hormones appear to be due to their effects on DNA transcription and, thus, protein synthesis.

powder, BioReagent, suitable for cell culture

potency: 5-50 ng/mL

To prepare 20 µg/ml stock solution, dissolve in 50 ml sterile culture medium per mg thyroxine.

ship: ambient store at: room temp

T1775-100MG	100 mg
T1775-500MG	500 mg
T1775-1G	1 g

L-Thyroxine sodium salt pentahydrate

Sodium levothyroxine; 3-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-L-alanine sodium salt; T_4
[6106-07-6] $HOC_6H_2I_2OC_6H_2I_2CH_2CH(NH_2)COONa \cdot 5H_2O$ $C_{15}H_{10}I_4NNaO_4 \cdot 5H_2O$ FW 888.93

L-Thyroxine (T_4) and triiodo-L-thyronine (T_3) are iodine-containing hormones produced from thyroglobulin in the thyroid follicular cells. The stimulation of metabolic rate and regulation of growth and development by these hormones appear to be due to their effects on DNA transcription and thus, protein synthesis.

γ-irradiated, lyophilized powder, BioXtra, suitable for cell culture

≥98%

potency: 5-50 ng/mL

To prepare 20 µg/ml stock solution, add 50 ml sterile culture medium.

solubility
H_2O
cell culture medium ..... 0.1 mg/mL
ship: ambient store at: –20°C

T0397-1MG	1 mg

3,3′,5-Triiodo-L-thyronine sodium salt

O-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine sodium salt; Liothyronine; T_3
[55-06-1] $C_{15}H_{11}I_3NNaO_4$ FW 672.96

T3 is a thyroid hormone that increases rates of proteins synthesis, stimulates the breakdown of cholesterol, and effects embryonic development. In cell culture, T3 regulates cell differentiation and protein expression.

potency: 0.02-50 µg/mL

powder, BioReagent, suitable for cell culture

≥95%

To prepare 20 µg/ml stock solution: add 1ml 1N NaOH per mg 3,3′, 5-triiodo-L-thyronine; gently swirl to dissolve. To this, add 49 ml sterile medium per ml 1N NaOH added.

ship: ambient store at: –20°C

T6397-100MG	100 mg
T6397-250MG	250 mg
T6397-1G	1 g

γ-irradiated, powder, BioXtra, suitable for cell culture

To prepare 20 µg/ml stock solution: add 1 ml 1 N NaOH; gently swirl to dissolve; add 49 ml sterile medium.

ship: ambient store at: –20°C

T5516-1MG	1 mg

Hybridoma Reagents

Conditioned Media, Media Supplements and Reagents

AAT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 75 µM adenine, 0.8 µM aminopterin, 16 µM thymidine.

endotoxin ..... tested
ship: dry ice store at: –20°C

A5539-1VL	1 vial

Aminopterin

4-Amino-PGA; 4-Aminopteroyl-L-glutamic acid; 4-Aminofolic acid
[54-62-6] $C_{19}H_{20}N_8O_5$ FW 440.41

Folic acid antagonist. Aminopterin is actively transported into cells by the folate transporter. In the cell, it is converted to a high molecular weight polyglutamate metabolite by folylpolyglutamate synthase that, in turn, binds to dihydrofolate reductase and inhibits its activity. Aminopterin-polyglutamate is degraded intracellularly by γ-glutamyl hydrolase.

More potent, but more toxic, than methotrexate.

ε^{1M}_{261nm}, 0.1 M NaOH 25,700
ε^{1M}_{282nm}, 0.1 M NaOH 24,500
ε^{1M}_{373nm}, 0.1 M NaOH 8,100

powder, BioReagent, suitable for cell culture

~98%

solubility
DMSO
2 M NaOH ..... 50 mg/mL
color ..... yellow
ship: ambient store at: –20°C

A3411-10MG	10 mg
A3411-25MG	25 mg
A3411-100MG	100 mg

Hybri-Max™, 50 ×, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Aminopterin concentrate supplement for hybridoma cell culture applications.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 0.4 µM aminopterin.

endotoxin ..... tested
ship: dry ice store at: –20°C

A5159-10VL	10 vials

AT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10ml sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration:75 µM adenine, 16 µM thymidine.

endotoxin ..... tested
ship: ambient store at: –20°C

A7422-1VL	1 vial

5-Azacytidine

Ladakamycin; 4-Amino-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one
[320-67-2] $C_8H_{12}N_4O_5$ FW 244.20

A potent growth inhibitor and cytotoxic agent; inhibits DNA methyltransferase, an important regulatory mechanism of gene expression, gene activation and silencing.

Hybri-Max™, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration: 10 µM 5-azacytidine.

endotoxin ..... tested
ship: ambient store at: –20°C

A1287-1VL	1 vial

8-Azaguanine

2-Amino-6-oxy-8-azapurine; 2-Amino-6-hydroxy-8-azapurine; Guanazolo
[134-58-7] $C_4H_4N_6O$ FW 152.11

8-AzaG
Hybri-Max™, γ-irradiated, powder, BioXtra, suitable for hybridoma

For use in hybridoma cell culture applications as a myeloma selection agent.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration for 10 mg/vial: 130 µM 8-azaguanine. Vial content provided on Certificate of Analysis.

ship: ambient store at: –20°C

A5284-10VL	10 vials

Azaserine-Hypoxanthine 50x

O-Diazoacetyl-L-serine-hypoxanthine
Hybri-Max™, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Qualified for use in hybridoma cell culture applications as a selection agent.

Reconstitute contents of vial with 10 mL sterile cell culture medium. Stock solution is sufficient to prepare 500 mL medium. Final working concentration: 5.7 µM azaserine, 100 µM hypoxanthine.

endotoxin ..... tested
ship: dry ice store at: –20°C

A9666-1VL	1 vial

HAT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 100 µM hypoxanthine, 0.4 µM aminopterin, 16 µM thymidine.

endotoxin ..... tested
ship: dry ice store at: –20°C

H0262-10VL	10 vials

HMT Media Supplement (50×) Hybri-Max™

γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

HMT Media Supplement is a cocktail of hypoxanthine, methotrexate and thymidine for use in hybridoma selection and cell culture applications.

Reconstitute contents of vial with 10ml sterile cell culture medium. Stock solution is sufficient to prepare 500ml medium. Final working concentration: 100 µM hypoxanthine, 0.4 µM methotrexate, 16 µM thymidine.

endotoxin ..... tested
ship: ambient store at: –20°C

H8016-10VL	10 vials

HT Media Supplement (50×) Hybri-Max™

lyophilized powder, γ-irradiated, BioXtra, suitable for hybridoma

HT Media supplement is a cocktail hypoxanthine and thymidine qualified for use in hybridoma selection and cell culture applications.

Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 100 µM hypoxanthine, 16 µM thymidine.

endotoxin ..... tested
ship: ambient store at: –20°C

H0137-10VL	10 vials

Hypoxanthine

6-Hydroxypurine
[68-94-0] $C_5H_4N_4O$ FW 136.11

powder, BioReagent, suitable for cell culture

Use to prepare hybridoma selection and maintenance media.

≥99%
solubility
1 M NaOH ..... 25 mg/mL
ship: ambient store at: room temp

H9636-1G	1 g
H9636-5G	5 g
H9636-25G	25 g

Poly(ethylene glycol)

PEG
[25322-68-3] $H(OCH_2CH_2)_nOH$

Recommended for cell fusion.

autoclaved
endotoxin ..... tested
vd ..... >1 (vs air) ait ..... 581 °F
vp ..... <0.01 mmHg (20 °C)

Hybri-Max™, mol wt 1,300-1,600, waxy solid, BioReagent, suitable for hybridoma
ship: ambient store at: room temp

P7777-5G	5 g

Hybri-Max™, mol wt 3,000-3,700, waxy solid, BioReagent, suitable for hybridoma
ship: ambient store at: room temp

P2906-5G	5 g

Methotrexate hydrate

L-Amethopterin hydrate; L-4-Amino-N^{10}-methylpteroylglutamic acid hydrate; MTX hydrate; Methylaminopterin hydrate; 4-Amino-10-methylfolic acid hydrate; Antifolan hydrate
[133073-73-1] $C_{20}H_{22}N_8O_5 \cdot xH_2O$ FW 454.44 (Anh)

Potent inhibitor of dihydrofolate reductase[1] and agent for antitumor studies.[2,3] Use to inhibit dihydrofolate reductase in DHFR-based protein expression systems.

Lit. cited: 1. Sasso, S.P., et al., *Biochim. Biophys. Acta* **1207**, 74 (1994)
2. Huennekens, F.M., *Adv. Enzyme Regul.* **34**, 397 (1994)
3. Nagy, A., et al., *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6373 (1993)

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥98% (HPLC)

Methotrexate used to inhibit dihyrofolate reductase and select for DHFR overexpresssion.

Recommended for use in cell culture and molecular biology applications at 0.01-300 µM in nucleoside-free cell culture medium to select for DHFR expression.

solubility
H_2O ..... insoluble
ship: ambient store at: −20°C

M8407-100MG	100 mg
M8407-500MG	500 mg

Polyethylene glycol solution

PEG solution
[25322-68-3]

Packaged in sealed ampules under nitrogen.

Solution is ready-to-use. If a less concentrated solution is desired, dilute with sterile DPBS without calcium (D5773). Some precipitate may appear after being exposed to cooler, but should disappear as the solution warms. Solution may be frozen if desired but should first be aliquotted to avoid repeated freeze/thaw cycles.

endotoxin ..... tested

Hybri-Max™, 50 % (w/v), average mol wt 1,450, sterile-filtered, BioReagent, suitable for hybridoma

Contains 50% (w/v) polyethylene glycol (Av. Mol. Wt. 1450) in DPBS without calcium.

Recommended for use in a normal fusion protocol requiring 50% PEG.
ship: ambient store at: 2-8°C

P7181-5X5ML	5 × 5 mL

Hybri-Max™, average mol wt 1,450, 50 % (w/v), sterile-filtered, BioReagent, suitable for hybridoma

Contains 50% (w/v) polyethylene glycol (Av. Mol. Wt. 1450) and 10% DMSO (v/v) in DPBS without Calcium.

Recommended for use in a normal fusion protocol requiring 50% PEG and 10% DMSO.
ship: ambient store at: 2-8°C

P7306-5X5ML	5 × 5 mL

Red Blood Cell Lysing Buffer Hybri-Max™

RBC Lysing Buffer
liquid, sterile-filtered, suitable for hybridoma

Red Blood Cell Lysing Buffer has been developed for use in hybridoma protocols to remove red blood cells from mouse splenocyte suspensions before fusion. It is also useful in systems where it may be desirable to remove red blood cells from cell suspensions, such as whole blood.

Recommended for use by adding 1 mL of buffer to a cell pellet (cell pellet = 1 spleen or 100-200 million cells). Gently mix for 1 minute. Dilute the buffer with 15-20 mL of medium or salt solution. Centrifuge at 250-500 × *g* for 7 minutes and decant the supernatant. Cells may be diluted and prepared for counting or fusion. If lysis is incomplete, steps 1-4 may be repeated.

Contains 8.3 g/L ammonium chloride in 0.01 M Tris-HCl buffer.

Note: This product is intended for the removal of red blood cells from mice. This product may not be appropriate for the lysis of red blood cells of other animals. The suitability of the product in any application other than mouse splenocytes must be determined by the researcher.

endotoxin ..... tested
ship: ambient store at: room temp

R7757-100ML	100 mL

6-Thioguanine

2-Amino-6-mercaptopurine; 2-Amino-6-purinethiol
[154-42-7] $C_5H_5N_5S$ FW 167.19

Hybri-Max™, 50 ×, γ-irradiated, lyophilized powder, BioXtra, suitable for hybridoma

Used in hybridoma formation applications as a selection agent.

~98%

Reconstitute contents of vial with 10 ml sterile cell culture medium. Stock solution is sufficient to prepare 500 ml medium. Final working concentration: 30 µM 2-amino-6-mercaptopurine.

endotoxin ..... tested
ship: ambient store at: room temp

A4660-2.5MG	2.5 mg

Thymidine

dT; Thymine deoxyriboside; 1-(2-Deoxy-β-D-ribofuranosyl)thymine; 1-(2-Deoxy-β-D-ribofuranosyl)-5-methyluracil; 2′-Deoxythymidine
[50-89-5] $C_{10}H_{14}N_2O_5$ FW 242.23

powder, BioReagent, suitable for cell culture

Use in HT and HAT cocktails for hybridoma fusion, selection and cloning.

≥99%
ship: ambient store at: room temp

T1895-1G	1 g
T1895-5G	5 g
T1895-10G	10 g
T1895-25G	25 g

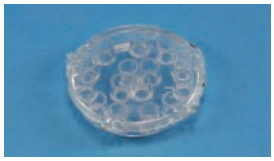
Equipment

Cloning cylinders, glass

Individual colonies of transfected cells can be isolated and picked from a plate containing many clones. Isolated clones can be dissociated and passaged free from surrounding cells or pulsed with 50-100 uL growth medium which can be analyzed for secreted products. The sterile cloning cylinders are greased on one end to allow the cylinder to seal to the plate. Cells are then dissociated within the cylinder, resuspeneded, and transferred to a new vessel as a pure colony.

sterile

Packaged in 60 mm dish with silicone grease on one end



volume 150 µL

8 mm ×8 mm

dish = 15 cylinders

ship: ambient store at: room temp

C1059-1EA	1 ea

volume 250 µL

10 mm ×10 mm

dish = 10 cylinders

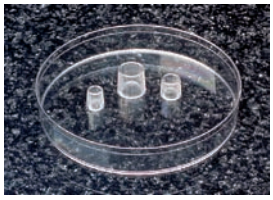
ship: ambient store at: room temp

C2059-1EA	1 ea

Scienceware® cloning cylinders, polystyrene

To isolate a clone, sterile grease (not included) is applied to bottom edge of cylinder and inverted over clone of choice. Provides an isolation chamber to trypsinize and recover clonal colonies.

sterile



40 of each size

box = 3 bags

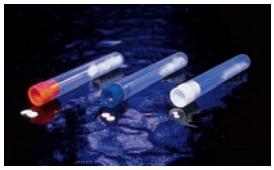
ship: ambient store at: room temp

Z370789-1PAK	1 pkg

Scienceware® cloning discs

Paper discs transfer cells quickly and easily to a 24 well plate for growth. Discs eliminate leak problems associated with cloning cylinders. Risk of contamination and drying out is reduced because cells are exposed only a fraction of the time it would take to apply a cloning cylinder.
Contains 2 vials of 50 cloning discs.

sterile; γ-irradiated



3 mm (1/8 in.)

ship: ambient store at: room temp

Z374431-100EA	100 ea

5 mm (3/16 in.)

ship: ambient store at: room temp

Z374458-100EA	100 ea

6 mm (1/4 in.)

ship: ambient store at: room temp

Z374466-100EA	100 ea

ITS, SITE, SPIT, and SPITE Supplements

Insulin-transferrin-sodium selenite media supplement

ITS Supplement
γ-irradiated, lyophilized powder, BioXtra, suitable for cell culture

Each vial contains: ≥21 mg insulin from bovine pancreas; ≥19 mg human transferrin (substantially iron free), and 25 µg sodium selenite.

ITS is a mixture of bovine insulin, human transferrin, and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham's F-12, DME/F-12, MEM) with sodium pyruvate.

Insulin-transferrin-sodium selenite supplement is used for serum-free cell culture applications.

Each vial sufficient to prepare 5 liters of medium.

ship: ambient store at: −20°C

I1884-1VL	1 vial
I1884-5X1VL	5 × 1 vial

ITS+1 Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Insulin-transferrin-sodium selenite, linoleic-BSA is used for serum-free cell culture applications.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 50 mg/ml bovine serum albumin and 470 µg/ml linoleic acid.

endotoxin ..... tested

ship: ambient store at: 2-8°C

I2521-5ML	5 mL

ITS+3 Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Insulin-transferrin-sodium selenite, linoleic;oleic-BSA is used for serum-free cell culture applications.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 470 µg/ml linoleic acid, 470 µg/ml oleic acid and 50 mg/ml bovine serum albumin. Contains 2 moles each of linoleic acid and oleic acid per mole of albumin.

endotoxin ..... tested

ship: ambient store at: 2-8°C

I2771-5ML	5 mL

ITS Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

ITS is a mixture of bovine insulin, human transferrin, and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham?s F-12, DME/F-12, MEM) with sodium pyruvate.

Insulin-transferrin-sodium selenite supplement is used for serum-free cell culture applications.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), and 0.5 µg/ml sodium selenite.

endotoxin ..... tested

ship: ambient store at: 2-8°C

I3146-5ML	5 mL

SITE+3 Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 0.2 mg/ml ethanolamine, 470 µg/ml linoleic acid, 470 µg/ml oleic acid and 50 mg/ml bovine serum albumin. Contains 2 moles each of linoleic acid and oleic acid per mole of albumin.

endotoxin ..... tested

ship: ambient store at: 2-8°C

S5295-5ML	5 mL

SITE Liquid Media Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite and 0.2 mg/ml ethanolamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

S4920-5ML	5 mL

SPITE Medium Supplement (100×)

liquid, sterile-filtered, BioReagent, suitable for cell culture

SPITE is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, sodium pyruvate and ethanolamine. It is designed for cell cultures in which media without sodium pyruvate are used.

For suspension cell cultures

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

Contains 1.0 mg/ml insulin from bovine pancreas, 0.55 mg/ml human transferrin (substantially iron-free), 0.5 µg/ml sodium selenite, 12 mg/ml pyruvate and 0.2 mg/ml ethanolamine.

endotoxin ..... tested

ship: ambient store at: 2-8°C

S5666-5ML	5 mL

Lipids and Lipid Carriers

Arachidonic acid

Eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoic acid; *cis,cis,cis,cis*-5,8,11,14-Eicosatetraenoic acid
[506-32-1] $CH_3(CH_2)_4(CH{=}CHCH_2)_4CH_2CH_2CO_2H$ $C_{20}H_{32}O_2$ FW 304.47

density ..... 0.922 g/mL, 25 °C

n_D^{20} 1.4872

from porcine liver, BioReagent, suitable for cell culture, ≥99% (GC)

liquid

Sealed ampule.

solubility

absolute ethanol ..... 10 mg/mL

color ..... clear colorless to very faintly yellow

ship: dry ice store at: −20°C

A3555-10MG	10 mg
A3555-50MG	50 mg
A3555-100MG	100 mg
A3555-1G	1 g

Arachidonic acid NEW

Eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraenoic acid; *cis,cis,cis,cis*-5,8,11,14-Eicosatetraenoic acid
[506-32-1] $CH_3(CH_2)_4(CH{=}CHCH_2)_4CH_2CH_2CO_2H$ $C_{20}H_{32}O_2$ FW 304.47

density ..... 0.922 g/mL, 25 °C

n_D^{20} 1.4872

from non-animal source, ≥98.5% (GC)

liquid

Sealed ampule

ship: dry ice store at: −20°C

A3611-100MG	100 mg
A3611-1G	1 g

Cholesterol

3β-Hydroxy-5-cholestene; 5-Cholesten-3β-ol
[57-88-5] $C_{27}H_{46}O$ FW 386.65

Major component of all biological membranes; ~25% of total brain lipid is cholesterol.

density ..... 1.067 g/mL, 25 °C

Cholesterol *(continued)*

Synthetic cholesterol
cGMP, ≥98%, SyntheChol™ synthetic

Synthesized from material of non-animal origin

ship: ambient store at: –20°C

C1231-10G	10 g
C1231-100G	100 g

Cholesterol

3β-Hydroxy-5-cholestene; 5-Cholesten-3β-ol
[57-88-5] $C_{27}H_{46}O$ FW 386.65

Major component of all biological membranes; ~25% of total brain lipid is cholesterol.

density ..... 1.067 g/mL, 25 °C

powder, BioReagent, suitable for cell culture, ≥99%

solubility
TWEEN® ..... soluble
ethanol ..... soluble
serum ..... soluble
H_2O ..... 0.002 mg/mL

ship: ambient store at: –20°C

C3045-5G	5 g
C3045-25G	25 g
C3045-100G	100 g

Cholesterol-Water Soluble

Cholesterol–methyl-β-cyclodextrin
powder, BioReagent, suitable for cell culture

Contains approx. 40 mg of cholesterol per gram; balance methyl-β-cyclodextrin.

Package size based on cholesterol

solubility
H_2O ..... 300 mg/mL

ship: ambient store at: –20°C

C4951-30MG	30 mg

Cod liver oil fatty acid methyl esters

BioReagent, suitable for insect cell culture

Natural distribution of saturated and unsaturated fatty acids as methyl esters. With 15-25% ω-3 (octadecatetraenoic, eicosapentaenoic and docosahexaenoic) methyl esters.

Prepared by transmethylation of cod liver oil.

ship: ambient store at: 2-8°C

C2294-1G	1 g
C2294-5G	5 g

α-Cyclodextrin

Cyclohexaamylose; Cyclomaltohexaose; α-Schardinger dextrin
[10016-20-3] $C_{36}H_{60}O_{30}$ FW 972.84

powder, BioReagent, suitable for cell culture, ≥98%

Useful for the solubilization of nonpolar macromolecules such as fatty acids, lipids, and cholesterol for use in cell culture applications.

solubility
H_2O ..... 10 mg/mL

ship: ambient store at: room temp

C4680-1G	1 g
C4680-5G	5 g

β-Cyclodextrin

Cycloheptaamylose; Cyclomaltoheptaose; Schardinger β-Dextrin; Caraway
[7585-39-9] $C_{42}H_{70}O_{35}$ FW 1134.98

$[\alpha]_D^{20}$ +162±3°, c = 1.5 in H_2O

powder, BioReagent, suitable for cell culture, ≥98%

Use to solubilize non-polar compounds such a fatty acids, lipids and cholesterol.

solubility
1 M NaOH ..... 50 mg/mL

ship: ambient store at: room temp

C4805-5G	5 g
C4805-25G	25 g
C4805-100G	100 g

γ-Cyclodextrin

Cyclooctaamylose; Cyclomaltooctaose; Schardinger γ-Dextrin
[17465-86-0] $C_{48}H_{80}O_{40}$ FW 1297.12

powder, BioReagent, suitable for cell culture, ≥98%

A molecule used to solublize non-polar molecules such a cholesterol for use in cell culture.

solubility
1 M NaOH ..... 25 mg/mL, clear to slightly hazy

ship: ambient store at: room temp

C4930-100MG	100 mg
C4930-1G	1 g

Fatty Acid Supplement

liquid, sterile-filtered, BioReagent, suitable for cell culture

Prepared with 100 mg/ml of bovine serum albumin in DPBS (D 5652). Contains 2 moles of linoleic and 1 mole oleic acid per mole of albumin.

Recommended for use with epithelial-derived cells at 0.5 to 1.0 ml per liter of medium.

ship: ambient store at: 2-8°C

F7175-5ML	5 mL

(2-Hydroxypropyl)-β-cyclodextrin

[128446-35-5]

The solubility of lipophilic drugs increases linearly with the concentration of hydroxypropyl-β-cyclodextrin (HBC) in aqueous solution because of the complex between HBC and the drug. This guest-host type complex is formed between the drug and the non-polar cavity in the HBC that results in enhanced solubility. Solutions may be lyophilized to produce freely soluble powders. Non-toxic in rabbits and mice.

powder, BioReagent, suitable for cell culture

Solutions may be stored for several months at 4°C. Solid should be stored tightly sealed at room temperature.

solubility
H_2O ..... 100 mg/mL
extent of labeling ..... 4-10 (determined by NMR)

ship: ambient store at: room temp

C0926-5G	5 g
C0926-10G	10 g

Linoleic acid

cis-9,*cis*-12-Octadecadienoic acid
[60-33-3] $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7CO_2H$ $C_{18}H_{32}O_2$ FW 280.45
density ..... 0.902 g/mL, 25 °C
n_D^{20} 1.466

liquid, BioReagent, suitable for cell culture

Fatty acid that is typically bound to a carrier molecule such as BSA or cyclodextrin for use in cell culture.

≥99%

Sealed ampule.

solubility
ethanol .....
NaOH ..... 1 M

ship: ambient store at: −20°C

L1012-100MG	100 mg
L1012-1G	1 g
L1012-5G	5 g

Linoleic Acid-Albumin from bovine serum albumin

γ-irradiated, lyophilized powder, BioReagent, suitable for cell culture

Binding of linoleic acid to BSA improves its stability and solubility in culture media.

Contains 1.1-2.3 moles linoleic acid per mole BSA

ship: ambient store at: 2-8°C

L8384-500MG	500 mg

Linoleic Acid-Water Soluble

powder, BioReagent, suitable for cell culture

Contains approx. 30 mg of linoleic acid per gram; balance methyl-β-cyclodextrin.
Package size based on linoleic acid content.

Methyl-β-cylodextran binding improves the solubility and delivery of Linoleic acid in culture media.

solubility
H_2O ..... 50 mg/mL

ship: ambient store at: −20°C

L5900-10MG	10 mg

Lipid Medium Supplement

liquid, sterile-filtered, BioReagent, suitable for cell culture, suitable for insect cell culture

Originally developed for the serum-free growth of *Spodoptera frugiperda (Sf9)* cells in IPL-41 Insect Medium.

ship: ambient store at: 2-8°C

L2273-100ML	100 mL

Lipid Mixture (1000×)

liquid, sterile-filtered, BioReagent, suitable for insect cell culture

composition
cholesterol 4.5 g/L
cod liver oil fatty acids (methyl esters) 10 g/L
polyoxyethylenesorbitan monooleate 25 g/L
D-α-tocopherol acetate 2.0 g/L

ship: ambient store at: 2-8°C

L5146-100ML	100 mL

Lipid Mixture 1, Chemically Defined

liquid, sterile-filtered, BioReagent, suitable for cell culture

Contains non-animal derived fatty acids (2 µg/ml arachidonic and 10 µg/ml each linoleic, linolenic, myristic, oleic, palmitic and stearic), 0.22 mg/ml cholesterol from New Zealand sheep's wool, 2.2 mg/ml TWEEN® 80, 70 µg/ml tocopherol acetate and 100 mg/ml Pluronic® F-68 solubilized in cell culture water.

Recommended for use in cell culture at 1 to 10 mL per liter of medium.

ship: ambient store at: 2-8°C

L0288-100ML	100 mL

Lipids Cholesterol Rich from adult bovine serum

Adventitious viral agents ..... none detected (9 CFR; 113.53)
IgG ..... none detected (RID)
Mycoplasma ..... none detected (Barile method)

liquid, sterile-filtered, BioReagent, suitable for cell culture, suitable for insect cell culture

Aqueous lipoprotein solution

composition
Cholesterol 9.0-11.0 g/L
Protein 15.0-25.0 g/L

endotoxin ..... ≤6.0 EU/mg cholesterol
Microbial content ..... none detected (USP XXII)

ship: ambient store at: 2-8°C

L4646-20ML	20 mL
L4646-100ML	100 mL

lyophilized powder, BioReagent, suitable for cell culture

Low-salt bovine lipoproteins supplemented with bovine albumin.

Recommended use at 5 to 10 ml/L in cell culture medium.

composition
Cholesterol 60-80 mg/g
Protein 600-800 mg/g

Reconstitute at 75 mg/ml in Dulbecco's Phosphate Buffered Saline.

endotoxin ..... ≤2.0 EU/mg cholesterol

ship: ambient store at: −20°C

C7305-1G	1 g

Methyl-β-cyclodextrin

[128446-36-6]

powder, BioReagent, suitable for cell culture

Use to increase the solubility of non-polar substances such as fatty acids, lipids, vitamins and cholesterol for use in cell culture applications.

Solutions may be obtained by stirring 30 min at room temperature. Alternatively, sonication with cooling may be employed. Solutions may be stored for several months at 4°C. Solid should be stored tightly sealed at room temperature.

solubility
H_2O ..... 50 mg/mL

extent of labeling ..... 1.5-2.1 methyl per mol glucose

ship: ambient store at: room temp

C4555-1G	1 g
C4555-5G	5 g
C4555-10G	10 g

Oleic acid

Elainic acid; *cis*-9-Octadecenoic acid
[112-80-1] $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ $C_{18}H_{34}O_2$ FW 282.46

Activates protein kinase C in hepatocytes.

Uncouples oxidative phosphorylation. Inhibits 2,4-dinitrophenol–stimulated ATPase. Action reversed by adding serum albumin.

density ..... 0.89 g/mL, 25 °C vp ..... 1 mmHg (176 °C)
n_D^{20} 1.459

BioReagent, suitable for cell culture

Oleic acid is a mono-unsaturated omega-9 fatty acid created by dehydrogenation of stearic acid. It is then further dehydrogenated and elongated to create members of the n-9 family of fatty acids. Oleic acid is not an essential fatty acid (EFA); however, providing it to cells in culture reduces energy demand.

≥99% (GC)

Sealed ampule.

Unsaturated fatty acids are subject to oxidation and peroxidation. Consider complexed fatty acid supplements.

solubility
ethanol .....
density ..... 0.89 g/mL, 25 °C
ship: ambient store at: –20°C

O1383-1G	1 g
O1383-5G	5 g
O1383-25G	25 g

Oleic Acid-Water Soluble

Oleic Acid–Cyclodextrin Complex

powder, BioReagent, suitable for cell culture

composition
Oleic acid ~30 mg/g (balance methyl-β-cyclodextrin)

Methyl-β-cylodextran binding improves the solubility and delivery of Oleic acid in culture media.

solubility
H_2O ..... 50 mg/mL, clear to slightly hazy
ship: ambient store at: –20°C

O1257-10MG	10 mg

Lipids Solubility Quick Reference Table

The following is a table with recommendations for solubilizing some of our lipids for cell culture use. The list highlights items that we have found to be troublesome. In addition, we have included molecular weights and storage temperatures.

Name	Mol Wt.	Ethanol	Storage Temp	Cat. No.
Cholesterol	386.65	soluble soluble soluble 0.002 mg/mL	–20°C	C3045-5G C3045-25G C3045-100G
Linoleic acid	280.45	soluble 1 M	–20°C	L1012-100MG L1012-1G L1012-5G
Oleic acid	282.46	soluble	–20°C	O1383-1G O1383-5G O1383-25G

Mouse Embryo Culture Products

Mouse Embryo Tested Media and Salts

M16 Medium

With sodium bicarbonate and lactic acid, without penicillin and streptomycin., liquid, sterile-filtered

Presence of particulates in solution will not affect product's performance.

M2 and M16 Medium are common media for in vitro culture of preimplantation stage embryos. It is a modified Krebs-Ringer bicarbonate solution, which is very similar to Whitten's Medium. M16 contains pyruvate and lactate as energy sources since preimplantation embryos do not utilize glucose efficiently.

suitable for mouse embryo

Supplement with 0.06 g/L potassium penicillin-G and 0.05 g/L streptomycin sulfate.

This product is tested for its ability to support the development of one-cell mouse embryos to expanded blastocysts. Minimum requirement is 80% development to blastocyst.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M7292-50ML	50 mL
M7292-100ML	100 mL

M2 medium

With HEPES, without penicillin and streptomycin, liquid, sterile-filtered, suitable for mouse embryo

Presence of particulates in solution will not affect product's performance.

Recommended for manipulation of mouse embryos at ambient temperature. M2 and M16 Medium are common media for in vitro culture of preimplantation stage embryos. It is a modified Krebs-Ringer bicarbonate solution, which is very similar to Whitten's Medium. M16 contains pyruvate and lactate as energy sources since preimplantation embryos do not utilize glucose efficiently.

M2 Medium is a further modification of M16 that substitutes HEPES buffer in place of some of the bicarbonate. M2 is used for collecting and handling embryos for prolonged periods outside a CO2 incubator.

Supplement with 0.06 g/L potassium penicillin-G and 0.05 g/L streptomycin sulfate.

This product is tested for its ability to support the development of one-cell mouse embryos to expanded blastocysts. Minimum requirement is 80% development to blastocyst.

endotoxin ..... tested
ship: ambient store at: 2-8°C

M7167-50ML	50 mL
M7167-100ML	100 mL

Tyrode's Salts

With sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture

Complete and ready to use formulation of Tyrode's salt solution for cell washing.

endotoxin ..... tested
ship: ambient store at: room temp

T2397-100ML	100 mL
T2397-500ML	500 mL
T2397-6X500ML	6 × 500 mL
T2397-1L	1 L
T2397-6X1L	6 × 1 L

Tyrode's Solution, Acidic

liquid, sterile-filtered, suitable for mouse embryo

For removal of the zona pellucida.

endotoxin ..... tested
pH ..... 2.5±0.3
ship: dry ice store at: –20°C

T1788-100ML	100 mL

Mouse Embryo Tested Reagents

Albumin from bovine serum

Bovine albumin; BSA
[9048-46-8]

mol wt ~66 kDa

powder, BioXtra

suitable for mouse embryo cell culture

≥96% (agarose gel electrophoresis)

ship: ambient store at: 2-8°C

A3311-10G	10 g
A3311-50G	50 g
A3311-100G	100 g

D-(+)-Glucose

Dextrose
[50-99-7] $C_6H_{12}O_6$ FW 180.16

suitable for mouse embryo, ≥99.5% (GC)

Mixed anomers

This glucose has been qualified for use in mouse embryo in vitro work.

powder

ship: ambient store at: room temp

G6152-100G	100 g
G6152-500G	500 g
G6152-1KG	1 kg

Gonadotropin from pregnant mare serum

eCG; PMSG; Equine gonadotropin; Pregnant mare serum gonadotropin
[9002-70-4]

Two chain glycoprotein hormone; stimulates the growth of ovarian follicles and the formation of the corpus luteum.

lyophilized powder, BioXtra, suitable for mouse embryo cell culture

Recommended for superovulation induction in mice.

aseptically processed

activity: 1,500-6,000 IU/mg

mol wt ~64 kDa

solubility
H_2O .....
saline ..... 1 mL/vial
ship: dry ice store at: –20°C

G4527-50IU	50 I.U.

HEPES

4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)
[7365-45-9] $C_8H_{18}N_2O_4S$ FW 238.30

pKa (25 °C) 7.5
useful pH range ..... 6.8 - 8.2

BioXtra, suitable for mouse embryo cell culture, ≥99.5% (titration)

This product has been qualified for use in cell and mouse embryo culture.

HEPES is an organic zwitterionic buffering agent effective in the physiological pH range of 6.8 to 8.2 (pKa 7.55). It is typically used in cell culture at concentration between 5mM to 30mM.

powder

Useful pH range: 6.8 - 8.2

$A_{290}^{33\%}$, H_2O ..... ≤0.05
heavy metals (as Pb) ..... ≤5 ppm
ship: ambient store at: room temp

H6147-25G	25 g
H6147-100G	100 g
H6147-500G	500 g

Hyaluronidase from bovine testes

Hyaluronoglucosaminidase; Hyaluronate 4-glycanohydrolase
[37326-33-3]

These enzymes randomly cleave β-N-acetylhexosamine-[1→4] glycosidic bonds in hyaluronic acid, chondroitin, and chondroitin sulfates.

Type IV-S, powder, mouse embryo tested, activity: 750-1500 units/mg solid

Recommended for dissolving cumulus mass in the isolation of mouse embryos.

aseptically processed

mol wt ~55 kDa (four subunits of 14 kDa each)

composition
Protein 80-105% (biuret)

One unit will cause a change in A_{600} nm of 0.330 per min at pH 5.7 at 37°C.

ship: ambient store at: –20°C

H4272-30MG	30 mg

Mineral oil

[8042-47-5]
n_D^{20} 1.467

light oil (neat), BioReagent, suitable for mouse embryo cell culture

Recommended for overlaying mouse embryo cultures to prevent drying.

This product is not sterile. Mineral oil is difficult to sterilize. It can only be sterilized by careful filtration of the warmed oil. It is assumed antibiotics will be added to the cell culture medium. Otherwise, Prod. No. M5310 should be used, as it is sterile filtered.

density ..... 0.84 g/mL, 25 °C
ship: ambient store at: room temp

M8410-5ML	5 mL
M8410-100ML	100 mL
M8410-500ML	500 mL
M8410-1L	1 L

light oil ((neat)), BioXtra, suitable for mouse embryo cell culture

Commonly used and recommended for overlaying mouse embryo cultures to prevent drying during the procedure.

suitable for mouse embryo cell culture

sterile-filtered

density ..... 0.82-0.88 g/mL, 25 °C
ship: ambient store at: room temp

M5310-100ML	100 mL
M5310-500ML	500 mL
M5310-1L	1 L

Polyvinylpyrrolidone

PVP; Polyvidone; Povidone
[9003-39-8] $(C_6H_9NO)_n$

Polyvinylpyrrolidone is a component of Denhardt's Solution and is included at a concentration of 1% (w/v) in the standard 50X stock solution.

powder, BioXtra, suitable for mouse embryo

Polyvinylpyrrolidone (PVP), Polyvidone, is a water-soluble polymer made from the monomer N-vinylpyrrolidone. PVP is soluble in water and other polar solvents. PVP is a component of nucleic acid (DNA & RNA) extraction buffers. Polyvinylpyrrolidone is a component of Denhardt's Solution and is included at a concentration of 1% (w/v) in the standard 50X stock solution.

viscosity

viscosity number 28-32(lit.)
solubility
H_2O ..... 100 mg/mL
ship: ambient store at: room temp

P0930-50G	50 g
P0930-100G	100 g

Protease from *Streptomyces griseus*

Actinase E; Pronase E
[9036-06-0]

Protease is typically used in nucleic acid isolation procedures in incubations of 0.5-3.0 hours supplemented with 0.2% sodium dodecyl sulfate and 10 mM EDTA.

A mixture of at least three proteolytic activities including an extracellular serine protease. In general, serine proteases display a wide range of substrate specificities, which are believed to be mediated by an active site composed of one Asp, one His, and a Ser residue in the molecule. This enzyme prefers to hydrolyze peptide bonds on the carboxyl side of glutamic or aspartic acid.

Collected from culture broth of *S. griseus* and purified by successive column procedures.

Completely inactivated by heating above 80 °C for 15-20 minutes.

powder, BioReagent, suitable for mouse embryo cell culture, activity: ≥3.5 units/mg solid

An unusually non-specific protease.

Contains approximately 25% calcium acetate.

One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

starch ..... essentially free
ship: ambient store at: –20°C

P8811-100MG	100 mg
P8811-1G	1 g
P8811-5G	5 g

Sodium chloride solution

[7647-14-5] NaCl ClNa FW 58.44

solution (0.9%), BioXtra, suitable for cell culture

Sodium chloride is widely used in biochemistry and molecular biology research. It is a component of phosphate buffered saline (Product No. P3813) and βC buffer (Product Nos. S0902 and S8015). Sodium chloride is a component of all media formulations. This product has been qualified for use in preparation of in vitro solutions.

sterile-filtered

Prepared in tissue culture grade water.

Packaged with septum caps.

endotoxin ..... tested
ship: ambient store at: room temp

S8776-10X10ML	10 × 10 mL
S8776-100ML	100 mL

Sodium DL-lactate solution

DL-Lactic acid sodium salt
[72-17-3] $CH_3CH(OH)COONa$ $C_3H_5NaO_3$ FW 112.06

synthetic, syrup, BioXtra, suitable for mouse embryo cell culture, 60 % (w/w)

Lactic acid is an α hydroxyl carboxylic acid produced from pyruvate by the enzyme lactate dehydrogenase (LDH). This mixed isomer lactate has been qualified for use in mouse embryo culture. Lactate regulates pyruvate uptake and metabolism in the preimplantation mouse embryo.

ship: ambient store at: 2-8°C

L7900-100ML	100 mL

Sodium pyruvate

α-Ketopropionic acid sodium salt; 2-Oxopropanoic acid sodium salt; Pyruvic acid sodium salt
[113-24-6] $CH_3COCOONa$ $C_3H_3NaO_3$ FW 110.04

powder, BioXtra, suitable for mouse embryo

Sodium pyruvate is used by cells as an easily accessible carbohydrate source. Additionally, it is involved with amino acid metabolism and initiates the Kreb's cycle. Sodium pyruvate attenuates oxidative stress in vitro. The 100 mM solution should be diluted 1:100 for most cell culture applications. This product has been qualified for use in mouse embryo cultures.

≥99%
solubility
H_2O ..... 100 mg/mL
ship: ambient store at: 2-8°C

P4562-5G	5 g
P4562-25G	25 g
P4562-100G	100 g

Streptomycin sulfate salt

[3810-74-0] $C_{21}H_{39}N_7O_{12} \cdot 1.5H_2O_4S$ FW 728.69

Mode of Action: Inhibits prokaryote protein synthesis. Binds to S12 protein of 30S ribosomal subunit, preventing the transition from initiation complex to chain-elongating ribosome, causing miscoding or inhibiting initiation.
Mode of Resistance: Mutation in *rps*L (gene for S12 ribosomal protein) prevents binding of streptomycin to ribosome. Aminoglycoside phosphotransferase also inactivates.
Antimicrobial spectrum: Gram-negative and Gram-positive bacteria.

powder, BioXtra, suitable for mouse embryo cell culture

Recommended for use in embryo culture at 50 mg/L. Stock solutions should be sterile-filtered and stored at 2-8 °C for up to a month or at -20 °C for extended periods. Solutions are stable at 37 °C for 3 days. Use to inhibit bacterial protein synthesis at the level of initiation. Use to study mechanisms of streptomycin resistance. Use together with penicillin and other agents to inhibit bacterial contamination in cell culture applications.

potency: ≥720 I.U. per mg
ship: ambient store at: 2-8°C

S1277-5G	5 g
S1277-50G	50 g

Water

[7732-18-5] H_2O FW 18.02

density ..... 1.000 g/mL, 3.98 °C
n_D^{20} 1.34

for embryo transfer, sterile-filtered, BioXtra, suitable for mouse embryo

For use in embryo manipulation.

endotoxin ..... tested
ship: ambient store at: room temp

W1503-100ML	100 mL
W1503-500ML	500 mL

Mycoplasma Detection and Elimination

The maintenance of contamination-free cell lines is essential to cell-based research. Among the biggest contaminant concerns are mycoplasma contamination. Although mycoplasma do not usually kill contaminated cells, they are difficult to detect and can cause a variety of effects on cultured cells, including altered metabolism, slowed proliferation and chromosomal aberrations. In short, mycoplasma contamination compromises the value of those cell lines in providing accurate data for life science research.
The sources of mycoplasma contamination in the laboratory are very challenging to completely control. As certain mycoplasma species are found on human skin, they can be introduced through poor aseptic technique. Additionally, they can come from contaminated supplements such as fetal bovine serum, and most importantly from other contaminated cell cultures. Once mycoplasma contaminates a culture, it can quickly spread to contaminate other areas of the lab. Strict adherence to good laboratory practices such as good aseptic technique are key, and routine testing for mycoplasma is highly recommended for successful control of mycoplasma contamination. There are products that represent a complete toolkit for myplasma contamination detection and elimination. The three most popular methods for detection include mycoplasma culture, DNA staining method and PCR.

LookOut® Mycoplasma PCR Detection Kit

configured for Optimized for use with JumpStart™ Taq DNA Polymerase, D9307.

The reaction tubes included with the kit are pre-coated with appropriate dNTPs, primers, and loading dye. Total assay time is greatly reduced compared to general protocols that require individual loading of reaction tubes.

The LookOut® Mycoplasma PCR Detection Kit utilizes the polymerase chain reaction (PCR), which is established as the method of choice for highest sensitivity in the detection of *Mycoplasma*, *Acholeplasma*, and *Ureaplasma* contamination in cell cultures and other cell culture derived biologicals. Detection requires less than 2 mycoplasma genomes per microliter of sample.

Not suitable for clinical diagnostic use. Will not detect clinically relevant species such as *M. pneumoniae* and *U. urealyticum*

Components

Pre-coated test reaction tubes
Pre-coated positive control reaction tubes
Rehydratin Buffer
Cap for reaction tubes
ship: ambient store at: 2-8°C

MP0035-1KT	1 kit

LookOut® Mycoplasma PCR Detection Kit

Cat. No. MP0035

Storage Temperature 2-8 °C

Product Description

The LookOut® Mycoplasma PCR Detection Kit utilizes the polymerase chain reaction (PCR), which is established as the method of choice for highest sensitivity in the detection of *Mycoplasma*, *Acholeplasma*, and *Ureaplasma* contamination in cell cultures and other cell culture derived biologicals. Detection requires less than 2 mycoplasma genomes per microliter of sample.

The primer set is specific to the highly conserved 16S rRNA coding region in the mycoplasma genome. This allows for detection of all mycoplasma species tested so far and usually encountered as contaminants in cell cultures. Eukaryotic and bacterial DNA are not amplified.

Because the reaction tubes included with the kit are pre-coated with appropriate dNTPs and primers, the total assay time is greatly reduced compared to general protocols that require individual loading of reaction tubes. The reaction tubes also contain DNA to serve as an internal control. For the internal control DNA, a successfully performed reaction is indicated by a distinct 481 bp band on the agarose gel. For convenience the gel loading buffer and dye are already included in the reaction tubes. After thermal cycling the PCR can be loaded directly on the agarose gel.

Mycoplasma Detection and Elimination: LookOut® Mycoplasma PCR Detection Kit

Components

Test Reaction Tubes—Cat. No. T0701 (transparent tubes pre-coated with primers, dNTPs, internal control DNA, and gel loading buffer/dye)	3 strips of 8 tubes each
Positive Control Reaction Tubes—Cat. No. P9123 (pink tubes pre-coated with primers, dNTPs, internal control DNA, non-infectious DNA fragments of *Mycoplasma orale* genome, prepared by PCR, and gel loading buffer/dye)	1 strip of 8 tubes
Mycoplasma PCR Rehydration Buffer—Cat. No. M4694	1.2 mL
Caps for PCR Tubes—Cat. No. C0242	4 strips of 8 caps

Equipment and Reagents Required But Not Provided

- JumpStart™ Taq DNA Polymerase, Catalog Number D9307
- Microcentrifuge tubes, Catalog Number T0447
- GenElute™ Blood Genomic DNA Kit, Catalog Number NA2000 (optional, for DNA extraction and purification)
- 1.2% Agarose Gel

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Kit components are stable during shipping at ambient temperature. Upon receipt, store at 2-8 °C. The kit is stable until the expiration date stated on the label.

Procedure

A. Preparation of Sample Material

Cell lines should be pre-cultured in the absence of mycoplasma active antibiotics for several days to maximize test sensitivity. Samples should be derived from cultures that are at 90-100 % confluence. PCR inhibiting substances may accumulate in the medium of older cultures. For a sample from an older culture, a DNA extraction is strictly recommended prior to testing. The GenElute™ Blood Genomic DNA Kit (Catalog Number NA2000) is recommended.

To avoid false positive results, the use of deionized, DNA-free water, aerosol-preventive filter tips, and gloves is recommended.

Cell culture supernatants can be tested directly without prior preparation. Stable templates for PCR analysis at a later date can be prepared by boiling the supernatant of cell cultures or other biologicals for 5 minutes as follows:

1. Transfer 100 ml of supernatant from the test culture to a sterile microcentrifuge tube (Catalog Number T0447). The lid should be tightly sealed to prevent opening during heating.
2. Boil or incubate the sample supernatant at 95 °C for 5 minutes.
3. Briefly centrifuge (5 seconds) the sample supernatant to pellet cellular debris before adding to the PCR mixture. The templates are stable at 2-8 °C for at least 1 week.

B. PCR Setup

Total volume for each PCR is 25 µl. It is recommended to perform a positive (step 4) and a negative control reaction.

1. Polymerase/Rehydration Buffer Preparation - Determine the total volume of Polymerase/Rehydration Buffer required for the reactions (see Table 1). Calculations should also include an additional reaction volume (23 µl) to compensate for pipetting losses. Pipette the required volume of Rehydration Buffer into a clean microcentrifuge tube (Catalog Number T0447) and add the required volume of DNA polymerase. One unit of DNA polymerase is required per reaction. For JumpStart Taq DNA polymerase with 2.5 units/µl, a volume of 0.4 µl is required per reaction. Mix the Polymerase/ Rehydration Buffer carefully by flicking the tube. DO NOT VORTEX!

Table 1

	Test Reaction	Positive Control	Negative Control
Polymerase/Rehydration Buffer	23 µL	25 µL	23 µL
Sample Volume	2 µL		
DNA-free Water		2 µL	

2. Reaction Tube Rehydration - Remove strip of Test Reaction Tubes (transparent) from bag and cut off the appropriate number of tubes. Replace remaining tubes in bag and seal. Peel off protective film from tubes. Add 23 µl of the prepared Polymerase/Rehydration Buffer to each test reaction tube.

3. Sample Addition - Add 2 µl of DNA-free water to the negative control and 2 µl of sample to each test reaction tube. Close tubes with Caps for PCR Tubes included in kit. Label tubes as appropriate.

4. Positive Control Preparation - Remove strip of Positive Control Reaction Tubes (pink) from bag and cut off the appropriate number of tubes. Replace remaining tubes in bag and seal. Peel off protective film from tubes. Label tubes as appropriate. Add 25 µl of the prepared Polymerase/Rehydration Buffer to each tube. Close tubes with Caps for PCR Tubes included in kit.

5. Incubation - Mix contents of each tube thoroughly by flicking tubes. DO NOT VORTEX! Ensure the liquid collects at the bottom of the PCR tube before incubating at room temperature for 5 minutes. Proceed immediately to thermal cycling.

C. Thermal Cycler Profile

The programming process of your cycler is explained in the instrument manual.

1. The incubation time depends on the DNA polymerase used. Hot start enzymes need to be activated at 94 °C. Please see DNA polymerase data sheet for duration. No activation step is required for JumpStart Taq DNA polymerase.

Thermal Cycler Program

1 cycle	94 °C for 2 minutes
40 cycles	94 °C for 30 seconds
	55 °C for 30 seconds
	72 °C for 40 seconds

Cool down to 4-8 °C

D. Agarose Gel

1. Use 1.2% standard agarose gel with 5 mm comb.
2. Directly load 8 µL for each PCR into a separate lane. The loading buffer is included in the PCR mixture.
3. Stop electrophoresis after migration of 3 cm (depending on the electrophoresis chamber used e.g., run for 20 minutes at 100 V).

Results

Gel Evaluation (Figure 1)

1. The internal control DNA and negative control samples show a distinct 481 bp band. Internal controls should appear in every lane indicating a successfully performed PCR. This band may be less intense with increased amounts of amplicons formed, caused by mycoplasma DNA loads of >5 x 10^6 copies/ml.
2. The positive control shows a band at 270 bp and depending on the activity of the DNA polymerase used, an additional band at 481 bp due to the internal control.
3. Mycoplasma positive samples show bands in the range of 270 ± 8 bp.

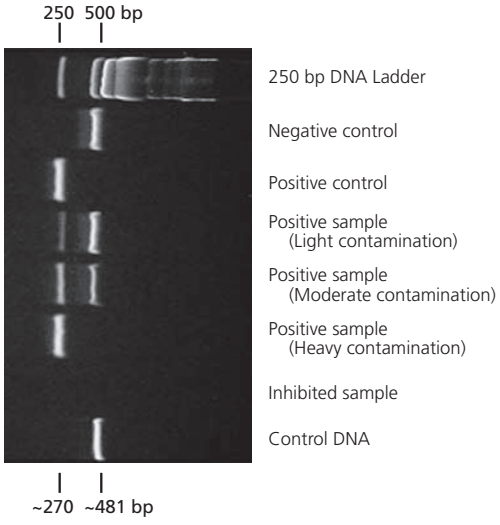


Figure 1. Relevant Amplicon Bands

Troubleshooting

1. No amplification of the control DNA may be due to the following:
- insufficient activity of DNA Taq polymerase
- reaction buffer is unsuitable for polymerase
- programming error
- pipetting error

2. Before rerunning of a negative and a positive control, check thermocycler program and pipetting scheme. DNA polymerase concentration can be raised up to 2.5 units/reaction. The Rehydration Buffer included in this kit can be replaced by the specific buffer provided with the polymerase; however, the magnesium concentration must then be adjusted to 3.0 mM.
3. This kit has been designed for high sensitivity and therefore, is prone to nonspecific annealing, resulting in bands of various lengths that are less intense being produced, but not indicative of positive results. Possible primer self-annealing produces another band of 80-90 bp, but also does not affect the precision or results of the test.
4. If the PCR of a sample is inhibited, PCR inhibitors can easily be removed from the sample by performing a DNA extraction with the GenElute™ Blood Genomic DNA Kit (Catalog Number NA2000).

Use of the PCR process requires a license.

LookOut® mycoplasma qPCR detection kit

NEW

qPCR Probe Kit for high quality and reliable quantitative detection of mycoplasma DNA in research, industrial application and product testing. Usable for direct testing of cell cultures and biologicals in combination with cell culture enrichment.

The LookOut Mycoplasma PCR Detection Kit utilizes the polymerase chain reaction (PCR), which is established as the method of choice for highest sensitivity in the detection of Mycoplasma and Acholeplasma contamination in cell cultures and other cell culture derived biologicals.

The primer/probe system detects the highly conserved 16S rRNA operon coding region of the mycoplasma genome. The kit is highly specific and does not detect eukaryotic DNA. The detection spectrum includes most mycoplasma species identified as cell culture contaminants (see Table 2). The kit contains the nucleotide dUTP instead of dTTP and is, therefore, suitable for UNG pretreatment.

This kit has been tested and optimized for use with JumpStart™ Taq DNA Polymerase, Catalog Number D9307. It is highly recommended that this product be used with the kit. Use of other Taq polymerase products may require reaction optimization to achieve proper results.

Not suitable for clinical diagnostic use.

ship: ambient store at: 2-8°C

MP0040-1KT	1 kit

Venor™ GeM Mycoplasma Detection Kit, PCR-based

Kit employs PCR technology for rapid and reliable detection of mycoplasma DNA in cell cultures and virus stocks.

1 kit sufficient for 25 tests

Does not include *Taq* Polymerase. Optimized for use with D9307, *Taq* DNA Polymerase

Components

Positive Control 1 vial
Negative Control 1 vial
PCR 10X Reaction Buffer 1 vial
Primer/Nucleotide Mix 1 vial
ship: ambient store at: 2-8°C

MP0025-1KT	1 kit

LookOut® Mycoplasma Erase

NEW

LookOut Mycoplasma Erase is a solution for cleansing and elimination of mycoplasma contamination from laboratory surfaces and apparatus, including clean benches, incubators, work benches, cell storage boxes, and liquid nitrogen containers.

store at: room temp

Mycoplasma erase spray

ship: ambient store at: room temp

L1420-500ML	500 mL

Mycoplasma erase spray refill

ship: ambient store at: room temp

L1545-1000ML	1000 mL

4′,6-Diamidino-2-phenylindole dihydrochloride

2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride; DAPI dihydrochloride
[28718-90-3] $C_{16}H_{15}N_5 \cdot 2HCl$ FW 350.25

Cell permeable fluorescent minor groove-binding probe for DNA. Binds to the minor groove of double-stranded DNA (preferentially to AT rich DNA), forming a stable complex which fluoresces approximately 20 times greater than DAPI alone.

DAPI is several times more sensitive than ethidium bromide for staining DNA in agarose gels. It may be used for photofootprinting of DNA, to detect annealed probes in blotting applications by specifically visualizing the double-stranded complex, and to study the changes in DNA and analyze DNA content during apoptosis using flow cytometry. DAPI staining has also been shown to be a sensitive and specific detection method for mycoplasma.

fluorescence ..... λ_{ex} 340 nm, λ_{em} 488 nm (nur DAPI)
fluorescence ..... λ_{ex} 364 nm, λ_{em} 454 nm (DAPI-DNA-Komplex)

powder, BioReagent, suitable for cell culture, ≥98% (HPLC and TLC)

Protect from light.

solubility
PBS ..... insoluble
H_2O .. 20 mg/mL (Heat or sonication may be required. Solutions stored in the dark at room temperature or 4 °C should be stable for 2 to 3 weeks.)
ε_{263nm}, H_2O 30
ship: ambient store at: 2-8°C

D8417-1MG	1 mg
D8417-5MG	5 mg
D8417-10MG	10 mg

Mycoplasma Agar

powder, suitable for microbiology

Recommended for the cultivation of mycoplasma

Powder contains (g/L): 10.0 bacterial peptone, 10.0 LAB-LEMCO powder, 5.0 sodium chloride, 0.5 mineral supplement, 10.0 agar.

solubility
H_2O .. 35.5 g/L (Boil to dissolve the agar and adjust pH to 7.8±0.2. Autoclave at 121°C, 15 psi for 15 minutes. Store prepared medium at 0-5°C.)
ship: ambient store at: room temp

M0660-500G	500 g

Mycoplasma Broth

powder, suitable for microbiology

Recommended for the cultivation of mycoplasma.

Mycoplasma Broth is a basal medium which, after enrichment with yeast extract, horse serum, and antibiotics, will support the growth of Mycoplasma sp. The components contained in this basal medium do not exhibit inhibitory or toxic effects when used to culture mycoplasma. A special mineral supplement has been added to improve the growth and colony characteristics of the organism.

Powder contains (g/L): 10.0 bacterial peptone, 10.0 LAB-LEMCO powder, 5.0 sodium chloride, 0.5 mineral supplement.

solubility
H_2O .. 25.5 g/L (Autoclave at 121°C, 15 psi for 15 minutes. Store prepared medium at 0-5°C.)
ship: ambient store at: room temp

M0535-250G	250 g
M0535-500G	500 g

Mycoplasma Control Slides

Cells are grown on coverslips which have been mounted on microscope slides. Use caution when handling control slides as cells are exposed to facilitate staining.

For use in Mycoplasma stain kit.

Components

negative control slides 10
positive control slides 10
ship: ambient store at: 2-8°C

M1414-1SET	1 set

Mycoplasma Detection

Materials

- Indicator cells, Vero (ATCC® CCL 81 or ECACC 84113001) or 3T6-Swiss albino (ATCC® CCL 96 or ECACC 86052701)
- Leighton tubes or glass cover slips/culture dishes
- Cell culture medium (growth medium)
- Methanol
- Glacial acetic acid
- Bisbenzimide (Catalog Number B1155) or DAPI (Catalog Number D8417)
- Mounting Solution McIlvanine's Buffer: Glycerol [1:1] (Catalog Number M7534)
- Fluorescence microscope (see Procedure: Examining cultures)

In situ DNA fluorescence is a very efficient method of screening for mycoplasma contamination in cell cultures. Bisbenzimide (Hoechst 33258) and DAPI (4′,6-Diamidino-2-phenylindole) are DNA fluorochromes which bind specifically to the Adenine-Thymidine (A-T) regions of DNA. Cultures contaminated with mycoplasma will have small, uniformly shaped fluorescent bodies evident in the extranuclear and intracellular spaces. Nuclei of cultured cells will also fluoresce.

Artifacts may fluoresce and interfere with interpretation. They will appear larger in size than mycoplasma and irregular in shape. Using healthy, log-phase indicator cells and test cells will reduce interference caused by artifacts.

Procedure

Culturing samples and indicator cells

1) Seed indicator cells at low density in a Leighton tube or on a glass coverslip in a culture dish containing tissue culture medium. Incubate for 24 hours at the conditions appropriate for the culture medium (typically 37 °C at 5% or 2% CO_2). Prepare enough cultures to inoculate with control and test samples.
2) To separate indicator cell cultures, add 0.1 ml of test samples. Negative control: Indicator cell cultures inoculated with 0.1 ml of culture medium. Positive control: If a positive control is desired, infect a culture of the indicator cells with 0.1 ml of a viable culture of mycoplasma species.
3) Allow all cultures to incubate for an additional 4 days.

NOTE: It is important to stain and examine cultures before they reach confluency. Adjust incubation time and inoculum density according to the growth characteristics of the test and indicator cells

Fixing Cells

1) Prepare Carnoy's fixative fresh on the day of use. Solution consists of 3 parts methanol to 1 part glacial acetic acid. Prepare enough solution to fix all cultures. Approximately 15 ml of fixative is required per culture.
2) Without decanting growth medium, add approximately 5 ml of Carnoy's fixative to each culture and allow to stand 2 minutes.
3) Decant and add 5 ml of fixative to the cultures and allow to stand 5 minutes.
4) Decant fixative, add 5 ml of fresh fixative, and allow to stand 5 minutes.
5) Finally, decant fixative and allow growth surface to air dry approximately 5 minutes.

Staining and Mounting Cells

1) Prepare working concentration of fluorochrome stain (Bisbenzimide) by dissolving to a concentration of 0.25-0.5 mg/ml in distilled water. Concentration of stock solution should be 50 mg/ml and stored in the dark. Stock solution should be sterile and discarded if performance deteriorates. **Note:** DAPI may be substituted for bisbenzimide. Solubilize DAPI in Phosphate Buffered Saline (PBS) at 0.1 mg/ml. Stain cells for 15-30 minutes.
2) Completely immerse the growth surface in the stain solution and allow to stand for 30 minutes.
3) Rinse twice with distilled water.
4) Mount growth surface, cell side down, with a drop of mounting solution on a microscope slide. Slides may be preserved by sealing the edges of the cover slip and slide with clear nail polish. Slides should be protected from light and heat. These will last several weeks without quenching if properly stored.

Sigma offers a Mycoplasma Stain Kit (MYC1) which contains all the reagents necessary to perform the Hoechst staining procedure described. The kit also contains positive and negative control slides for comparison with test slides.

Examining Cultures

A fluorescence microscope capable of epifluorescence is needed for visualizing the stain preparations. A typical system includes fluorescent microscope with a 53/44 barrier filter and a BG-3 exciter filter. A total magnification of 500x (40x;12.5) is usually sufficient to visualize mycoplasma, but higher magnification may be used.

References

1. Chen, T.R., *Exp. Cell. Res.,* **104,** 255-262, 1977.
2. Hay, R.J. et al., *Nature,* **339,** 487-488, 1989.
3. McGarrity, G.J. et. al., In: Methods in Mycoplasmology Vol. 2 Tully and Razin (eds). Academic Press, Inc., New York, NY, 487-488, 1983.

Mycoplasma Stain Kit

The Mycoplasma Stain Kit is designed for in situ detection of mycoplasma and other prokaryotic organisms in cell cultures. This procedure is based on the Hoechst Stain method cited by the Tissue Culture Association (TCA Procedure No. 75361).

1 kit sufficient for 100 tests

Components

Hanks' Balanced Salt Solution without phenol red and sodium bicarbonate 3×35 mL
Hoechst Stain Solution 10 mL
Mounting Medium 10 mL
negative control slides 10
positive control slides 10
ship: ambient store at: 2-8°C

MYC1-1KT	1 kit

Mounting Medium

For use in Mycoplasma stain kit.

Contains sodium phosphate and citric acid in glycerol.
ship: ambient store at: room temp

M1289-10ML	10 mL

bisBenzimide H 33258

HOE 33258; 2-[2-(4-Hydroxyphenyl)-6-benzimidazoyl]-6-(1-methyl-4-piperazyl) benzimidazole trihydrochloride; 2′-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi(1H-benzimidazole) trihydrochloride; BBIH; Hoechst 33258
[23491-45-4] $C_{25}H_{24}N_6O \cdot 3HCl$ FW 533.88

Membrane-permeable, fluorescent DNA stain with low cytotoxicity that intercalate in A-T regions of DNA.

Useful for staining DNA, chromosomes and nuclei. May be used for fluorescence microscopy or flow cytometry.
Excitation max. = 346 nm
Emission max. = 460 nm

▶ **powder, BioReagent, suitable for cell culture, ≥98% (HPLC and TLC)**

passes application test for fluorescence
solubility
H_2O ..... 10 mg/mL
ship: ambient store at: −20°C

B1155-25MG	25 mg
B1155-100MG	100 mg

Hoechst Stain solution

Bisbenzimide H 33258
[23491-45-4]

A fluorescent DNA stain qualified for use in Mycoplama staining.

Contains 0.5µg/ml Hoechst bisbenzimide 33258 fluorochrome stain and thimerosal.
ship: ambient store at: 2-8°C

H6024-10ML	10 mL

LookOut® Mycoplasma Elimination Kit

The kit is comprised of a combination of biological agents that reliably and completely eliminate mycoplasma contamination. The initial treatment of this eradication procedure is adequate for mycoplasma elimination in most applications. The second step suppresses and inactivates any remaining mycoplasma using a follow-up antibiotic treatment.

The LookOut Mycoplasma Elimination Kit has been developed to quickly and efficiently eliminate mycoplasma contamination from cell cultures.

1 kit sufficient for 5 mL, mycoplasma elimination

Components

Initial treatment
Final treatment
ship: ambient store at: 2-8°C

MP0030-1KT	1 kit

LookOut® DNA Erase

LookOut DNA Erase may be applied to steel, glass, ceramic, plastic, rubber, or precious metals including laboratory equipment and pipettors. It should not be applied on light and non-ferrous metals. For other sensitive surfaces, test in a small area before applying.

LookOut® DNA Erase is a potent, ready-to-use solution for rapid DNA decontamination of surfaces in laboratories. This reagent is characterized by its high efficiency. The decontamination spray completely destroys DNA within 60 seconds of surface treatment. The solution contains a unique combination of DNA destroying and surface active agents.

Both the 250 ml spray reagent (Product Number L8917) and the 1 L refill (Product Number L9042) are supplied ready-to-use.

Spray reagent for DNA decontamination of equipment and surfaces.
ship: ambient store at: room temp

L8917-250ML	250 mL

Refill
ship: ambient store at: room temp

L9042-1L	1 L

LookOut® Mycoplasma Elimination Kit

Cat. No. MP0030

Storage Temperature 2-8 °C

Product Description

The LookOut® Mycoplasma Elimination Kit has been developed to quickly and efficiently eliminate mycoplasma contamination from cell cultures. The potential effects of mycoplasma contamination in biological products are of major concern in research, diagnostics, and biotechnological environments. The effects of mycoplasma are decreased quantity of product produced, decreased quality of the product, and lack of consistency and reproducibility. Current methods for the inactivation or elimination of mycoplasma in cell cultures are antibiotic-based. Antibiotic therapies do not always result in successful elimination of contaminants. Moreover antibiotics exhibit cytotoxic properties in that they are capable of modifying the metabolism of eukaryotic cells and they promote the development of resistant mycoplasma strains.

The LookOut Mycoplasma Elimination Kit is a combination of biological agents that reliably and completely eliminate mycoplasma contamination. The initial treatment of this eradication procedure is adequate for mycoplasma elimination in most applications. The second step suppresses and inactivates any remaining mycoplasma using a follow-up antibiotic treatment. The cytotoxic properties of the reagents are minimal and the development of resistant strains is highly unlikely due to the drastically reduced mycoplasma concentration after the initial treatment. In comparison to other products for the elimination, inactivation or suppression of mycoplasma, the LookOut Mycoplasma Elimination Kit has not been shown to cause any changes in normal cell characteristics and is more effective and the least cytotoxic method (overall cytotoxicity demonstrated <20% over a diverse groups of cell lines).

The LookOut Mycoplasma Elimination Kit is suitable for the elimination of *Mollicutes* and related organisms (*Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma*) in cell and virus cultures. It is not effective for treating mycoplasma contamination in *Chlamydia* cultures.

Components

Mycoplasma Elimination Initial Treatment—Cat. No. M4569 (amber vial/yellow cap, vial contains 500 mL)	1 vial
Mycoplasma Elimination Final Treatment—Cat. No. M4444 (amber vial/red cap, each vial contains 500 mL)	3 vials

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit components are shipped at ambient temperature and should be stored at 2-8 °C. Protection from light is recommended. When stored properly the kit is stable until the expiration date stated on the label.

Procedure

1. Transfer 4.5 ml of prewarmed medium (37 °C) to a cell culture flask or dish.
2. Add the vial contents (500 µl) of the Mycoplasma Elimination Initial Treatment (Catalog Number M4569) to the medium. Mix carefully.
3. Split and passage the cells as thinly as possible given the nature of the cell line.
   a. Add 5 ml of cell suspension to medium/elimination reagent mixture.
   b. Avoid cell aggregates. Use single cell suspension during treatment. Confirm microscopically. Trypsinize or triturate as necessary.
   c. FBS concentration of 5% (v/v) is recommended.
4. Grow cells until 80-90% confluent.
5. Passage cells at usual rate. Add the contents of one vial (500 µl) of Mycoplasma Elimination Final Treatment (Catalog Number M4444) to 9.5 ml of passaged cells in fresh medium. Resume routine FBS concentration.
6. Repeat Final Treatment (step 5) through two additional passages. At this time cultures are mycoplasma free.

Related Products

LookOut® Mycoplasma PCR Detection Kit (MP0035)
LookOut® DNA Erase (Spray for surface cleaning) (L8917)
LookOut® DNA Erase Refill (L9042)}

Miscellaneous Reagents and Supplements

Acetylcholine chloride

ACh
[60-31-1] $(CH_3)_3N^+CH_2CH_2OCOCH_3Cl^-$ $C_7H_{16}ClNO_2$ FW 181.66

Endogenous neurotransmitter at cholinergic synapses; amplifies action potential of the sarcolemma thereby inducing muscle contractions.

suitable for cell culture

~99%

ship: ambient store at: room temp

A2661-25G	25 g
A2661-100G	100 g

N-Acetyl-D-glucosamine

2-Acetamido-2-deoxy-D-glucose; D-GlcNAc
[7512-17-6] $C_8H_{15}NO_6$ FW 221.21

BioReagent, suitable for cell culture

ship: ambient store at: −20°C

A3286-5G	5 g
A3286-25G	25 g
A3286-100G	100 g

Adenine hemisulfate salt

Adenine sulfate salt; 6-Aminopurine hemisulfate salt
[321-30-2] $C_5H_5N_5 \cdot 1/2H_2SO_4$ $C_5H_5N_5 \cdot 0.5H_2O_4S$ FW 184.17

powder, BioReagent, suitable for cell culture

≥99%

solubility
0.5 M HCl ..... 10 mg/mL

ship: ambient store at: room temp

A3159-5G	5 g
A3159-25G	25 g
A3159-100G	100 g
A3159-500G	500 g

Adenine hydrochloride

6-Aminopurine hydrochloride
[6055-72-7] $C_5H_5N_5 \cdot HCl$ FW 171.59

powder, BioReagent, suitable for cell culture

≥99%

solubility
H_2O ..... 50 mg/mL

ship: ambient store at: room temp

A9795-1G	1 g
A9795-5G	5 g
A9795-25G	25 g

Adenosine 5′-triphosphate disodium salt hydrate

ATP disodium salt
[34369-07-8] $C_{10}H_{14}N_5Na_2O_{13}P_3 \cdot xH_2O$ FW 551.14 (Anh)

P_2 purinergic agonist; increases activity of Ca^{2+}-activated K^+ channels; substrate for ATP-dependent enzyme systems

crystalline, BioReagent, suitable for cell culture, ≥99%

microbial

solubility
H_2O ..... 50 mg/mL

ship: ambient store at: −20°C

A6419-1G	1 g
A6419-5G	5 g
A6419-10G	10 g

Adonitol

Adonite; Ribitol
[488-81-3] $C_5H_{12}O_5$ FW 152.15

BioReagent, suitable for cell culture

Useful for studies of the thermo-tolerance of cells in cell culture.

ship: ambient store at: room temp

A9790-25G	25 g

Agarose, low gelling temperature

2-Hydroxyethyl agarose
[39346-81-1]

BioReagent, suitable for cell culture, suitable for insect cell culture

A low gelling temperature derivative with unique gelling properties. Gels form at <30 °C, remelt at temperatures in excess of 60 °C. Gels exhibit excellent clarity and are particularly useful for the preparation of media containing heat-labile materials.

solubility
H_2O ..... 10 mg/mL (with heat)

ship: ambient store at: room temp

A9045-5G	5 g
A9045-10G	10 g
A9045-25G	25 g
A9045-50G	50 g
A9045-100G	100 g
A9045-250G	250 g

4% Agarose gel

sterile-filtered, suitable for insect cell culture

Matrix useful for protein MALDI-MS.

ship: ambient store at: room temp

A6689-40ML	40 mL

L-2-Aminobutyric acid

L-α-Aminobutyric acid
[1492-24-6] $C_4H_9NO_2$ FW 103.12

BioReagent, suitable for cell culture

L-α-Aminobutyric acid (AABA) is an isomer of the non-natural amino acid aminobutyric acid with activity in the γ-glutamyl cycle that regulates glutathione biosynthesis. Recently AABA has been studied as a supplement to in vitro maturation medium (NCSU 23 medium) for culture of oozytes and embryos. This product has been qualified for use in cell culture. AABA is also used as a substitute amino acid for alanine in studies on peptide function.

ship: ambient store at: room temp

A2536-1G	1 g
A2536-5G	5 g

Calcium L-lactate hydrate

L-Lactic acid hemicalcium salt; Sarcolactic acid hemicalcium salt; (*S*)-(+)-2-Hydroxypropanoic acid hemicalcium salt
[41372-22-9] $[CH_3CH(OH)COO]_2Ca \cdot xH_2O$ $C_6H_{10}CaO_6 \cdot xH_2O$ FW 218.22 (Anh)

powder, BioReagent, suitable for cell culture

≥85%
solubility
H_2O ..... 50 mg/mL
ship: ambient store at: 2-8°C

L4388-10G	10 g
L4388-50G	50 g
L4388-250G	250 g

Carboxyethyl-γ-aminobutyric acid

CEGABA
[4386-03-2] $C_7H_{13}NO_4$ FW 175.18

Cell growth promoter

Hybri-Max™, powder, γ-irradiated, BioXtra, suitable for hybridoma

A polyamine derivative that stimulates the growth of hybridoma cells.

Stimulates hybridoma growth.
solubility
H_2O ..... (Store solution at −20°C.)
cell culture medium ..... 1.5 mg/mL (Store solution at −20°C.)
endotoxin ..... tested
ship: ambient store at: room temp

C5556-15MG	15 mg

Casein from bovine milk

[9000-71-9]

Numerous experimental applications including use as a blocking agent in immunochemistry, recovery of enzyme activity from SDS extracted samples, and as a substrate for protease and kinase assays.

BioReagent, suitable for insect cell culture
ship: ambient store at: room temp

C6554-500G	500 g

vitamin free, BioReagent, suitable for insect cell culture
ship: ambient store at: room temp

C5679-500G	500 g

Cobalt(II) chloride hexahydrate

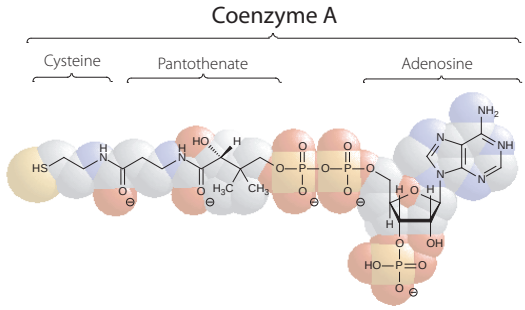
Cobaltous chloride hexahydrate
[7791-13-1] $CoCl_2 \cdot 6H_2O$ $Cl_2Co \cdot 6H_2O$ FW 237.93
vp ..... 40 mmHg (0 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture
ship: ambient store at: room temp

C8661-25G	25 g
C8661-100G	100 g

Coenzyme A sodium salt hydrate

CoA Na_2
$C_{21}H_{36}N_7O_{16}P_3S \cdot xNa^+ \cdot yH_2O$ FW 767.53 (FA/Anh)



Coenzyme A (CoA, CoASH or HSCoA) is the key cofactor in first step of the TCA cycle, responsible for transferring the acetyl group from pyruvate oxidation to oxaloacetate yielding citrate.
Coenzyme A is also a critical cofactor in fatty acid metabolism. Coenzyme A carries fatty acids through the catabolic/oxidation process in the mitochondria and transfers acetyl groups during the elongation process of fatty acid synthesis in the cytosol.

BioReagent, suitable for cell culture

Use to facilitate acyl group transfers especially in fatty acid and lipid metabolism reactions.

Enzymatically assayed using phosphotransacetylase. Approx. 95% CoA-SH
ship: ambient store at: −20°C

C4780-10MG	10 mg
C4780-25MG	25 mg
C4780-100MG	100 mg

Concanavalin A from *Canavalia ensiformis* (Jack bean)

Con A
[11028-71-0]

Con A is not blood group specific but has an affinity for terminal α-D-mannosyl and α-D-glucosyl residues. Ca^{2+} and Mn^{2+} ions are required for activity. Con A dissociates into dimers at pH 5.6 or below. Between pH 5.8 and pH 7.0, Con A exists as a tetramer; above pH 7.0 higher aggregates are formed. Con A exhibits mitogenic activity which is dependent on its degree of aggregation. Succinylation results in an active dimeric form which remains a dimer above pH 5.6.

Where reported, agglutination activity is expressed in µg/ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, containing Ca^{2+} and Mn^{2+} of a 1 mg per mL solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hr incubation at 25 °C.
solubility
H_2O ..... , slightly hazy
PBS ..... 5 mg/mL, slightly hazy

Type IV-S, lyophilized powder, aseptically processed, BioReagent, suitable for cell culture

salt ..... contains (Contains buffer salts and NaCl)
ship: ambient store at: −20°C

C5275-5MG	5 mg

Type IV-S, lyophilized powder, γ-irradiated, BioReagent, suitable for cell culture

salt ..... essentially free

ship: ambient store at: −20°C

C0412-5MG	5 mg

Copper(II) chloride dihydrate

Cupric chloride dihydrate
[10125-13-0] $CuCl_2 \cdot H_2O$ $Cl_2Cu \cdot 2H_2O$ FW 170.48

powder, BioReagent, suitable for cell culture

ship: ambient store at: room temp

C3279-100G	100 g
C3279-500G	500 g

Copper(II) sulfate pentahydrate

Cupric sulfate pentahydrate
[7758-99-8] $CuSO_4 \cdot 5H_2O$ $CuO_4S \cdot 5H_2O$ FW 249.69

vp ..... 7.3 mmHg (25 °C)

BioReagent, suitable for cell culture, ≥98%

ship: ambient store at: room temp

C8027-500G	500 g
C8027-1KG	1 kg

Corn meal

BioReagent, suitable for insect cell culture

May also be used in corn-meal agar for studies of yeast differentiation.

ship: ambient store at: room temp

C6304-1KG	1 kg

Demecolcine solution

N-Deacetyl-N-methylcolchicine solution; Colcemid™ solution
[477-30-5]

10 µg/mL in HBSS, ACF Qualified, BioXtra

Used for cell synchronization by arresting them at metaphase. Qualified for use in animal component free applications.

sterile-filtered

liquid

ship: ambient store at: 2-8°C

D1925-10ML	10 mL
D1925-100ML	100 mL

2′-Deoxyadenosine monohydrate

Adenine deoxyriboside; 9-(2-Deoxy-β-D-ribofuranosyl)adenine
[16373-93-6] $C_{10}H_{13}N_5O_3 \cdot H_2O$ FW 269.26

powder, BioReagent, suitable for cell culture

ship: ambient store at: room temp

D8668-1G	1 g
D8668-5G	5 g

2′-Deoxycytidine hydrochloride

1-(2-Deoxy-β-D-ribofuranosyl)cytosine hydrochloride; Cytosine deoxyriboside hydrochloride
[3992-42-5] $C_9H_{13}N_3O_4 \cdot HCl$ FW 263.68

BioReagent, suitable for cell culture

ship: ambient store at: room temp

D0776-250MG	250 mg
D0776-1G	1 g
D0776-10G	10 g

2′-Deoxyguanosine monohydrate

Guanine-2′-deoxyriboside; 9-(2-Deoxy-β-D-ribofuranosyl)guanine
[312693-72-4] $C_{10}H_{13}N_5O_4 \cdot H_2O$ FW 285.26

powder, BioReagent, suitable for cell culture, 99-100%

solubility

1 M NH4OH ..... 50 mg/mL

ship: ambient store at: room temp

D0901-100MG	100 mg
D0901-250MG	250 mg
D0901-1G	1 g

2-Deoxy-D-ribose

2-Deoxy-D-erythropentose; Thyminose; 2-Deoxy-D-arabinose
[533-67-5] $C_5H_{10}O_4$ FW 134.13

BioReagent, suitable for cell culture

ship: ambient store at: 2-8°C

D5899-10MG	10 mg
D5899-1G	1 g

Ferric citrate

Iron(III) citrate
[3522-50-7] $C_6H_5FeO_7$ FW 244.94

BioReagent, suitable for cell culture

Provides a source of iron used in cell culture applications. Provides iron in a less toxic form than free iron salts.

ship: ambient store at: room temp

F3388-250G	250 g
F3388-1KG	1 kg

Fetuin from fetal calf serum

[9014-81-7]

lyophilized powder, BioReagent, suitable for cell culture

A glycoprotein derived from FBS that is used as Fetuin has been used at a concentration of 500 mg/ml to supplement serum free F12 medium (along with insulin, transferrin and 2-mercaptoethanol) in culture of embryonal carcinoma cells.

Prepared by ammonium sulfate fractionation of fetal bovine serum by the method of Pederson, K.O., *J. Phys. and Colloid Chem.*, 51, 164 (1947).

solubility

H_2O ..... 1 mg/mL

free N-acetylneuraminic acid ..... ≤0.3%

ship: ambient store at: 2-8°C

F3385-100MG	100 mg
F3385-1G	1 g
F3385-5G	5 g
F3385-25G	25 g

Fetuin *(continued)*

γ-irradiated, BioXtra, suitable for cell culture

This product is cell culture tested (0.5 g/L) and is appropriate for use in cell culture applications.

ship: ambient store at: 2-8°C

F6131-250MG	250 mg

Flavin adenine dinucleotide disodium salt hydrate

FAD; FAD-Na_2; Riboflavin 5′-adenosine diphosphate disodium salt
[84366-81-4] $C_{27}H_{31}N_9Na_2O_{15}P_2 \cdot xH_2O$ FW 829.51 (Anh)

BioReagent, suitable for cell culture

A redox cofactor of flavoprotins.

≥95%

ship: ambient store at: −20°C

F8384-100MG	100 mg
F8384-1G	1 g

D-(+)-Galactosamine hydrochloride

2-Amino-2-deoxy-D-galactopyranose hydrochloride; D-Chondrosamine hydrochloride
[1772-03-8] $C_6H_{13}NO_5 \cdot HCl$ FW 215.63

BioReagent, suitable for cell culture

≥99%

Glucosamine ..... essentially free

ship: ambient store at: room temp

G1639-100MG	100 mg
G1639-1G	1 g
G1639-5G	5 g

D-(+)-Galactose

[59-23-4] $C_6H_{12}O_6$ FW 180.16

powder, anhydrous, BioReagent, suitable for cell culture, suitable for insect cell culture

solubility
H_2O ..... 100 mg/mL
Glucose ..... ≤2%

ship: ambient store at: room temp

G5388-100G	100 g
G5388-500G	500 g
G5388-1KG	1 kg

Gluconolactone

δ-Gluconolactone; 1,2,3,4,5-Pentahydroxycaproic acid δ-lactone; D-(+)-Dextronic acid δ-lactone; D-(+)-Gluconic acid δ-lactone
[90-80-2] $C_6H_{10}O_6$ FW 178.14

meets USP testing specifications

ship: ambient store at: room temp

G2164-100G	100 g
G2164-1KG	1 kg

D-(+)-Glucose solution

[50-99-7] $C_6H_{12}O_6$ FW 180.16

Provides the primary energy source for cell metabolism.

solution
endotoxin ..... tested

45% in H_2O, autoclaved, BioXtra, suitable for cell culture

ship: ambient store at: room temp

G8769-100ML	100 mL

100 g/L in H_2O, autoclaved, BioXtra, suitable for cell culture

ship: ambient store at: room temp

G8644-100ML	100 mL

D-Glucuronic acid sodium salt monohydrate

Sodium D-glucuronate
[207300-70-7] $C_6H_9NaO_7 \cdot H_2O$ FW 234.14

BioReagent, suitable for cell culture

ship: ambient store at: room temp

G7906-10G	10 g

L-Glutamic acid monosodium salt hydrate

Glu; (*S*)-2-Aminopentanedioic acid; Monosodium glutamate; MSG; L-Glutamic acid monosodium salt
$C_5H_8NNaO_4 \cdot xH_2O$ FW 169.11 (Anh)

Agonist at kainate, NMDA, and quisqualate receptors; an excitatory amino acid neurotransmitter.

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

ship: ambient store at: room temp

G5889-100G	100 g
G5889-500G	500 g
G5889-1KG	1 kg

L-Glutamic acid potassium salt monohydrate

Potassium L-glutamate; Glu; L-α-Aminoglutaric acid potassium salt; L-2-Aminopentanedioic acid potassium salt; (*S*)-2-Aminopentanedioic acid
[6382-01-0] $C_5H_8KNO_4 \cdot H_2O$ FW 203.23

Agonist at kainate, NMDA, and quisqualate receptors; an excitatory amino acid neurotransmitter.

BioReagent, suitable for insect cell culture, ≥99% (TLC)

ship: ambient store at: room temp

G1149-100G	100 g
G1149-500G	500 g

L-Glutamine Solution 200 mM NEW

L-Glutamine solution
[56-85-9]

29.23 mg/mL in saline, solution, suitable for cell culture

for research or for further manufacturing use

ship: dry ice store at: −20°C

59202C-100ML	100 mL
59202C-500ML	500 mL

L-Glutathione oxidized disodium salt

GSSG
(γ-Glu-Cys-Gly) (γ-Glu-Cys-Gly) [Disulfide bridge: 2_a-2_b] [103239-24-3]
$C_{20}H_{30}N_6Na_2O_{12}S_2$ FW 656.59

BioReagent, suitable for cell culture

For use in cell culture applications as an antioxidant and heavy metal detoxification agent.

≥98%

ethanol ..... essentially free

ship: ambient store at: −20°C

G2140-250MG	250 mg
G2140-1G	1 g
G2140-5G	5 g

Guanosine

9-(β-D-Ribofuranosyl)guanine; Guanine-9-β-D-ribofuranoside
[118-00-3] $C_{10}H_{13}N_5O_5$ FW 283.24

BioReagent, suitable for cell culture

For use in cell culture applications as a precursor of GMP, GDP, GTP and cGMP.

≥98%

ship: ambient store at: room temp

G6264-1G	1 g
G6264-5G	5 g
G6264-25G	25 g

Halocarbon oil 27

[9002-83-9]

ship: ambient store at: room temp

H8773-100ML	100 mL

Halocarbon oil 700

[9002-83-9]

ship: ambient store at: room temp

H8898-50ML	50 mL
H8898-100ML	100 mL

Heparin sodium salt from porcine intestinal mucosa

[9041-08-1]

Grade I-A, activity: ≥180 USP units/mg, powder, BioReagent, suitable for cell culture

solubility
H_2O ..... 50 mg/mL

ship: ambient store at: room temp

H3149-10KU	10000 units
H3149-25KU	25000 units
H3149-50KU	50000 units
H3149-100KU	100000 units
H3149-250KU	250000 units
H3149-500KU	500000 units
H3149-1MU	1000000 units

Hydrochloric acid solution

[7647-01-0] HCl FW 36.46

1.0 N, BioReagent, suitable for cell culture

sterile-filtered

ship: ambient store at: room temp

H9892-100ML	100 mL

Iron(III) nitrate nonahydrate

Ferric nitrate nonahydrate
[7782-61-8] $Fe(NO_3)_3 \cdot 9H_2O$ $FeN_3O_9 \cdot 9H_2O$ FW 404.00

BioReagent, suitable for cell culture

Iron(II) sulfate heptahydrate is used in cell culture applications generally bound to transferrin, citrate or other iron transport molecules.

ship: ambient store at: room temp

F8508-100G	100 g

Iron(III) phosphate

Ferric phosphate
[58782-48-2] $FePO_4$ FeO_4P FW 150.82

BioReagent, suitable for insect cell culture

Compound is a mixture of ferric phosphate and sodium citrate.

Iron(II) sulfate heptahydrate is used in cell culture applications generally bound to transferrin, citrate or other iron transport molecules.

ship: ambient store at: room temp

F1523-500G	500 g

Iron(II) sulfate heptahydrate

Ferrous sulfate heptahydrate
[7782-63-0] $FeSO_4 \cdot 7H_2O$ $FeO_4S \cdot 7H_2O$ FW 278.01

density ..... 1.898 g/mL, 25 °C
vp ..... 14.6 mmHg (25 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

Iron(II) sulfate heptahydrate is used in cell culture applications generally bound to transferrin, citrate or other iron transport molecules.

ship: ambient store at: room temp

F8633-250G	250 g
F8633-1KG	1 kg

Lactalbumin enzymatic hydrolysate

Lactalbumin Hydrolysate Solution 50X

liquid, cell culture tested, insect cell culture tested NEW

Prepared with 166.5 g/L of lactalbumin enzymatic hydrolysate (LAH) in cell culture grade water. LAH is specifically formulated for use with insect cell culture media.

For research or for further manufacturing use

ship: ambient store at: 2-8°C

58901C-100ML	100 mL

powder, BioReagent, suitable for cell culture

Use as an amino acid, peptide rich supplement in mammalian and microbial cell culture applications.

nitrogen analysis ..... ~6.25% amino, ≥11.0% total

ship: ambient store at: 2-8°C

L9010-500G	500 g
L9010-1KG	1 kg

Lactoferrin from human milk

Lactoferrin is an iron binding protein. It is structurally similar to transferrin, the plasma iron transport protein; but lactoferrin has a much higher affinity for iron (250 fold). It is very abundant in colostrum and small amounts can also be found in tears, saliva, mucous secretions and in the secondary granules of neutrophils. It is made by mucosal epithelium and neutrophils and is released by these cells in response to inflammatory stimuli. Bacterial growth is inhibited by its ability to sequester iron and also permeabilize bacterial cell walls by binding to lipopolysaccharides through its N-terminus. Lactoferrin can inhibit viral infection by binding tightly to the viral envelope protein. This prevents cell-virus fusion by blocking the binding domain. Lactoferrin appears to activate host defense systems in part by stimulating the release of interleukin-8, a neutrophil activator. It may also be involved in antibody and interleukin synthesis, lymphocyte proliferation and complement activation.

mol wt ~90 kDa

▸ powder, BioReagent, suitable for cell culture

Associated gene(s): LTF (4057)

~90% (SDS-PAGE)

solubility
H_2O ..... 1 mg/mL
color ..... pink
ship: ambient store at: 2-8°C

L4894-5MG	5 mg
L4894-25MG	25 mg
L4894-100MG	100 mg

α-Lactose monohydrate

4-O-β-D-Galactopyranosyl-α-D-glucose; β-D-Gal-(1→4)-α-D-Glc; Milk sugar
[5989-81-1] $C_{12}H_{22}O_{11} \cdot H_2O$ FW 360.31

▸ BioReagent, suitable for cell culture

β-lactose ..... ≤4%
Glucose ..... ≤0.05%
ship: ambient store at: room temp

L2643-500G	500 g
L2643-5KG	5 kg

Lectin from *Phaseolus vulgaris* (red kidney bean)

Phaseolus vulgaris agglutinin; PHA

PHA consists of two molecular species, an erythroagglutinin (PHA-E) which has low mitogenic activity and high erythroagglutinin activity, and leucoagglutinin (PHA-L) which has high mitogenic and leucoagglutinating activity, but very low erythroagglutinating activity.[1,2]
PHA-E is not blood group specific, but agglutination can be inhibited by certain oligosaccharides.[3] PHA-P is the protein form of PHA prior to separation and purification of erythroagglutinin and leucoagglutinin.[4] PHA-M is the mucoprotein form.[4] Conjugates are prepared from the corresponding purified lectins.

Lit. cited: 1. Yachnin, A. and Svenson, R.H., *Immunology* **22**, 871 (1972)
2. Felsted, et al., *J. Biol. Chem.* **252**, 2967 (1977)
3. Kornfeld, R. and Kornfeld, S., *J. Biol. Chem.* **245**, 2536 (1970)
4. Rigas, D.A. and Osgood, E.E., *J. Biol. Chem.* **212**, 607 (1955)

▸ Phytohemagglutinin PHA-P, lyophilized powder (contains buffer salts and NaCl), BioReagent, suitable for cell culture

mitogenic activity: ≤10 µg per mL

Agglutination activity is expressed in µg/ml and is determined from serial dilutions of a 1 mg/ml solution. This activity is the lowest concentration to agglutinate a suspension of 2% human erythrocytes in phosphate buffered saline, pH 6.8 after 1 hr incubation at 25°C. Mitogenic activity is determined by BrdU incorporation in HeLa cell cultures.

solubility
PBS ..... 5 mg/mL, clear to slightly hazy, colorless to faintly yellow (pH 6.8)
color ..... white to off-white
ship: ambient store at: 2-8°C

L1668-5MG	5 mg

▸ Phytohemagglutinin PHA-M, lyophilized powder, BioReagent, suitable for cell culture

agglutination activity: <40 µg per mL

Agglutination activity is expressed in µg/mL and is determined from serial dilutions of a 1 mg/mL solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes in phosphate buffered saline, pH 6.8, after 1 hr incubation at 25 °C.

salt ..... free
ship: ambient store at: 2-8°C

L8902-5MG	5 mg
L8902-25MG	25 mg
L8902-100MG	100 mg

▸ Leucoagglutinin PHA-L, lyophilized powder, BioReagent, suitable for cell culture

leucocyte agglutination activity tested

agglutination activity: ≤1.0 µg per mL

mitogenic activity: ≤5 µg per mL

Agglutination activity is expressed in µg/ml and is determined from serial dilutions of a 1 mg/ml solution. This activity is the lowest concentration to agglutinate a suspension of either human erythrocytes (2% in phosphate buffered saline, pH 6.8) or human leukocytes (10^7 per mL in saline) after 1 hr incubation at 25 °C.

salt ..... free
ship: ambient store at: 2-8°C

L4144-5MG	5 mg

Lectin from *Phytolacca americana* (pokeweed)

Pokeweed mitogen; PWM

PWM is extracted from pokeweed roots. This protein has hemagglutinating, leukoagglutinating, and mitogenic properties.[1] PWM is not blood group specific, but has affinity for N-acetyl-β-D-glucosamine oligomers.[1]

Mitogenic activity was determined by BrdU incorporation in HeLa cell cultures.

Lit. cited: 1. Yokoyama, K., et al., *Biochim. Biophys. Acta* **538**, 384 (1978)

▸ lyophilized powder, BioReagent, suitable for cell culture

[63231-57-2]

Partially purified TCA precipitate.
Essentially salt-free.

aseptically filled

mitogenic activity: <5 µg per mL
ship: ambient store at: 2-8°C

L8777-5MG	5 mg
L8777-10MG	10 mg

Lectins

Lectin from *Canavalia ensiformis* (Cat. Nos. C5275, C0412)

CONCANAVALIN A
Concanavalin A is supplied as a highly purified, essentially salt-free, aseptically-filled, lyophilized powder packaged in a 5 ml amber serum vial containing 5 mg. To reconstitute, add 1.0 ml sterile phosphate buffered saline or tissue culture medium to the vial and gently rotate. Solution may appear hazy. Reconstituted product may be further diluted to desired working concentration using sterile buffer prior to use. Filtration should be avoided to prevent any product loss.

Lectin from *Phasolus vulgaris* (Cat. Nos. L4144, L9132)

LEUCOAGGLUTININ (PHA-L), PHYTOHEMAGGLUTININ (PHA-P)
PHA-P and PHA-L are supplied as essentially salt-free, aseptically-filled, lyophilized powders at 5 mg per vial. To reconstitute, add 1.0 ml Dulbecco's Phosphate Buffered Saline or tissue culture medium to the vial and gently rotate. Solution may appear slightly hazy. Reconstituted product may be further diluted to desired working concentration using sterile buffer prior to use. Filtration should be avoided to prevent any product loss.

Cat. No.	Lectin	Mol. Wt. (kDa)	Subunits	Specificity Blood Group	Specificity Sugar	Mitogenic Activity
L5640	**Agaricus bisporus**	58.5	–	–	β-gal(1→3)galNAc	
L0881	**Arachis hypogaea**	120	4	T	β-gal(1→3)galNAc	
L6135	Biotin					
L7381	FITC					
L7759	Peroxidase					
L3766	TRITC					
L2507	Agarose					
L3515	**Artocarpus integrifolia**	42	4	T	α-gal→OMe	(+)
L5147	Agarose					
	Bandeiraea simplicifolia					
L2380	BS-I	114	4	A,B	α-gal, α-galNAc	
L3759	Biotin					
L9381	FITC					
L5264	TRITC					
	BS-1-A_4	114	4	A	α-gal, α-galNAc	
L0890	FITC					
L3019	BS-I-B_4	114	4	B	α-gal	
L2140	Biotin					
L2895	FITC					
L5391	Peroxidase					
	Caragana arborescens	60; 120[g]	2;4	–	galNAc	
L9637	Biotin					
L3141	**Cicer arietinum**	44	2	–	fetuin	
L2638	**Codium fragile**	60	4	–	galNAc	
C7275	**Concanavalin A**	102	4	–	α-man, α-glc	(+)
C2272	Biotin					
C7898	Ferritin					
C7642	FITC					
L5021	Gold, 10 nm					
L3642	Gold, 20 nm					
L6397	Peroxidase					
C6904	Agarose					
C9017	Sepharose®					

Cat. No.	Lectin	Mol. Wt. (kDa)	Subunits	Specificity Blood Group	Specificity Sugar	Mitogenic Activity
L3885	**Succinyl-Concanavalin A**	51	2	–	α-man, α-glc	(+)[b]
L9385	FITC					
L2766	**Datura stramonium**	86	2(αβ)[a]	–	$(glcNAc)_2$	
L2785	**Dolichos biflorus**	140	4	A^1	α-galNAc	
L6533	Biotin					
L9142	FITC					
L1287	Peroxidase					
L9658	TRITC					
L9894	Agarose					
L5390	**Erythrina cristagalli**	56.8	2(αβ)[a]	–	β-gal(1→4)glcNAc	
L8275	**Galanthus nivalis**	52	4	Rabbit[c]	non-reduc. α-man	
L8775	Agarose					
L1395	**Glycine max**	110	4	–	galNAc	(+)[d]
L2650	Peroxidase					
L4511	TRITC					
L6635	**Helix aspersa**	79	–	A	galNAc	
L8764	Biotin					
L3382	**Helix pomatia**	79	6	A	galNAc	
L6512	Biotin					
L1034	FITC					
L6387	Peroxidase					
L1261	TRITC					
L8639	Agarose					
L9637	**Lens culinaris**	49	2	–	α-man	
L4143	Biotin					
L9262	FITC					
L4018	Agarose					
L0511	Sepharose®					
L2263	**Limulus polyphemus**	400	18	–	NeuNAc	
L2886	**Lycopersicon esculentum**	71	–	–	$(glcNAc)_3$	(-)[e]
L0651	Biotin					
L0401	FITC					
L8025	**Maackia amurensis**	130	2(αβ)	O	sialic acid	(+)
	Phaseolus coccineus	112	4	–	–	
L4389	TRITC					
	Phaseolus vulgaris					
L8629	PHA-E	128	4	–	oligosaccharide	(+)
L6139	TRITC					
L2769	PHA-L	126	4	–	oligosaccharide	(+)
L8754	PHA-P					
L2646	PHA-M					
L9379	**Phytolacca americana**	32[f]	–	–	$(glcNAc)_3$	(+)
L5380	**Pisum sativum**	49	4(αβ)[a]	–	α-man	(+)
L0770	FITC					
L9895	**Pseudomonas aeruginosa**	13-13.7	–	–	gal	(+)[d]
	Psophocarpus tetragonolobus	35	1	–	galNAc, gal	
L3014	Biotin					
L3264	FITC					
L3139	Peroxidase					
L3389	TRITC					
	Ricinus communis					
L7886	Agglutinin, RCA_{120}	120	4	–	β-gal	
L2390	Agarose					
L9514	Ricin, A chain					
L4022	Ricin, A chain, deglycosylated					
L6890	**Sambucus nigra**	140	4(αβ)[a]	–	$\alpha NeuNA_c(2\rightarrow6)gal/galNA_c$	(+)[d]
L4266	**Solanum tuberosum**	50; 100[g]	1;2	–	$(glcNA_c)_3$	

Cat. No.	Lectin	Mol. Wt. (kDa)	Subunits	Specificity Blood Group	Specificity Sugar	Mitogenic Activity
L9254	**Tetragonolobus purpureas**	120(A), 58(B), 117(C)	4;2;4	H	α-L-fuc	
L3134	Biotin					
L9640	**Triticum vulgaris**	36	2	–	$(glcNAc)_2$, NeuNAc	(+)
L5142	Biotin					
L9884	Evans Blue					
L4895	FITC					
L1894	Gold, 10 nm					
L3892	Peroxidase					
L5266	TRITC					
L1882	Agarose					
	Ulex europaeus					
L5505	UEA I	68	–	H	α-L-fuc	
L8262	Biotin					
L9006	FITC					
L8146	Peroxidase					
L4889	TRITC					
	Vicia villosa	139	4[a]	A_1+T_n	galNAc	
L9388	Agarose					
L7513	Isolectin B_4	143	4	T_n	galNAc	
L2662	**Viscum album**	115[h]	4(αβ)[a]	–	β-gal	
L8258	**Wisteria floribunda**	68	2	–	galNAc	
L1516	Biotin					
L1641	FITC					
L2016	Reduced	34	1	(150)	galNAc	
L1766	Biotin					

Notes:

a Subunits are of different molecular weights
b Non-agglutinating and mitogenic
c Agglutinates rabbit, but not human, erythrocytes
d Mitogenic for neuraminidase-treated lymphocytes
e Inhibits mitogenic activity of PHA
f Data given for PWM Pa2
g Concentration-dependent mol. wt. change
h Data given for VAA (I)

Lipopolysaccharides (LPS)(Cat. Nos. L2654, L4391, L4516, L4641, L6529, L7770, L6143, L7895)

Product Description

LPS is a major constituent of the cell wall of most Gram-negative bacteria. It is a highly immunogenic antigen with the ability to enhance immune responses to soluble antigens.[1,2] LPS also acts as a specific mitogen for bone marrow derived B lymphocytes from mice,[3] rabbits,[4] chickens,[5] cows,[6] hamsters,[7] and humans.[8]

Recommended Usage

Lipopolysaccharides are supplied as lyophilized, g-irradiated powders. To reconstitute, add 1 ml sterile balanced salt solution or tissue culture medium to the vial (1 mg) and gently swirl until the powder dissolves. Reconstituted product may be further diluted to desired working concentrations using sterile balanced salt solution or tissue culture medium.

Product Storage

Prior to reconstitution vial should be stored at 2-8 °C. After reconstitution aliquots may be stored frozen at -20 °C. Repeated freezing and thawing are not recommended.

NOTE: Lipopolysaccharide compounds are highly pyrogenic. Avoid inhalation of any LPS and prevent these compounds from entering the bloodstream.

References

1. Landy, M. and Baker, P., *J. Immunol.*, **97,** 670, 1966.
2. Skidmore, B. et al., *J. Immunol.*, **144,** 770, 1975.
3. Andersson, J. et al., *Cellular Immunol.*, **4,** 381, 1972.
4. Shek, P. et al., *Int. Arch. Allergy Appl. Immunol.*, **46,** 753, 1974.
5. Weber, W., *Transpl. Rev.*, **24,** 113, 1975.
6. Hammarstrom, L., *Nature,* **263,** 60, 1976.
7. Streilein, J. and Hart, D., *Infect. Immun.*, **14,** 463, 1976.
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Lipopolysaccharides from *Escherichia coli* 0111:B4

γ-irradiated, BioXtra, suitable for cell culture

LPS is a major constituent of the cell wall of most gram negative bacteria. It is a highly immunogenic antigen with the ability to enhance immune responses to soluble antigens. LPS also acts as a specific mitogen for bone marrow derived B lymphocytes from mice, rabbits, chickens, cows, hamsters, and humans.

purified by gel-filtration chromatography

lyophilized powder

Lipopolysaccharides are supplied as lyophilized, γ-irradiated powders. To reconstitute, add 1 ml sterile balanced salt solution or tissue culture medium to the vial (1 mg) and gently swirl until the powder dissolves. Reconstituted product may be further diluted to desired working concentrations using sterile balanced salt solution or tissue culture medium.

solubility
H_2O ..... 5 mg/mL, slightly hazy
Protein ..... <1% (Lowry)

Lit. cited: 1. Landy, M. and Baker, P., J., *Immunology* **97**, 670 (1966)
2. Skidmore, B., et al., *Immunology* **114**, 770 (1975)

ship: ambient store at: 2-8°C

L4391-1MG	1 mg

Lipopolysaccharides from *Escherichia coli* 026:B6

γ-irradiated, BioXtra, suitable for cell culture

LPS is a major constituent of the cell wall of most gram negative bacteria. It is a highly immunogenic antigen with the ability to enhance immune responses to soluble antigens.[1,2]

purified by gel-filtration chromatography

lyophilized powder

Lipopolysaccharides are supplied as lyophilized, γ-irradiated powders. To reconstitute, add 1 ml sterile balanced salt solution or tissue culture medium to the vial (1 mg) and gently swirl until the powder dissolves. Reconstituted product may be further diluted to desired working concentrations using sterile balanced salt solution or tissue culture medium.

solubility
H_2O ..... 5 mg/mL, slightly hazy
Protein ..... <5% (Lowry)

Lit. cited: 1. *Immunology* **97**, 670 (1966)
2. Skidmore, B., et al.,, *Immunology* **114**, 770 (1975)

ship: ambient store at: 2-8°C

L2654-1MG	1 mg

Lipopolysaccharides from *Escherichia coli* 055:B5

γ-irradiated, BioXtra, suitable for cell culture

LPS is a major constituent of the cell wall of most gram negative bacteria. It is a highly immunogenic antigen with the ability to enhance immune responses to soluble antigens.[1,2]

purified by gel-filtration chromatography

lyophilized powder

Lipopolysaccharides are supplied as lyophilized, γ-irradiated powders. To reconstitute, add 1 ml sterile balanced salt solution or tissue culture medium to the vial (1 mg) and gently swirl until the powder dissolves. Reconstituted product may be further diluted to desired working concentrations using sterile balanced salt solution or tissue culture medium.

solubility
H_2O ..... 5 mg/mL, slightly hazy
Protein ..... <3% (Lowry)

Lit. cited: 1. Landy, M. and Baker, P., , *Immunology* **97**, 670 (1966)
2. Skidmore, B., et al.,, *Immunology* **114**, 770 (1975)

ship: ambient store at: 2-8°C

L6529-1MG	1 mg

Lipopolysaccharides from *Salmonella enterica* serotype enteritidis

γ-irradiated, BioXtra, suitable for cell culture

Lipopolysaccharides is used in cell culture and cell binding applications.

purified by gel-filtration chromatography

lyophilized powder

Protein ..... <1% (Lowry)

ship: ambient store at: 2-8°C

L7770-1MG	1 mg

Lipopolysaccharides from *Salmonella enterica* serotype minnesota

γ-irradiated, BioXtra, suitable for cell culture

Lipopolysaccharides is used in cell culture and cell binding applications.

purified by gel-filtration chromatography

lyophilized powder

Protein ..... <1% (Lowry)

ship: ambient store at: 2-8°C

L4641-1MG	1 mg

Lipopolysaccharides from *Salmonella typhosa*

γ-irradiated, BioXtra, suitable for cell culture

Lipopolysaccharides is used in cell culture and cell binding applications.

purified by gel-filtration chromatography

lyophilized powder

Protein ..... <1% (Lowry)

ship: ambient store at: 2-8°C

L7895-1MG	1 mg

Manganese sulfate monohydrate

Manganese(II) sulfate monohydrate
[10034-96-5] $MnSO_4 \cdot H_2O$ $MnO_4S \cdot H_2O$ FW 169.02

meets USP testing specifications

ship: ambient store at: room temp

M8179-100G	100 g
M8179-500G	500 g

Manganese(II) chloride tetrahydrate

[13446-34-9] $MnCl_2 \cdot 4H_2O$ $Cl_2Mn \cdot 4H_2O$ FW 197.91

BioReagent, suitable for insect cell culture

ship: ambient store at: room temp

M5005-100G	100 g

Manganese(II) sulfate monohydrate

[10034-96-5] $MnSO_4 \cdot H_2O$ $MnO_4S \cdot H_2O$ FW 169.02

BioReagent, suitable for cell culture

ship: ambient store at: room temp

M1144-100G	100 g

Melanin

[8049-97-6]

Synthetic, BioReagent, suitable for cell culture

Melanin may be useful as a photoprotectant in cell culture.

Prepared by oxidation of tyrosine with hydrogen peroxide.

ship: ambient store at: –20°C

M0418-100MG	100 mg
M0418-1G	1 g
M0418-5G	5 g

2-Mercaptoethanol

Thioethylene glycol; 2-Hydroxyethylmercaptan; BME; β-Mercaptoethanol
[60-24-2] $HSCH_2CH_2OH$ C_2H_6OS FW 78.13

BME is suitable for reducing protein disulfide bonds prior to polyacrylamide gel electrophoresis and is usually included in a sample buffer for SDS-PAGE at a concentration of 5%. Cleaving intermolecular (between subunits) disulfide bonds allows the subunits of a protein to separate independently on SDS-PAGE. Cleaving intramolecular (within subunit) disulfide bonds allows the subunits to become completely denatured so that each peptide migrates according to its chain length with no influence due to secondary structure.

concentration ..... 14.3 M (pure liquid)
density ..... 1.114 g/mL, 25 °C
vp ..... 1 mmHg (20 °C)
n_D^{20} 1.500
lel 18%
vd ..... 2.69 (vs air)

liquid, BioReagent, suitable for cell culture

solubility
H_2O ..... 1 mL/mL

ship: ambient store at: room temp

M7522-100ML	100 mL
M7522-250ML	250 mL
M7522-500ML	500 mL

Methyl cellulose

[9004-67-5]

viscosity 15 cP, 2% in H_2O (25 °C)(lit.), BioReagent

Use to create semi-solid matrices, as a cell culture gel, for culture of colony forming cells such as CFU-G; CFU-GM; cells and other cells used in toxicity assays. Low viscosity, not for plaquing assays or cloning.

suitable for cell culture

ship: ambient store at: room temp

M7027-100G	100 g
M7027-250G	250 g

Methyl 4-hydroxybenzoate

Methyl paraben; *p*-Hydroxybenzoic acid methyl ester
[99-76-3] $HOC_6H_4CO_2CH_3$ $C_8H_8O_3$ FW 152.15

BioReagent, suitable for insect cell culture

Use in studies on sterol hormones such as testerone biosynthesis.

ship: ambient store at: room temp

H3647-100G	100 g
H3647-1KG	1 kg

N1 Medium Supplement (100×)

solution, sterile-filtered, suitable for cell culture

Contains 0.5 mg/ml insulin from bovine pancreas, 0.5 mg/ml human transferrin (partially iron-saturated), 0.5 µg/ml sodium selenite, 1.6 mg/ml putrescine, and 0.73 µg/ml progesterone.

Use as a supplement to produce complete N1 neural cell growth media or closely related media.

Prepared in Earle's Balanced Salt Solution (EBSS) without phenol red.

endotoxin ..... tested

ship: ambient store at: 2-8°C

N6530-5ML	5 mL

Nickel(II) chloride hexahydrate

[7791-20-0] $NiCl_2 \cdot 6H_2O$ $Cl_2Ni \cdot 6H_2O$ FW 237.69

BioReagent, suitable for cell culture

ship: ambient store at: room temp

N6136-100G	100 g
N6136-500G	500 g

β-Nicotinamide adenine dinucleotide phosphate sodium salt hydrate

Triphosphopyridine nucleotide sodium salt hydrate
[698999-85-8] $C_{21}H_{27}N_7NaO_{17}P_3 \cdot xH_2O$ FW 765.39 (Anh)

Electron acceptor

Packaged by solid weight.

This is the common form of NADP. Do not confuse with 3'–NADP or α-NADP.

powder, BioReagent, suitable for cell culture

≥98% (HPLC)

≥98% (spectrophotometric assay)

Meets or exceeds NRC specifications and is comparable to any commercial preparation available.

solubility
H_2O ..... 50 mg/mL

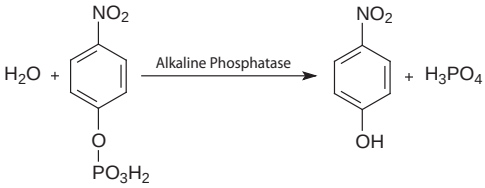
ship: ambient store at: –20°C

N3139-100MG	100 mg
N3139-250MG	250 mg
N3139-1G	1 g

4-Nitrophenyl phosphate disodium salt hexahydrate

p-Nitrophenyl phosphate disodium hexahydrate; pNPP disodium hexahydrate; Disodium 4-nitrophenyl phosphate hexahydrate; di-Sodium 4-nitrophenyl phosphate
[333338-18-4] $O_2NC_6H_4OP(O)(ONa)_2 \cdot 6H_2O$ $C_6H_4NNa_2O_6P \cdot 6H_2O$ FW 371.14

Chromogenic substrate for the determination of acid and alkaline phosphatases.[1,2] The reaction produces a soluble end product that is yellow in color. The reaction may be stopped with 3 N NaOH and read at 405 nm.



pNPP substrates develop the yellow end-product, p-nitrophenol, when hydrolyzed by alkaline phosphatase. Since this system forms a soluble end product, it is not recommended for blotting or histochemistry.

Lit. cited: 1. J.-P. Bretaudiere and T. Spillman, H.U. Bergmeyer, ed., *Meth. Enzym. Anal.* 3rd ed., Weinheim **4**, 75 (1984)
2. O.H. Lowry, *Meth. Enzymol.* **4**, 366 (1957)

powder, BioReagent, suitable for cell culture, ≥97%
solubility
H_2O ..... 100 mg/mL
ship: ambient store at: –20°C

N4645-1G	1 g
N4645-5G	5 g

Oxaloacetic acid

Oxobutanedioic acid; 2-Oxosuccinic acid; Oxalacetic acid; Ketosuccinic acid
[328-42-7] $HOOCCH_2COCOOH$ $C_4H_4O_5$ FW 132.07

≥97%
ship: ambient store at: –20°C

O4126-1G	1 g
O4126-5G	5 g
O4126-25G	25 g
O4126-100G	100 g

powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥97%

Use as a TCA (Krebs cycle) intermediate supplement in insect cell culture applications. Enhances insect cell growth and productivity.
solubility
H_2O ..... 100 mg/mL
ship: ambient store at: –20°C

O7753-5G	5 g
O7753-25G	25 g
O7753-100G	100 g

Hybri-Max™, powder, suitable for hybridoma

Use as a TCA (Krebs cycle) intermediate supplement in hybridoma cell culture applications. Enhances hybridoma growth and productivity.
solubility
H_2O ..... 100 mg/mL
endotoxin ..... tested
ship: ambient store at: –20°C

O9504-5G	5 g
O9504-25G	25 g

Peptone from animal tissue

[73049-73-7]

from meat, powder, suitable for cell culture

Enzymatic hydrolysate

from
solubility
H_2O ..... 50 mg/mL
ship: ambient store at: room temp

P5905-100G	100 g
P5905-500G	500 g
P5905-1KG	1 kg

L-α-Phosphatidylcholine

L-α-Lecithin; 1,2-Diacyl-*sn*-glycero-3-phosphocholine; 3-*sn*-Phosphatidylcholine; Azolectin
[8002-43-5]

A major structural phospholipid in brain, comprising approx. 15% of total lipid; primarily localized to gray matter.

solution, from soybean, Type III-S, suitable for cell culture

≥98%

Prepared Chromatographically.
Prepared by a modification of the procedure of Singleton, W.S., et al., J. Am. Oil Chem. Soc., 42, 53 (1965).
concentration ..... 100 mg/mL in chloroform
ship: ambient store at: –20°C

P3782-50MG	50 mg
P3782-100MG	100 mg
P3782-500MG	500 mg
P3782-1G	1 g

Poly(2-hydroxyethyl methacrylate) [Poly-HEMA] (Cat. No. P3932)

Product Description

Poly(2-hydroxyethyl methacrylate) may be used as a surface coating agent to reduce or eliminate adhesion of cells to growth surfaces. This may be applicable when it is necessary to maintain cells as suspension cultures.

Product Use

1) Prepare a stock solution by dissolving the product in 95% ethanol at 120 mg/ml with vigorous shaking. Incubate overnight at 37 °C to enhance solubility. If any undissolved material remains, centrifuge at 2500 rpm for 30 minutes.
2) Dilute stock solution with 95% ethanol to produce coatings of varying thicknesses. Thicker coats yield greater attachment inhibition. Optimal attachment inhibition must be determined by the investigator.
3) Coat vessels with 0.1 ml/cm2 of surface and allow to air dry in a sterile environment. Vessels are ready to use.

References

1. Folkman, J. and Moscona, A., *Nature* **273**, 345-349, 1978.

Poly(2-hydroxyethyl methacrylate)

Poly(2-HEMA); Poly-HEMA
[25249-16-5] $(C_6H_{10}O_3)_n$

Water-swellable polymer. Hydrogel.

density ..... 1.15 g/mL, 25 °C

BioReagent, powder, suitable for cell culture

Used to inhibit cell adhesion to growth surfaces in culture vessels.

solubility

ethanol ..... 120 mg/mL

ship: ambient store at: room temp

P3932-10G	10 g
P3932-25G	25 g

Potassium chloride solution

[7447-40-7] KCl ClK FW 74.55

0.075 M, sterile-filtered, BioXtra, suitable for cell culture

Hypotonic solution used to enlarge cells for adequate spreading of metaphase chromosomes.

endotoxin ..... tested

ship: ambient store at: room temp

P9327-100ML	100 mL

Pristane

2,6,10,14-Tetramethylpentadecane
[1921-70-6] $(CH_3)_2CH(CH_2)_3CH(CH_3)(CH_2)_3CH(CH_3)(CH_2)_3CH(CH_3)_2$ $C_{19}H_{40}$
FW 268.52

density ..... 0.783 g/mL, 20 °C
n_D^{20} 1.438

from synthetic, liquid, sterile-filtered, BioReagent

Used to induce disease models for study including arthritis as a non-antigenic arthritogenic adjuvant and plasmacytomas.

Pristane is a natural saturated terpenoid alkane used in research on the pathogenesis of autoimmune diseases such as arthritis and lupus.

≥95%

Packaged in flame sealed ampules.

endotoxin ..... tested

ship: ambient store at: room temp

P9622-10X1ML	10 × 1 mL
P9622-5X5ML	5 × 5 mL

Propionic acid

Propanoic acid; Propanyl acid; Acid C_3
[79-09-4] CH_3CH_2COOH $C_3H_6O_2$ FW 74.08

density ..... 0.993 g/mL, 25 °C
n_D^{20} 1.386
vd ..... 2.55 (vs air)
vp ..... 2.4 mmHg (20 °C)
ait ..... 955 °F
lel 12.1%

BioReagent, suitable for insect cell culture, ~99%

Use in insect cell culture to inhibit mold growth.

density ..... 0.99 g/mL, 25 °C

ship: ambient store at: room temp

P5561-1L	1 L

Prostaglandin E_1

(11α,13E,15S)-11,15-Dihydroxy-9-oxoprost-13-enoic acid; PGE_1; Alprostadil
[745-65-3] $C_{20}H_{34}O_5$ FW 354.48

γ-irradiated, powder, BioXtra, suitable for cell culture

For use in cell culture applications for the study of prostaglandin regulated cell signaling and gene regulation.

solubility

ethanol ..... 1 mg/mL

ship: ambient store at: −20°C

P7527-1MG	1 mg

synthetic, powder, BioReagent, suitable for cell culture

Prostaglandin E1 may be used in cell culture to protect human umbilical vein endothelial cells (HUVEC) from injury induced by hydrogen peroxide.

≥98% (HPLC)

potency: 0.25-100 ng/mL

ship: ambient store at: −20°C

P8908-10MG	10 mg

Prostaglandin E_2

(5Z,11α,13E,15S)-11,15-Dihydroxy-9-oxoprosta-5,13-dienoic acid; PGE_2; Dinoprostone
[363-24-6] $C_{20}H_{32}O_5$ FW 352.47

Most biologically active prostaglandin. PGE_2 induces cervical ripening and parturition; mediates bradykinin-induced vasodilation; regulates adenylyl cyclase. Tumor cells that over-express cyclooxygenase 2 display increased invasiveness, angiogenesis, and resistance to apoptosis that may be due to the PGE_2-induced expression of angiogenic factors and stabilization of the anti-apoptotic protein, survivin.

powder -20 °C; stock-frozen in working aliquots, avoid repeated freeze/thaw

potency: 0.25-100 ng/mL

γ-irradiated, powder, BioXtra, suitable for cell culture

For use in cell culture applications for the study of prostaglandin regulated cell signaling and gene regulation.

solubility

ethanol ..... 1 mg/mL

ship: ambient store at: −20°C

P6532-1MG	1 mg

synthetic, powder, BioReagent, suitable for cell culture

≥99% (TLC)

ship: ambient store at: −20°C

P0409-1MG	1 mg
P0409-5MG	5 mg
P0409-10MG	10 mg

Putrescine dihydrochloride

1,4-Diaminobutane dihydrochloride; 1,4-Butanediamine dihydrochloride; Tetramethylenediamine dihydrochloride
[333-93-7] $NH_2(CH_2)_4NH_2 \cdot 2HCl$ $C_4H_{12}N_2 \cdot 2HCl$ FW 161.07

Binds to the polyamine modulatory site of the NMDA receptor and potentiates NMDA-induced currents; precursor of spermidine.

≥97% (TLC)

▸ powder, BioReagent, suitable for cell culture

solubility
H_2O ..... 100 mg/mL
ship: ambient store at: room temp

P5780-5G	5 g
P5780-25G	25 g

▸ γ-irradiated, lyophilized powder, BioXtra, suitable for cell culture

solubility
cell culture medium ..... 0.16 mg/mL
ship: ambient store at: room temp

P6024-1MG	1 mg

D-(+)-Raffinose pentahydrate

O-α-D-Galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside; Melitriose; Melitose
[17629-30-0] $C_{18}H_{32}O_{16} \cdot 5H_2O$ FW 594.51

▸ powder, BioReagent, suitable for cell culture

≥98.0%
solubility
H_2O ..... 50 mg/mL
ship: ambient store at: room temp

R7630-100G	100 g
R7630-500G	500 g

Select Yeast Extract

[8013-01-2]

Water soluble portion of autolyzed yeast with intact B-complex vitamins. Yeast extract is a mixture of amino acids, peptides, water soluble vitamins and carbohydrates and can be used as additive for culture media.

For general bacteriological use with a variety of microorganisms.

▸ powder, BioReagent, suitable for cell culture, suitable for insect cell culture

Spray dried, autolyzed yeast extract.
solubility
H_2O ..... 10% (remains clear after heating to 40°C.)
ship: ambient store at: room temp

Y1000-500G	500 g
Y1000-1KG	1 kg

▸ BioReagent, suitable for cell culture, powder

Spray dried, autolyzed yeast extract

May contain black specks.
solubility
H_2O ..... 10 mg/mL, hazy
ship: ambient store at: room temp

Y0500-500G	500 g
Y0500-1KG	1 kg

Silver nitrate

[7761-88-8] $AgNO_3$ FW 169.87

solid
vd ..... 5.8 (vs air)

▸ BioReagent, suitable for plant cell culture, >99% (titration)

Used with sodium thiosulfate to produce a silver thiosulfate solution (STS) containing the ethylene inhibitor ion $[Ag(S_2O_3)_2]^{-3}$.
ship: ambient store at: room temp

S7276-25G	25 g
S7276-100G	100 g

Sodium chloride solution NEW

[7647-14-5] NaCl ClNa FW 58.44

▸ 5 M

Prepared with 292.2 g/L of sodium chloride in tissue culture grade water.

292.2 g/L sodium chloride

for research or for further manufacturing use
ship: ambient store at: room temp

59222C-500ML	500 mL
59222C-1000ML	1000 mL

Sodium hydroxide solution

[1310-73-2] NaOH HNaO FW 40.00

vp ..... 3 mmHg (37 °C)

▸ 1.0 N, BioReagent, suitable for cell culture

Qualified for use as a general titration base for cell culture applications.

sterile-filtered
ship: ambient store at: room temp

S2770-100ML	100 mL

Sodium propionate

Propionic acid sodium salt
[137-40-6] CH_3CH_2COONa $C_3H_5NaO_2$ FW 96.06

▸ BioReagent, suitable for insect cell culture

Used as a mold inhibitor in insect cell culture applications.
ship: ambient store at: room temp

P5436-100G	100 g

Sodium pyruvate

α-Ketopropionic acid sodium salt; 2-Oxopropanoic acid sodium salt; Pyruvic acid sodium salt
[113-24-6] $CH_3COCOONa$ $C_3H_3NaO_3$ FW 110.04

▸ powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

solubility
H_2O ..... 100 mg/mL
ship: ambient store at: 2-8°C

P5280-25G	25 g
P5280-100G	100 g
P5280-500G	500 g

Sodium succinate dibasic hexahydrate

Succinic acid disodium salt; Butanedioic acid disodium salt
[6106-21-4] $NaOOCCH_2CH_2COONa \cdot 6H_2O$ $C_4H_4Na_2O_4 \cdot 6H_2O$ FW 270.14

BioReagent, suitable for cell culture, suitable for insect cell culture

Use as a TCA (Krebs cycle) supplement in cell culture applications.

ship: ambient store at: room temp

S9637-100G	100 g
S9637-500G	500 g

Soy Hydrolysate UF Solution 50X NEW

Soy protein acid hydrolysate liquid, sterile-filtered

For research or for further manufacturing use

Prepared with 250 g/L soy hydrolysate ultrafiltate in tissue culture grade water.

ship: ambient store at: room temp

58903C-100ML	100 mL

Succinic acid

Butanedioic acid
[110-15-6] $HOOCCH_2CH_2COOH$ $C_4H_6O_4$ FW 118.09

BioReagent, suitable for cell culture, suitable for insect cell culture

Use as a TCA (Krebs cycle) supplement in insect cell culture applications.

ship: ambient store at: room temp

S9512-100G	100 g
S9512-500G	500 g

1-Thioglycerol

α-Thioglycerol; 3-Mercapto-1,2-propanediol; α-Monothioglycerol
[96-27-5] $HSCH_2CH(OH)CH_2OH$ $C_3H_8O_2S$ FW 108.16

density 1.25 g/mL, 25 °C
n_D^{20} 1.527

liquid, BioReagent, suitable for cell culture, ≥97% (titration)

Cell culture studies of embryonic cortical and hippocampal neurons, mouse bone marrow mast cell lines, and human B cell lines have utilized 1-thioglycerol as a component of the culture medium to stimulate proliferation.

ship: ambient store at: 2-8°C

M6145-25ML	25 mL
M6145-100ML	100 mL
M6145-250ML	250 mL

Thymidine

dT; Thymine deoxyriboside; 1-(2-Deoxy-β-D-ribofuranosyl)thymine; 1-(2-Deoxy-β-D-ribofuranosyl)-5-methyluracil; 2′-Deoxythymidine
[50-89-5] $C_{10}H_{14}N_2O_5$ FW 242.23

powder, BioReagent, suitable for cell culture

Use in HT and HAT cocktails for hybridoma fusion, selection and cloning.

≥99%

ship: ambient store at: room temp

T1895-1G	1 g
T1895-5G	5 g
T1895-10G	10 g
T1895-25G	25 g

Thymine

2,4-Dihydroxy-5-methylpyrimidine; 5-Methyluracil
[65-71-4] $C_5H_6N_2O_2$ FW 126.11

BioReagent, suitable for cell culture

≥99% (HPLC)

ship: ambient store at: room temp

T0895-5G	5 g
T0895-25G	25 g

D-(+)-Trehalose dihydrate

α-D-Glucopyranosyl-α-D-glucopyranoside; α,α-Trehalose
[6138-23-4] $C_{12}H_{22}O_{11} \cdot 2H_2O$ FW 378.33

from *Saccharomyces cerevisiae*, powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99%

Use as a cryoprotectant in a variety of cell freezing media.

from *Saccharomyces cerevisiae*

solubility
H_2O 50 mg/mL

ship: ambient store at: room temp

T0167-10G	10 g
T0167-25G	25 g
T0167-100G	100 g

Uracil

2,4-Dihydroxypyrimidine; 2,4-Pyrimidinediol; 2,4(1*H*,3*H*)-Pyrimidinedione
[66-22-8] $C_4H_4N_2O_2$ FW 112.09

BioReagent, suitable for cell culture

ship: ambient store at: room temp

U1128-25G	25 g
U1128-100G	100 g

Urea

Carbamide; Carbonyldiamide
[57-13-6] NH_2CONH_2 CH_4N_2O FW 60.06

Used for the denaturation of proteins and as a mild solubilization agent for insoluble or denatured proteins. Useful for renaturing proteins from samples already denatured with 6 M guanidine chloride such as inclusion bodies. May be used with guanidine hydrochloride and dithiothreitrol (DTT) in the refolding of denatured proteins into their native or active form.

density 1.335 g/mL, 25 °C

BioReagent, suitable for cell culture

ship: ambient store at: room temp

U4128-5KG	5 kg

Uridine

Uracil-1-β-D-ribofuranoside; 1-β-D-Ribofuranosyluracil
[58-96-8] $C_9H_{12}N_2O_6$ FW 244.20

powder, BioReagent, suitable for cell culture

≥99%

solubility
H_2O 50 mg/mL

ship: ambient store at: room temp

U3003-5G	5 g
U3003-50G	50 g

Water

[7732-18-5] H_2O FW 18.02

density ..... 1.000 g/mL, 3.98 °C
n_D^{20} 1.34

sterile-filtered, BioReagent, suitable for cell culture

For use in the preparation of cell culture media, and cell suspension and washing solutions.

This product is a very low endotoxin (less than 0.1 eu/mL), 0.2 micron filtered.

endotoxin ..... ≤1.0 EU/mL
ship: ambient store at: room temp

W3500-100ML	100 mL
W3500-500ML	500 mL
W3500-6X500ML	6 × 500 mL
W3500-24X500ML	24 × 500 mL
W3500-1L	1 L
W3500-6X1L	6 × 1 L
W3500-2L	2 L

Water - Cell Culture Grade NEW

Water
[7732-18-5] H_2O FW 18.02

density ..... 1.000 g/mL, 3.98 °C
n_D^{20} 1.34

Meets all USP and EP criteria for Water for Injection (WFI). It has undergone purification via activated carbon, reverse osmosis, deionization, ultraviolet disinfection, and distillation. For use in the preparation of cell culture media, and cell suspension and washing solutions.

for research or for further manufacturing use

meets criteria for WFI, endotoxin $\leq$ 0.25 EU/mL
ship: ambient store at: room temp

59900C	

Yeast Extract Hy-Yest® 412

Select Yeast Extract
[8013-01-2]

Water soluble portion of autolyzed yeast with intact B-complex vitamins. Yeast extract is a mixture of amino acids, peptides, water soluble vitamins and carbohydrates and can be used as additive for culture media.

For general bacteriological use with a variety of microorganisms.

Hy-Yest® 412

Recommended for use when low salt conditions are required.

powder

nitrogen analysis ..... ~5.2% amino, ~10.7% total
ship: ambient store at: room temp

Y1001-250G	250 g
Y1001-1KG	1 kg

Yeast Extract Ultrafiltrate (50×)

liquid, BioReagent, suitable for insect cell culture

Prepared using 200 g/L yeast extract in deionized water. Ultrafiltered using a 10,000 dalton cutoff filter.

ship: ambient store at: 2-8°C

Y4375-100ML	100 mL

Zinc chloride

[7646-85-7] $ZnCl_2$ Cl_2Zn FW 136.30

vp ..... 1 mmHg (428 °C)

BioReagent, suitable for cell culture, suitable for insect cell culture
ship: ambient store at: room temp

Z0152-100G	100 g
Z0152-500G	500 g
Z0152-1KG	1 kg

Zinc sulfate heptahydrate

[7446-20-0] $ZnSO_4 \cdot 7H_2O$ $O_4SZn \cdot 7H_2O$ FW 287.56

BioReagent, suitable for cell culture
ship: ambient store at: room temp

Z0251-100G	100 g
Z0251-500G	500 g

ECACC Cell Lines

ECACC® Cell Lines

ECACC® and Sigma-Aldrich® Distribution Partnership

In recent years, Sigma-Aldrich has been successfully distributing ECACC (European Collection of Cell Cultures) cell lines within Europe. Customers have benefited from:

- The ease of ordering ECACC's cell lines locally
- A reliable and speedy delivery service
- The guarantee that the cell lines are authenticated and have undergone stringent quality control, including mycoplasma testing
- A local technical support service

ECACC and Sigma-Aldrich are pleased to announce that this service is now fully extended to the USA. Supply of over 1500 cell lines from ECACC, will be supplied from Sigma-Aldrich inventory held in St. Louis or on an "on demand basis". On demand products ship in less than three weeks. ECACC and Sigma-Aldrich believe that once again this emphasizes the synergy between the two organizations and demonstrates how they can work together to serve the cell culture community in a progressive manner.

ECACC Cell Line: Currently ECACC cell lines are supplied in the U.S., Canada, Mexico, Europe, Australia, New Zealand, India, Brazil, South Africa, China and Korea. Check your local office for availability in other countries.

Note: The volume per vial for ECACC cell lines is 1 mL, with a standard cell count of 3×10^6 cells/vial.

ECACC® Cell Line Collections

General Collection

Name	Description	Cat. No.
104C1	Guinea pig fetal cells transformed	90110524-1VL
108CC15	Mouse neuroblastoma x Rat glioma hybrid	08062516-1VL
108CC5	Mouse neuroblastoma x Rat glioma hybrid	08062521-1VL
108CC5-BU	Mouse neuroblastoma x Rat glioma hybrid	08062566-1VL
108CC5-BU-5	Mouse neuroblastoma x Rat glioma hybrid	08062567-1VL
108CC5-BU-8	Mouse neuroblastoma x Rat glioma hybrid	08062569-1VL
108CC5T-BU-1	Mouse neuroblastoma x Rat glioma hybrid	08062570-1VL
108CC5T-BU-4	Mouse neuroblastoma x Rat glioma hybrid	08062571-1VL
108CC5-TG-1	Mouse neuroblastoma x Rat glioma hybrid	08062543-1VL
108CC5-TG-2	Mouse neuroblastoma x Rat glioma hybrid	08062544-1VL
108CC5-TG-3	Mouse neuroblastoma x Rat glioma hybrid	08062545-1VL
108CC5-TG-4	Mouse neuroblastoma x Rat glioma hybrid	08062546-1VL
10T½	Mouse embryo fibroblast	99072801-1VL
1156QE	Human embryonal carcinoma	06072609-1VL
1184	Human skin fibroblast	90011883-1VL
1221	Human skin fibroblast, thioguanine resistant	90011873-1VL
1306	Human skin fibroblast	90011887-1VL
1411H	Human yolk sac carcinoma	06011805-1VL
142BR	Human skin fibroblast	90011806-1VL
143B	Human bone osteosarcoma TK-	91112502-1VL
149BR	Human skin fibroblast	90011807-1VL
153BR	Human skin fibroblast	90011808-1VL
155BR	Human skin fibroblast	90011809-1VL
161BR	Human skin fibroblast	90011810-1VL

Name	Description	Cat. No.
A 172	Human glioblastoma	88062428-1VL
174BR	Human skin fibroblast	90011895-1VL
175BR	Human skin fibroblast	90011863-1VL
1777N Rpmet	Human embryonal carcinoma	06011801-1VL
180BR	Human skin fibroblast	90011862-1VL
1BR3	Human skin fibroblast	90011801-1VL
1BR.3.G	Human skin fibroblast, transformed	90020507-1VL
1BR.3.GN	Human skin fibroblast, transformed	90020509-1VL
1BR.3.N	Human skin fibroblast, transformed	90020508-1VL
208F	Rat Fischer fibroblast	85103116-1VL
G-292 Clone A141B1	Human Caucasian osteosarcoma	90110522-1VL
293 GTP-AC-free	Human Embryo Kidney, serum-free	05011003-1VL
293-Hektor	Human Embryo Kidney, serum-free	05030204-1VL
293 N3S	Human embryo kidney	92052131-1VL
293-PERV-PK-CIRCE	Human embryo kidney, infected with endogenous retrovirus of pigs	97051411-1VL
293TGPRT+R1	Packaging cell line for HIV based vectors	04072121-1VL
293TGPRT+R1-A	Packaging cell line for HIV based vectors	04072120-1VL
311	Drosophila embryo	90070547-1VL
328/7	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062530-1VL
328/8	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062531-1VL
328/9	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062533-1VL
328/10	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062536-1VL

Name	Description	Cat. No.
328/11	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062538-1VL
328/12	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062540-1VL
328/14	Mouse neuroblastoma x mouse L cell fibroblast hybrid	08062541-1VL
3.334 SC5/8	Rat x Mouse hybrid	86082107-1VL
33B	Rat nervous tissue oligodendroglioma	85081901-1VL
G-361	Human Caucasian malignant melanoma	88030401-1VL
3T3(+3)	Mouse	89022402-1VL
3T3-F442A	Mouse pre-adipocytes	00070654-1VL
3T3-MSV	Mouse Swiss 3T3 embryo, Moloney-MSV transformed	90092701-1VL
3T3 Swiss Albino	Mouse Swiss Albino embryo fibroblast	85022108-1VL
3T6 Swiss Albino	Mouse Swiss Albino embryo fibroblast	86120801-1VL
G-401	Human Caucasian rhabdoid tumour (formerly classified as Wilm' s tumour)	87042204-1VL
G-402	Human Caucasian renal leiomyoblastoma	90112715-1VL
45.6.TG1.7	Mouse BALB/c myeloma	90110506-1VL
46Br.1G1	Human skin fibroblast, SV40 T antigen transformed	92091814-1VL
46BR.1N	Human skin fibroblast	92100623-1VL
577MF	Human forehead metastasis of malignant teratoma	06011802-1VL
59M	Human ovarian tumour epithelial	89081802-1VL
60H9(9)D10.E6	Human IgG1 secreting B cell	94111519-1VL
707B1011C3	Mouse DBA/2 Friend leukaemic lymphoblast	87120401-1VL
707.fl	Mouse friend Leukaemia	99072807-1VL
70Z/3	Mouse pre-B lymphocyte	91092002-1VL
745C2	Mouse erythroleukaemic lymphoblast	87030901-1VL
81.3	Human lymphoblastoid	85100105-1VL
8305C	Human thyroid anaplastic carcinoma, undifferentiated.	94090183-1VL
833KE	Human embryonal carcinoma	06072611-1VL
84BR	Human skin fibroblast	90011805-1VL
8505C	Human thyroid carcinoma, undifferentiated.	94090184-1VL
92BR	Donkey testis	90011874-1VL
A-72	Canine Golden Retriever tumour	89050908-1VL
A10-85	Rat glioma	93040117-1VL
A15	Rat, BDIX, glioma	93040110-1VL
A2	Hamster Chinese ovary	89030316-1VL
A2058	Human caucasian metastatic melanoma	91100402-1VL
A2H	Hamster Chinese ovary	85011441-1VL
A373-C6	Human malignant melanoma IL-1 sensitive	97011321-1VL
A6	South African clawed toad kidney	89072613-1VL
A673	Human rhabdomyosarcoma	85111504-1VL
A.704	Human kidney adenocarcinoma	93020513-1VL
A9	Mouse C3H/An areolar and adipose tissue	85011426-1VL
A9(Hamprecht)	Mouse Fibroblast (L cells)	08062526-1VL
A9HT	A9 tumour induced C3H mouse	88042804-1VL
AC2	Mouse lymphoblast	86061902-1VL

Name	Description	Cat. No.
ACHN	Human renal adenocarcinoma	88100508-1VL
Aedes aegypti	Mosquito larvae	87091801-1VL
AGLCL	Human Caucasian normal B cell	89120566-1VL
AK-D	Fetal cat lung normal	89071903-1VL
AKR-2B	Mouse, AKR, embryo	91061307-1VL
Ampli-GPE	Mouse NIH Swiss embryo, transfected with retroviral DNA	95050229-1VL
ANGM-CSS	Human glioblastoma	08040401-1VL
Anr4	Rat F344/Ducrj liver	90071808-1VL
AR42J	Rat exocrine pancreatic tumour	93100618-1VL
ARH 77	Human plasma cell leukaemia	88121201-1VL
ARL-6	Rat Wistar liver hepatoma	87050701-1VL
ARLJ 301-3	Rat F334/Ducrj liver	90071806-1VL
AsPC-1	Human pancreas adenocarcinoma ascites metastasis	96020930-1VL
AT-1	Rat Dunning R-3327,dorsal prostatic adenocarcinoma	94101449-1VL
AT-2.1	Rat prostatic cancer	94101450-1VL
AT-3.1	Rat dorsal prostatic adenocarcinoma	94101451-1VL
AT6.1	Rat dorsal prostatic adenocarcinoma	94101452-1VL
ATDC5	Mouse 129 teratocarcinoma AT805 derived	99072806-1VL
ATHOS	Porcine thyroid epithelial	93122325-1VL
AtT20	Mouse LAF1 pituitary gland tumour	87021902-1VL
AtT-20/D16v-F2	Mouse pituitary tumour	94050406-1VL
AV3 (HeLa derivative)	Human Negroid cervix carcinoma	88102402-1VL
B12	Rat nervous tissue glial	85042303-1VL
B2.Sp	Bovine spleen	90030906-1VL
B50	Rat nervous tissue neuronal	85042302-1VL
B65	Rat nervous tissue neuronal	85042305-1VL
B82	Mouse C3H/An sc areolar and adipose tissue	85011408-1VL
B82 (Hamprecht)	Mouse Fibroblast (L cells)	08062522-1VL
B92	Rat nervous tissue glial	85042304-1VL
BAE-1	Bovine aortic endothelium	88031149-1VL
K-BALB (K-234)	Mouse A31 embryo Kirsten sarcoma, transformed	89092101-1VL
BAOEC	Bovine lung endothelium	86123102-1VL
BB	Fish Brown Bullhead posterior trunk tissue	87101201-1VL
BC3H1	Mouse brain tumour	86093001-1VL
BCL1 Clone CW13.20-3B3	Mouse BALB/c B cell leukaemia	90061904-1VL
BE(2)-C	Human Caucasian neuroblastoma	95011817-1VL
BE10-7	Rat, BDIX, brain, pre-malignant	93040125-1VL
BE10-Intermediate	Rat, BDIX, fetal brain, pre-malignant	93040119-1VL
BE10-Late	Rat, BDIX, fetal brain, malignant	93040120-1VL
BE11 (Early)	Rat, BDIX, fetal brain	93040121-1VL
BE(2)-M17	Human Caucasian neuroblastoma	95011816-1VL
b.End5	Mouse Balb/c brain endothelioma	96091930-1VL
BF-2	Fish Bluegill fry caudal trunk	87032603-1VL
BFA	Bovine aorta endothelium fetal	87022601-1VL
BHK21C13-2P	Hamster Syrian kidney	84111301-1VL
BHK21C13-3P	Hamster Syrian kidney	84100501-1VL
BHK21-Hektor	Hamster Syrian kidney, serum-free	05062301-1VL
BHK21-InVitrus	Hamster Syrian kidney, serum-free	05062302-1VL
BHK 21 STRAIN 31	Hamster Syrian kidney	93120840-1VL

ECACC® Cell Line Collections: General Collection

Name	Description	Cat. No.
BHK 21 STRAIN 35	Hamster Syrian kidney	93120841-1VL
BHK 21 STRAIN 38	Hamster Syrian kidney	93120842-1VL
BHK/AC9	Hamster Syrian kidney	85040103-1VL
BHK 21 CL13(IZS)	Hamster Syrian kidney	93120815-1VL
BHK TK-	Hamster Syrian kidney	85011423-1VL
BHL-89	Human B-type EBNA negative lymphoma	98070109-1VL
BICR 3	Alveolus squamous cell carcinoma	06032203-1VL
BICR 6	Hypopharynx squamous cell carcinoma	05070501-1VL
BICR 10	Buccal mucosa squamous carcinoma	04072103-1VL
BICR 16	Tongue squamous carcinoma	06031001-1VL
BICR 18	Larynx squamous cell carcinoma (lymph node metastasis)	06051601-1VL
BICR 22	Tongue squamous carcinoma (lymph node metastasis)	04072106-1VL
BICR 31	Tongue squamous carcinoma	04072107-1VL
BICR 56	Tongue squamous cell carcinoma	06031002-1VL
BICR 78	Oral alveolus squamous carcinoma	04072111-1VL
BICR 82	Human Upper Jaw (Maxilla) Carcinoma	04072112-1VL
BICR/M1Rk	Rat Marshall mammary tumour	96112021-1VL
BL-3	Bovine leukaemic lymphoblast	86062401-1VL
U-BLC1	Human bladder transitional cell carcinoma	06013102-1VL
BLO-11	Mouse Moblo/y muscle hereditary emphysema	90112702-1VL
BPAEC	Bovine lung endothelium	86123101-1VL
BRISTOL 8	Human B lymphocyte	85011436-1VL
BRL-1	Rat epithelial cells	94121649-1VL
BRL 3A	Buffalo Rat liver	85111503-1VL
BS1-B4	Mouse 3T3 Swiss Albino embryo fibroblast	85011410-1VL
BT	Bovine turbinate, BVDV negative	89031603-1VL
Bu25 TK-	Human cervical carcinoma	85022104-1VL
BUD-8	Human Caucasian skin fibroblast	90031532-1VL
Bu (IMR-31)	Buffalo lung	89051704-1VL
BW1J	Mouse hepatoma	94101901-1VL
BW5147.G.1.4.OUAR.1	Mouse AKR/J T cell lymphoma	88100507-1VL
BW5147.G.1.4.OUA/R.1	Mouse AKR/J T cell lymphoma	85082302-1VL
C-4I	Human Caucasian Cervical carcinoma	89051702-1VL
C127I	Mouse RIII mammary tumour	90060504-1VL
C1300 CLONE NA	Mouse neuroblastoma	93120817-1VL
C16 (HeLa derivative)	Human Negroid cervix carcinoma	84121902-1VL
C17.2	Mouse multipotent neural progenitor or stem-like cells	07062902-1VL
C2	Rat hepatoma [HGPRT-]	94101906-1VL
C211	Human Caucasian skin, Cri du Chat syndrome	90112604-1VL
C22 (Clara)	Mouse lung epithelial progenitor cell	07021401-1VL
C2-Rev 7	Rat hepatoma [HGPRT-]	94101907-1VL
C32	Human amelanotic melanoma	87090201-1VL
C32TG	Human Amelanotic Melanoma	96020925-1VL
C3H/MCA clone 16	Mouse C3H embryo	90110523-1VL
C57/B1	Mouse C57BL/6J melanoma epithelial	85011438-1VL

Name	Description	Cat. No.
C6-2-3	Rat glioma x rat glioma hybrid	08062528-1VL
C6-4-2	Rat glioma x rat glioma hybrid	08062524-1VL
C6-BU-1	Rat glioma x rat glioma hybrid	08062563-1VL
C8166	Human T cell leukaemia	88051601-1VL
CA46	Human Burkitt's Lymphoma	95010509-1VL
CAKI 2	Human Caucasian kidney carcinoma	93120819-1VL
CALU 1	Human Caucasian lung epidermoid carcinoma	93120818-1VL
CAR	Goldfish fin tissue	89072611-1VL
Ca Ski	Human Caucasian cervical epidermoid carcinoma	87020501-1VL
CC-1	Rat Wistar liver, normal	93070901-1VL
CCD 13Lu	Human Negroid lung	90112704-1VL
CCD 16Lu	Human Caucasian lung	86090301-1VL
CCD-18Co	Human colon fibroblast, normal, ethnicity: Black	90070503-1VL
CCD 18Lu	Human Negroid lung	90112706-1VL
CCD 25Lu	Human Caucasian lung	90112710-1VL
CCD-29Lu	Human lung emphysema	90110516-1VL
CCD-32Lu	Human Caucasian lung	90110517-1VL
CCD-33Lu	Human Caucasian lung	90110515-1VL
CCD-34Lu	Human Negroid lung	90110514-1VL
CCD-37Lu	Human Caucasian lung	90110513-1VL
CCD-39Lu	Human Caucasian lung	90110512-1VL
CCF-STTG1	Human Caucasian astrocytoma	90021502-1VL
CCO	Channel Catfish ovary	95060212-1VL
CCRF S-180 II	Mouse CFW sarcoma 180	90011901-1VL
CCRF-CEM	Human Caucasian acute lymphoblastic leukaemia	85112105-1VL
CCRF-HSB-2	Human Caucasian acute lymphoblastic leukaemia	85112801-1VL
CCRF-SB	Human Caucasian acute lymphoblastic leukaemia	89090405-1VL
CESS	Human lymphoblast	92110206-1VL
CF2Th	Canine thymus	90110521-1VL
CFPAC-1	Human Caucasian pancreatic adenocarcinoma	91112501-1VL
CGR8	Mouse embryonic stem cell	07032901-1VL
ChaGo-K-1	Human lung bronchus carcinoma	96020948-1VL
CHH-1	Fish Chum heart	92110412-1VL
CHL	Hamster Chinese lung	87111906-1VL
CHO/dhFr-	Hamster Chinese ovary	94060607-1VL
CHO-K1/SF	Hamster Chinese ovary (MEM adapted)	93061607-1VL
CHP-100	Human neuroblastoma epidural tumour of spine	06122001-1VL
CHP-134	Human neuroblastoma tumour mass of left adrenal gland	06122002-1VL
CHP 4 (W.W.)	Human Negroid skin asymptomatic Galactosemia	90102537-1VL
CHSE-214 Cell Line	Salmon embryo, normal	91041114-1VL
CII	Human x mouse hybrid	86072801-1VL
Citrullinemia	Human Caucasian skin	90102532-1VL
CL 21	Mouse / Human hybrid, SV-40 transformed	86080602-1VL
CLC	Carp leukocyte	95070628-1VL
Clone M-3	Mouse (CxDBA)F1 melanoma	87081806-1VL
6-23 (clone 6)	Rat medullary thyroid carcinoma	87042206-1VL
Clone 9	Rat Sprague Dawley liver	88072203-1VL

Name	Description	Cat. No.
Clone 81	Cat kidney fibroblast, MSV transformed	90031403-1VL
Clone 707	Mouse DBA/2 Friend leukaemic lymphoblast	86052101-1VL
Clone C6/36	Mosquito larvae, Aedes albopictus	89051705-1VL
Clone 15 HL-60	Human caucasian promyelocytic leukaemia	98052918-1VL
CL-S1	Mouse BALB/c mammary alveolar nodules, pre-neoplastic	90110502-1VL
CMT 93	Mouse rectum carcinoma	89111413-1VL
CMT64/61	Mouse C57BL/1CRF lung carcinoma	86082105-1VL
CMT93/69	Mouse C57BL/1CRF rectum carcinoma	86082106-1VL
CNC 127I	Mouse RIII mammary tumour	87091701-1VL
COLO 201	Human Caucasian colon adenocarcinoma	87091201-1VL
COLO 206F	Human colon carcinoma	93052620-1VL
COLO 320DM	Human Caucasian colon adenocarcinoma	87061205-1VL
COLO 320 DMF	Human colon carcinoma	93051118-1VL
COLO 320HSR	Human Caucasian colon adenocarcinoma	87101501-1VL
COLO 668	Human lung oat cell carcinoma	87061209-1VL
COLO 677	Human lung small cell carcinoma	93072109-1VL
COLO 679	Human skin melanoma	87061210-1VL
COLO 684	Human uterus adenocarcinoma	87061203-1VL
COLO 685	Human uterus adenocarcinoma	87061206-1VL
COLO 720 E	Human adenocarcinoma	93072111-1VL
COLO 720 L	Human normal B lymphocytes	93052623-1VL
COLO 741	Human colon carcinoma	93052621-1VL
COLO 775	Human leukaemia	94072236-1VL
COLO 792	Human malignant melanoma	93052616-1VL
Colo 794	Human melanoma	94072237-1VL
COLO 800	Human melanoma	93051123-1VL
COLO 839	Human Caucasian B lymphocyte	93051119-1VL
COLO 853	Human malignant melanoma	93052606-1VL
COLO 857	Human melanoma	93051120-1VL
COLO 858	Human melanoma	93052613-1VL
COR-L 23/CPR	Human Caucasian lung, large cell carcinoma	96042336-1VL
COR-L105	Human Caucasian lung adenocarcinoma	92031918-1VL
COR-L23	Human Caucasian lung large cell carcinoma	92031919-1VL
COR-L23/R	Human Caucasian lung large cell carcinoma	96042339-1VL
COR-L23/5010	Human Caucasian lung, large cell carcinoma, drug resistant	96042338-1VL
COR-L23/R23-	Human Caucasian lung large cell carcinoma,drug-resistant revertant	96042337-1VL
COR-L24	Human lung small cell carcinoma	96020717-1VL
COR-L26	Human large cell lung cancer bone marrow aspirate	96020740-1VL
COR-L279	Human lung small cell carcinoma	96020724-1VL
COR L303	Human lung small cell carcinoma	96020722-1VL
COR-L311	Human lung small cell carcinoma	96020721-1VL
COR-L32	Human small cell carcinoma	96020744-1VL
COR-L321	Human lung carcinoma	96020756-1VL
COR-L47	Human Caucasian small cell lung carcinoma	92031915-1VL
COR-L51	Human Caucasian lung carcinoma	92031916-1VL
COR-L64	Human bone marrow, small cell lung carcinoma patient	96020749-1VL
COR-L88	Human Caucasian lung small cell carcinoma	92031917-1VL
COR-L95	Human lung small cell carcinoma	96020733-1VL
COV318	Human ovarian epithelial-serous carcinoma, peritoneal ascites	07071903-1VL
COV362	Human ovarian epithelial-endometroid carcinoma	07071910-1VL
COV362.4	Human ovarian epithelial-endometroid carcinoma, pleural effusion	07071904-1VL
COV413A	Human ovarian epithelial-serous carcinoma, sigmoid	07071905-1VL
COV413B	Human ovarian epithelial-serous carcinoma, bladder dome	07071906-1VL
COV434	Human ovarian granulosa tumour	07071909-1VL
COV504	Human ovarian epithelial-serous carcinoma, pleural effusion	07071902-1VL
COV644	Human ovarian epithelial-mucinous carcinoma, primary tumour	07071908-1VL
CP5	Bos taurus (Holstein-Fresian) articular cartilage progenitor	08052101-1VL
CPAE	Bovine pulmonary artery endothelium	86111401-1VL
CRE BAG2	Mouse NIH 3T3 fibroblast, Moloney murine leukaemia virus transfected	95082219-1VL
CRFK (BVD Ag negative)	Cat kidney	86093002-1VL
CRI-D11	Rat NEDH islet tumour	88031604-1VL
CRI-D2	Rat NEDH islet tumour	88031605-1VL
Cri du Chat	Human Caucasian skin deletion in chromosome 5	90102533-1VL
CRI-G1	Rat NEDH islet tumour	87052701-1VL
CRI-G5	Rat NEDH islet tumour	88031603-1VL
CSE-119	Fish Coho salmon fibroblast	95122019-1VL
CTAC	Dog thyroid gland adenocarcinoma	90071812-1VL
CTLL-2	Mouse C57b1/6 T cell	93042610-1VL
CTX TNA2	Rat Astrocyte, Transfected	98102213-1VL
D17	Canine Poodle primary osteosarcoma	89090403-1VL
D98/AH2 Clone B (HeLa derivative)	Human Negroid cervix carcinoma	85112701-1VL
DBTRG.05MG	Human glioblastoma	93061119-1VL
DDT1-MF2	Syrian Hamster leiomyosarcoma (Mesocricetus Auratus)	89051703-1VL
DEDE	Hamster Chinese lung female	87091502-1VL
Detroit 510	Human Caucasian skin Galactosemia	90102531-1VL
Detroit 525	Human Skin Turners Syndrome	90121002-1VL
Detroit 529	Human, Down syndrome	87021203-1VL
Detroit 532	Human Caucasian, skin, Down syndrome	87032602-1VL
Detroit 539	Human Caucasian skin Down syndrome	91031102-1VL
Detroit 551	Human Caucasian embryo skin	86082802-1VL
Detroit 562	Human Caucasian pharynx carcinoma	87042205-1VL
Detroit 573	Human Caucasian skin, B/D translocation	90112601-1VL

ECACC® Cell Line Collections: General Collection

Name	Description	Cat. No.
DF	Mouse / Human Hybrid, NIH-3T3 transfectant	86072802-1VL
DH14	Drosophila embryo	90070551-1VL
DH15	Drosophila embryo	90070549-1VL
DH33	Drosophila embryo	90070559-1VL
DH82	Canine Monocyte-macrophage	94062922-1VL
DHD/K12/TRb	Rat colonic carcinoma	90062901-1VL
DI TNC1	Rat Astrocyte transfected	98102214-1VL
DK	Canine kidney	93120836-1VL
DLD-1	Human colon adenocarcinoma	90102540-1VL
DMS 53	Human Caucasian lung small cell carcinoma	95062823-1VL
DMS 79	Human lung small cell carcinoma	95062824-1VL
DMS 92	Human Caucasian lung small cell carcinoma, metastasis to bone marrow	95062825-1VL
DMS 153	Human Caucasian lung small cell carcinoma, metastasis to liver	95062827-1VL
DMS 273	Human lung, small cell carcinoma	95062830-1VL
DMS 454	Human Caucasian lung, small cell carcinoma	95062832-1VL
DOK	Human Caucasian dysplastic oral keratinocyte	94122104-1VL
DON	Hamster Chinese lung	87091501-1VL
DSL6A/C1	Rat pancreatic carcinoma	94022431-1VL
Duck Embryo	Duck Pekin embryo	89051503-1VL
Ea.4	Saltmarsh caterpillar *Estigmena acrea* (Subclone 4)	96073116-1VL
EB-3	Human Negroid Burkitt's lymphoma	90121003-1VL
EBTr	Bovine fetal trachea	87090202-1VL
ECV304	Human urinary bladder carcinoma	92091712-1VL
E.Derm	Horse dermis	88032803-1VL
E.H.IV (Elaine IV)	Human Caucasian infectious mononucleosis lymphocytes	90121307-1VL
EIII	Human x mouse hybrid	86072804-1VL
EJ138	Human bladder carcinoma	85061108-1VL
EJG	Bovine adrenal medulla endothelial	93051803-1VL
EL4.BU.OU6	Mouse C57BL/6N ascites lymphoma lymphoblast	85121301-1VL
EL4.NOB-1	Mouse C57BL/6N ascites lymphoma lymphoblast	87020408-1VL
H-EMC-SS	Human chondrosarcoma	94042258-1VL
EMT6/P	Mouse Mammary, parent to drug resistant derivatives	96042344-1VL
EMT6/AR1	Mouse mammary, drug-resistant	96042327-1VL
EMT6/AR10.0	Mouse mammary, drug-resistant	96042326-1VL
EMT6/CPR	Mouse mammary, drug-resistant	96042318-1VL
EMT6/MTXR	Mouse mammary, drug-resistant	96042322-1VL
EMT6/VCR/R	Mouse mammary, drug-resistant	96042323-1VL
EMT6/VRP/R	Mouse mammary, drug-resistant	96042319-1VL
EoL-1 cell	Human eosinophilic leukaemia	94042252-1VL
Eos-HL-60	Human Caucasian promyelocytic leukaemia	96100920-1VL
ESK-4	Porcine kidney	93120821-1VL
EZZ (TOU II-4)	Human B cell lymphocyte, EBV transformed	93093003-1VL
F-36P	Human leukemia, myelodysplastic syndrome	99072808-1VL
F4N (MEL)	Mouse Erythroleukemia	96121718-1VL
F5-5.F1	Mouse Lymphocyte, friend leukemia	99072803-1VL
F9	Mouse 129 embryo teratocarcinoma	85061803-1VL
Fc2Lu	Cat lung	90112712-1VL
Fc3Tg	Cat tongue	90073002-1VL
FEA4 + PFSC/C1	Cat Domestic embryo fibroblast	90031402-1VL
FEL	Rabbit embryo skin	89020802-1VL
FER	Cat Domestic embryo fibroblast	90031401-1VL
FGC4	Rat hepatoma [alb-gpt plasmid]	94101904-1VL
FHM	Fish Fat Head minnow	88102401-1VL
FL (HeLa derivative)	Human Negroid cervix carcinoma	90111910-1VL
FM3A	Mouse C3H mammary carcinoma	87100804-1VL
FM3Ats C1.T85	Mouse C3H mammary carcinoma	87111904-1VL
FoLu	Fox Grey lung	90120501-1VL
FR	Rat Sprague-Dawley skin	87020503-1VL
fR2	Human breast epithelial, SV40 transformed	98031102-1VL
fR5	Human breast epithelial, SV40 transformed	98031103-1VL
FRTL-5	Rat thyroid	91030711-1VL
FT	Bull frog tongue	90102525-1VL
FTC-236	Human Thyroid Cancer Neck Lymph Node Metastasis	06030202-1VL
FTC-238	Human follicular thyroid carcinoma	94060902-1VL
G	Rat Dunning R-3327,prostate adenocarcinoma	94101453-1VL
G-7	Mouse Swiss-Webster myoblast	90110520-1VL
G-8	Mouse Swiss-Webster myoblast	89050906-1VL
GEEP	Goat x Sheep hybrid, skin.	92062401-1VL
GH1	Rat Wistar-Furth pituitary tumour	89072610-1VL
GH3	Rat Wistar-Furth pituitary tumour	87012603-1VL
GIRARDI HEART (HeLa derivative)	Human Negroid cervix carcinoma	93120822-1VL
GL-1	Lizard Gekko lung	89072612-1VL
GM1899A	Human lymphoblast, HPRT - deficient	98120701-1VL
GP2d	Human Caucasian colon adenocarcinoma	95090714-1VL
GP5d	Human Caucasian colon adenocarcinoma	95090715-1VL
GR-M	Human Caucasian melanoma	95032301-1VL
GRL101 (KC7)	Hamster Chinese ovary transfected with Lymnaea receptor GRL101	94071259-1VL
GRL101 (MIX)	Hamster Chinese ovary transfected with Lymnaea receptor GRL101	94071258-1VL
Grunt Fin (GF)	Fish Grunt fin tissue blue striped	88010601-1VL
GS-9L	Rat glioma	94110705-1VL
GS-109-V-20	Human Caucasian skin fibroblast Gardner's syndrome	90110505-1VL
GS-109-V-34	Human Caucasian skin fibroblast Gardner's syndrome	90110504-1VL
GS-109-V-63	Human Caucasian skin fibroblast Gardner's syndrome	90110503-1VL
H103	Human oral squamous cell carcinoma, tongue	06092001-1VL
H157	Human oral squamous cell carcinoma	07030901-1VL
H2.35	Mouse Hepatocyte, SV40 (tsA255) transformed	94050407-1VL

Name	Description	Cat. No.
H314	Human oral squamous cell carcinoma, floor of mouth	06092003-1VL
H33HJ-JA1	Human lymphoma	90112119-1VL
H357	Human oral squamous cell carcinoma, tongue	06092004-1VL
H376	Human oral squamous cell carcinoma, floor of mouth	06092005-1VL
H400	Human oral squamous cell carcinoma, alveolar process	06092006-1VL
H413	Human oral squamous cell carcinoma, buccal mucosa	06092007-1VL
H4II	Rat skin Hepatocellular carcinoma	89042702-1VL
H4-II-E-C3	Rat liver hepatoma	85061112-1VL
H4S	Rat liver hepatoma	89102001-1VL
H5	Rat hepatoma	94101905-1VL
H69	Human Caucasian lung small cell carcinoma	91091802-1VL
H69V	Human lung small cell carcinoma	91091803-1VL
H9	Human T cell	85050301-1VL
HaP-T1	Hamster Syrian adenocarcinoma	93121054-1VL
T/G HA-VSMC	Human Caucasian aorta, normal smooth muscle	94102702-1VL
HCA-7	Human colon adenocarcinoma	06061902-1VL
HCA-24	Human colon adenocarcinoma	06061903-1VL
HCA-46	Human colon adenocarcinoma	07031601-1VL
HCA-7 Colony 1	Human colorectal adenocarcinoma	09071501-1VL
HCA-7 Colony 3	Human colorectal adenocarcinoma	09071502-1VL
HCA-7 Colony 6	Human colorectal adenocarcinoma	09071504-1VL
HCA-7 Colony 11	Human colorectal adenocarcinoma	09071506-1VL
HCA-7 Colony 24	Human colorectal adenocarcinoma	09071509-1VL
HCA-7 Colony 26	Human colorectal adenocarcinoma	09071511-1VL
HCA-7 Colony 27	Human colorectal adenocarcinoma	09071513-1VL
HCA-7 Colony 29	Human Colon Carcinoma (subpopulation isolated from the HCA-7 cell line)	02091238-1VL
HCA-7 Colony 30	Human colorectal adenocarcinoma	09071516-1VL
HCT-8	Human ileocecal adenocarcinoma	90032006-1VL
HCT-15	Human colon adenocarcinoma	91030712-1VL
HEL 299	Human Negroid embryo lung	87042207-1VL
HEL92.1.7	Human Caucasian erythroleukaemia	92111706-1VL
HeLa B	Human Negroid cervix carcinoma	85060701-1VL
HeLa (AC-free)	Human Negroid cervical carcinoma, serum-free, animal component (AC) free	08011102-1VL
L-41(HeLa derivative)	Human Negroid cervix carcinoma	96121716-1VL
HeLa DH	Human cervix carcinoma	96112022-1VL
HeLa Ohio	Human Negroid cervix carcinoma	84121901-1VL
HeLa S3	Human Negroid cervix carcinoma	87110901-1VL
Hep2 (Clone 2B) (HeLa derivative)	Human Negroid cervix carcinoma	85011412-1VL
Hepa 1-6	Mouse hepatoma	92110305-1VL
Hepa-1c1c7	Mouse hepatoma	95090613-1VL
Hepa-E1	Eel liver	99072812-1VL
Hepa-T1	Eel liver	99072813-1VL

Name	Description	Cat. No.
HEPM	Human embryonic palatal mesenchyme	90120505-1VL
HF1	Rat hepatoma Hybrid [H5xFao]	94101908-1VL
HF1-5	Rat hepatoma Hybrid [H5xFao]	94101909-1VL
HF19	Human fetal lung fibroblast	85011418-1VL
HF2x653	Human (WI-L2-729-FF2) x mouse (P3X63/Ag8.653) heterohybridoma	90012609-1VL
HFFF2	Human Caucasian fetal foreskin fibroblast	86031405-1VL
HFL1	Human fetal lung fibroblast	89071902-1VL
HG261	Human Caucasian skin Fanconi's anaemia	90112603-1VL
HGC-27	Human gastric carcinoma	94042256-1VL
HKT-1097	Syrian Hamster kidney	98061003-1VL
HL	Human lung fibroblast	96121720-1VL
HL60 15-12	Human Caucasian promyelocytic leukaemia	88120805-1VL
HL60 Ast.3	Human Caucasian promyelocytic leukaemia	88120801-1VL
HL60 Ast.4	Human Caucasian promyelocytic leukaemia	88120802-1VL
HL60 M2	Human Caucasian promyelocytic leukaemia	88120803-1VL
HL60 M4	Human Caucasian promyelocytic leukaemia	88120804-1VL
HMT-3522 S1	Human Caucasian breast epithelial	98102210-1VL
HMT-3522 S2	Human Caucasian breast epithelial	98102211-1VL
HMT-3522 T4-2	Human Caucasian breast epithelial	98102212-1VL
HMVII	Human vaginal maligant melanoma	92042701-1VL
HOS	Human Caucasian osteosarcoma, TE85	87070202-1VL
HR5	Human cervix carcinoma, HeLa derivative, transformed	95091409-1VL
HR5-CL11	Human cervix carcinoma, HeLa derivative, transformed	95091411-1VL
HRA-16	Human rectal adenocarcinoma	06061905-1VL
HRA-19a1.1	Human colorectal adenocarcinoma	09071519-1VL
HRA-19a1.1 Alpha 2F	Human colorectal adenocarcinoma	09071520-1VL
HRA-19a1.1 Alpha2 B	Human colorectal adenocarcinoma	09071522-1VL
HRA-19a1.1 Alpha2Alpha1 B	Human colorectal adenocarcinoma	09071524-1VL
HRA-19a1.1 Alpha2Alpha1 E	Human colorectal adenocarcinoma	09071527-1VL
HRT-18	Human rectum adenocarcinoma	86040306-1VL
Hs 27	Human foreskin, fibroblast	94041901-1VL
Hs 68	Human newborn foreskin	89051701-1VL
Hs1.Tes	Human normal testis	97123004-1VL
Hs 578T	Human breast carcinoma	86082104-1VL
Hs 633T	Human fibrosarcoma MBA/8387	89050201-1VL
Hs 888Lu	Human Caucasian normal lung tissue	90112709-1VL
HSDM1C1	Mouse Swiss Albino fibrosarcoma	90112810-1VL
HSG (HeLa derivative)	Human Negroid cervix carcinoma	95031024-1VL
HS-Sultan	Human Caucasian plasma cell plasmacytoma	87012701-1VL
HT 1080	Human fibrosarcoma	85111505-1VL

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Name	Description	Cat. No.
HT115	Human colon carcinoma	85061104-1VL
HT 1197	Human Caucasian bladder carcinoma	87032403-1VL
HT 1376	Human Caucasian bladder carcinoma	87032402-1VL
HT29/219	Human Caucasian colon carcinoma	85061109-1VL
HT29 gluc C1	Human Caucasian colon adenocarcinoma	92012401-1VL
HT2 Clone A5E	Mouse helper T cell	92021401-1VL
HTC	Rat hepatoma	93120108-1VL
HTC (BUdR)	Rat liver hepatoma	85061110-1VL
HTK-	Human osteomyeloma	88022409-1VL
HtTA-1	Human cervix carcinoma, HeLa derivative, transformed	95091410-1VL
HUC-Fm	Human umbilical cord fibroblast	87100801-1VL
HuP-T3	Human pancreatic adenocarcinoma	93121055-1VL
HuP-T4	Human pancreatic adenocarcinoma	93121056-1VL
HUT-78	Human T cell lymphoma	88041901-1VL
I-10	Mouse BALB/c Leydig cell testicular tumour	89031605-1VL
IA-Xs SBR	Holtzmann rat small bowel adenocarcinoma	88090801-1VL
ICR-2A	Frog Grass embryo	89072615-1VL
IEC 6	Rat small intestine epithelial	88071401-1VL
IEC 18	Normal rat ileum	88011801-1VL
IgH-2	Iguana heart	90030804-1VL
II b	Mouse/human hybrid	86072805-1VL
C-4 II	Human Caucasian cervical carcinoma	88102701-1VL
H-4-II-E	Rat hepatoma Reuber H35	87031301-1VL
IIC9 (CH0)	Chinese hamster embryo fibroblast	00030814-1VL
IM 9	Human B lymphocyte	86051302-1VL
IMR 32	Human Caucasian neuroblastoma	86041809-1VL
IMR-33	Gerbil Fibroma	96020931-1VL
IMT 9	Indian Muntjac skin fibroblast	95060901-1VL
IPI-1	Boar Miniature male ileum, SV40 transformed	93100621-1VL
IPI-2I	Boar Miniature male ileum, SV40 transformed	93100622-1VL
Ishikawa (Heraklio) 02 ER-	Human endometrial adenocarcinoma	98032302-1VL
J45.01	Human acute T cell leukaemia	93031145-1VL
J558L	Mouse BALB/c myeloma	88032902-1VL
J.CaM1.6	Human T cell, mutant derivative of Jurkat	96060401-1VL
JEG3	Human choriocarcinoma	92120308-1VL
Jensen sarcoma	Rat sarcoma, asparagine requiring	90111913-1VL
JH4 clone 1	Guinea pig lung	86070201-1VL
JIII (HeLa derivative)	Human Negroid cervix carcinoma	93120824-1VL
JIYOYE	Human Negroid Burkitt's lymphoma	88071302-1VL
JM	Human T cell	86010201-1VL
JTC-19	Rat lung	87111902-1VL
JTC-27	Rat hepatoma	94042255-1VL
JVM-2	Human Caucasian B-prolymphocytic leukaemia, EBV-transformed	96090515-1VL
JVM-13	Human Caucasian B-prolymphocytic leukaemia, EBV transformed	96090516-1VL

Name	Description	Cat. No.
K562 AZQR	Human caucasian chronic myelogenous leukemia (drug resistant)	93112521-1VL
K562 cl.6	Human Caucasian chronic myelogenous leukaemia	85011407-1VL
K6H6/B5	Mouse x Human hybrid myeloma	89101606-1VL
KARPAS 45	Human T-cell Leukaemia	06072602-1VL
KARPAS 1106P	Human B-Cell Non-Hodgkin's Lymphoma	06072607-1VL
KARPAS 1718	Human splenic lymphoma	08072401-1VL
KARPAS 299	Human Non-Hodgkin's Ki-positive Large Cell Lymphoma	06072604-1VL
KATO-III	Human gastric carcinoma	86093004-1VL
KB (HeLa derivative)	Human Negroid cervix carcinoma	94050408-1VL
KC	Drosophila embryo	90070550-1VL
KCB 85015	Black Bear lung	93052110-1VL
KCB 89001	Sambar deer skin	93052109-1VL
KCB89018	Fox Red lung, MNNG transformed fibroblast	93052111-1VL
KG-1	Human Caucasian bone marrow myelogenous leukaemia	86111306-1VL
KG1a	Human Caucasian bone marrow acute myelogenous leukaemia	91030101-1VL
KHOS-240S	Human Caucasian osteosarcoma	86112810-1VL
KHOS-312H	Human Caucasian osteosarcoma	86112811-1VL
KHOS/NP	Human Caucasian osteosarcoma	84102903-1VL
KLN 205	Mouse squamous cell carcinoma	90110519-1VL
KNRK	Normal rat kidney, Kirsten MSV transformed	87061001-1VL
KU-812E	Human myelogenous leukaemia	90071803-1VL
KU-812 Cell Line	Human myelogenous leukaemia	90071807-1VL
KU-812F	Human myelogenous leukaemia	90071804-1VL
KYSE-30	Human Asian squamous cell carcinoma	94072011-1VL
KYSE-70	Human oesophageal squamous cell carcinoma	94072012-1VL
KYSE-150	Human oesophageal squamous cell carcinoma	94072015-1VL
KYSE-270	Human oesophageal squamous cell carcinoma	94072021-1VL
KYSE-410	Human oesophageal squamous cell carcinoma	94072023-1VL
L-2	Rat lung	90112811-1VL
L1210	Mouse DBA/2 lymphocytic leukaemia	87092804-1VL
L132 (HeLa derivative)	Human Negroid cervix carcinoma	89111004-1VL
L14	Porcine peripheral blood B cell	91012317-1VL
L23	Porcine peripheral blood B cell	91012318-1VL
L35	Porcine peripheral blood T cell	91012319-1VL
L45	Porcine peripheral blood T cell	91012320-1VL
L5178Y	Mouse DBA/2 lymphoma	87111908-1VL
L5178Y-R	Mouse DBA/2 Lymphoma	90062802-1VL
L5178Y-S	Mouse DBA/2 Lymphoma	93050408-1VL
L52	Porcine peripheral blood B cell	91012321-1VL
L6.C11	Rat skeletal muscle myoblast	92102119-1VL
L6.G8	Rat skeletal muscle myoblast	92102117-1VL
L6.G8.C5	Rat skeletal muscle myoblast	92121114-1VL
L8	Rat Skeletal muscle myoblast	95102434-1VL
L929/R	Mouse C3H/An connective tissue cisplatin resistant	04102001-1VL

Name	Description	Cat. No.
L929(NCTC)	Mouse C3H/An areolar and adipose tissue	85103115-1VL
L929S	Mouse areolar adipose tissue	86032004-1VL
LA-N-1	Human Neuroblastoma Bone Marrow Metastasis	06041201-1VL
LA-N-2	Human Neuroblastoma	06041202-1VL
LA-4	Mouse A/He lung adenoma	90040512-1VL
LA1-55n	Human Neuroblastoma	06041203-1VL
LA1-5s	Human Neural Crest-Derived Non-Neuronal Progenitor	06041204-1VL
LB173	Human lymphoblastoid, normal, EBV-transformed	98062316-1VL
LB182	Human lymphoblastoid, normal, EBV-transformed	98062317-1VL
LB185	Human lymphoblastoid, normal, EBV-transformed	98062318-1VL
LB198	Human lymphocytes, Trichothiodystrophy heterozygote, EBV-transformed	98062320-1VL
LB199	Human lymphocyte, trichothiodystrophy, EBV-transformed	98062321-1VL
LB205	Human lymphocytes, Xeroderma pigmentosum variant, EBV-transformed	98062329-1VL
LB242	Human Caucasian lymphoblastoid, Cockayne syndrome, EBV-transformed	98062324-1VL
LB253	Human lymphocytes, Xeroderma pigmentosum group C, EBV-transformed	98062330-1VL
LB277	Human lympoblastoid, Xeroderma pigmentosum,EBV-transformed	98062331-1VL
LB316	Human lymphoblastoid, Xeroderma pigmentosum, EBV-transformed	98062333-1VL
LB42	Human lymphocytes, normal, EBV-transformed	98062313-1VL
LB443	Human lymphoblastoid, trichothiodystrophy, EBV-transformed	98062322-1VL
LB541	Human lymphoblastoid, Ataxia telangiectasia variant, EBV-transformed	98062326-1VL
LB58	Human lymphoblastoid, Ataxia telangiectasia, EBV-transformed	98062325-1VL
LB687	Human lymphoblastoid, Xeroderma pigmentosum complementation, EBV-transformed	98062335-1VL
LB7	Human lymphocytes, normal, EBV-transformed	98062312-1VL
LB707	Human lymphoblastoid, Xeroderma pigmentosum heterozygote, EBV-transformed	98062336-1VL
LB708	Human lymphoblastoid, Xeroderma pigmentosum, EBV-transformed	98062337-1VL
LB750	Human lymphoblastoid, trichothiodystrophy, EBV-transformed	98062323-1VL
LB81	Human lymphoblastoid, Xeroderma pigmentosum heterozygote, EBV-transformed	98062327-1VL
LB823	Human lymphocytes, Xeroderma pigmentosum, EBV-transformed	98062338-1VL
LB83	Human lymphocytes, Xeroderma pigmentosum, EBV-transformed	98062328-1VL
LBRM-33-1A5	Mouse B10.BR lymphoma	86060302-1VL
LBRM TG6	Mouse Lymphoma Radiation-induced	96020937-1VL
LC540	Rat Fischer Leydig cell testicular tumour	89031604-1VL
LC-2/ad	Human lung adenocarcinoma	94072247-1VL
LL24	Human diploid lung	90073001-1VL
LL29 (AnHa)	Human Caucasian diploid lung	87112508-1VL
LL47 (MaDo)	Human Negroid diploid lung	90102538-1VL
LL86 (LeSa)	Human Caucasian diploid lung	90102541-1VL
LLC-PK1	Porcine kidney	86121112-1VL
LLC-WRC 256	Rat Walker carcinoma epithelial	87061101-1VL
LL/2(LLc1)	Mouse C57BL Lewis lung carcinoma	90020104-1VL
L-M	Mouse C34/An connective tissue	87032401-1VL
LS 123	Human colon adenocarcinoma	94120801-1VL
LS174T	Human Caucasian colon adenocarcinoma	87060401-1VL
LS180	Human Caucasian colon adenocarcinoma	87021202-1VL
LTK-	Mouse C34/An connective tissue	85011432-1VL
LUDLU-1	Human Caucasian lung squamous cell carcinoma	92012463-1VL
M-1	Mouse kidney cortical collecting duct, SV40 transformed	95092201-1VL
M1	Mouse myeloblast	93120826-1VL
M15	Mouse mesonephric epithelium, polyoma virus large T transformed	95102517-1VL
M3 Clone M-3	Mouse Cloudman S 91 melanoma	93120827-1VL
MAT-Lu	Rat dorsal prostate adenocarcinoma	94102735-1VL
MAT-LyLu	Rat prostate adenocarcinoma	94101454-1VL
MC116	Human lymphoma	90110501-1VL
MCA-RH 7777	Rat Buffalo hepatoma	90021504-1VL
McCoy	Mouse fibroblast	90010305-1VL
MDA-MB-157	Human Negroid breast medulla carcinoma	92020422-1VL
MDA-MB-361	Human Caucasian breast adenocarcinoma	92020423-1VL
MDBK Cell Line	Bovine kidney	90050801-1VL
MDCK	Canine Cocker Spaniel kidney	85011435-1VL
MDCK I	Canine Cocker Spaniel kidney	00062106-1VL
MDCK-Protein Free	Canine Cocker Spaniel kidney	02050101-1VL
MDST8	Human colon carcinoma	99011801-1VL
M. dunni (Clone III8C)	Mouse tail, normal fibroblast	94101211-1VL
MEF-1	Mouse embryo fibroblast, SV40 - transformed	98061101-1VL
MEG-01	Human megakaryoblastic leukaemia	94012401-1VL
Mero-14	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100101-1VL
Mero-25	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100102-1VL
Mero-41	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100103-1VL
Mero-48a	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100104-1VL
Mero-82	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100105-1VL

ECACC® Cell Line Collections: General Collection

Name	Description	Cat. No.
Mero-83	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100106-1VL
Mero-84	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100107-1VL
Mero-95	A human mesothelioma cell line derived from a malignant mesothelioma. Asbestos exposure	09100108-1VL
MES-SA	Human uterus sarcoma	95051030-1VL
MES-SA/Dx-5	Human uterus sarcoma	95051031-1VL
Meta 7	Mouse TS/A lung metastases	90062272-1VL
Meta 10	Mouse TS/A lung metastases	90062271-1VL
Meta 15	Mouse TS/A lung metastases	90062270-1VL
MEWO	Human malignant melanoma	93082609-1VL
MFE-280	Human caucasian endometrial adenocarcinoma	98050131-1VL
MFE-296	Human caucasian endometrial adenocarcinoma	98031101-1VL
MFM-223	Human caucasian mammary carcinoma	98050130-1VL
MH-S	Mouse alveolar macrophage, SV40 transformed	95090612-1VL
MH-22A	Mouse C3HA hepatocarcinoma	96121721-1VL
MH1C1	Rat liver hepatoma	85112702-1VL
MIA-Pa-Ca-2	Human Caucasian pancreatic carcinoma	85062806-1VL
MiCL1 (S+L-)	Mink lung, Moloney murine sarcoma virus infected	86020603-1VL
ML-1	Human acute myeloblastic leukaemia	88113007-1VL
MLg	Mouse ddY lung	90112707-1VL
MMT-060562	Mouse C57BL x A/F1 mammary tumour	90111911-1VL
MNNG/HOS (TE85, Clone F-5)	Human Caucasian osteosarcoma, chemically transformed	87070201-1VL
MOCHA	Mouse Skin Fibroblast	02111901-1VL
MOG-G-CCM	Human brain astrocytoma	86022702-1VL
MOG-G-UVW	Human brain astrocytoma	86022703-1VL
MOH (TOU II-3)	Human B lymphocyte, EBV transformed	93093002-1VL
MOLT-3	Human acute T lymphoblastic leukaemia	90021901-1VL
MOLT-4	Human acute T lymphoblastic leukaemia	85011413-1VL
MOP-8	Mouse, SV40 Polyoma transformed NIH/3T3 cells	92111707-1VL
MOPC 31C	Mouse BALB/c plasmacytoma, IgG secreting	90110707-1VL
MOPC 315	Mouse myeloma	85022106-1VL
MOR	Human lung adenocarcinoma	84112312-1VL
MOR/0.2R	Human lung adenocarcinoma, drug-resistant	96042335-1VL
MOR/0.4R	Human lung adenocarcinoma, drug-resistant	96042334-1VL
MOR/CPR	Human lung adenocarcinoma, drug-resistant	96042333-1VL
MOUSE L CELLS (TK+, HBS Ag+)	Mouse embryo fibroblast, Moloney Sarcoma Virus transformed	90110101-1VL
MPC-11	Mouse BALB/C Myeloma	91031103-1VL
MPK	Minipig kidney	87032604-1VL
MRC-7	Human fetal lung	85020203-1VL
MRC-9	Human Caucasian fetal lung	85020202-1VL
MRC-5 SV1 TG1	Human fetal lung, SV40 transformed	85042501-1VL
MRC-5 SV1 TG2	Human fetal lung, SV40 transformed	85042502-1VL
MRC-5 SV2	Human fetal lung, SV40 transformed	84100401-1VL
MRC-5 pd13	Human fetal lung	05011802-1VL
MSV.B	Mouse BALB/c embryonic fibroblast, M-MSV transformed	90031901-1VL
M-MSV-BALB/3T3	Mouse BALB/3T3 embryo, Moloney sarcoma virus transformed	90030802-1VL
MSVIF-TK+	Mouse BALB/c embryonic fibroblast, M-MSV transformed	90031902-1VL
MT-4	Human T cell	08081402-1VL
Mv.1.Lu	Mink lung	88050503-1VL
My-La CD4+	Human Caucasian CD4+ T Lymphocyte, mycosis fungoides	95051032-1VL
My-La CD8+	Human Caucasian T Lymphocyte, mycosis fungoides.	95051033-1VL
N115-BU-1	Mouse neuroblastoma, bromodeoxyuridine resistant	08062547-1VL
N115-BU-2	Mouse neuroblastoma, bromodeoxyuridine resistant	08062552-1VL
N115-BU-7	Mouse Neuroblastoma	08062554-1VL
N115-BU-8	Mouse Neuroblastoma	08062519-1VL
N115-BU-9	Mouse Neuroblastoma	08062558-1VL
N115-BU-10	Mouse Neuroblastoma	08062559-1VL
N18	Mouse neuroblastoma x Rat glioma hybrid	88112301-1VL
N18 (Hamprecht)	Mouse neuroblastoma	08062527-1VL
N18TG2	Mouse neuroblastoma	08062523-1VL
N1E-115	Mouse neuroblastoma	88112303-1VL
N1E-115-1	Mouse neuroblastoma	08062511-1VL
N1-S1	Rat hepatoma	90011902-1VL
N2102Ep Clone 2/A6	Human embryonal carcinoma	06011803-1VL
N4TG3	Mouse neuroblastoma	08062515-1VL
Namalwa	Human Burkitt's Lymphoma	87060801-1VL
NB4 1A3	Mouse C-1300 Neuroblastoma	89121405-1VL
NB69	Human neuroblastoma (Stage III)	99072802-1VL
NBT-II	Rat Wistar bladder tumour	93012901-1VL
NC-37	Human Caucasian peripheral blood lymphocyte	89111414-1VL
NCI-H292	Human Negroid lung, mucoepidermoid carcinoma	91091815-1VL
NCI-H358	Human Caucasian bronchioalveolar carcinoma	95111733-1VL
NCI-H510A	Human lung carcinoma, adrenal gland metastasis	96020944-1VL
NCI-H69/CPR	Human small cell lung cancer, drug-resistant	96042328-1VL
NCI-H69/LX10	Human small cell lung cancer, drug-resistant	96042331-1VL
NCI-H69/LX20	Human Caucasian small cell lung carcinoma	96042332-1VL
NCI-H69/LX4	Human Caucasian small cell lung carcinoma, drug-resistant	96042329-1VL
NCI-H69VCR/R	Human small cell lung cancer, drug-resistant	96042330-1VL
NCI-H727	Human lung non-small cell carcinoma	94060303-1VL
NCI-H929	Human Caucasian IgA-producing plasmacytoma	95050415-1VL
NCTC 2071	Mouse C3H/An connective tissue	90050803-1VL

Name	Description	Cat. No.
NCTC 4093	Mouse C57B1/KaLw embryo	90102530-1VL
NCTC 4206	Hamster Chinese peritoneum	91010303-1VL
NCTC clone 1469	Mouse C3H/An liver	88111403-1VL
NCTC clone 929	Mouse C3H/An connective tissue	88102702-1VL
ND-C	Mouse neuroblastoma x Rat neurone hybrid	92090913-1VL
ND15	Mouse neuroblastoma x Rat neurone hybrid	92090907-1VL
ND27	Mouse neuroblastoma x Rat neurone hybrid	92090912-1VL
ND3	Mouse neuroblastoma x Rat neurone hybrid	92090901-1VL
Neopu	Drosophila embryo	90070561-1VL
NFS-25 C-3	Mouse pre-B lymphoblast	93090713-1VL
NFS-5 C1	Mouse pre-B lymphoblast	88041904-1VL
NFS-70 C10	Mouse Pro-B lymphoblast	88041905-1VL
NG108-15	Mouse neuroblastoma x Rat glioma hybrid	88112302-1VL
NG115-401L	Mouse neuroblastoma x Rat glioma hybrid	87032003-1VL
NH15-CA2	Mouse neuroblastoma x rat glioma hybrid	08062525-1VL
NH17	Human T cell lymphoblastic leukaemia	92111101-1VL
NIH-3T3 4-2	Mouse fibroblast	86041101-1VL
NIH-3T3D4	Mouse Swiss NIH embryo	85111801-1VL
NMuMG	Mouse mammary gland, normal epithelial	94081121-1VL
NRK-49F	Rat kidney fibroblast	86101301-1VL
NRK-52E	Rat kidney epithelial	87012902-1VL
NS0-Turbodoma	Mouse myeloma, serum-free, without cholesterol	06020202-1VL
NS20Y	Mouse neuroblastoma	08062517-1VL
NS20Y-BU-2	Mouse neuroblastoma	08062502-1VL
NS20Y-BU-4	Mouse neuroblastoma	08062503-1VL
NS20Y-BU-5	Mouse neuroblastoma	08062504-1VL
NS20Y-BU-6	Mouse neuroblastoma	08062507-1VL
NS20Y-BU-7	Mouse neuroblastoma	08062510-1VL
NS20Y-TG	Mouse neuroblastoma	08062512-1VL
Nthy-ts1	Human thyroid follicular mutant epithelial	90011610-1VL
OAW42	Human ovarian tumour epithelial	85073102-1VL
OK	American Opossum, kidney	91021202-1VL
OV17R	Human ovarian adenocarcinoma (ascites) stage III	96020763-1VL
OV56	Human ovarian serous carcinoma (ascites) stage IV, poorly differentiated	96020759-1VL
OV58	Human ovarian serous cancer (ascites) stage III	96020760-1VL
OV7	Human ovarian cancer, stage III	96020764-1VL
OVNIS	Ovine thyroid epithelial	93122324-1VL
P1Bb1.1 (DBA/2)	Mouse DBA/2 mastocytoma	87121102-1VL
P1.HTR	Mouse DBA/2 mastocytoma	87121104-1VL
P1.HTR.TK-	Mouse DBA/2 mastocytoma	87121103-1VL
P22	Hamster Chinese ovary	89030317-1VL
P388.D1 (Clone 3124)	Mouse lymphoid macrophage	85011439-1VL
P3HR-1	Human Burkitt's Lymphoma	86010701-1VL
P3/NS1/1-Ag4.1	Mouse myeloma	85011427-1VL
P3U1	Mouse myeloma	85011417-1VL
P3X63Ag8	Mouse myeloma	85011401-1VL

Name	Description	Cat. No.
P3X63Ag8.653-Turbo-Doma	Mouse BALB/c myeloma, serum-free	03092502-1VL
P3X63Ag8U.1	Mouse Myeloma	94120802-1VL
P815-1-1	Mouse DBA/2 mastocytoma	85011406-1VL
PA-1	Human Caucasian ovary teratocarcinoma	90013101-1VL
PA317	Mouse fibroblast with herpes TK gene	89032007-1VL
PC-14	Human adenocarcinoma	90071810-1VL
PD5	Porcine kidney	93120830-1VL
PDVC 57B	Mouse Spindle Cell carcinoma	01022129-1VL
PEA-10	Mouse embryo fibroblast, LRP-deficient, heterozygous	97040310-1VL
PEA-13	Mouse embryo fibroblast, LRP-deficient, homozygous	97040311-1VL
PE/CA-PJ15	Human oral squamous cell carcinoma	96121230-1VL
PE/CA-PJ34 (clone C12)	Human basaloid squamous cell carcinoma	97062513-1VL
PE/CA-PJ41 (clone D2)	Human oral squamous cell carcinoma	98020207-1VL
PE/CA-PJ49	Human oral squamous cell carcinoma	00060606-1VL
PG	Fish Northern Pike ovaries	95070629-1VL
PG-4	Cat brain Moloney sarcoma virus-transformed	94102703-1VL
PG13	Mouse embryo fibroblast, GALV-based retrovirus	95110215-1VL
Phi-1	Rat Liver	85061101-1VL
PI.1 Ut	Racoon uterus	90111909-1VL
PLC/PRF/5	Human liver hepatoma, Alexander cell line	85061113-1VL
PNT1A (SERUM FREE)	Human post pubertal normal, immortalised with SV40, serum-free	07052901-1VL
PNT2 (SERUM FREE)	Human prostate normal, serum-free	07042701-1VL
PORTHOS	Porcine thyroid epithelial	93122323-1VL
Pr8/22	Mouse DBA/2 lymphoid neoplasm derivative; drug resistant	93112518-1VL
PSA1	Mouse SIM embryo teratocarcinoma	85011403-1VL
Psi-CRE	Mouse NIH 3T3 transformed with Moloney murine leukaemia mutants	94090902-1VL
Psi-CRIP	Mouse NIH 3T3 transformed with Moloney murine leukaemia mutants	94090901-1VL
PSN1	Human pancreatic adenocarcinoma	94060601-1VL
Ptk1	Potoroo kidney	91013163-1VL
Ptk2	Potoroo kidney	88031601-1VL
Pu-518	Mouse BALB/c lymphoid macrophage	85051501-1VL
PUTKO	Human K562 x Human P3HR-1 hybrid myeloid leukaemia	88042802-1VL
QIMR-WIL	Human myeloblastic leukaemia lymphoblast	86030601-1VL
QT 6	Quail Japanese fibrosarcoma	93120831-1VL
QT 35	Quail Japanese fibrosarcoma	93120832-1VL
R2C	Rat testis Leydig cell tumour	89031606-1VL
R74	Rat embryonic fibroblasts RSV-33 transformed	93120215-1VL
R9	Rat embryonic fibroblasts RSV-33 transformed	93120214-1VL

ECACC® Cell Line Collections: General Collection

Name	Description	Cat. No.
R9ab	Rabbit New Zealand white lung	88011502-1VL
RAB-9	Rabbit New Zealand white adult skin fibroblast	87042203-1VL
RaBE	Rat Bronchial Epithelial Cells (RaBE)	04100601-1VL
RaBE (Slow Growth)	Rat Bronchial Epithelial Cells (RaBE) Slow Growth	04100602-1VL
RAG	Mouse BALB/c renal adenocarcinoma	89040605-1VL
RAJI'A'	Human Negroid Burkitt's lymphoma	85022102-1VL
RAJI TK+	Human Negroid Burkitt's lymphoma	91112124-1VL
RAJI TK-	Human Negroid Burkitt's lymphoma	91112125-1VL
RAMOS-AW	Human Caucasian Burkitt's lymphoma	85062003-1VL
RAMOS-EHRB	Human Caucasian Burkitt's lymphoma	85030804-1VL
RAMOS (RA.1)	Human Caucasian Burkitt's lymphoma	91030710-1VL
RAT-2	Rat embryo	94050409-1VL
RB247C	Human Retinoblastoma	06070601-1VL
RBL-1	Rat basophilic leukaemia granulocyte	86061001-1VL
RC-4B/C1	Rat pituitary adenoma	94020810-1VL
RCC4 plus vector alone	Renal cell carcinoma cell line RCC4 stably transfected with an empty expression vector, pcDNA3, conferring neomycin resistance.	03112702-1VL
RCC4plusVHL	Renal cell carcinoma cell line RCC4 stably transfected with pcDNA3-VHL	03112703-1VL
RCE	Rabbit corneal epithelial cells, SV40 transformed	95081046-1VL
RCP	Rat Choroid Plexus	00031626-1VL
RD	Human Caucasian embryo rhabdomyosarcoma	85111502-1VL
RED.1	Mouse leukaemic lymphoblast	85092601-1VL
REM 885	Human EBV transformed lymphocytes Alzheimer	06070401-1VL
RENE 1	Rat Sprague-Dawley, primary endometrial, adenovirus transformed	92092510-1VL
RENE 2	Rat Sprague-Dawley, primary endometrial, adenovirus transformed	92092511-1VL
RENT 4	Rat Sprague-Dawley, primary endometrial, SV40 large T antigen transformed	92092512-1VL
RENT RO1	Rat Sprague-Dawley, primary endometrial, SV40 T antigen transformed	92092513-1VL
RENT RO3	Rat Sprague-Dawley, primary endometrial, SV40 T antigen transformed	92092514-1VL
RENT ts3.1	Rat Sprague-Dawley, primary endometrial, SV40 T antigen transformed	92092516-1VL
RENT tsS1.1	Rat Sprague-Dawley, primary endometrial, SV40 T antigen transformed	92092515-1VL
RFL-6	Rat Sprague-Dawley lung	86072501-1VL
Ri-1	Human Caucasian B-cell lymphoma	96090512-1VL
RIN-m	Rat islet cell tumour	95071701-1VL
RIN-5F	Rat islet cell tumour	95090402-1VL
RLC 27	Rat liver	90071811-1VL
4/4 RM-4	Rat Fischer visceral pleura mesothelium	90112711-1VL
RN 22	Rat Schwann cell, immature	93011414-1VL
RO82-W-1	Human follicular thryoid carcinoma	92030502-1VL
Rolf B1.T	Adult rat olfactory nerve ensheathing cells	03071601-1VL
RPMI 1788	Human Caucasian peripheral blood lymphocyte	85112106-1VL
RPMI 1846	Hamster Golden Syrian melanotic melanoma	93120834-1VL
RPMI 2650	Human nasal septum quasidiploid tumour	88031602-1VL
RPMI 6666	Human Caucasian lymphoblast, Hodgkins disease	87081807-1VL
RPMI 7666	Human Caucasian lymphoblast	87012703-1VL
RPMI 8226	Human myeloma	87012702-1VL
RR1022	Rat Amsterdam Schmidt-Ruppin sarcoma	90102526-1VL
RR-CHOKI	Hamster Chinese ovary	92052129-1VL
RT 1	Rat Lewis fibrosarcoma	94011416-1VL
RT112/84	Human bladder carcinoma epithelial	85061106-1VL
RT4	Human Caucasian bladder transitional-cell carcinoma	91091914-1VL
RT4/31	Human bladder transitional cell papilloma	86082102-1VL
RT4-D6P2T	Rat Schwann cell, immature	93011415-1VL
RTG-2	Trout Rainbow gonad tissue	90102529-1VL
22Rv1	Human Xenograft Prostate	05092802-1VL
S1814.PB5	Human embryo fibroblast	85011402-1VL
SAF-1	Gilt head seabream caudal fin	00122301-1VL
SBAC	Bovine adrenal cortex, normal	91021201-1VL
SC-1	Mouse feral embryo	86060301-1VL
SC 11	Mouse Marsupial (*Sminthopsis crassicaudata*) kidney	90011892-1VL
SCC-4	Human tongue squamous carcinoma	89062002-1VL
SCC-9	Human squamous carcinoma of the tongue	89062003-1VL
54SCC2	Rat glioma x mouse L cell fibroblast hybrid	08062560-1VL
54SCC3	Rat glioma x mouse L cell fibroblast hybrid	08062561-1VL
54SCC7	Rat glioma x mouse L cell fibroblast hybrid	08062518-1VL
Sci-1	Human Caucasian B Cell Lymphoma	96090513-1VL
SCL 4.1/F7	Rat Schwann cell	93031204-1VL
SCP	Ovine brain choroid plexus	89101302-1VL
Sf21	*Spodoptera frugiperda* pupal ovarian tissue	05022801-1VL
Sf21 TitreHigh AC free	*Spodoptera frugiperda* pupal ovarian tissue, serum-free	05030202-1VL
Sf9 TitreHigh AC free	*Spodoptera frugiperda* pupal ovarian tissue, serum-free	05011001-1VL
Sf 1Ep	Rabbit epidermis, cottontail	90092702-1VL
SHK-1	Atlantic salmon head kidney	97111106-1VL
SHOK	Hamster Syrian embryo	90071805-1VL
SHP-77	Human Caucasian lung small cell carcinoma, large cell variant	98110201-1VL

Name	Description	Cat. No.
SIRC	Rabbit Cornea	89090404-1VL
SJMRF	Buffalo	92020421-1VL
SK-N-AS	Human neuroblastoma	94092302-1VL
SK-N-BE(2)	Human Caucasian neuroblastoma	95011815-1VL
SK-N-DZ	Human neuroblastoma	94092305-1VL
SK-N-F1	Human neuroblastoma	94092304-1VL
SK-HEP-1	Human liver adenocarcinoma	91091816-1VL
SK LU 1	Human Caucasian lung adenocarcinoma	93120835-1VL
SK-N-MC	Human neuroepithelioma, metastasis to supra-orbital area	90022302-1VL
SK MES 1	Human Caucasian lung squamous carcinoma	93120837-1VL
SK-PN-DW	Human primitive neuroectodermal tumour	94092301-1VL
SNU-C2B	Human Colorectal Carcinoma	96020932-1VL
Sp1K	Dolphin kidney	89120705-1VL
SP2/0-Ag14 (AC-free)	Mouse x mouse hybridoma non-secreting, serum-free, animal component (AC) free	08060101-1VL
Sp2/0-Ag14-TurboDoma	Mouse x Mouse hybridoma, non-producing, serum-free	03092501-1VL
SR-4987	Mouse bone marrow stromal cells	94102704-1VL
SSE-5	Fish Sockeye Salmon embryo	95122021-1VL
SSN-1	Fish striped snakehead fry, spontaneous production of c-type retrovirus	96082808-1VL
ST	Porcine testis, fetal	92040221-1VL
STE-137	Fish steelhead trout embryo	95122020-1VL
STO	Mouse SIM embryo fibroblast	86032003-1VL
3A sub E	Human placenta (post crisis); SV40 transformed	90110508-1VL
Subclone 707 BUF	Mouse Friend leukaemic lymphoblast	91112122-1VL
Subclone 707 DAP8	Mouse Friend leukaemic lymphoblast	91112129-1VL
SUP-T1	Human T cell lymphoblastic Lymphoma	95013123-1VL
SV3T3B	Mouse SIM embryo fibroblast	85011409-1VL
SVCT-MI2	Human Breast Epithelial, SV40 transformed	98031105-1VL
SV-T2	Mouse BALB/3T3 clone A31	90030801-1VL
SW 13	Human Caucasian adrenal cortex adenocarcinoma	87031801-1VL
SW 48	Human colon adenocarcinoma	89012702-1VL
SW 1116	Human Caucasian colon adenocarcinoma	87071006-1VL
SW 1417	Human colon adenocarcinoma	90102543-1VL
SW 1463	Human Caucasian rectum adenocarcinoma	90112713-1VL
SW 403	Human Caucasian colon adenocarcinoma	87071008-1VL
SW 626	Human Caucasian ovarian metastasis of a primary colon adenocarcinoma	91091203-1VL
SW 837	Human Caucasian rectum adenocarcinoma	91031104-1VL
SW 948	Human Caucasian colon adenocarcinoma	91030714-1VL
T24/83	Human bladder carcinoma	85061107-1VL
T7	Mouse Type-II lung epithelial cell	07021402-1VL
TA3 Hauschka	Mouse mammary carcinoma	85061102-1VL
TAK3	Human B cell lymphocyte, EBV transformed	93093004-1VL
TB1 Lu (NBL-12)	Bat lung	90020805-1VL
TE 671	Human Caucasian rhabdomyosarcoma	89071904-1VL
TE671 Subline No.2	Human Caucasian rhabdomyosarcoma	94052610-1VL
TGP 49	Mouse pancreatic acinar carcinoma	94022427-1VL
TGP 52	Mouse islet cell tumour	94022429-1VL
TGP 54	Mouse pancreatic islet cell tumour	94022430-1VL
TGP 55	Mouse pancreatic small cell anaplastic carcinoma	95062833-1VL
TH-1 subline B1	Turtle heart	90102528-1VL
L-M (TK-)	Mouse C34/An connective tissue	90083001-1VL
TK6	Human lymphoblast, thymidine kinase heterozygote	95111735-1VL
TK6TGR	Human lymphoblast	87020507-1VL
TK-TS 13	Hamster Syrian kidney	93120838-1VL
TM3	Mouse BALB/c testis, Leydig cell	91060526-1VL
TM4	Mouse BALB/c testis sertoli cell	88111401-1VL
TOU (TOU I-2)	Human B lymphocyte, EBV transformed	93093001-1VL
3A(tPA-30-1)	Human placenta, SV40 transformed	96020926-1VL
TPS	Fish Rainbow Trout *Pronephric Stroma*	96112839-1VL
TR33B	Rat Wistar-Furth oligodendroglioma	85040102-1VL
TR6Bc1	Mouse C3H.He Schwannoma	85042301-1VL
TRA-171	Insect larvae, mosquito	90120514-1VL
TT	Human Caucasian thyroid medullary carcinoma	92050721-1VL
Tufted Deer	Tufted deer lung	96011147-1VL
U266B1	Human myeloma	85051003-1VL
U937(CD59+)	Human Caucasian histiocytic lymphoma	95102435-1VL
UMR-106	Rat osteosarcoma	90111314-1VL
UMR-108	Rat Sprague-Dawley osteosarcoma	91060527-1VL
UM-RC-2	Human renal carcinoma	08090511-1VL
UM-RC-3	Human renal carcinoma	08090512-1VL
UM-RC-6	Human renal carcinoma	08090513-1VL
UM-RC-7	Human renal carcinoma	08090514-1VL
UM-UC-1	Human bladder transitional cell carcinoma	06080301-1VL
UM-UC-3	Human Bladder Transitional Cell Carcinoma	96020936-1VL
UM-UC-4	Human adenocarcinoma lymph node metastasis	08090501-1VL
UM-UC-5	Human squamous cell carcinoma of the bladder	08090502-1VL
UM-UC-6	Human transitional cell carcinoma of the bladder	08090503-1VL
UM-UC-7	Human transitional cell carcinoma of the bladder	08090504-1VL
UM-UC-9	Human transitional cell carcinoma of the bladder	08090505-1VL
UM-UC-10	Human transitional cell carcinoma of the bladder	08090506-1VL
UM-UC-11	Human transitional cell carcinoma of the bladder	08090507-1VL
UM-UC-13	Human transitional cell carcinoma lymphatic metastasis	08090508-1VL

ECACC® Cell Line Collections: General Collection

Name	Description	Cat. No.
UM-UC-14	Human transitional cell carcinoma of the renal pelvis	08090509-1VL
UM-UC-16	Human transitional cell carcinoma of the bladder	08090510-1VL
UT-1	Hamster Chinese ovary	87100701-1VL
V79	Hamster Chinese lung male	86041102-1VL
V79-4	Hamster Chinese lung	93010723-1VL
V79-HG04	Hamster Chinese embyro lung fibroblast transformed	87020406-1VL
VA-ES-BJ	Human skin epithelioid sarcoma	94092303-1VL
VP229	Human Breast Cancer	05092804-1VL
VP267	Human Breast Cancer	05092805-1VL
VP303	Human Breast Cancer	05092806-1VL
Vx2	Rabbit tumour	90030912-1VL
WB2054M	Rat colon carcinoma	91011802-1VL
WEHI 3B	Mouse BALB/c myelomonocyte	86013003-1VL
WEHI 3BD	Mouse BALB/c granulocytic leukaemia	92110401-1VL
WEHI 164 Cell Line	Mouse BALB/c fibrosarcoma	87022501-1VL
WEHI-231	Mouse B cell lymphoma	85022107-1VL
WEHI-274.1	Mouse monocyte	91061231-1VL
WEHI-279	Mouse B cell lymphoma	94110901-1VL
WERI	Human Retinoblastoma	06070602-1VL
WI 38VA13 Subline 2RA	Human Caucasian fetal lung, SV40 transformed	85062512-1VL
WiDr	Human colon adenocarcinoma	85111501-1VL
WIL2 NS	Human Caucasian B lymphocyte	90112121-1VL
WIL2.NS.6TG	Human Caucasian B lymphocyte	93031001-1VL
WILCL	Human Caucasian normal B cell	89120565-1VL
WISH (HeLa derivative)	Human Negroid cervix carcinoma	88102403-1VL
WI 26 VA4	Human Caucasian fetal lung, SV40 transformed	89101301-1VL
WKD (HeLa derivative)	Human Negroid cervix carcinoma	93120839-1VL
WM-115	Human melanoma	91061232-1VL
WM 266-4	Human melanoma	91061233-1VL
WR19L	Mouse lymphoma	95050414-1VL
WRC	Rat Walker carcinoma	88031148-1VL
WRL 68 (HeLa derivative)	Human Negroid cervix carcinoma	89121403-1VL
WS1	Human Negroid skin embryo	88021104-1VL
WX310	Mouse tumour of unknown lineage	85022818-1VL
X1/5	Human (HeLa) with tTA	95051229-1VL
XC	Rat Wistar sarcoma	88120601-1VL
XrS6	Hamster Chinese ovary	92052128-1VL
XrS6-hamKu80	Hamster Chinese ovary, transformed with hamster Ku80 cDNA	96012301-1VL
Y1	Mouse LAF1 adrenal cortex tumour	85062807-1VL
Y3.AG.1.2.3	Rat LOU myeloma	85110502-1VL
Y79	Human Caucasian retinoblastoma	86093003-1VL
YAC-1	Mouse lymphoma	86022801-1VL
YO	Rat myeloma	85110501-1VL
ZR-75-1	Human Caucasian breast carcinoma	87012601-1VL
ZR-75-30	Human Negroid breast carcinoma	88113004-1VL

Hybridoma Collection

Name	Source	Isotype	Description	Cat. No.
07-4D	Mouse x Mouse Hybridoma	IgG	Reactivity: Anti-Desmocollins, bovine	91121239-1VL
111A3	Mouse x Bovine Heterohybridoma	None	Application: Fusion partner in the production of heterohybridomas	85062504-1VL
11-5F	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Desmoplakins	91121236-1VL
13C4	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Shigella-like Toxin I (SLTI)	95032701-1VL
16-23	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-HLA-DR3 and DR52a	99043001-1VL
17D	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD4, ovine	91060551-1VL
1B8-F11	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Bovine Herpes Virus 1	95022202-1VL
1D10(ATT) Clone A6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-α1-Antitrypsin (also known as α1-AT or AAT)	02043018-1VL
20.27	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD1, sheep and cattle	93070779-1VL
20.76	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Leukocyte Molecules of 35kDa, sheep	93100510-1VL
25-32	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD44, ovine	91060546-1VL
2A2(PGM1) Clone A6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Phosphoglucomutase-1 (PGM1)	02043024-1VL
2D12	Mouse x Mouse Hybridoma	IgG2a, kappa	Reactivity: Anti-Yellow Fever Virus	91051509-1VL
2G2B(AAT) Clone 4D4	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-α1-Antitrypsin (also known as α1-AT or AAT)	02043016-1VL
2H6-C2	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-Bovine Herpes Virus 1 (BHV-1)	95022203-1VL
32-2B	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Desmoglein	91121237-1VL
33-3D	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Desmoglein	91121238-1VL
347C2	Mouse x Bovine Heterohybridoma	Bovine IgG1	Reactivity: Anti-F5 (K99) pilus antigen of E.coli	93071515-1VL
347C2b/D4	Mouse x Bovine Heterohybridoma	Bovine IgG1	Reactivity: Anti-F5 (K99) pilus antigen of E.coli	93071503-1VL
348C6	Mouse x Bovine Heterohybridoma	Bovine IgG1	Reactivity: Anti-F5 (K99) pilus antigen of E.coli	85062511-1VL
351D2/D7	Mouse x Bovine Heterohybridoma	Bovine IgG1	Reactivity: Anti-F5 (K99) pilus antigen of E.coli	93071501-1VL
357-101-4	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-TNF-α, human	92030603-1VL
357-167-24	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-TNF-α, human	92030604-1VL
359-81-11	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-TNF-β human	92030606-1VL
359-238-8	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-TNF-β human	92030605-1VL

Name	Source	Isotype	Description	Cat. No.
36F	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD2, ovine	91060547-1VL
3.C2	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-Myelin, fish	92092320-1VL
3C9-D11-H11	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Porcine Pparvovirus (PPV)	92022115-1VL
455	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Epidermal Growth Factor (EGF) Receptor	92090408-1VL
4.6.5a114K	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-Madin-Darby canine kidney cells	93062218-1VL
4G5(PGM1) Clone B7	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Phosphoglucomutase-1 (PGM1)	02043023-1VL
4H8(GC) Clone 4D4	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-group specific component (GC)	02043019-1VL
50-11-10	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-CD5, Human	94020814-1VL
11-5.2.1.9	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-I-Ak	89050905-1VL
52-3D	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Desmocollins	91121235-1VL
537-B1-1	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Lymphocytes/endothelium, sheep	93102701-1VL
53B3	Mouse x Bovine Heterohybridoma	None	Application: Fusion partner	85062501-1VL
54B3	Mouse x Bovine Heterohybridoma	None	Reactivity: Non Ig secreting	93071512-1VL
55-6	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-HIV-1 gp120	97040308-1VL
6.23.58K	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-Madin-Darby canine kidney cells	93062219-1VL
6D8 MB4	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-gp50 region of Pseudorabies virus	95022201-1VL
6G5(AAT) Clone 1C9	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-α1-Antitrypsin (also known as α1-AT or AAT)	02043017-1VL
6H7(PGM1) Clone D10	Mouse x Mouse Hybridoma	Not specified	Reactivity: Anti-Phosphoglucomutase-1 (PGM1)	02043022-1VL
73B	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45RA, ovine	91060554-1VL
7.4.C4	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-Fish Leukocytes, red blood cells and endothelial cells	92092321-1VL
7C2	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD8, ovine	91060550-1VL
7D4	Mouse x Rat Heterohybridoma	Rat IgM, kappa	Reactivity: Anti-Interleukin-2 receptors, murine	88111402-1VL
86D	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-T Cell Receptor (TcR) gamma/delta, ovine	91060557-1VL
8A12	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Guinea Pig Major Basic Protein	94091319-1VL
8C9	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-CD36, human	99072805-1VL
8D12	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Guinea Pig Major Basic Protein	94091320-1VL
94A1	Mouse x Bovine Heterohybridoma	Bovine IgG1	Application: Fusion partner	85062503-1VL
A1H4	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-Madin-Darby canine kidney cell E-cadherin (uvomorulin)	93062220-1VL
A2B5 clone 105	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Retinal Neuron Glycolipid, chick	90070501-1VL
10(AAT) Clone A	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-α1-Antitrypsin (also known as α1-AT or AAT)	02043015-1VL
AFRC 17D1	Horse x Mouse/Horse Hybridoma	None	Application: Fusion partner for hybridoma production	94120825-1VL
AFRC 56D2	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120826-1VL
AFRC 58D6	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120827-1VL
AFRC 78A3	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120828-1VL
AFRC 84B4	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120829-1VL
AFRC 84C6	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120830-1VL
AFRC 85A2	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120831-1VL
AFRC 85B5	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120832-1VL
AFRC 85D6	Horse x Mouse/Horse Hybridoma	Horse IgG	Reactivity: Anti-Haemagglutinin, influenza	94120833-1VL
AFRC IAH-CC1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45, bovine	95072101-1VL
AFRC IAH-CC101	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-WC1, bovine/porcine	99011338-1VL
AFRC IAH-CC104	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD11b, bovine	95072105-1VL
AFRC IAH-CC13	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-CD1, bovine	88022403-1VL
AFRC IAH-CC14	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD1, bovine	88022404-1VL
AFRC IAH-CC15	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-WC1, bovine	88022410-1VL
AFRC IAH-CC17	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD5, bovine	88022406-1VL
AFRC IAH-CC171	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD45, bovine	99011340-1VL
AFRC IAH-CC20	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD1, bovine	91080605-1VL
AFRC IAH-CC21	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-WC3, bovine	88022411-1VL
AFRC IAH-CC29	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD5, bovine	88022407-1VL
AFRC IAH-CC30	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD4, bovine	88022412-1VL
AFRC IAH-CC32	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-L-Selectin	95072102-1VL
AFRC IAH-CC37	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-WC3, bovine	92021415-1VL
AFRC IAH-CC38	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD6, bovine	88022413-1VL
AFRC IAH-CC39	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-WC1, bovine	91080607-1VL
AFRC IAH-CC42	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD2, bovine	88022408-1VL

ECACC® Cell Line Collections: Hybridoma Collection

Name	Source	Isotype	Description	Cat. No.
AFRC IAH-CC51	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-WC3, bovine	88022414-1VL
AFRC IAH-CC55	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-WC4, bovine	91080604-1VL
AFRC IAH-CC56	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-B cells, bovine	91080609-1VL
AFRC IAH-CC57	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-WC4, bovine	91080608-1VL
AFRC IAH-CC58	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD8, bovine	91080606-1VL
AFRC IAH-CC62	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD26 (formerly WC10), bovine	99011334-1VL
AFRC IAH-CC63	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD8, bovine	91080603-1VL
AFRC IAH-CC76	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45R, bovine	95072103-1VL
AFRC IAH-CC8	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD4, bovine	88022405-1VL
AFRC IAH-CC94	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD11b, bovine	95072104-1VL
AFRC IAH-CC98	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-WC6, bovine	99011337-1VL
AFRC MAC 1	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102263-1VL
AFRC MAC 2	Rat x Rat Hybridoma	IgG2c	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102264-1VL
AFRC MAC 3	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102265-1VL
AFRC MAC 4	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102266-1VL
AFRC MAC 5	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102267-1VL
AFRC MAC 6	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102268-1VL
AFRC MAC 7	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102269-1VL
AFRC MAC 8	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102270-1VL
AFRC MAC 9	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102271-1VL
AFRC MAC 10	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102274-1VL
AFRC MAC 11	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	85102273-1VL
AFRC MAC 12	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030301-1VL
AFRC MAC 13	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030302-1VL
AFRC MAC 14	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030303-1VL
AFRC MAC 15	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030304-1VL
AFRC MAC 17	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030306-1VL
AFRC MAC 18	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030307-1VL
AFRC MAC 19	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030308-1VL
AFRC MAC 20	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Glycoprotein of *Chlamydomonas reinhardii*	86030309-1VL
AFRC MAC 22	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, rabbit	92102336-1VL
AFRC MAC 23	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, rabbit	92102338-1VL
AFRC MAC 24	Rat x Rat Hybridoma	IgM	Reactivity: Anti-LDL, rabbit	92102337-1VL
AFRC MAC 25	Rat x Rat Hybridoma	IgM	Reactivity: Anti-LDL, rabbit	92102330-1VL
AFRC MAC 26	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, rabbit	92102345-1VL
AFRC MAC 27	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, rabbit	92102339-1VL
AFRC MAC 28	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-LDL, rabbit	92102331-1VL
AFRC MAC 29	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, rabbit	92102332-1VL
AFRC MAC 31	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, rabbit	92102333-1VL
AFRC MAC 48	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Phytochrome	85060403-1VL
AFRC MAC 49	Rat x Rat Hybridoma	IgG1	Reactivity: Anti-Phytochrome	85060404-1VL
AFRC MAC 50	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Phytochrome	85102259-1VL
AFRC MAC 51	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Phytochrome	85060405-1VL
AFRC MAC 52	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Phytochrome	85060406-1VL
AFRC MAC 54	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Phytochrome	85060407-1VL
AFRC MAC 57	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Lipopolysaccharide	86030310-1VL
AFRC MAC 60	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Abscisic acid, plant growth hormone	86052303-1VL
AFRC MAC 61	Rat x Rat Hybridoma	IgG2c	Reactivity: Anti-Abscisic acid, plant growth hormone	86052301-1VL
AFRC MAC 63	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Peribacteroid membrane from *Pisum sativum*	86030311-1VL
AFRC MAC 65	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Peribacteroid membrane from *Pisum sativum*	86030313-1VL
AFRC MAC 74	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Nitrate Reductase	86112690-1VL
AFRC MAC 75	Rat x Rat Hybridoma	IgG1	Reactivity: Anti-Nitrate Reductase	85102260-1VL
AFRC MAC 76	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Nitrate Reductase	85102261-1VL
AFRC MAC 77	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Nitrate Reductase	85102262-1VL
AFRC MAC 78	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Nitrate Reductase	85102272-1VL
AFRC MAC 79	Rat x Rat Hybridoma	IgG	Reactivity: Anti-Nitrate Reductase	86112691-1VL
AFRC MAC 127	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-LDL, human	92102334-1VL
AFRC MAC 128	Rat x Rat Hybridoma	IgM	Reactivity: Anti-LDL, Human	92102335-1VL

Name	Source	Isotype	Description	Cat. No.
AFRC MAC 129	Rat x Rat Hybridoma	IgG1	Reactivity: Anti-LDL, Human	92102340-1VL
AFRC MAC 130	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, Human	92102342-1VL
AFRC MAC 131	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-LDL, Human	92102343-1VL
AFRC MAC 132	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-LDL, Human	92102341-1VL
AFRC MAC 133	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-LDL, Human	92102344-1VL
AFRC MAC 156	Mouse x Rat Hybridoma	Rat IgG2a	Reactivity: Anti-Cytokinin, plant	93090996-1VL
AFRC MAC 157	Mouse x Rat Hybridoma	Rat IgG2a	Reactivity: Anti-Cytokinin, plant	93090997-1VL
AFRC MAC 158	Mouse x Rat Hybridoma	Rat IgM	Reactivity: Anti-Cytokinin, plant	93090998-1VL
AFRC MAC 159	Mouse x Rat Hybridoma	Rat IgM	Reactivity: Anti-Isopentenyladenosine (2iPA), plant	93090999-1VL
AFRC MAC 160	Mouse x Rat Hybridoma	Rat IgG2a	Reactivity: Anti-Cytokinin, plant	93091001-1VL
AFRC MAC 188	Rat x Rat Hybridoma	IgG1	Reactivity: Anti-LDL receptor, rabbit	92102372-1VL
AFRC MAC 252	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Abscisic acid, plant growth hormone	01071008-1VL
AFRC MAC 256	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Maize membrane auxin binding protein	89120557-1VL
AFRC MAC 257	Mouse x Rat Heterohybridoma	Rat IgM	Reactivity: Anti-Maize membrane auxin binding protein	89120558-1VL
AFRC MAC 258	Rat x Rat Hybridoma	IgM	Reactivity: Anti-Maize membrane auxin binding protein	89120559-1VL
AFRC MAC 259	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Maize membrane auxin binding protein	89120560-1VL
AFRC MAC 260	Mouse x Rat Heterohybridoma	Rat IgG2c	Reactivity: Anti-Maize membrane auxin binding protein	89120561-1VL
AFRC MAC 61.6	Rat x Rat Hybridoma	IgG2c	Reactivity: Anti-Abscisic acid, plant growth hormone	01010804-1VL
50 AZA SEL	Mouse x Sheep Heterohybridoma	None	Application: Fusion partner	85062505-1VL
B1D6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Annexin II, human	98042014-1VL
B3/25	Mouse x Mouse Hybridoma	IgG	Reactivity: Anti-Haematopoietic Cell, human	94050507-1VL
BBA4	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Trypanosoma brucei basal bodies	00020912-1VL
BC9	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-TNF, bovine	95030301-1VL
BF-11	Rat x Mouse Hybridoma	Rat IgG2a	Reactivity: Anti-Erythropoietin, human	95050413-1VL
BMAC-1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45 determinant 2b, human	89062103-1VL
BMAC-3	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45 determinant 2a, human	89062105-1VL
C1	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-HLA DQ2	99061825-1VL
C3B9	Mouse x Mouse Hybridoma	IgG12b	Reactivity: Anti-Acetylated α-Tubulin	00020913-1VL
C7	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-LDL Receptors, human & bovine	88090802-1VL
CAR-2/3F4	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Thioredoxin, human	97011602-1VL
CAR-XP10	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Thioredoxin, human (monomeric and polymeric forms)	97011601-1VL
CIIC1	Mouse x Mouse Hybridoma	IgG2a, kappa	Reactivity: Anti-Type II collagen	89083001-1VL
Clone 197	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-T19, ovine	91060548-1VL
Clone 218	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD49d, ovine	91060545-1VL
CLVIII-3A6-E3	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-L. panamensis-Amastigote & Promastigote	08041720-1VL
CT14-B2	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-c-Myc gene product, human	85102205-1VL
CT9-B7	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-c-Myc gene product, human	85102206-1VL
DA6.147	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II α-chain, human	90110605-1VL
DA6.231	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II β-chain, human	90110606-1VL
Du 1-29	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-L-Selectin, ovine	91060553-1VL
7E10(GC) Clone A	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-group specific component (GC)	02043021-1VL
1E7(GC) Clone B	Mouse x Mouse Hybridoma	IgG1, IgGM	Reactivity: Anti-group specific component (GC)	02043020-1VL
ED1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Macrophage, rat	89040701-1VL
ED17	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Myeloid Antigen 55kDa, rat	95110627-1VL
ED2	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Macrophage, rat	89040702-1VL
ED3	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Macrophage, rat	89040703-1VL
ED7	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD11b (CR3), rat	95110624-1VL
ED8	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD11b (CR3), rat	95110625-1VL
ED9	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Myeloid Protein, rat	95110626-1VL
F10-89-4	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD45 determinant 1, human	89062101-1VL
F10-150	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD11a (LFA-1), ovine	91060552-1VL
F10-150.58	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-LFA-1, sheep	93100509-1VL
F12	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Haemolysin, *Aeromonas* species	92032412-1VL
F15-42-1-5	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Thy-1, human	89062107-1VL
F16-4-4-11	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class I RT1-A, rat (monomorphic determinant)	89062109-1VL
F17-23-2	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC Class II RT1-B Antigen, rat (polymorphic determinant)	89062110-1VL

ECACC® Cell Line Collections: Hybridoma Collection

Name	Source	Isotype	Description	Cat. No.
F3-87-8-1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CNS specific membrane glycoprotein, human	89062108-1VL
F8-11-13	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45R, human	89062102-1VL
FC4.14F8	Unknown	IgG1	Reactivity: Anti-Leukocyte Molecules, sheep	93100516-1VL
FL.1	Mouse x Mouse Hybridoma	IgG2b, kappa	Reactivity: Anti-Protein-Tyrosine Phosphatase	92092319-1VL
FW 11.10.3	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD44 v4, human	93070776-1VL
FW 11.24.7.36	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD44 v9, human	93070775-1VL
FW 11.9.2.2	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD44 v6, human	93070778-1VL
FW14.14.18	Unknown	IgG1	Reactivity: Anti-α-6 Integrin, human	93100512-1VL
FW3-47	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD29, ovine	91060559-1VL
FW3.132.6	Unknown	IgG1	Reactivity: Anti-Leukocyte Molecules, sheep	93100514-1VL
FW3.151	Unknown	IgG2b	Reactivity: Anti-Leukocytes, 44kDa antigen, sheep	93100515-1VL
FW 4.101	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD29 (β1 integrin)	93070777-1VL
GA4	Mouse x Sheep Heterohybridoma	None	Application: Fusion partner	85062502-1VL
GAD-1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Glutamic acid decarboxylase	92020601-1VL
H129-121.6.8	Mouse x Rat Heterohybridoma	Rat IgG2b	Reactivity: Anti-Transferrin Receptor, mouse	85103103-1VL
H129-19.6.8	Mouse x Rat Heterohybridoma	IgG2a, kappa	Reactivity: Anti-L3T4, mouse	85103101-1VL
H139-52	Mouse x Rat Heterohybridoma	Rat IgG1, kappa	Reactivity: Anti-κ-Chain, mouse	85103111-1VL
H1a	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-IgM, grey seal	93031701-1VL
H24b	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG, grey seal	93031702-1VL
H25B10	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Hepatitis B Surface Antigen	90120615-1VL
H49a	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG1, grey seal	93031703-1VL
H6/31HL	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-IgD, mouse	85102214-1VL
H81-208.22.6	Mouse x Mouse Hybridoma	IgG2a, kappa	Reactivity: Anti-I-Ek	85062136-1VL
H9-14.8	Mouse x Mouse Hybridoma	IgG2a, kappa	Reactivity: Anti-I-Ek	85103106-1VL
H97-102.11.3	Mouse x Mouse Hybridoma	IgG2a, kappa	Reactivity: Anti-H-2Dd	85062137-1VL
HA cl. 55	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-Measles Virus Haemagglutinin	95040311-1VL
HFN 36.3	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Fibronectin	89062006-1VL
HFN 7.1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Fibronectin, human	89062001-1VL
HK-PEG-1	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-Influenza Virus	90112229-1VL
HP 6053	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-κ-Chain, human	94050510-1VL
HP 6054	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-λ-Light chain, human	93092704-1VL
HyGPD-YK-1-1	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-Muscle Glyceraldehyde-3-phosphate Dehydrogenase, rabbit	92120704-1VL
IC-01	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Galactocerebroside, monoglactosyldiglyceride psychosine	93042001-1VL
IC-07	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-Galactocerebroside, chicken	93062301-1VL
IC-08	Mouse x Mouse Hybridoma	IgG	Reactivity: Anti-Oligodendrocytes and Schwann cells, bovine	93062302-1VL
IC-09	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-Oligodendrocytes and Schwann cells, bovine	93062303-1VL
IgG-6A6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-VLDL receptor	97040309-1VL
IgG-B16	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Oxysterol Binding Protein	94080428-1VL
IL-A105	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD8, bovine	91072535-1VL
IL-A11	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD4, bovine	91072511-1VL
IL-A16	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD11c, bovine	91072514-1VL
IL-A19	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC class I, bovine	91072515-1VL
IL-A2	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG, bovine	91072510-1VL
IL-A21	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC class II, bovine	91072516-1VL
IL-A24	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Myeloid Cells, bovine	91072517-1VL
IL-A29	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-WC1, bovine	91072519-1VL
IL-A30	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgM (monomorphic epitope), bovine	91072520-1VL
IL-A42	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD2, bovine	91072521-1VL
IL-A43	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD2, bovine	91072522-1VL
IL-A46	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Myeloid Cells, bovine	91072523-1VL
IL-A50	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-IgM (polymorphic determinant), bovine	91072524-1VL
IL-A57	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD6, bovine	91072526-1VL
IL-A58	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Ig light chain, bovine	91072527-1VL
IL-A59	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Ig light chain, bovine	91072528-1VL
IL-A60	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG1, bovine	91072529-1VL
IL-A71	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-IgA, bovine	91072532-1VL

Name	Source	Isotype	Description	Cat. No.
IL-A88	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC class I, bovine	91072534-1VL
IX-2H7-E10	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-L. (L) amazonensis-less with L. major	08041722-1VL
J5	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-T Cell Activation Antigen, bovine	91072536-1VL
JB1	-	IgG3	Reactivity: Anti-Aggregated HFE protein, Human	01091808-1VL
JW183/5/1	Mouse + Human Transfectoma	IgG2	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	87080703-1VL
JW184/2/1	Mouse + Human Transfectoma	IgG4	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	87080704-1VL
JW393/A	Mouse + Human Transfectoma	IgA2	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	87080702-1VL
JW8/5/13	Mouse + Human Transfectoma	IgE	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	87080706-1VL
KM703	Rat x Mouse Hybridoma	Rat IgG2a	Reactivity: Anti-Pgp-1, murine homologue of CD44	94090613-1VL
L180/1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD58 (LFA-3), ovine	91060558-1VL
LDS 73/LW1	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Glutathione S-Transferase subunit YC2	96050401-1VL
LI-3H2-G6	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-Leishmania (L) donovani	08041714-1VL
LR-3	Rat x Mouse Hybridoma	Rat IgM	Reactivity: Anti-Heat Stable Antigen, mouse	93062221-1VL
Lu2	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-DR11	99043003-1VL
Lu6	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-HLA DRII	99043004-1VL
LXVIII-4D8-E3	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-L. panamensis-Amastigote & Promastigote	08041712-1VL
LY9	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-HLA DR7, DRw10,DRw53	99061826-1VL
M290	Mouse x Rat Hybridoma	Rat IgG2a	Reactivity: Anti-α-E (α-M290) integrin subunit, mouse	93012001-1VL
Mc 39-16	Mouse x Rat Heterohybridoma	Rat IgG2a	Reactivity: Anti-A31 idiotype, murine	89072609-1VL
MCP21	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Proteasomes, human	96030418-1VL
MH01	Human x Mouse Hybridoma	Human IgM	Reactivity: Anti-Neurones	93062401-1VL
MN4-91-6.E2	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-RT1-A MHC class I antigen (polymorphic determinant), rat	89062111-1VL
MT3/B4	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-rh Interferon α-2 (recombinant human) and related subtypes	92030607-1VL
MT4/E4	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interferon α-2, human and some related subtypes	92030608-1VL
Myb2-7	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-myb gene products, avian	84110801-1VL
Myb2-32	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-myb gene products, avian	84101003-1VL
Myb2-37	Mouse x Mouse Hybridoma	IgG2b, kappa	Reactivity: Anti-myb gene products, avian	84110802-1VL
Mycl-3C7	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-c-Myc gene product, human	85102203-1VL
Mycl-8F9	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-c-Myc gene product, human	85102204-1VL
Mycl-9E10	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-c-Myc gene product, human	85102202-1VL
NA1/34.HLK	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD1a, human	85102243-1VL
NI 1-_-364/3B3-14	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interleukin-1, human	92030611-1VL
NI 1-_-364/8C4-8	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interleukin-1, human	92030612-1VL
NI G 386/3B6-12	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Granulocyte Colony Stimulating Factor (G-CSF), human	92030614-1VL
NI G 386/3D1-16	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Granulocyte Colony Stimulating Factor (G-CSF), human	92030613-1VL
NI 3 416/6C6-6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interleukin-3, human	92030615-1VL
NI 3 416/7B5-5	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interleukin-3, human	92030616-1VL
NI GM 465/7A6-37	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-GM-CSF, human	92030620-1VL
NP cl.120	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Measles Virus Nucleoprotein	95040312-1VL
NP cl.25	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Measles Virus Nucleoprotein	95051114-1VL
NRbM	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgM, rabbit	91073008-1VL
OKM1	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD11b, human	88012702-1VL
OKT1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD5, human	86022705-1VL
OKT10	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD38, human	87021903-1VL
OKT11	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD2, human	86050802-1VL
OKT3	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD3, human	86022706-1VL
OKT4	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD4, human	87012602-1VL
OKT5	Mouse x Mouse Hybridoma	IgG2	Reactivity: Anti-Suppresser/cytotoxic T cells, human	88070705-1VL
OKT6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD1a, human	87091001-1VL
OKT8	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD8, human	85112802-1VL
OKT9	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD71, human	86022707-1VL
OX-1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45, rat	84112004-1VL
OX-2	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Thymocyte, brain, B cells, dendritic cells, rat	84112005-1VL
OX-3	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Ia (polymorphic), rat	84112006-1VL

ECACC® Cell Line Collections: Hybridoma Collection

Name	Source	Isotype	Description	Cat. No.
OX-7	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Thy-1.1	84112008-1VL
OX-12	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-κ-Chain, rat	88051301-1VL
OX-14	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG2b, rat	87112401-1VL
OX-17	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Ia-E, rat	84112010-1VL
OX-18	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class I, rat	84112011-1VL
OX-19	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD5, rat	84112012-1VL
OX-20	Rat x Rat Hybridoma	IgG1	Reactivity: Anti-κ-Chain, mouse	88070801-1VL
OX-21	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-C3b Inactivator, human	91060417-1VL
OX-22	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45, rat	84112013-1VL
OX-24	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Factor H, human	00010403-1VL
OX-26	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Transferrin receptor, CD71, rat	84112014-1VL
OX-27	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC Class I (polymorphic), rat	84112015-1VL
OX-29	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD45, rat	88070802-1VL
OX-30	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD45, rat	86100901-1VL
OX-32	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD45R, rat	88051302-1VL
OX-33	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45, rat	86100902-1VL
OX-34	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD2, rat	86100903-1VL
OX-38	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD4, rat	88051303-1VL
OX-39	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD25, rat	86100905-1VL
OX-40	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD134, rat	86100906-1VL
OX-41	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-Macrophage subset, rat	88051304-1VL
OX-43	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-macrophages, red blood cells and endothelium, rat	87081804-1VL
OX-44	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD53, rat	92102355-1VL
OX-45	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-BCM1, rat	86100910-1VL
OX-47	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Lymphocytes (activated), rat	90071611-1VL
OX-49	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD44, rat	91062501-1VL
OX-50	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD44, rat	91062502-1VL
OX-52	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-pan T cell, rat	87081805-1VL
OX-53	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD2, rat	89081707-1VL
OX-54	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD2, rat	88070803-1VL
OX-55	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD2, rat	88070804-1VL
OX-57	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD43, rat	91051424-1VL
OX-58	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD43, rat	91051425-1VL
OX-59	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG, rat	92102356-1VL
OX-60	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgD, rat	92102357-1VL
OX-61	Mouse x Mouse Hybridoma	IgG3	Reactivity: Anti-CD26, rat	91100409-1VL
OX-68	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD4, rat	94011007-1VL
OX-74	Mouse x Mouse Hybridoma	IgG	Reactivity: Anti-CD43, rat	91051426-1VL
OX-75	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-CD43, rat	91051427-1VL
OX-78	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-BCM1, mouse	92102358-1VL
OX-81	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interleukin-4, rat	95040518-1VL
OX-85	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-L-Selectin, rat	95111405-1VL
OX-86	Rat x Mouse Hybridoma	Rat IgG1	Reactivity: Anti-CD134, mouse	96110601-1VL
OX-87	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Interleukin 4, rat	95102516-1VL
OX-89	Rat x Mouse Hybridoma	Rat IgG1	Reactivity: Anti-OX40 ligand (CD134 ligand), mouse	03062501-1VL
OX-90	Rat x Mouse Hybridoma	Rat IgG2a	Reactivity: Anti-CD200 (OX2), mouse	03062502-1VL
OX-101	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD47, rat	03062503-1VL
OX-102	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD200 receptor, rat	03062504-1VL
OX-104	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD200 (OX2), human	03062505-1VL
OX-6 Cell Line	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Ia, rat	84112007-1VL
OX-8 Cell Line	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD8, rat	84112009-1VL
OX-35 Cell Line	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD4, rat	89041184-1VL
OX-42 Cell Line	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD11, rat	87081803-1VL
OX-62 Cell Line	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-dendritic cells, rat	91061805-1VL
OX-36 clone 3	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-CD4, rat	91051422-1VL
PAP 7	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Papaya Proteinase 4	86080701-1VL
PAP 8	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Papaya Proteinase omega	86080702-1VL

Name	Source	Isotype	Description	Cat. No.
PC615.3	Mouse x Rat Hybridoma	Rat IgG1	Reactivity: Anti-T cell IL-2 receptor, murine	88041902-1VL
PNMR 120E	-	IgM	Reactivity: Anti-Neural Crest (Quail) and human neuroendocrine tissue	01080926-1VL
PNMR 160D	-	IgM	Reactivity: Anti-Neural Crest (Quail) and human neuroendocrine tissue	01080923-1VL
PNMR OE	-	IgM	Reactivity: Anti-Neural Crest (Quail) and human neuroendocrine tissue	01080925-1VL
PNV35B	-	IgM plus IgG	Reactivity: Anti-Osteoclasts (human foetal), ECV304 and U937 cells	01080924-1VL
PS/2	Mouse x Rat Hybridoma	Rat IgG2b	Reactivity: Anti-VLA-4 integrin-like cell adhesion molecule, murine	93091302-1VL
R1	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-HLA-DQ	99043005-1VL
R4-6A2	Mouse x Rat Hybridoma	Rat IgG1	Reactivity: Anti-Interferon-γ murine	91032502-1VL
R73	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-T cell receptor (TcR), rat	90091904-1VL
RFAL3	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-CD10, human	87100702-1VL
S2	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-HLA DR13	99043002-1VL
S2.1	Pig x Pig Hybridoma	IgA	Reactivity: Anti-Swine Testis (ST) cell antigen	92040116-1VL
S2.2	Pig x Pig Hybridoma	IgA	Reactivity: Anti-Swine Testis (ST) cell antigen	92040117-1VL
SJK-132-20	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-DNA Polymerase, human	88041903-1VL
SJK-287-38	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-DNA Polymerase, human	90112228-1VL
SW73.2	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-MHC class II, ovine	93113021-1VL
T11D7	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Thy-1.1	92021209-1VL
T11D7e2	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Thy-1.1	92021210-1VL
TAT-1	Mouse x Mouse Hybridoma	IgG12b + IgG2a	Reactivity: Anti-α-Tubulin	00020911-1VL
TFT B1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Ricin β-chains	94102701-1VL
THG1-24	Mouse + Human Transfectoma	IgG1	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	87120202-1VL
THG3-B5-13	Mouse + Human Transfectoma	IgG3 G3m(g)	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	88022402-1VL
THG3-MP-2-19-3-8	Mouse + Human Transfectoma	IgG3 G3m(b)	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	88012701-1VL
THM 7.3.5	Mouse + Human Transfectoma	IgM	Reactivity: Anti-NP (4-hydroxy-3 nitrophenacetyl)	87120201-1VL
TM1	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Leu-5	92100624-1VL
P-tyr-1	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Phosphotyrosine	95092903-1VL
U28	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-IgG2a, mouse	93022518-1VL
VPM 5	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-CD1, ovine	93113022-1VL
VPM 6	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-IgG heavy chain, ovine	93113023-1VL
VPM 8	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Ig light chain, ovine	93113024-1VL
VPM 18	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD45, ovine	93113025-1VL
VPM 19	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class I, heavy chain, ovine	93113026-1VL
VPM 36	Mouse x Mouse Hybridoma	IgG1, kappa	Reactivity: Anti-MHC class II DQ chain, ovine	93113027-1VL
VPM 37	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-DRβ chain, ovine	93113028-1VL
VPM 38	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II α-chain, ovine	93113029-1VL
VPM 40	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II DQβ chain, ovine	93113030-1VL
VPM 41	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II DQβ chain, ovine	93113031-1VL
VPM 43	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II DRβ chain, ovine	93113032-1VL
VPM 44	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II DQβ chain, ovine	93113033-1VL
VPM 45	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC class II DQβ chain, ovine	93113034-1VL
VPM 46	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC class II β chain, ovine	93113035-1VL
VPM 47	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-MHC class II DR α-chain, ovine	93113036-1VL
VPM 48	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-MHC class II α- chain, ovine	93113037-1VL
VPM 54	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-DR α-chain, ovine	93113038-1VL
W3/13	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD43, rat	84112001-1VL
W3/15	Mouse x Mouse Hybridoma	-	Reactivity: Anti-Thymocytes/erythrocytes, rat	88051305-1VL
W3/25	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-CD4, rat	84112002-1VL
W6/32	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-HLA-A,B,C	84112003-1VL
W6/32HK	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-HLA	86012801-1VL
W6/32HL	Mouse x Mouse Hybridoma	IgG2a	Reactivity: Anti-HLA-A,B,C	85102222-1VL
WM1	Mouse x Mouse Hybridoma	IgG1, lambda	Reactivity: Anti-C3, human	92021211-1VL
XCIII-1F8-D7	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Leishmania (L) pifanoi Amastigote	08041723-1VL
XCIII-3D8-D8	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Leishmania (L) pifanoi -Amastigote	08041724-1VL
XCIV-1H2-A8	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-Leishmania (L) tropica	08041728-1VL

Name	Source	Isotype	Description	Cat. No.
XXIX-1G12-G7	Mouse x Mouse Hybridoma	IgG1	Reactivity: Anti-Leishmania (L) infantum	08041715-1VL
Y13-238	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-p21 Protein, Harvey Murine Sarcoma Virus	89111411-1VL
Y13-259	Rat x Rat Hybridoma	IgG1	Reactivity: Anti-p21 Protein, Harvey Murine Sarcoma Virus	89111412-1VL
YBM 15.1.6	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-MAC-1, mouse	89040602-1VL
YBM 6.1.10	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-MAC-1, mouse	89040601-1VL
YL1/2	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Tubulin	92092402-1VL
YN1/1.7.4	Rat x Rat Hybridoma	-	Reactivity: Anti-Murine Activated Lymphocyte Antigen-2 (MALA-2)	90110706-1VL
YOL1/34	Rat x Rat Hybridoma	IgG2a	Reactivity: Anti-Tubulin	85102247-1VL
YTA 3.1.2	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-L3T4 epitope B, mouse	89040603-1VL
YTS 121.5.2	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Lyt-1, mouse	87072283-1VL
YTS 154.7.7.10	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Thy-1, mouse	87072285-1VL
YTS 156.7.7	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Lyt-3 epitope B, mouse	89040604-1VL
YTS 169.4.2.1	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-Lyt-2, mouse	87072284-1VL
YTS 191.1.1.2	Rat x Rat Hybridoma	IgG2b	Reactivity: Anti-L3T4, mouse	87072282-1VL
Z2	Mouse x Mouse Hybridoma	IgG2b	Reactivity: Anti-IgG2a, mouse	93022522-1VL
Z26	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-IgG2a, mouse	93022519-1VL
Z34	Mouse x Mouse Hybridoma	IgA	Reactivity: Anti-IgG2a and IgG2b (allotype dependent), mouse	93022521-1VL
Z41	Mouse x Mouse Hybridoma	IgM	Reactivity: Anti-IgG2a, mouse	93022520-1VL

Hybridoma II

Name	Source	Isotype	Description	Cat. No.
111A3	Mouse x Bovine Heterohybridoma	None	Application: Fusion partner in the production of heterohybridomas	85062504-1VL
53B3	Mouse x Bovine Heterohybridoma	None	Application: Fusion partner	85062501-1VL
94A1	Mouse x Bovine Heterohybridoma	Bovine IgG1	Application: Fusion partner	85062503-1VL
AFRC 17D1	Horse x Mouse/Horse Hybridoma	None	Application: Fusion partner for hybridoma production	94120825-1VL
50 AZA SEL	Mouse x Sheep Heterohybridoma	None	Application: Fusion partner	85062505-1VL
GA4	Mouse x Sheep Heterohybridoma	None	Application: Fusion partner	85062502-1VL

Cell Line Authentication

Authentication Techniques

Whatever the scope of work to be carried out it is important to know that the work is being conducted using the correct reagents. This is no less important for cell cultures, since if cell cultures are not what they are reported to be then work can be invalidated and resources wasted. There is now considerable evidence of gross cross-contamination of cell lines, in particular with HeLa (Catalog Number 93021013) where up to 16 lines have been offered to ECACC® with DNA profiles identical to HeLa. These include Hep 2, WISH, INT 407, Chang liver and Giradi heart. To minimize the risk of working with contaminated cell lines it is advisable to obtain cells from a recognized source such as a culture collection that will have confirmed the identity of the cells as part of the banking process.

Tests used to authenticate cell cultures include iso-enzyme analysis, karyotyping/cytogenetic analysis, and more recently molecular techniques of DNA profiling. While most of the techniques above are generalized tests and are applicable to all cell lines additional specific tests may also be required to confirm the presence of a product or antigen of interest.

Iso-Enzyme Analysis

Iso-enzymes are a series of enzymes present in different species that have similar catalytic properties, but differ in their structure. By studying the iso-enzymes present in cell lines it is possible to identify the species from which the cell line was derived. The technique is also used as a means of excluding the possibility of gross cross-contamination of the cell line with another culture of a different species.

The principles upon which iso-enzyme analysis is based are:

- Each iso-enzyme has multiple gene loci coding for different polypeptides with identical enzyme activity (e.g. lactate dehydrogenase, LD)
- Electrophoretic migration rates change dependent on sub-unit composition e.g. LD has five possible iso-forms (LD 1-5)
- Different species have different combinations of these iso-forms
- Using a typical panel of 4 iso-enzymes a composite picture is built up enabling the species of origin to be determined by the use of reference tables

DNA Fingerprinting

DNA fingerprinting enables the following:

- Identification of individual cell lines from the same species
- Confirmation of the identity of cell banks compared to reference master stocks
- Detection of cross-contamination

Multi-locus DNA fingerprinting and multiplex—PCR DNA profiling are the methods used routinely as part of ECACC's routine cell banking procedures.

Multi-Locus DNA Fingerprinting

- Uses multi-locus Jeffrey's probes 33.15 or 33.6, along with Southern blotting technology producing a complex banding pattern
- Probes cross-hybridize with most common species
- Has the disadvantage that the profiles require visual interpretation and comparison with other samples can be subjective

Multiplex-PCR (STR) DNA Profiling

- Uses a set of primers (9 used at ECACC) recognizing micro-satellites using PCR and automated DNA sequencing techniques
- Primers are species specific and are used only for human cell lines
- Produces a color-coded banding pattern, that translates into a digital code that can easily be stored on a database and compared to other stored profiles

Authentication—An Essential Part of Any Cell Culture Operation

Are You Confident You Are Working With The Cell Line You Think You Are?

Isabel Atkin and Bryan Bolton
European Collection of Cell Cultures
Health Protection Agency, Porton Down, Wiltshire, UK

Authentication, the process of determining the true origin and identity of cell lines, should concern everyone using cell cultures. The consequences of working with a cell line that is mis-identified or cross-contaminated with cells from a different origin can be devastating. Results invalidated, products devalued, years of research made irrelevant. Imagine discovering the hepatocytes you are using to study liver dysfunction are actually cervical carcinoma cells. Or your extensive work on a 'spontaneously transformed' human umbilical cord cell line is invalidated, as it is in fact a human bladder carcinoma epithelial cell line. This has been a bleak reality for some scientists.

Instances of cross-contamination of cell lines are more common than generally appreciated. Estimates suggest that up to 1 in 5 experiments in fields such as microbiology and cancer employ the wrong cells. In a study of 252 human cell lines 18% were found to be cross-contaminants (Macleod et al., 1999). Last year a study of over 500 human leukemia-lymphoma cell lines showed 15% were not unique (Drexler et al., 2003).

Can you afford not to take the precaution of authenticating your cell lines? A vivid demonstration of the importance of authentication has been provided through the progressive realization, since the late 1960s, that a large number of human cell lines assigned unique identities and tissues of origin were, in fact, all cervical carcinoma HeLa cells. Table 1 lists cell lines that have been identified as HeLa contaminants. In addition, Table 2 shows examples of contaminated cell lines not involving HeLa.

Despite wide publicity of the HeLa contamination story, and the cell lines affected, there remains a lack of awareness of the dangers of cross-contamination of cell lines and the value of authentication. A search of literature published in 2003 to spring 2004 revealed researchers continuing to use cell lines, identified as HeLa contaminants, for purposes dictated by their original, incorrect designation. For example the KB cell line, an established HeLa contaminant, was referred to as being an oral carcinoma cell line and used for periodontal disease studies. Likewise, the WISH cell line, originally considered to be derived from human amnion cells, but since shown to be a HeLa contaminant, has been used to study amnion pathophysiology. Many cell lines are morphologically very similar, without use of recognized authentication techniques cross-contamination can remain undetected, potentially for years. Even where expression of certain markers suggests a cell line is unique this may not be the case.

Table 1. Cell lines determined to be HeLa contaminants listed by ECACC®

Cell Line Name	ECACC® Cat. No.
AV3 (HeLa Derivative)	88102402
C16 (HeLa Derivative)	84121902
Chang Liver (HeLa Derivative)	88021102
Clone 1-5c-4 (HeLa Derivative)	88021103
D98/AH2 Clone B (HeLa Derivative)	85112701
FL (HeLa Derivative)	90111910
Girardi Heart (HeLa Derivative)	93120822
Hep2 (Clone 2B) (HeLa Derivative)	85011412
HEP2 (HeLa Derivative)	86030501
INT 407 (HeLa Derivative)	85051004
Jill (HeLa Derivative)	93120824
KB (HeLa Derivative)	94050408
L-41 (HeLa Derivative)	96121716
L132 (HeLa Derivative)	89111004
WISH (HeLa Derivative)	88102403
WKD (HeLa Derivative)	93120839
WRL 68 (HeLa Derivative)	89121403

For example, ED(67), originally considered a trophoblast-like cell line derived from human chorionic villus, appeared to be phenotypically distinct, but has been shown to be genetically identical to the HeLa cell line (Kniss et al., 2002). Suspicions were aroused when the cells were shown to lack properties of primary cells from the same origin i.e. primary villus cytotrophoblasts; secretion of human chorionic gonadotropin could not be detected. HeLa cells had never been grown in the laboratory; the origin of the HeLa contamination was traced to the WISH cell line.

Table 2. Examples of Contaminated Cell Lines Not Involving HeLa Cell Lines

Cell Line	Originally Referred to As:	Determined to Be:
ECV304	'Spontaneously transformed' human umbilical cord endothelial cells	Genetically identical to T24 — a human bladder carcinoma epithelial cell line (Macleod et al., 1999)
TSU-Pr1	A unique human prostate cell line	A derivative of the bladder carcinoma cell line T24 (van Bokhoven et al., 2001)
JCA-1	A unique human prostate cell line	A derivative of the bladder carcinoma cell line T24 (van Bokhoven et al., 2001)
JROECL 47	A unique human esophageal adenocarcinoma cell line	An admixture of the human colon adenocarcinoma cell line HCT 116 (Wijnhoven et al., 2000)

Reduce the Risk

Every cell culture laboratory is at risk from cross-contamination and misidentification of cells. The common practice of exchanging cell lines between research laboratories, the use of several different cell lines in a single facility, and the greater the number of individuals in contact with a cell line all contribute to the risk. As do a high throughput of short-term research staff, limited number of trained cell culturists, and little in the way of defined cell culture disciplines and quality control.

Take measures to protect the quality and relevance of your work by authenticating the cell lines you use. ECACC advises adherence to three key practices to enhance confidence and reliability in the cell lines you work with:

Start With Authenticated Cell Lines

This can be achieved by obtaining cell lines from a well documented and quality controlled source such as a recognized culture collection. If a cell line is only available by transfer between research laboratories, request evidence of its providence. It records are not available authenticate the cell line prior to use.

Ensure Good Cell Culture Practice is in Place

If high quality practical cell culture training is not readily available in-house, why not consider the cell culture training courses offered by ECACC®?

Re-authenticate Cell Lines at Regular Intervals

If you do not have the resources to authenticate cell lines at your laboratory use an established authentication service. If you are generating new cell lines it is advisable to define their DNA profiles at an early stage. Results from the study of 500 leukemia-lymphoma cell lines, mentioned previously, implied that most contamination occurs during the initial establishment of a cell line (Drexler et al., 2003). The EBV (Epstein-Barr virus) lymphocyte immortalization and cell banking service at ECACC routinely stores blood samples on filter paper and compares DNA from the end lymphoblastoid cell lines generated with DNA from the original blood donors.

Methods of Authentication Used at ECACC®

ECACC authenticates all cell lines deposited into its cell collection and offers a cell line authentication service. A combination of isoenzyme analysis, multi-locus DNA fingerprinting, and Short Tandem Repeat (STR) multiplex PCR analysis (also known as multiplex DNA fingerprinting) is used to authenticate cell lines.

Isoenzyme analysis utilizes the property that isoenzymes have similar substrate specificity, but different molecular structures. This affects their electrophoretic mobility, thus producing specific mobility patterns for each species. It is a good tool for identifying different species. However, this technique will not distinguish between cell lines from the same species. To complement this multi-locus DNA fingerprinting should be performed for non-human cell lines.

For multi-locus DNA fingerprinting, DNA is extracted from a cell line, digested by restriction enzymes and subsequently separated by gel electrophoresis. Multi-locus probes are applied to the separated DNA and visualized by chemiluminescence to produce the DNA fingerprint of the cell line. Multi-locus DNA fingerprinting is used to differentiate cell lines both between species and within a species. However, multi-locus DNA fingerprinting is prone to inter-run variation, so that to differentiate DNA from two different cell lines it is necessary to run them side by side on the same gel. This means that one gel cannot be compared with an archived banding pattern on another gel. Therefore DNA fingerprinting is primarily a confirmatory technique, but still remains the only practicable method available for the routine authentication of non-human cell lines.

STR PCR profiling, the third authentication technique used at ECACC, is a powerful tool developed following advances in forensic techniques for DNA profiling. This technique generates multiple PCR amplicons, each incorporating a unique fluorescent marker. When these different amplicons are separated by chromatography, the relative distribution of emission peaks creates a unique profile or 'signature' that can be recorded digitally. This signature is reproducible and therefore can be compared with archived profiles. STR analysis enables ECACC to compare the profile of newly accessioned human cell lines with all the human cell lines already deposited in the collection. The existence of a large database of STR multiplex PCR profiles of human cell lines within the ECACC cell collection is an invaluable resource.

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Mycoplasma Screening

Are your Cell Lines Free From Mycoplasma Contamination?

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Consider the effects Mycoplasma contamination has on the properties and functions of a cell line and how this might affect your research:

- Affect uptake across cell membranes
- Interfere with membrane receptor function
- Cause morphological change
- Influence amino acid and nucleic acid metabolism
- Induce cell transformation

In addition to the effects mycoplasmas might have on an individual cell line, the introduction of a contaminated culture can devastate a cell culture facility due to its ability to spread rapidly through all cell cultures, causing an outbreak situation.

The Solution

The best way to avoid the introduction of Mycoplasma is to obtain your cell lines from a recognized culture collection such as ECACC. However, it is still necessary to carry out regular testing of cell lines in routine culture and at the time of cell banking, so that any contamination can be quickly identified and removed from the facility. ECACC provides a Mycoplasma testing service, which is used by many of our customers. Three Mycoplasma detection methods are currently routinely employed at ECACC, each having particular strengths and weaknesses (see Table 1). Reliance on a single detection method for anything other than screening purposes is not advisable, and if a cell line has not been tested for some time ECACC recommends testing by all three methods.

Table 1. Mycoplasma Detection Methods

Method	Sensitivity	Species Range	Speed	US FDA Approval
PCR	Low/Medium	Uncertain	1 day	No
Indirect DNA Stain	Medium	All	2–3 days	Yes
Culture Isolation	High	Majority	3–4 weeks	Yes

Testing for Mycoplasma

The protocols outlined opposite are routinely used by ECACC for testing all manufactured cell banks. A more detailed version of these protocols is provided in the popular ECACC and Sigma-Aldrich joint publication "Fundamental Techniques in Cell Cultures–A laboratory handbook." For further information visit **sigma-aldrich.com** or **ecacc.org.uk**.

Detection of Mycoplasma by Indirect DNA Stain (Hoechst 33258)

DNA staining methods such as Hoechst stain, are suitable for the detection of Mycoplasma in both cell cultures and cell culture reagents and can give results within 24 hours (Figure 1). However, direct staining is relatively insensitive, with a detection limit of 10^6 colony forming units (CFU) ml^{-1}. Co-culturing the test sample with an indicator cell line such as Vero (ECACC Cat. No. 84113001) can improve the sensitivity to 10^4 CFU ml^{-1} by increasing the available surface area upon which mycoplasmas can adhere.

Detection of Mycoplasma by Culture

Detection of mycoplasmas using both direct culture and an enrichment step, is regarded as the reference method, with a theoretical detection level of 10 CFU ml^{-1} (Figure 2). This method is suitable for the detection of mycoplasmas in both cell cultures and reagents, with results available within four weeks. However, it is worth noting that certain strains of *Mycoplasma hyorhinis* cannot be cultured *in vitro*. *Mycoplasma sp.* colonies have a characteristic "fried egg" appearance.

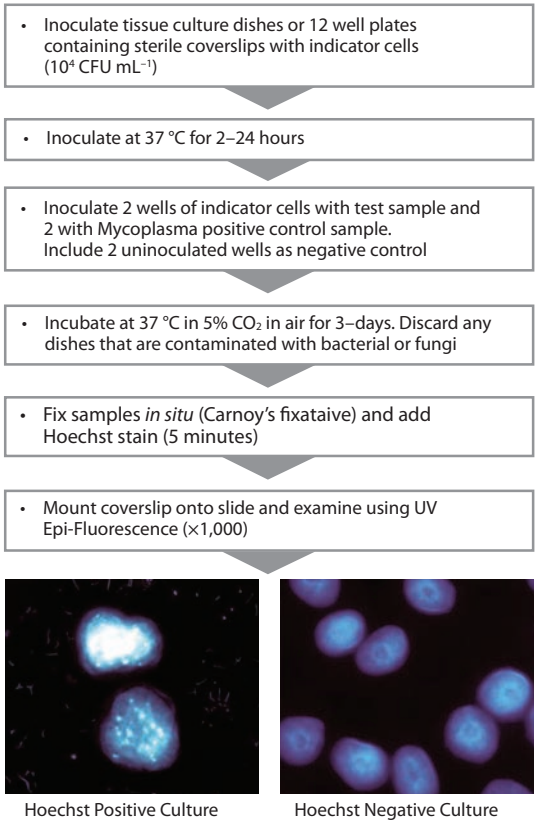

Figure 1. Detection of Mycoplasma by Indirect DNA Stain

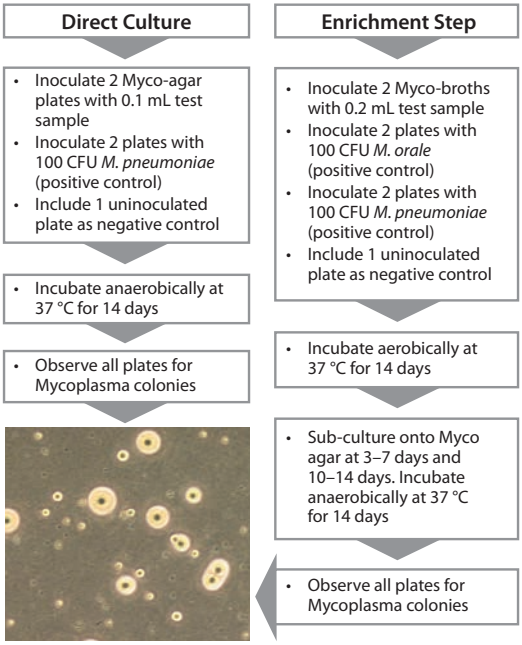

Figure 2. Detection of Mycoplasma by Cell Culture Isolation

Important Notes for Both Methods

1. These test procedures should be carried out in a microbiological laboratory away from the cell culture laboratory.
2. *M. pneumoniae* is a potential pathogen and must be handled in class II microbiological safety cabinet operating to ACDP Category 2 Conditions (the ACDP is a regulatory body of the UK).
3. Hoechst stain is toxic and should be handled and discarded with care.

Mycoplasma Screening: Frequently Asked Questions

Focus on Mycoplasma

What Are Mycoplasma?

Mycoplasma are the smallest (0.2-2 mm in diameter) and most unusual of the prokaryotes. The microorganism's characteristics include:

- The absence of a rigid peptidoglycan cell wall and intracytoplasmic membrane
- The presence of small genome
- Reproduction by binary fission
- A requirement for cholesterol for membrane function and growth and the use of UGA codon for tryptophan

Mycoplasma contain the minimum macromolecular constituents required for self-replication in a cell-free medium, and so are intimately dependent upon the host for nutrients and protection. At least 10^8 colony-forming units of mycoplasma per milliliter of medium can be present within a cell culture.[1,2,3]

ECACC has concentrated on answering the predominant queries relating to mycoplasma.

There is No Visual Indication That My Cell Line is Contaminated With Mycoplasma. Do I Still Need to Check for Mycoplasma?

Yes. Mycoplasma can rapidly spread throughout the laboratory. All cells from normal or neoplastic tissues, primary or continuous, fibroblastic or epithelial, and monolayer or suspection are susceptible to contamination. It is often difficult to detect mycoplasma because the contaminated culture may grow well and appear to be normal under a light microscope. Moreover, there is normally an absence of visual signs such as turbidity, cytopathic effect, and pH change.

What Changes in the Cell Does Mycoplasma Contamination Cause?

Mycoplasma can cause alterations in the rate of cell growth,[4] induction of morphological changes, cause chromosome aberrations,[5] changes in amino acid and nucleic acid metabolism, induction of transformation, induction of apoptosis, induction of cytokines and oxidative radicals, macrophage activation, inhibition of antigen presentation, and induction of signal transduction.[6] In addition, mycoplasma can also interfere with membrane receptor function and penetrate the host cells.[7]

What Are the Sources of Mycoplasma Contamination?

Exogenous mycoplasma arise from a variety of sources which include:

- Cross-contamination from already infected cell cultures from unknown sources. This is the most common cause of mycoplasma contamination
- Serum products
- Aerosols created from pipetting
- Multiple users of the laboratory who come into contact with contaminated cultures and spread it through usage of laboratory instrumentation, media, and reagents
- General environment and products such as hand lotion dispensers and ethanol sprays

What Screening Methods Can be Used to Detect Mycoplasma?

Many different methods are employed to detect mycoplasma contamination in cultures, and each has advantages and disadvantages with respect to cost, time reliability, sensitivity and specificity. The methods used include: culture isolation (Figure 1),[1] indirect DNA stain (Figure 2),[2] immunological methods,[8] nucleic acid hybridization,[9] the use of MycoAlert® to detect the activity of mycoplasmal enzymes, electron microscopy and PCR.[10,11] Reliance on a single detection method is not advisable. If mycoplasma contamination is detected, it is important to find the source of the problem. ECACC conducts stringent quality control procedures for all its cell lines and can offer to test cultures for you.

How Do I Submit a Sample for Mycoplasma Testing at ECACC®?

A biohazard form and sample submission form (located on the ECACC website) must be completed and accepted by ECACC before any materials can be sent. ECACC will then contact you to confirm acceptance of the material and supply you with a reference number, which should be quoted in all further correspondence. Samples sent without a biohazard form will not be accepted for testing.

ECACC will accept cell cultures either growing or as frozen ampules. If a frozen ampule is sent then details of the media used must accompany it. This will be subjected to a handling fee to cover costs of establishing the cell lines in culture. Growing cultures must have been passaged twice in the absence of antibiotics and cryoprotectants.

Cultures should be growing for three days since the last medium change. Notification of the time of the last medium change must be made so that the minimum growth time can be completed at ECACC if necessary. The sample volume required is a 25 cm^2 flask or frozen ampule.

Flasks should be sent topped up to the neck with medium, in a non-vented flask, sealed to prevent leakage and packed in absorbent material for the event of spillage. Frozen ampules should be sent with adequate dry ice to keep them frozen for the journey.

Packages should be clearly labeled with the storage temperature, sender, contact information, the quotation number provided by ECACC and should be addressed to the ECACC QC Department. Packaging and transportation should be in accordance with UN 602 regulation. If you use a courier service to deliver your samples, please contact the company involved for advice on their own approved packaging.

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Depositing Cell Lines

Depositing Cell Lines in the Collections

The General and Hybridoma Cell Collections at ECACC have developed over the last 20 years. This development has followed requests from customers for particular cell types and donation of cultures by numerous investigators. Depositing cultures with ECACC promotes the distribution and use of cell lines throughout the scientific community and encourages the development of new cell-based technologies. Through donating cell lines to ECACC a depositor is internationally recognized as the originator and has their work secured for posterity.

ECACC is continuing to expand the diversity of its collections and is currently acquiring primary and permanent cell lines from epithelial, mesenchymal, endothelial, neuronal and embryonal tissues. In addition, ECACC is continuing to add to its human cell collection to include normal, and disease states, various tumor cell types: breast, lung, colon, pancreas, skin, cervical and gonadal, and cells from transgenic animals.

Depositing a cell line into the General Collection at ECACC is not only an easy process but is also **free of charge**. Depositors benefit from storing their cell lines in ECACC's state-of-the-art on-site storage facility that contains over 40 storage vessels. ECACC has one of the largest liquid nitrogen repositories for animal cells in Europe. It provides depositors with the guarantee of confidentiality, security, monitoring, annual reporting of stock status, stringent quality control testing, and the dedication of ECACC's trained staff who are on site 24 hours a day.

All cell lines deposited in ECACC's General Collection are supplied strictly for research use only as specified in ECACC's Standard Terms and Conditions of Sale. ECACC does not own its cell lines and serves in the role of custodian. Consequently ECACC will observe any conditions or restrictions placed on the distribution of a cell line by the Depositor. In some cases the Depositor's permission to release is obtained for each individual customer. In the event ECACC is approached with a request for commercial use of a cell line, such a request will always be referred to the Depositor.

Upon receipt of a cell line, ECACC will create a bank of the new cell line within its quarantine laboratories. Quality control measures are then in place to ensure viability of the bank, authentication, and the absence of microbial contamination. On successful completion of all quality control checks the cell line is allocated a unique accession number and included in the catalog with the approval of the depositor. The cell line is then made available to the scientific community in a tightly controlled manner. Advertisements within ECACC's Cell Line Catalogs, website, and other promotional materials encourage worldwide distribution and applications.

For full details on "How to deposit a cell line(s)," contact ECACC at ecacc@hpa.org.uk or contact your local Sigma-Aldrich office.

ECACC® Technical Information

The ECACC® Cryostorage Facility: Development of a cGMP Safe Depository

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The Need for Second Site cGMP Storage

Cultured mammalian cells, often following genetic manipulation, are increasingly associated in some way with the manufacture and/or testing of a therapeutic substance that is manufactured according to cGMP. The current direction of US and EU regulations is towards extending the requirements of cGMP further "upstream" of the manufacturing process, to include clinical trial materials and the early provenance of cell lines used in a cGMP operation. Consequently any cell line that is likely to be used in a cGMP process should be banked as a Master Cell Bank (MCB), and validated early in the research and development phases of a project.

Subsequently, all the work conducted using cells from this MCB represents a cumulative investment, which eventually can assume a very high value. In order to secure such an investment the owners of the MCB are advised to arrange a second site, back-up storage for either a part of the original MCB or a direct derivative. Cells recovered from back-up storage must be fit for use in the same cGMP operation as the primary stock so the second site storage conditions need to be cGMP compliant.

For many years ECACC has provided second site storage for valuable third party cell lines. In recognition of the increasing need for a Safe Deposit facility that is able to support the needs of cGMP operations, ECACC has set up, and is now validating a dedicated cGMP Safe Deposit facility.

The ECACC Safe Deposit Facility for cGMP Cell Banks

The cGMP Safe Deposit vessel is being established within ECACC's new, state-of-the-art cryostorage facility located on the Health Protection Agency Porton Down site. This facility is serviced by two 10,000 L liquid nitrogen tanks providing >100% reserve capacity. The inventory storage vessels are configured for automatic replenishment and each vessel is monitored by an electronic telemetry alarm system. This facility benefits from the latest safety advances including oxygen sensors linked to powerful air ventilation. A 24 hour security service operates at the Porton Down site.

The cGMP Safe Deposit vessel is a Custom Biogenic Systems V3000 Isothermal model designed so that the liquid nitrogen is contained in a "jacket" which surrounds the storage compartment. This design allows vapor phase storage, which minimizes the opportunity for cross contamination between vials through the liquid nitrogen medium. In addition, the temperature distribution within an isothermal vessel covers a narrower range, and can be more closely controlled when compared to a conventional vapor phase vessel.

The "conventional" design of a liquid nitrogen storage vessel requires that the liquid nitrogen is delivered into the actual storage compartment of the vessel. The inventory is then stored either submerged in a large volume of liquid nitrogen (liquid phase storage), or otherwise in the gaseous space above the surface of a smaller volume of liquid nitrogen (vapor phase storage). Liquid phase storage guarantees a constant storage temperature of -196 °C, but presents a number of operational and biological risks the most outstanding of which is the possibility that contaminants may pass between vials through the liquid nitrogen medium. Such a mode of contamination was demonstrated when hepatitis B virus contaminated units of human bone marrow stored in liquid nitrogen at a UK blood laboratory. Consequently vapor phase storage is considered more suitable for a cGMP Safe Depository that will contain multiple cell lines.

In a conventional liquid nitrogen storage vessel, vapor phase storage has the disadvantage that a temperature gradient inevitably extends from the surface of the liquid nitrogen to the upper regions of the compartment just beneath the lid. This temperature gradient expands and contracts as the liquid nitrogen fill level fluctuates. Provided the temperature does not exceed -135 °C, such fluctuations are unlikely to affect the cell stocks. This value represents the glass transition temperature of water below which molecular movement ceases and all biological activity is suspended. Nevertheless broad, fluctuating temperature gradients make it more difficult to control this critical threshold and therefore should be minimized. Isothermal inventory storage vessels have been identified as a means of achieving this.

Validation of the cGMP Safe Deposit Isothermal Vessel

The cGMP isothermal storage vessel has been temperature mapped to determine the temperature at different locations in the storage compartment. These determinations have been repeated at different points in the liquid nitrogen fill cycle. The effects of opening the lid and the subsequent recovery times have also been determined. Similar mapping has been applied to a conventional vessel for the purpose of comparison.

Operation of the ECACC cGMP Safe Depository

Only cell stocks that have been tested and shown to be free of microbial contaminants will be stored in the cGMP Safe Depository. In particular, candidate Safe Deposits must be thoroughly tested for mycoplasma contamination. It is more difficult, perhaps impracticable to eliminate the possibility of any virus contaminant, which is why vapor phase storage is so important.

Features of the ECACC® cGMP Safe Depository

- The operational procedures for Isothermal V3000 Storage Vessel have been validated
- Vapor phase storage to minimize the opportunity of cross-contamination
- Security and restricted access
- Confidentiality
- Staff on site 24 hours each day
- Maintenance and supervision by specialist, trained staff
- Eurotherm Chessel telemetry alarm system
- Continuous temperature logging, both electronic and manual
- Regular resuscitation of standard control "Monitor" cell lines that are stored in the Safe Deposit vessels
- Event logging and reporting
- Annual reporting of stock status, stock movements, the maintenance of storage conditions and the results from monitor cell lines

Conclusion

ECACC has been storing cell banks for almost 20 years and has one of the largest liquid nitrogen repositories for animal cells, in Europe. Recent initiatives will enable ECACC to make this expertise available to those who wish to secure cGMP cell stocks. For further details, including prices visit **ecacc.org.uk** or**sigma-aldrich.com**.

References

1. Tedder, M., et al., Hepatitis B transmission from contaminated Cryopreservation tank. *Lancet,* **346,** 137-140, 1995.

Cryopreservation and Storage of Cell Lines

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Benefits That Cryopreservation Offers

Cryopreservation is invaluable when dealing with cells of limited life span. It allows cells to be stored at ultra-low temperature for future use without having to resort to the continuous cultivation of cell lines. Other main advantages include:

- Reduced risk of microbial contamination
- Reduced risk of cross-contamination with other cell lines
- Reduced risk of genetic drift and morphological changes
- Work conducted using cells at a consistent passage number
- Reduced costs

The Requirements for Successful Cryopreservation

A large amount of development work has been undertaken to ensure successful cryopreservation and resuscitation of a wide variety of cell lines of different cell types. The basic principal of cryopreservation is to slow freeze and quick thaw. The most reliable and reproducible way to achieve a slow freeze at a rate of -1 °C to -3 °C per minute is with the use of a programmable rate controlled freezer. The cost in acquiring such equipment is often beyond the budget for the majority of research laboratories. An alternative approach is to freeze passively by keeping the ampules for 24 hours in a Nalgene® Mr. Frosty (Sigma Cat. No. C1562) filled with isopropyl alcohol at -80 °C.

Cryopreservation also depends upon the use of a high concentration of serum/protein (>20% should be used and in many cases serum is used at 90%) and cryoprotectants such as dimethyl sulphoxide (DSMO, Sigma Cat. No. D2650) or glycerol (Sigma Cat. No. G2025). Both cryoprotectants help to prevent the cells from rupturing due to the formation of ice crystals. DMSO is the most common cryoprotectant used at a final concentration of 10%; however, this is not always appropriate because DMSO induces differentiation in some cell lines (e.g., HL60, ECACC Cat. No. 98070106). In such cases, glycerol is often used as the alternative (refer to ECACC data sheet for details of the correct cryoprotectant for a particular cell line). It is essential that immediately prior to cryopreservation cultures should be healthy with a viability of >90% and in the log phase of growth. The latter parameter can be achieved by using pre-confluent cultures (i.e., cultures that are below their maximum cell density).

Ultra-Low Temperature Storage of Cell Lines

Following controlled rate freezing, cells can be cryopreserved in a suspended state for an indefinite period provided a temperature of less than -135 °C is maintained. ECACC strongly discourages the idea of long-term storage at -80 °C. Such ultra-low temperatures can only be attained by specialized electric freezers or more usually by immersion in liquid or vapor phase nitrogen. The advantages and disadvantages of each are summarized below:

Table 1. Comparison of Ultra-low Temperature Storage Methods for Cell Lines

Methods	Advantages	Disadvantages
Electric (–135 °C) Freezer	• Ease of maintenance • Steady temperature • Low running costs	• Requires liquid nitrogen back-up • Mechanically complex • Storage temperatures high relative to liquid nitrogen
Liquid Phase Nitrogen	• Steady ultra-low (–196 °C) temperature • Simplicity and mechanical reliability	• Requires regular supply of liquid nitrogen • High running costs • Risk of cross-contamination via the liquid nitrogen
Vapor Phase Nitrogen	• No risk of cross-contamination from liquid nitrogen • Low temperatures achieved • Simplicity and reliability	• Requires regular supply of liquid nitrogen • High running costs • Temperature fluctuations between –135 °C and –190 °C

Ask ECACC®...

Focus on Cryopreservation

ECACC receives a diverse range of technical inquiries. We have focused on the freezing of cell lines which account for a large proportion of the queries we deal with.

How Should I Store My Frozen Cells on Arrival?

On arrival, frozen vials should be transferred immediately to vapor phase LN_2 or liquid phase LN_2 if vapor phase not available. Do not use a -80 °C freezer as an alternative as this can reduce the viability of the cells.

Why is Vapor Phase LN_2 Preferred to Liquid Phase LN_2?

If ampules are immersed in Liquid Phase LN_2 it increases the risk of LN_2 seeping into the vial, this would cause potential problems of cross-contamination and increased risk of the ampule exploding when thawed.

What Safety Precautions Should I Take When Thawing Ampules?

It is important to first check the Material Safety Data Sheet (MSDS) that is supplied with the cells to insure that the correct level of containment is observed for the cell lines you are using. When handling the ampules it is important to wear insulated safety gloves and fullface visor in addition to a lab coat.

When Should I Freeze Down a Bank of Cells?

Once in culture it is advisable to freeze down a token bank of 3-5 ampules as soon as possible. This will provide a source for Master banks from which you can create working stocks to use. This tiered banking system will minimize risks associated with maintaining cells in permanent culture such as microbial contamination, genetic drift, loss of characteristics of interest, or senescence of finite life span cell lines.

How Can I Get the Correct Freezing Rate?

Cells should be frozen slowly at a rate of -1 to -3 °C per minute to prevent cell damage. To achieve this you can either use a rate programmable freezer or an isopropanol bath, such as a "Mr. Frosty" (Sigma Cat. No. C1562). Ampules should then be transferred directly to LN_2 storage.

What Quality Control Testing Should I Perform on Frozen Stocks?

After freezing down a bank it is important to thaw 2-3 ampules to check for cell count/viability and freedom from bacteria, fungi, and mycoplasma. This testing should be performed on all master and working banks produced prior to use. It is also important to confirm that Master and Working banks are genetically identical by DNA profiling. ECACC performs this same level of QC testing on all available cell lines together with any additional testing as required (such as BVDV testing for Bovine cell lines).

Name	Cat. No.
Cell Freezing Medium-DMSO 1×, sterile-filtered, suitable for cell culture	C6164-50ML C6164-6X50ML
Freezing container, Nalgene® Mr. Frosty	C1562-1EA

What Concentration of Cells Can I Expect Per Vial?

The standard cell count per vial is 3×10^6 cells/vial. For exceptionally large cells, counts may decrease. Suspension cell lines, generally smaller cells, may contain as many as $4\text{-}5 \times 10^6$ cells/vial to assure optimal vialbility upon thaw.

Further Information

For further information and protocols on cell culture methods visit the ECACC and Sigma-Aldrich Web sites.

Labware

Labware

Cell Counting

Bright-Line™ Hemacytometer

H-shaped moat forms two cell-counting areas. The surface features enhanced Neubauer rulings. Replacement cover slips sold separately.

Supplied with two cover slips.

ship: ambient store at: room temp

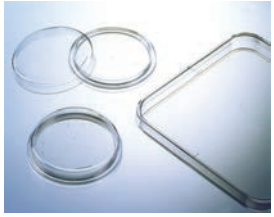
Z359629-1EA	1 ea

Bright-Line™ Hemacytometer replacement cover slip

ship: ambient store at: room temp

Z375357-1EA	1 ea

Cell Culture Dishes



Name	Description	Inside	Mfr. No.	Cat. No.
Corning® CellBIND® surface culture dishes	-	D × H 35 × 10 mm surface area 8 cm^2	Corning 3294	CLS3294-210EA
	-	D × H 60 × 15 mm surface area 21 cm^2	Corning 3295	CLS3295-126EA
	-	D × H 100 × 20 mm surface area 55 cm^2	Corning 3296	CLS3296-5EA CLS3296-40EA
Corning® IVF culture dish	-	diam. 60 mm	Corning 3260	CLS3260-500EA
Corning® square bioassay dishes	without handles, TC treated, square	H 25 mm surface area 500 cm^2	Corning 431110	CLS431110-4EA CLS431110-16EA
	without handles, not TC treated, square	H 18 mm surface area 500 cm^2	Corning 431111	CLS431111-4EA CLS431111-16EA
	with handles, not TC treated, square	H 18 mm surface area 500 cm^2	Corning 431272	CLS431272-16EA
	low profile, without handles, not TC treated, square	H 12.5 mm	Corning 431301	CLS431301-20EA
Corning® non-treated culture dishes	-	D × H 60 × 15 mm surface area 21 cm^2	Corning 430589	CLS430589-500EA
	-	D × H 100 × 20 mm surface area 55 cm^2	Corning 430591	CLS430591-100EA CLS430591-500EA
	-	D × H 150 × 25 mm surface area 148 cm^2	Corning 430597	CLS430597-60EA
	-	D × H 35 × 10 mm surface area 8 cm^2	Corning 430588	CLS430588-500EA

Name	Description	Inside	Mfr. No.	Cat. No.
Corning® tissue-culture treated culture dishes	-	D × H 35 × 10 mm surface area 8 cm^2	Corning 430165	CLS430165-100EA CLS430165-500EA
	-	D × H 60 × 15 mm surface area 21 cm^2	Corning 430166	CLS430166-20EA CLS430166-100EA CLS430166-500EA
	-	D × H 60 × 15 mm (with 2 mm grid) surface area 21 cm^2	Corning 430196	CLS430196-100EA CLS430196-500EA
	-	D × H 150 × 25 mm surface area 148 cm^2	Corning 430599	CLS430599-5EA CLS430599-60EA
	-	D × H 80.5 × 20 mm surface area 55 cm^2	Corning 430293	CLS430293-480EA
	-	D × H 100 × 20 mm surface area 55 cm^2	Corning 430167	CLS430167-20EA CLS430167-100EA CLS430167-500EA
Corning® Ultra-low attachment culture dishes	60mm petri dish, low-attachment	D × H 60 × 15 mm surface area 21 cm^2	Corning 3261	CLS3261-20EA
	100mm petri dish, low-attachment	D × H 100 × 20 mm surface area 55 cm^2	Corning 3262	CLS3262-20EA
Greiner petri dishes	vented, square	W × L × D 120 × 120 × 17 mm	Greiner 688102	Z617679-240EA
	vented	diam. × H 60 × 15 mm	Greiner 628102	Z643084-600EA
	vented	diam. × H 94 × 16 mm	Greiner 633180	Z617636-480EA
	vented	diam. × H 94 × 15 mm	Greiner 633185	Z643114-480EA
	vented	diam. × H 145 × 20 mm	Greiner 639102	Z652539-120EA
	vented, heavy-duty plastic	diam. × H 100 × 15 mm	Greiner 663102	Z666246-420EA
	vented, heavy-duty plastic	diam. × H 100 × 20 mm	Greiner 664102	Z666254-360EA
	vented, gridded contact plate	diam. × H 65 × 15 mm	Greiner 629180	Z666149-600EA
	vented, 3 compartments	diam. × H 94 × 16 mm	Greiner 637102	Z666173-480EA
	unvented	diam. × H 94 × 16 mm	Greiner 632180	Z666165
Petri dishes, polystyrene	beveled stacking rings	60 × 15 mm	Excel Scientific D-901	P5481-500EA
	beveled stacking rings	100 × 15 mm	Excel Scientific D-910	P5731-500EA
	beveled stacking rings	100 × 20 mm	Excel Scientific D-905	P5606-400EA
	vertical stacking rings	100 × 15 mm	Excel Scientific D-900	P5856-500EA
	ringless for easy sliding	150 × 15 mm	Excel Scientific D-902	P5981-100EA
	compartmentalized I-dish with beveled stacking rings	100 × 15 mm	-	P6106-500EA
	compartmentalized with vertical stacking rings	100 × 15 mm	-	P6481-500EA
Activated culture dishes	Culture dishes	O.D. × H 60 × 15 mm	-	Z358924-20EA

Cell Culture Flasks

CellSTACK® Culture Chambers

Description	Surface Area (cm^2)	Working Volume (mL)	Case Of	Corning No.	Cat. No.
1 chamber	636	125-190	8	3268	CLS3268-8EA
2 chamber	1272	250-380	5	3269	CLS3269-1EA CLS3269-5EA
5 chamber	3180	625-950	2	3319	CLS3319-2EA
5 chamber	3180	625-950	8	3313	CLS3313-8EA
10 chamber	6360	1250-1900	2	3270	CLS3270-2EA
10 chamber	6360	1250-1900	6	3271	CLS3271-6EA
40 chamber	25440	5000-7600	2	3272	CLS3272-2EA
1 chamber	636	125-190	8	3330	CLS3330-8EA
2 chamber	1272	250-380	5	3310	CLS3310-5EA
5 chamber	3180	625-950	2	3311	CLS3311-2EA
10 chamber	6360	1250-1900	2	3312	CLS3312-2EA
10 chamber	6360	1250-1900	6	3320	CLS3320-6EA
40 chamber	25440	5000-7601	2	3321	CLS3321-2EA

Corning® CellSTACK® Accessories

Description	Sterile	Corning No.	Cat. No.
Vent cap, 9.5 mm I.D tubing, 7 cm length, Pall Acro 50, PVDF filter	Yes	3281	CLS3281-5EA
Vent cap, 9.5 mm I.D tubing, 7 cm length, Pall Bacterial Air Vent	Yes	3284	CLS3284-1EA CLS3284-4EA
Universal cap with vented overcap	Yes	3332	CLS3332-4EA
Stacking device, ABS	No	3331	CLS3331-5EA
fill cap, 3.2 mm I.D tubing, female luer lock with male luer plug	Yes	3282	CLS3282-5EA
fill cap, 6.4 mm I.D tubing, 70 cm length, male MPC coupling with female end cap	Yes	3333	CLS3333-1EA CLS3333-4EA
fill cap, 33 mm threaded cap with 9.5 mm I.D tubing and 7.94 mm barbed fitting	Yes	3283	CLS3283-5EA
fill cap, female MPC coupling, 6.4 mm I.D barbed fitting with male end cap	Yes	3328	CLS3328-1EA CLS3328-4EA
fill cap, female MPC coupling with male end cap, 9.5 mm I.D barbed fitting with male end cap	Yes	3329	CLS3329-1EA CLS3329-4EA
fill cap, male MPC coupling, with male end cap, 6.4 mm I.D barbed fitting with female end cap	Yes	3334	CLS3334-1EA CLS3334-4EA
fill cap, male MPC coupling with male end cap, 9.5 mm I.D barbed fitting with female end cap	Yes	3339	CLS3339-1EA CLS3339-4EA
screw cap, not vented	Yes	3969	CLS3969-6EA

Corning® CellBIND® RoboFlask® for Automation

Surface Treatment	Surface Area (cm^2)	Maximum Volume (mL)	Working Volume (mL)	Cap	Cat. No.
TC-Treated	92.6	70	20-30	(septum)	CLS3070-100EA
CellBIND® surface	92.6	70	20-30	(septum)	CLS3067-100EA

Corning® Cell Culture Flasks

Size	Maximum Volume (mL)	Working Volume	Cap	Neck	Corning No.	Cat. No.
flask rectangular						
surface area 25 cm^2	10	5-7.5 mL	high-density polyethylene (vent)	canted neck	3815	CLS3815-24EA
	10	5-7.5 mL	(phenolic-style)	angled neck	430372	CLS430372-100EA CLS430372-500EA
	10	5-7.5 mL	(plug seal)	canted neck	430168	CLS430168-100EA CLS430168-500EA
	60	5-7.5 mL	(vented)	canted neck	430639	CLS430639-20EA CLS430639-200EA
surface area 75 cm^2	60	15-22.5 mL	high-density polyethylene (vent)	canted neck	3814	CLS3814-24EA
	60	15-22.5 mL	(phenolic-style)	canted neck	430725	CLS430725-20EA CLS430725-100EA
	60	15-22.5 mL	(plug seal)	canted neck	430720	CLS430720-5EA CLS430720-100EA
	60	15-22.5 mL	(vented)	canted neck	3290	CLS3290-5EA CLS3290-100EA
	60	15-22.5 mL	(vented)	canted neck	430641	CLS430641-5EA CLS430641-20EA CLS430641-100EA
surface area 100 cm^2	30	20-30 mL	(vented)	angled neck (low profile)	3816	CLS3816-60EA
	30	20-30 mL	(vented)	angled neck (low profile)	3073	CLS3073-6EA CLS3073-60EA
surface area 150 cm^2	210	30-45 mL	(phenolic-style)	canted neck	430824	CLS430824-50EA
	210	30-45 mL	(plug seal)	canted neck	430823	CLS430823-5EA CLS430823-50EA
	210	30-45 mL	(vented)	canted neck	3291	CLS3291-50EA
	210	30-45 mL	(vented)	canted neck	430825	CLS430825-5EA CLS430825-50EA

Cell Culture Flasks: Corning® Cell Culture Flasks

Size	Maximum Volume (mL)	Working Volume	Cap	Neck	Corning No.	Cat. No.
surface area 175 cm^2	250	35-52.5 mL	(plug seal)	angled neck	431079	CLS431079-50EA
	250	35-52.5 mL	(phenolic-style)	angled neck	3298	CLS3298-50EA
	250	35-52.5 mL	(phenolic-style)	angled neck	431085	CLS431085-50EA
	250	35-52.5 mL	(vented)	angled neck	3292	CLS3292-50EA
	250	35-52.5 mL	(vented)	angled neck	431080	CLS431080-5EA CLS431080-50EA
	250	35-52.5 mL	(vented)	angled neck	431328	CLS431328-84EA
surface area 225 cm^2	370	45-67.5 mL	(plug seal)	angled neck	431081	CLS431081-25EA
	370	45-67.5 mL	(vented)	angled neck	3293	CLS3293-25EA
	370	45-67.5 mL	(vented)	angled neck	431082	CLS431082-5EA CLS431082-25EA
surface area 235 cm^2	250	47-70.5 mL	(vented)	angled neck	431346	CLS431346-7EA CLS431346-42EA
flask rectangular (with bar code)						
surface area 175 cm^2	250	35-52.5 mL	(vented)	angled neck	431306	CLS431306-84EA
surface area 92.6 cm^2	70	20-30 mL	(flat top)	-	3059	CLS3059-50EA
	70	20-30 mL	(flat top)	-	3071	CLS3071-100EA
	70	20-30 mL	(septum)	-	3070	CLS3070-100EA
	70	20-30 mL	(septum)	-	3069	CLS3069-50EA
	70	20-30 mL	(septum)	-	3067	CLS3067-100EA
flask triangular						
surface area 25 cm^2	10	5-7.5 mL	(phenolic-style)	angled neck	3055	CLS3055-500EA
	10	5-7.5 mL	(vented)	angled neck	3056	CLS3056-200EA
surface area 75 cm^2	90	15-22.5 mL	(phenolic-style)	straight neck	3275	CLS3275-100EA
	90	15-22.5 mL	(vented)	straight neck	3276	CLS3276-100EA

Corning® CellBIND® Surface HYPER*Flask*® Cell Culture Vessels

	Maximum Volume (mL)	Surface Area (cm^2)	Working Volume (mL)	Cap	Flask	Neck	Corning No.	Cat. No.
	560	1720	560	(plug seal)	rectangular	straight neck	10024	CLS10024-24EA
NEW	560	1720	560	(plug seal)	rectangular	straight neck	10020	CLS10020-4EA

Greiner Culture Flasks

Surface Area (cm^2)	Capacity (mL)	Material	Mfr Designation	Cat. No.
25	50	cap (filter), angled neck	Greiner 690195	C6731-200EA
25	50	angled neck	Greiner 690160	C6231-200EA
25	50	angled neck (measuring grid)	Greiner 690170	C6356-200EA
25	50	angled neck (filter cap)	Greiner 690175	C6481-200EA
75	250	angled neck (plug cap)	Greiner 658170	C7106-120EA
75	250	angled neck (filter cap)	Greiner 658175	C7231-120EA
175	550	flask hi-profile, angled neck	Greiner 661190	C4357-40EA
182	550	lo-profile, angled neck	Greiner 660160	C7356-50EA
182	550	lo-profile, filter cap	Greiner 660175	C7481-50EA

Nunc® Culture Flasks

Name	Size (cm^2)	Cap	Case Of (ea)	Mfr Designation	Cat. No.
Nunc® EasYFlasks™	area 25	vented/close	200	Nunc 156340	F7177-1CS
	area 25	filter	200	Nunc 156367	F7302-1CS
	area 75	vented/close	100	Nunc 156472	F7427-1CS
	area 75	filter	100	Nunc 156499	F7552-1CS
	area 175	vented/close	30	Nunc 159920	F7802-30EA
	area 175	filter	30	Nunc 159910	F7677-1CS
Nunc® Triple flasks	area 500	vented/close cap	32	Nunc 132867	F8542-1CS
	area 500	filter cap	32	Nunc 132913	F8667-1CS

Shaker and Fernbach Flasks

Corning®

Flask Capacity	Neck Diam.	Graduations (mL)	Cat. No.
cap polypropylene (vented, Fernbach design)			
3,000 mL	60.58 mm	25	CLS431252-4EA
cap polypropylene (Easy-Grip vent)			
125 mL	26 mm	25	CLS431143-1EA CLS431143-50EA
125 mL	26 mm	25	CLS431405-50EA
250 mL	25 mL	25	CLS431407-1EA CLS431407-50EA
250 mL	31 mm	25	CLS431144-1EA CLS431144-50EA
500 mL	43 mm	50	CLS431145-1EA CLS431145-25EA
500 mL	43 mm	50	CLS431401-25EA
1 L	43 mm	50	CLS431403-25EA
1,000 mL	43 mm	50	CLS431147-1EA CLS431147-25EA
2,000 mL	48 mm	50	CLS431255-6EA
2,000 mL	48 mm	50	CLS431256-6EA
cap polypropylene (Easy-Grip flat)			
125 mL	26 mm	25	CLS431404-1EA CLS431404-50EA
125 mL	26 mm	25	CLS430421-25EA CLS430421-50EA
250 mL	31 mm	25	CLS430183-1EA CLS430183-25EA CLS430183-50EA
250 mL	31 mm	25	CLS431406-1EA CLS431406-50EA
500 mL	43 mm	50	CLS431408-1EA CLS431408-25EA
500 mL	43 mm	50	CLS430422-1EA CLS430422-25EA
1,000 mL	43 mm	50	CLS431146-25EA

Nalgene®

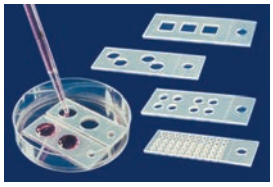
Neck	Capacity	Size	Graduations (mL)	Cat. No.
Beaded neck	flask capacity 250 mL	diam. 26.9 mm	25	F0152-4EA
Beaded neck	capacity 2,000 mL	neck diam. 46.7 mm	200	F0527-2EA
Straight neck	flask capacity 500 mL	diam. 32.8 mm	50 (Marked every 25mL at 400 - 500mL range)	F0277-4EA
Straight neck	flask capacity 1,000 mL	diam. 30.5 mm	-	F0402-2EA

Spinner Flasks

Name	Description	Capacity	Neck Diam. (mm)	Side Arm Diam. (mm)	Cat. No.
Corning® disposable spinner flasks	Sidearms, 2	125 mL	70	25	CLS3152-1EA CLS3152-12EA
	Sidearms, 2	500 mL	100	45	CLS3153-1EA CLS3153-12EA
	Sidearms, 2	1 L	(NA)	54	CLS3561-6EA
	Sidearms, 2	3 L	(NA)	54	CLS3563-4EA
Corning® ProCulture™ spinner flask complete, baffled, angled side-arms	Sidearms, 2	125 mL	70	32	CLS4500125-1EA
	Sidearms, 2	250 mL	70	32	CLS4500250-1EA
	Sidearms, 2	500 mL	100	45	CLS4500500-1EA
	Sidearms, 2	3 L	100	45	CLS45003L-1EA
	Sidearms, 2	6 L	100	45	CLS45006L-1EA
	Sidearms, 2	8 L	100	45	CLS45008L-1EA
	Sidearms, 2	15 L	100	45	CLS450015L-1EA
	Sidearms, 2	36 L	100	45	CLS450036L-1EA
Corning® ProCulture™ spinner flask complete, baffled, wide mouth, angled side-arms	Sidearms, 2	3 L	120	45	CLS45023L-1EA
	Sidearms, 2	6 L	120	45	CLS45026L-1EA
	Sidearms, 2	8 L	120	45	CLS45028L-1EA
	Sidearms, 2	15 L	120	45	CLS450215L-1EA
	Sidearms, 2	36 L	120	45	CLS450236L-1EA
Corning® ProCulture™ spinner flask complete, baffled, extra wide mouth, angled sidearms	Sidearms, 2	3 L	140	45	CLS45043L-1EA
	Sidearms, 2	6 L	140	45	CLS45046L-1EA
	Sidearms, 2	8 L	140	45	CLS45048L-1EA
	Sidearms, 2	15 L	140	45	CLS450415L-1EA
	Sidearms, 2	36 L	140	45	CLS450436L-1EA
Corning® ProCulture™ spinner flask only, baffled, vertical sidearms	Sidearms, 4	8 L	100	45	CLS45108L-1EA
	Sidearms, 4	8 L	120	45	CLS45128L-1EA
	Sidearms, 4	15 L	100	45	CLS451015L-1EA
	Sidearms, 4	15 L	120	45	CLS451215L-1EA
	Sidearms, 6	15 L	140	45	CLS451415L-1EA
	Sidearms, 6	36 L	100	45	CLS451036L-1EA
	Sidearms, 6	36 L	120	45	CLS451236L-1EA
	Sidearms, 6	36 L	140	45	CLS451436L-1EA

Culture Chambers, Coverslips, and Slides

Culturewell™ Systems



Description	Size (mm)	Cat. No.
2 wells	diam. × D 15 × 1.0	C7360-20EA
2 wells	diam. × D 15 × 2.0	C7485-20EA
2 wells	L × W × D 18 × 18 × 2.0	P9990-4EA
4 wells	diam. × D 9 × 1.0	C7610-20EA
8 wells	diam. × D 6 × 1.0	C4732-20EA
50 wells	diam. × D 3 × 1.0	C7735-20EA
2 wells	diam. × D 15 × 2.0	C6735-10EA
4 wells	diam. × D 9 × 1.0	C6860-10EA
50 wells	diam. × D 3 × 1.0	C7110-10EA
coverslips only (5 pouches of 4 coverslips)	-	C8110-1PAK

Nunclon® Chambers

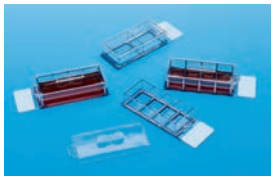
Nunclon® D Multidishes

Useful in all areas of cell culture e.g. scale-up and cloning. SonicSeal slide wells for cell culture in situ on Permanox® slide. Permanox is not auto-fluorescent. Raised well rims to lower risk of cross contamination. Excellent optical quality. Certified surface treatment ensures optimal condition for cell attachment and growth.

sterile; γ-irradiated

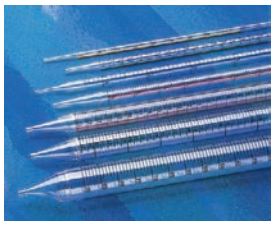
Description	Pkg Qty	Mfr Designation	Cat. No.
4 well round	case of 120 (4 per bag)	Nunc 176740	D6789-1CS
12 well round	case of 75 (individually wrapped)	Nunc 150628	D6315-1CS
24 well round	case of 75 (individually wrapped)	Nunc 142475	D7039-1CS
48 well round	case of 75 (individually wrapped)	Nunc 150687	D6440-1CS

Nunc® Lab-Tek® Chamber Slide™ Systems



Description	Mfr Designation	Cat. No.
1 well on glass	Nunc 154453	C6307-1PAK C6307-1CS
2 wells on glass	Nunc 154461	C6557-1PAK C6557-1CS
4 wells on glass	Nunc 154526	C6807-1PAK C6807-1CS
8 wells on glass	Nunc 154534	C7057-1PAK C7057-1CS
1 well	Nunc 154739	S6440-1PAK
2 wells	Nunc 154852	S6565-1PAK
4 wells	Nunc 154917	S6690-1PAK
8 wells	Nunc 154941	S6815-1PAK
1 well on Permanox®	Nunc 177410	C6432-1PAK
2 wells on Permanox®	Nunc 177429	C6682-1PAK C6682-1CS
4 wells on Permanox®	Nunc 177437	C6932-1PAK C6932-1CS

Pipettes



Name	L (mm)	Capacity (mL)	Graduations (mL)	Color-Coded Stripe	Cat. No.
Corning® Costar® Stripette® serological pipettes, individually plastic wrapped	278	1	±0.01	yellow	CLS4011-100EA CLS4011-500EA CLS4011-1000EA
	278	1	±0.01	yellow	CLS4012-200EA
	344	2	±0.01	green	CLS4021-100EA CLS4021-500EA CLS4021-1000EA
	348	5	±0.1	blue	CLS4051-50EA CLS4051-200EA
	344	10	±0.1	orange	CLS4101-50EA CLS4101-200EA
	344	10	±0.1	orange	CLS4492-200EA
	344	25	±0.2	red	CLS4251-50EA CLS4251-200EA
	370	50	±0.5	purple	CLS4501-100EA
	344	100	±1	aqua	CLS4484-100EA
Corning® Costar® Stripette® serological pipettes, individually paper/plastic wrapped, triple bagged, clean room pack style	278	1	±0.01	yellow	CLS7041-1000EA
	277	2	±0.01	green	CLS7042-1000EA
	348	5	±0.1	blue	CLS7045-200EA
	344	10	±0.1		CLS7015-200EA
	344	25	±0.2	red	CLS7016-200EA
	370	50	±0.5	purple	CLS7017-100EA
	348	100	±1	aqua	CLS7000-100EA
Corning® Costar® Stripette® serological pipettes, bulk packed	278	1	±0.01	yellow	CLS4010-1000EA
	277	2	±0.01	green	CLS4020-1000EA
	348	5	±0.1	blue	CLS4050-500EA
	344	10	±0.1	orange	CLS4100-500EA
	344	25	±0.2	red	CLS4250-200EA
	370	50	±0.5	purple	CLS4500-100EA
Corning® Costar® Stripette® serological pipettes, individually paper/plastic wrapped	278	1	±0.01	yellow	CLS4485-50EA CLS4485-100EA CLS4485-1000EA
	277	2	±0.01	green	CLS4486-50EA CLS4486-100EA CLS4486-1000EA
	348	5	±0.1	blue	CLS4487-50EA CLS4487-200EA
	344	10	±0.1	orange	CLS4488-50EA CLS4488-200EA
	344	25	±0.2	red	CLS4489-25EA CLS4489-50EA CLS4489-200EA
	370	50	±0.5	purple	CLS4490-25EA CLS4490-100EA
	348	100	±1	aqua	CLS4491-10EA CLS4491-100EA

Multiwell Membrane Plates and Inserts

Scaffdex Inserts

CellCrown™ inserts

NEW

CellCrown is a disposable insert, which immobilizes a sample in its place on a 6, 12, 24, 48, and 96-well plate. Fibers, foils, membranes, textiles, tissue and block like samples can be fixed with CellCrown in a firm, adjusted position in the well. The sample can be left in the well during the change of media by means of a pipette between the wall of the well and the CellCrown. The lid of the well plate can be closed while CellCrown is used. The insert can be removed from the well plate and unloaded after cell culturing without damaging the sample. CellCrown is made of medical grade polycarbonate and the product range includes sterile products ready to use as well as non-sterile products to be sterilized by the user. Sterilization can be by gamma or e-beam radiation, autoclaving, ethylene oxide gas and plasma sterilization.

- Use on top of small scaffolds and tissue samples
- Place sample particles on top of the filter
- Use as a mold for hydrogels
- Culture two cell types simultaneously, one on top of the filter and one below

Sterilization Conditions:
Temperature.....121 °C / 250 °F
Time.....15 min.
Drying time.....15 min.

CellCrown 6

CellCrown 12 and 24

CellCrown 48

CellCrown 96

Insert	Sterile	Cat. No.
6 well plate inserts	No	Z681792-3EA
6 well plate inserts	Yes	Z681806-3EA
6 well plate inserts with 5.0 µm nylon filter	Yes	Z681814-3EA
6 well plate inserts with 1.0 µm polycarbonate filter	Yes	Z681822-3EA
12 well plate inserts	No	Z681830-6EA
12 well plate inserts	Yes	Z681849-6EA
12 well plate inserts with 5.0 µm nylon filter	Yes	Z681857-6EA
12 well plate inserts with 1.2 µm nylon filter	Yes	Z681865-6EA
12 well plate insert with 8 µm PET filter	Yes	Z681873-6EA
24 well plate inserts	No	Z681881-12EA
24 well plate inserts	Yes	Z681903-12EA
24 well plate inserts with 5.0 µm nylon filter	Yes	Z681911-12EA
24 well plate inserts with 1.2 µm nylon filter	No	Z681938-12EA
24 well plate inserts with 8 µm PET filter	Yes	Z681946-12EA
48 well plate inserts, 6-well strips	No	Z681954-4EA
48 well plate inserts, 6-well strips	Yes	Z681962-4EA
48 well plate inserts with 8 µm PET filter, 6-well strips	Yes	Z681970-4EA
48 well plate inserts with 1 µm PET filter, 6-well strips	Yes	Z681989-4EA
96 well plate inserts, 8-well strips	No	Z681997-6EA
96 well plate inserts, 8-well strips	Yes	Z682004-6EA

CellVessel™ inserts

NEW

CellVessel is made of bioabsorbable multifilament (8-filaments) poly-96L/4D-lactide copolymer fiber. Scaffdex's braided scaffold is made for research purposes, only and it should not be used in any kind of clinical settings. Poly-96L/4D-lactide copolymer has been tested with various cell types and it works especially well with fibroblasts. CellVessel scaffolds are provided as gamma sterilized and with three widths. The shape of the cross-section of CellVessel varies a little bit from complete round to oval, depending on the size. If further sterilization is needed before cell culturing, ethylene oxide or ethanol rinse to avoid further decreasing of inherent viscosity and strenth properties is suggested. Should CellVessels be cut into wanted lengths by the use, a light heat treatment of the ends of the two bigger sizes should be done to avoid the unloading of the structure during testing.

fiber L:100 mm

Fiber L (mm)	Diam. (mm)	Sterile	Cat. No.
100	1.7	Yes	Z682020-1EA
100	2.4	Yes	Z682039-1EA
100	2.9	Yes	Z682047-1EA

CellCeram™ insert

NEW

diam. 10 mm × thickness 3 mm, sterile

Scaffdex ceramic scaffold CellCeram is made of bioabsorbable hydroxyapatite and ß-tricalciumphosphate composite containing 60% hydroxyapatite and 40% ß-tricalciumphosphate. Scaffdex CellCeram is indicated for research purposes only, not for clinical use. CellCeram has a foam type structure with average porosity of 83%. Average pore size is 200-400 µm with overall range of 100-800 µm. It also contains some microporosity and interconnected pores. CellCeram is available with diameter of 10 mm and height of 3 mm. It fits to 24- and 48 well plates. CellCrown™24 and CellCrown™48 can be used as assisting devices to maintain the firm position of CellCeram on the bottom of the well. CellCeram is available as single packed and sterile, sterilized by gamma irradiation. CellCeram is for single use, only. It is recommended to put CellCeram scaffolds into cell culturing media one day before initiation of the cell culturing for the sufficient wetting of the scaffold.

ship: ambient store at: room temp

Z682012-1EA	1 ea

Corning® Transwell®

Corning® Transwell®-COL collagen-coated membrane inserts

Transwell-COL has a transparent, collagen-treated PTFE membrane that promotes cell attachment and spreading, and allows cells to be visualised during culture. The membrane has an equimolar mixture types I and III. Transwell-COL units are individually blister packed. Cluster plates are included.

sterile

pkg = 1 plates inner packaging

Pore Size (µm)	Membrane Diam. (mm)	Surface Area (cm^2)	Corning No.	Cat. No.
0.4	6.5	0.3	3495	CLS3495-24EA
	12	1.1	3493	CLS3493-24EA
	24	4.7	3491	CLS3491-24EA
3.0	6.5	0.3	3496	CLS3496-24EA
	12	1.1	3494	CLS3494-24EA
	24	4.7	3492	CLS3492-24EA

Corning® Transwell® polyester membrane inserts

Transwell® cell culture inserts are convenient, easy-to-use permeable support devices for the study of both anchorage-dependent and anchorage-independent cell lines They feature a 10 mm, thin, microscopically transparent polyester membrane that is tissue culture treated for optimal cell attachment and growth. Provides excellent cell visibility under phase contrast microscopy and allows assessment of cell viability and monolayer formation.
sterile

Pore Size (µm)	Membrane Diam. (mm)	Surface Area (cm^2)	Corning No.	Pkg. Qty.	Cat. No.
0.4	6.5	0.33	3470	packaged 12 inserts/plate. 4 plates/case	CLS3470-48EA
	12	1.1	3460	packaged 12 inserts/plate. 4 plates/case	CLS3460-48EA
	24	4.5	3450	packaged 6 inserts/plate. 4 plates/case	CLS3450-24EA
3.0	6.5	0.3	3472	packaged 12 inserts/plate. 4 plates/case	CLS3472-48EA
	12	1.1	3462	packaged 12 inserts/plate. 4 plates/case	CLS3462-48EA
	24	4.5	3452	packaged 6 inserts/plate. 4 plates/case	CLS3452-24EA

Corning® Transwell® polycarbonate membrane inserts

Transwell® cell culture inserts are convenient, easy-to-use permeable support devices for the study of both anchorage-dependent and anchorage-independent cell lines. They feature a 10 um thin transparent polycarbonate membrane available in a variety of pore sizes. Transwell are treated for optimal cell attachment. Cells must be stained for cell visibility. The PC membrane is compatible with most organic fixatives and stains.
lid ..... Yes
tissue-culture treated ..... Yes

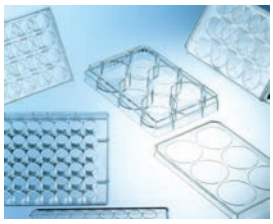
Pore Size (µm)	Membrane Diam. (mm)	Surface Area (cm^2)	Tissue-Culture Treated	Corning No.	Pkg Qty	Cat. No.
0.4	6.5	0.3	Yes	3413	inner package 12 plates	CLS3413-48EA
	12	1.1	Yes	3401	case of 48 (packaged 12 inserts/12 well plate; 4 plates per case)	CLS3401-48EA
	24	4.67	Yes	3412	inner package, case of 24	CLS3412-24EA
	75	44	Yes	3419	inner package 1 plates	CLS3419-12EA
3.0	6.5	0.3	Yes	3415	inner package 12 plates	CLS3415-48EA
	12	1.1	Yes	3402	inner package 12 plates	CLS3402-48EA
	24	4.5	Yes	3414	inner package 6 plates	CLS3414-24EA
	75	44	Yes	3420	inner package 1 plates	CLS3420-12EA
5.0	6.5	0.3	Yes	3421	4 plates per case 12 inserts per 24-well plate	CLS3421-48EA
8.0	6.5	0.3	Yes	3422	inner package 12 plates	CLS3422-48EA
	24	4.5	Yes	3428	inner package 6 plates	CLS3428-24EA

Corning® HTS Transwell® 96 well permeable support

membrane thickness:10.0 µm
clear flat bottom wells
polystyrene
sterile
surface treatment ..... TC-Treated
lid ..... Yes

Pore Size (µm)	Membrane	Corning No.	Case Of	Cat. No.
0.4	polycarbonate	3381	1	CLS3381-1EA
	polycarbonate	3391	5	CLS3391-5EA
3.0	polycarbonate	3385	2	CLS3385-2EA
	polycarbonate	3386	8	CLS3386-8EA
8.0	polyethylene	3374	2	CLS3374-2EA
	polyethylene	3384	8	CLS3384-8EA
5.0	polycarbonate	3387	8	CLS3387-8EA
	polycarbonate	3388	2	CLS3388-2EA

Multiwell Plates



Corning® Multiwell Plates

Name	Description	Corning No.	Cat. No.
Corning® Universal-BIND™ Plate	96 well	2503	CLS2503-50EA
Corning® Sulfhydryl-BIND™ 96 well plate	96 well	2509	CLS2509-50EA
Corning® 96 well EIA/RIA plate	96-well	-	CLS3366-100EA
Corning® 96 Well Plates	NBS™	3990	CLS3990-25EA
	non-treated	3367	CLS3367-50EA
Corning® 96 well plates, half-area	non-treated	3695	CLS3695-25EA CLS3695-100EA
	non-treated	3693	CLS3693-25EA CLS3693-100EA
	non-binding surface	3993	CLS3993-25EA
Corning® 96 Well Plates, Clear Bottom	high binding	3601	CLS3601-100EA
	non-treated	3631	CLS3631-100EA
Corning® 96 well plates, opaque bottom	non-treated	3912	CLS3912-100EA
	high-binding	3922	CLS3922-100EA
	non-treated	3789	CLS3789-100EA
	non-treated	3792	CLS3792-100EA
Corning® 96 well special optics plates	non-treated	3615	CLS3615-100EA
Corning® 96 well UV plates	flat bottom	3635	CLS3635-50EA
Corning® 96 well storage plates, blocks & mats	0.5 mL, V-bottom	3956	CLS3956-50EA
	1 mL, round bottom, 100/case	3959	CLS3959-100EA
	1 mL, round bottom, 25/case	3958	CLS3958-25EA
	2 mL, V-bottom	3960	CLS3960-25EA
Corning® 384 Well Plates	black, 384-well	3985	CLS3985-100EA
	112 µl. black	3712	CLS3712-20EA CLS3712-100EA
Corning® 384 Well Plates, Low Volume	50µl, white, nonbinding surface	3673	CLS3673-100EA
	50µl, black, nonbinding surface	3676	CLS3676-100EA
Corning® 1536 well plate	solid black, non-treated plate	3936	CLS3936-50EA
	solid white, non-treated plate	3937	CLS3937-50EA

Greiner Multiwell Plates

Name	Description	Greiner	Cat. No.
Greiner suspension culture multiwell plates	96 well plate, round bottom with lid	650185	M3562-60EA
	96 well, flat bottom with lid	655185	M3687-60EA
Greiner multiwell plates for culture scale up	6 wells (TC treated with lid)	657160	M8562-100EA
	6 wells (suspension culture with lid)	657185	M9062-100EA
	12 wells (TC treated with lid)	665180	M8687-100EA
	12 wells (suspension culture with lid)	665102	M9187-100EA
	24 wells (TC treated with lid)	662160	M8812-100EA
	24 wells (suspension culture with lid)	662102	M9312-100EA
	48 wells (TC treated with lid)	677180	M8937-100EA
	48 wells (suspension culture with lid)	677102	M9437-100EA

Microcarrier Beads

Name	Description	Bead Size (µm)	Cat. No.
Sigma®-Solohill microcarrier beads	Collagen coated	125 - 212	Z378674-20G Z378674-100G
	FACT coated	125 - 212	Z378682-20G
	ProNectin® F coated	125 - 212	Z378666-10G Z378666-100G
Cytodex® microcarrier beads	Cytodex 1	60 - 87	C0646-5G C0646-25G C0646-100G
	Cytodex 3	60 - 87 (min. 70%)	C3275-10G
Cultispher®	-G standard porosity	130 - 380	M9418-10G
	-S standard porosity, High thermal stability	130 - 380	M9043-10G

Cryogenic Vials and Accessories

Name	Description	Capacity (mL)	Corning No.	Cat. No.
Corning® cryogenic vials, internal thread	bottom conical, seal washer, self standing yes	1.2	430487	CLS430487-500EA
	bottom round, seal washer, self standing yes	2.0	430488	CLS430488-500EA
	bottom round, seal washer, self standing yes	2.0	431386	CLS431386-50EA CLS431386-250EA
	bottom round, seal washer, self standing No	2.0	430489	CLS430489-50EA CLS430489-500EA
	bottom round, seal washer, self standing No	4.0	430490	CLS430490-500EA
	bottom round, seal washer, self standing yes	4.0	430491	CLS430491-500EA
	bottom round, seal washer, self standing No	5.0	430492	CLS430492-500EA
	bottom round, seal washer, self standing yes	5.0	430656	CLS430656-500EA
Corning® cryogenic vials, external thread	conical bottom, self standing Yes	1.2	430658	CLS430658-500EA
	round bottom, self standing Yes	2.0	430659	CLS430659-500EA
	round bottom, self standing No	2.0	430661	CLS430661-500EA
	round bottom, self standing Yes	4.0	430662	CLS430662-500EA
	round bottom, self standing Yes	5.0	430663	CLS430663-500EA
Corning® cryogenic vial, external thread with plug seal cap	round bottom not self-standing	2.0	430289	CLS430289-500EA

Scrapers, Spreaders, Innoculating Loops

Scrapers

Blade L (cm)	Handle L (cm)	Corning No.	Cat. No.
1.8	25	3010	CLS3010-1EA CLS3010-10EA CLS3010-100EA
3.0	39	3011	CLS3011-10EA CLS3011-100EA
1.9	18	3008	CLS3008-100EA

Spreaders

Name	Description	Material	Cat. No.
Lazy-L-Spreader™	10 per pouch	polystyrene	Z376779-1PAK
Lazy-L-Spreader™	individually wrapped	polystyrene	Z723193-500EA

Innoculation

Name	Size	Color	Pkg Qty	Cat. No.
Inoculating Loops, calibrated	× L 1 µL × 70 mm	Platinum wire	1 ea	I0889-1EA
	× L 10 µL × 70 mm	Platinum wire	1 ea	I0764-1EA
Nunc® inoculating loops	1 µL	colorless	pkg of 600 (50 sleeves of 12 each)	I7648-1PAK
	1 µL	colorless	sleeve of 50	I7773-1000EA
	10 µL	blue	pkg of 600 (50 sleeves of 12 each)	I7898-1PAK
	10 µL	blue	sleeve of 50	I7523-1000EA

Sterilizer

Name	Description	Chamber D (cm)	H × W × D (cm)	AC	Cat. No.
Sterilizer, dry bead	Steri 250	8	14.5 × 12.5 × 13.5	240 V	Z378569EU-1EA Z378569-1EA
Sterilizer, dry bead	Steri 350	14	20.5 × 12.5 × 13.5	240 V	Z378585-1EA

Microscopes and Cameras

Microscopes

Jenco™ Compound Microscopes NEW

Science teachers cannot expend their entire budget on microscopes or sacrifice quality on such an integral tool, so Jenco keeps prices low, quality high and offers selection to provide them affordable options. Jenco brand microscopes virtually eliminate downtime and our optics facilitate student throughput.

Choose from different frames for the right reason and teach science with the right equipment for your students.

- Illumination can be a mirror, a rechargeable LED, an incandescent bulb or halogen bulb
- Eye pieces come in monocular heads, or teaching heads for assisting students in finding specimens and also binocular heads for easy viewing with both eyes
- Objectives are offered in multiple quality and quantity levels
- Video systems are available to project images on a screen

Industry leading 5 year warranty

3-plug

Left to Right: Z735086, Z735299, Z735418

Description	Jenco No.	Cat. No.
brightfield, achromatic lens, monocular, fixed stage; LED or 20 W incandescent or fluorescent	CP06-L	Z735418-1EA
brightfield, achromatic lens, monocular, mechanical stage; LED incandescent or fluorescent	CP135-M3	Z735515-1EA
brightfield, achromatic lens, monocular, mechanical stage; LED or other illumination	CP135-M4	Z735620-1EA
brightfield, achromatic lens, binocular, mechanical stage; LED or other illumination	CP135-B4	Z735744-1EA
EU 2-prong plug brightfield, semi-planachromatic lens, monocular; 20w halogen bulb	CP-111-2	Z737178-1EA
EU 2-prong plug brightfield, semi-planachromatic lens, binocular; 20w halogen bulb	CP-112-2	Z737275-1EA
EU 2-prong plug brightfield, achromatic lens, monocular, fixed stage; led or 20w incandescent or fluorescent	CP06-L-2	Z737380-1EA
EU 2-prong plug brightfield, achromatic lens, monocular, mechanical stage; led incandescent or fluorescent	CP135-M3-2	Z737496-1EA
EU 2-prong plug brightfield, achromatic lens, monocular, mechanical stage; led or other illumination	CP135-M4-2	Z737607-1EA
EU 2-prong plug brightfield, achromatic lens, binocular, mechanical stage; led or other illumination	CP135-B4-2	Z737712-1EA

Jenco™ Compound Microscopes, BC Series

NEW

Jenco listened to the needs of experienced researchers and designed the BC Series Upright Compound microscopes to perform in your demanding environment. The BC Series incorporates advanced technology into a durable, cost effective solution for your microscope needs.

- Modern frame with enhanced stability for image documentation
- Ergonomic single hand focus/stage control increase workflow, minimize fatigue
- Robust stainless steel & brass gear train focus mechanism endures high throughput well beyond its 5 year warranty
- Brilliant images resolved by superb optics; choose Infinity or 160mm objectives
- Versatile performance - multiple microscopic methods available, including brightfield, darkfield, phase contrast and simple polarization

Key Specifications:

- Optical body - Seidentopf design inclined 30°; 55 to 75 mm interpupillary adjustment, 360° rotation
- Nosepiece - Quintuple ball bearing, reversed, with positive click stops & smooth operation
- Eye pieces - 10X wide field; Focal Length 25mm, Field Number 20 mm.
- Mechanical stage - 8.25"(209mm) X 5.5"(140mm) with vernier scale; Low-positioned right hand coaxial control knobs for one hand focusing; removable spring clip slide holder
- Focus mechanism - adjustable tension control to prohibit drift and adjustable up-stop to protect slides, specimen and objective lenses; markings at 0.002mm increments
- Condenser - 1.25 N.A. 2 element Abbe with iris diaphragm and rack and pinion focusing
- Kohler Illumination, field diaphragm, 20 watt, 6 volt Halogen bulb with electronic dimmer
- Dimensions - Trinocular: (WHD) (in/cm) 7.8 x 16.5 x 10.9 / 20.0 x 41.9 x 27.8
- Binocular: (WHD) (in/cm) 7.8 x 16.2 x 10.9 / 20.0 x 40.9 x 27.8
- Gross Weight: (lb/kg) 20/9.1

Note: Brightfield Microscope Includes: the four objectives in the table, dust cover, immersion oil, two color filters, spare halogen lamp fuse and instruction manual

3 Plug

Description	Jenco No.	Cat. No.
EU 2-prong plug brightfield, planachromatic lens, binocular, modern ergonomic frame; 20w halogen bulb	BC-211-2	Z736945-1EA
EU 2-prong plug brightfield, planachromatic lens, trinocular, modern ergonomic frame; 20w halogen bulb	BC-311-2	Z737062-1EA
brightfield, planachromatic lens, binocular, modern ergonomic frame; 20 W Halogen bulb	BC-211	Z734977-1EA
brightfield, planachromatic lens, trinocular, modern ergonomic frame; 20 W halogen bulb	BC-311	Z735086-1EA

Jenco™ Compound Microscopes, CP Series

NEW

The CP-300 series is designed to make your microscopy task easier while offering the benefit of cost efficiency. This series provides value and versatility with many models from which to choose. The CP-300 series remains the most student proof with focus stop, condenser lock and a durable metal frame. Anti-fungal agents increase longevity. Customize models by changing objectives, heads or illumination systems.

Durable: An investment that will last for years, these models have glass condensers (won't scratch over time), all metal focusing mechanisms and metal frames backed by a 5 year warranty

Versatile: More choices on the low cost side, including semi-planachromatic objectives and monocular heads

Affordable: You don't have to invest your time in set up and maintenance or sacrifice quality because these microscopes were built to last with cost in mind

Key Specifications:

- Optical body, Seidentopf design inclined 30° with 55 to 75 mm interpupillary adjustment, 360° rotation possible
- Reversed, Quadruple ball bearing nosepiece with positive click stops & smooth operation; 10X wide field eye pieces; Focal Length 25mm Field No. 20mm
- Integrated mechanical stage with vernier and slideholder large enough for two slides. (WxD) (in/cm)5.5 x 4.78 /140 x 124
- Coaxial stage control knobs, ergonomic low-positioned, right hand
- All metal focus mechanism with adjustable tension control to prohibit drift and an adjustable up-stop to protect slides, specimen and objective lenses; coarse/fine markings at 0.002mm increments
- 1.25 N.A. 2 element Abbe condenser with iris diaphragm and rack and pinion focusing
- Kohler Illumination, field diaphragm 20 watt, 6 volt Halogen bulb with electronic dimmer110/220V Switchable
- Dimensions: Binocular: (WHD) (in/cm) 8 x 14. x 8.75/20.3 x36 x 22
- Gross Weight: (lb/kg)16 / 7.3

Microscope Includes: dust cover, immersion oil, two color filters, spare halogen lamp and fuse

3 Plug

not available in EU

Description	Jenco No.	Cat. No.
brightfield, semi-planachromatic lens, binocular; 20 W halogen bulb	CP-112	Z735299-1EA
brightfield, semi-planachromatic lens, monocular; 20 W halogen bulb	CP-111	Z735183-1EA

Jenco™ Inverted Compound microscopes

NEW

Jenco introduces a high optical standard on a versatile frame at budget conscious levels. The long working distance condenser (50 mm) and objectives (six supplied) provide brilliant resolved images in both phase contrast and brightfield techniques. The condenser swings out to accommodate roller bottles or other large cultivation vessels.

- Affordable - Jenco delivers exacting performance for routine research without consuming your budget
- Versatile - trinocular body for image documentation; condenser design for observation of virtually all vessel types; mechanical stage with inserts for well plates and petri dishes; three objectives each for both phase contrast and brightfield
- Durable - an all metal frame with an all metal focusing mechanism ensures this instrument will perform well beyond the industry leading 5 Year Warranty
- 230V units are CE Compliant

Key Specifications:

- Optical body - Seidentopf design inclined 30°; 55 to 75 mm interpupillary adjustment, 360° rotation
- Nosepiece - quintuple, ball bearing
- Eye pieces - 10X, Focal Length 25mm, FN 20 mm.
- Focus mechanism - adjustable tension control to prohibit drift and adjustable up-stop to protect objective lenses; dial markings at 0.002mm increments
- Condenser - 1.25 N.A. 2 element Abbe with iris diaphragm and rack and pinion focusing; 55mm working distance, swing out working distance is 3 inches
- Kohler Illumination, field diaphragm, 30 watt, 6 volt Halogen bulb with electronic dimmer
- Mechanical stage: (WHD) (in/cm) 8.25 x 8.875 / 20.95 x 22.54; Right-hand Coaxial dropdown X-Y Control Knobs; Four Inserts for: well plates (13 x 8 cm and 8 x 5 cm) petri dish (6.8 cm diam.) and slides (7.5 x 3.5 cm and 7.5 x 2.5 cm)
- Dimensions: (WHD) (in/cm) 9 x 22.5 x 21.5 / 22.86 x 57.15 x 54.61; Gross Weight: (lb/kg) 50/22.7

Microscope Includes: the six objectives in the table, dust cover, three color filters, spare halogen lamp and fuse, phase centering telescope, phase annulus sliders and instruction manual

Z723975

Description	Jenco No.	Cat. No.
30W halogen bulb	CP-2A1	Z723975-1EA
30W halogen bulb EU 2-prong plug	CP-2A1-2	Z724084-1EA

Jenco™ Stereo Microscopes, ST-800 Series

The ST-800 series two power stereo helps students down the road because they select magnification by turning a knob on the head, replicating a process on more sophisticated zoom models. Teachers like them because the eyepieces are locked and don't walk, the all metal frames are built to last and the low voltage bulbs last for hundreds of hours and are easy to replace. Even models with rechargeable illuminators are available.

3-plug

Left to Right: Z735841, Z736295, Z736406

Description	Jenco No.	Cat. No.
binocular 10x-20x, illumination: halogen top; fluorescent bottom	ST-F801	Z735841-1EA

Jenco™ Stereo Microscopes, GL Series

NEW

- Save time - no need to refocus when zooming magnification, the GL Series is parfocal and its continuous zoom is available with detentes at 0.5X increments for precise measurements
- Accommodate all users - wide interpupillary adjustment (45 - 75mm) and diopters built in to the eyepieces allow virtually all individuals to work in comfort
- Minimize fatigue - side mounted zoom controls and low positioned focus control with tension adjustment reduce stress and let the user rest in a natural position
- Save Money for years to come - high contrast, flat images are now cost effective and Jenco's 5 year limited warranty protects your investment.

The remarkably clear, non-distorted 3D image resolved by the GL Series optics is a result of advanced technologies in optical design. The Jenco Greenough design produces lifelike images in a large field of view and presents erect images with astounding depth perception.

The Illuminated Stand

Pictured here, the Illuminated stand allows observation of both opaque and transparent specimens with individual controls for reflected and transmitted light. While the reflected or incident light is a 15W Halogen with electronic dimmer, the transmitted light can be either a 15W Halogen with dimmer or a "cool" 5W fluorescent bulb for heat sensitive samples. The individual lighting controls provide ideal conditions for photographing images in a fully illuminated field.

Note: All models include: a pair of 10X eye pieces, black/white reversible stage plate, dust cover, power cord and instruction manual.

3 Plug

Description	Jenco No.	Cat. No.
binocular, 7:1 zoom ratio 6.5X to 45X, dual halogen	GL7-280	Z736406-1EA
EU 2-prong plug binocular, 7:1 zoom ratio 6.5x to 45x, dual halogen	GL7-280-2	Z738387-1EA
binocular, 7:1 zoom ratio 6.5X to 45X, halogen/fluorescent	GL7-290	Z736511-1EA
EU 2-prong plug binocular, 7:1 zoom ratio 6.5x to 45x, halogen/fluorescent	GL7-290-2	Z738492-1EA

Cameras

Canon® EOS Rebel® T1i digital SLR camera body, no lens, for Jenco™ inverted compound microscopes

NEW

15.1Mp CMOS Sensor
HD 1080p, 720p, and VGA Video Capture
3 in. Clear View LCD with Live View
DIGIC 4 Image Processor
ISO Expandable to 12,800
EF-S 18-55mm f/3.5-5.6 IS Lens
Compatible w/ 60-plus EF & EF-S Optics

ship: ambient store at: room temp

Z570001-1EA 1 ea

Color video camera for Jenco™ inverted compound microscopes

NEW

5 Megapixel, CMOS, with USB 2.0 cable, optical adapter and measurement software

ship: ambient store at: room temp

Z569992-1EA 1 ea

Technical Information

Cell Culture Basics

Laboratory Design

Perhaps one of the most under-rated aspects of tissue culture is the need to design the facility to ensure that good quality material is produced in a safe and effi cient manner. Most tissue culture is undertaken in laboratories that have been adapted for the purpose and in conditions that are not ideal. However, as long as a few basic guidelines are adopted this should not compromise the work.

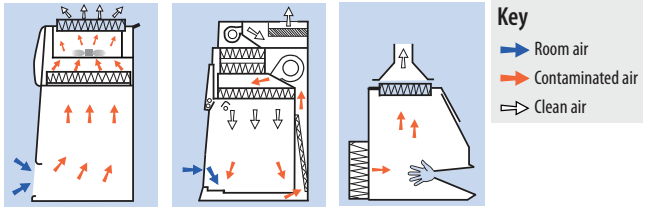
There are several aspects to the design of good tissue culture facilities. Ideally work should be conducted in a single use facility which, if at all possible, should be separated into an area reserved for handling newly received material (quarantine area) and an area for material which is known to be free of contaminants (main tissue culture facility). If this is not possible work should be separated by time with all manipulations on clean material being completed prior to manipulations involving the 'quarantine material'. Different incubators should also be designated. In addition, the work surfaces should be thoroughly cleaned between activities.

All new material should be handled as 'quarantine material' until it has been shown to be free of contaminants such as bacteria, fungi and particularly mycoplasma. Conducting tissue culture in a shared facility requires considerable planning and it is essential that a good technique is used throughout to minimise the risk of contamination occurring.

For most cell lines the laboratory should be designated to at least Category 2 based on the Advisory Committee on Dangerous Pathogens (ACDP) guidelines (ACDP, 1995)†. However, the precise category required is dependent upon the cell line and nature of the work proposed. The guidelines make recommendations regarding the laboratory environment including lighting, heating, the type of work surfaces and flooring and provision of hand washing facilities. In addition, it is recommended that laboratories should be run at air pressures that are negative to corridors to contain any risks within the laboratory.

Microbiological Safety Cabinets

A microbiological safety cabinet is probably the most important piece of equipment since, when operated correctly, it will provide a clean working environment for the product, while protecting the operator from aerosols. In these cabinets operator and/or product protection is provided through the use of HEPA (high efficiency particulate air) filters. The level of containment provided varies according to the class of cabinet used. Cabinets may be ducted to atmosphere or re-circulated through a second HEPA filter before passing to atmosphere.



Class 1 Class 2 Class 3

Source: ECACC

Environmental monitoring with Tryptose Soya Broth agar settle plates inside the cabinet for a minimum of four hours should be a good indicator of how clean a cabinet is. There should be no growth of bacteria or fungi on such plates.

In most cases a class II cabinet is adequate for animal cell culture. However, each study must be assessed for its hazard risk and it is possible that additional factors, such as a known virus infection or an uncertain provenance, may require a higher level of containment.

Centrifuges

Centrifuges are used routinely in tissue culture as part of the subculture routine for most cell lines and for the preparation of cells for cryopreservation. By their very nature centrifuges produce aerosols and, thus, it is necessary to minimize this risk. This can be achieved by purchasing models that have sealed buckets. Ideally the centrifuge should have a clear lid so that the condition of the load can be observed without opening the lid. This will reduce the risk of the operator being exposed to hazardous material if a centrifuge tube has broken during centrifugation. Care should always be taken not to over-fill the tubes and to balance them carefully. These simple steps will reduce the risk of aerosols being generated. The centrifuge should be situated where it can be easily accessed for cleaning and maintenance. Centrifuges should be checked frequently for signs of corrosion.

Incubators

Cell cultures require a strictly controlled environment in which to grow. Specialized incubators are used routinely to provide the correct growth conditions, such as temperature, degree of humidity and CO_2 levels in a controlled and stable manner. Generally they can be set to run at temperatures in the range of 28 °C (for insect cell lines) to 37 °C (for mammalian cell lines) and set to provide CO_2 at the required level (e.g., 5–10%). Some incubators also have the facility to control the O_2 levels. Copper-coated incubators are also now available. These are reported to reduce the risk of microbial contamination within the incubator due to the microbial inhibitory activity of copper. The inclusion of water bath treatment fluid (**Cat. No. S5525**) in the incubator water trays will also reduce the risk of bacterial and fungal growth in the water trays. However, there is no substitute for regular cleaning. (Note Sigma Clean **Cat. No. S5525** is harmful by inhalation, contact with skin or if swallowed and is also a severe irritant.)

Work Surfaces and Flooring

In order to maintain a clean working environment the laboratory surfaces, including benchtops, walls, and flooring, should be smooth and easy to clean. They should also be waterproof and resistant to a variety of chemicals (such as acids, alkalis, solvents, and disinfectants). In areas used for the storage of materials in liquid nitrogen, the floors should be resistant to cracking if any liquid nitrogen is spilled. In addition, the floors and walls should be continuous with a coved skirting area to make cleaning easier and reduce the potential for dust to accumulate. Windows should be sealed. Work surfaces should be positioned at a comfortable working height.

† Advisory Committee on Dangerous Pathogens (1995) Categorisation of Biological Agents According to Hazard and Categories of Containment, 4th edition, Health & Safety Executive (HSE) books, Sudbury, UK, (***www.hse.gov.uk***).

Plasticware and Consumables

Almost every type of cell culture vessel, together with support consumables such as tubes and pipettes, are commercially available as single use, sterile packed, plasticware. The use of such plasticware is more cost effective than recycling glassware, enables a higher level of quality assurance, and removes the need for validation of cleaning and sterilization procedures. Plastic tissue culture flasks are usually treated to provide a hydrophilic surface to facilitate attachment of anchorage dependent cells.

Care and Maintenance of Laboratory Areas

In order to maintain a clean and safe working environment, tidiness and cleanliness are key. Obviously all spills should be dealt with immediately. Routine cleaning should also be undertaken involving the cleaning of all work surfaces both inside and outside of the microbiological safety cabinet, the floors, and all other pieces of equipment e.g., centrifuges. Humidified incubators are a particular area for concern due to the potential for fungal and bacterial growth in the water trays. This will create a contamination risk that can only be avoided by regular cleaning of the incubator. All major pieces of equipment should be regularly maintained and serviced by qualified engineers, for example:

- Microbiological safety cabinets should be checked every six months to ensure that they are safe to use in terms of product and user protection. These tests confirm that the airfl ow is correct and that the HEPA fi lters are functioning properly.
- The temperature of an incubator should be regularly checked with a NAMAS (National Accreditation of Measurement and Sampling, UK), or equivalent calibrated thermometer and temperature adjusted as necessary.
- Incubator CO_2 and O_2 levels should also be regularly checked to ensure the levels are being maintained correctly.

Disinfection

Methods designed for the disinfection/decontamination of culture waste, work surfaces and equipment represent important means for minimizing the risk of harm.

The major disinfectants fall into four groups and their relative merits can be summarized as follows:

Hypochlorites:

- Good general purpose disinfectant
- Active against viruses
- Corrosive against metals and, therefore, should not be used on metal surfaces e.g., centrifuges
- Readily inactivated by organic matter and, therefore, should be made fresh daily
- Should be used at 1,000 ppm for general use surface disinfection, 2,500 ppm in discard waste pots for washing pipettes, and 10,000 ppm for tissue culture waste and spillages.When fumigating a cabinet or room using formaldehyde all the hypochlorites must first be removed as the two chemicals react together to produce carcinogenic products.

Phenolics:

- Not active against viruses
- Remain active in the presence of organic matter

Alcohols (e.g., ethanol, isopropanol):

- Effective concentrations: 70% for ethanol, 60–70% for isopropanol
- Their mode of activity is by dehydration and fixation
- Effective against bacteria. Ethanol is effective against most viruses, but not non-enveloped viruses
- Isopropanol is not effective against viruses

Aldehydes (e.g., glutaraldehyde, formaldehyde):

- Aldehydes are irritants and their use should be limited due to problems of sensitization
- Glutaraldehyde may be used in situations where the use of hypochlorites is not suitable e.g., cleaning of centrifuge bowls or materials constructed of stainless steel that may be attacked or corroded by using hypochlorite solutions.

Main Types of Cell Culture

Primary Cultures

Primary cultures are derived directly from excised, normal animal tissue and cultured either as an explant culture or following dissociation into a single cell suspension by enzyme digestion. Such cultures are initially heterogeneous, but later become dominated by fibroblasts. The preparation of primary cultures is labor intensive and they can be maintained *in vitro* only for a limited period of time. During their relatively limited life span primary cells usually retain many of the differentiated characteristics of the cell *in vivo*. IMPORTANT NOTE: Primary cultures by defi nition have not been passaged, as soon as they are passaged they become a cell line and are no longer primary. 'Primary' cells sourced from most suppliers are in fact low-passage cell lines.

Continuous Cultures

Continuous cultures are comprised of a single cell type that can be serially propagated in culture either for a limited number of cell divisions (approximately thirty) or otherwise indefinitely. Cell lines of a finite life are usually diploid and maintain some degree of differentiation. The fact that such cell lines senesce after approximately thirty cycles of division means it is essential to establish a system of Master and Working banks in order to maintain such lines for long periods.

Continuous cell lines that can be propagated indefinitely generally have this ability because they have been transformed into tumor cells. Tumor cell lines are often derived from actual clinical tumors, but transformation may also be induced using viral oncogenes or by chemical treatments. Transformed cell lines present the advantage of almost limitless availability, but the disadvantage of having retained very little of the original *in vivo* characteristics.

Culture Morphology

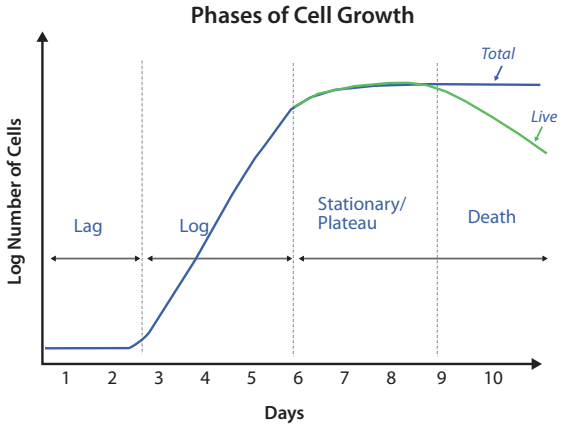
Morphologically, cell cultures take one of two forms, growing either in suspension (as single cells or small free floating clumps) or as a monolayer that is attached to the tissue culture flask. The form taken by a cell line reflects the tissue from which it was derived, e.g., cell lines derived from blood (leukemia, lymphoma) tend to grow in suspension, whereas, cells derived from solid tissue (lungs, kidney) tend to grow as monolayers. Attached cell lines can be classified as endothelial such as BAE-I (ECACC Number 88031149), epithelial such as HeLa (**Cat. No. 93021013**), neuronal such as SH-SY5Y (**Cat. No. 94030304**) or fibroblasts such as MRC-5 (**Cat. No. 84101801**), and their morphology reflects the area within the tissue of origin.

Phases of Cell Growth

It is important to know and record the growth characteristics of the cell line of use before starting any experiments. An alteration in cellular growth can indicate a signifi cant problem within the cell line and if undetected can have detrimental effects on experimental results.

A typical growth curve for cultured cells displays a sigmoid pattern of proliferation. The growth phases associated with normal cells are defi ned as:

1. Lag Phase – at this stage the cells do not divide. During this period the cells adapt to the culture conditions and the length of this phase will depend upon the growth phase of the cell line at the time of subculture and also the seeding density.
2. Logarithmic (Log) Growth Phase – cells actively proliferate and an exponential increase in cell density arises. The cell population is considered to be the most viable at this phase, therefore it is recommended to assess cellular function at this stage. Each cell line will show different cell proliferation kinetics during the log phase and it is therefore the optimal phase for determining the population doubling time.
3. Plateau (or Stationary) Phase – cellular proliferation slows down due to the cell population becoming confl uent. It is at this stage the number of cells in the active cell cycle drops to 0-10% and the cells are most susceptible to injury.
4. Decline Phase – cell death predominates in this phase and there is a reduction in the number of viable cells. Cell death is not due to the reduction in nutrient supplements but the natural path of the cellular cycle.



Source: ECACC

Note: Different cell lines have different timescales for each phase; this graph is provided as a general example of a typical growth curve.

The Cell Environment (Including Types of Culture Medium)

In general terms, cultured cells require a sterile environment and a supply of nutrients for growth. In addition, the culture environment should be stable in terms of pH and temperature. Over the last thirty years various defined basal media types have been developed and are now available commercially. Originally, balanced salt solutions were used to maintain contractility of mammalian heart tissue, and Tyrode's salt solution (**Cat. No. T2397**) was designed for use in work with primary mammalian cells. These have since been modified and enriched with amino acids, vitamins, fatty acids, and lipids. Consequently, media suitable for supporting the growth of a wide range of cell types are now available. The precise media formulations have often been derived by optimizing the concentrations of every constituent. Examples of the different media and their uses are given in **Table 1**.

Table 1. Different types of culture medium and their uses

Media Type	Examples	Uses
Balanced salt solutions	PBS, HBSS, EBSS DPBS (**Cat. Nos. D8537/D8662**) HBSS (**Cat. Nos. H9269/H9394**) EBSS (**Cat. No. E2888**)	Form the basis of many complex media
Basal media	MEM (**Cat. No. M2279**)	Primary and diploid cultures
	DMEM (**Cat. No. D5671**)	Modification of MEM containing increased level of amino acids and vitamins. Supports a wide range of cell types including hybridomas
	GMEM (**Cat. No. G5154**)	Glasgow's modified MEM was defined for BHK-21 cells
Complex media	RPMI 1640 (**Cat. No. R0883**)	Originally derived for human leukemic cells. It supports a wide range of mammalian cells including hybridomas
	Iscoves DMEM (**Cat. No. I3390**)	Further enriched modification of DMEM which supports high density growth
	Leibovitz L-15 (**Cat. No. L5520**, liquid)	Designed for CO_2 free environments
	Graces Insect medium (**Cat. No. G8142**) Schneiders Insect medium (**Cat. No. S0146**)	Designed for culturing insect cells
Serum-Free Media	CHO (**Cat. No. C5467**)	For use in serum-free applications
	Ham F10 and derivatives Ham F12 (**Cat. No. N4888**) DMEM/F12 (**Cat. No. D8062**)	**NOTE:** These media must be supplemented with other factors such as insulin,transferrin, and epidermal growth factor. These media are usually HEPES buffered
Insect cells	Serum-Free Insect Medium 1 (**Cat. No. S3777**)	Specifically designed for use with *Sf*9 insect cells

Basic Constituents of Classic Media

- Inorganic salts
- Carbohydrates
- Amino Acids
- Vitamins
- Fatty acids and lipids
- Proteins and peptides
- Serum

Each type of constituent performs a specific function as outlined below:

Inorganic Salts

The inclusion of inorganic salts in media performs several functions. Primarily they help to retain the osmotic balance of the cells and help regulate membrane potential by provision of sodium, potassium, and calcium ions. All of these are required in the cell matrix for cell attachment and as enzyme cofactors.

Buffering Systems

Most cells require pH conditions in the range 7.2–7.4 and close control of pH is essential for optimal culture conditions. There are major variations to this optimum. Fibroblasts prefer a higher pH (7.4–7.7), whereas, continuous transformed cell lines require more acid conditions pH (7.0–7.4).

Regulation of pH is particularly important immediately following cell seeding when a new culture is establishing and is usually achieved by one of two buffering systems; 1) a "natural" buffering system where gaseous CO_2 balances with the CO_3/HCO_3 content of the culture medium and 2) chemical buffering using a zwitterion such as HEPES (**Cat. No. H4034**).

Cultures using natural bicarbonate/CO_2 buffering systems need to be maintained in an atmosphere of 5–10% CO_2 in air, usually supplied in a CO_2 incubator. Bicarbonate/CO_2 is low cost, non-toxic, and also provides other chemical benefits to the cells.

HEPES (**Cat. No. H4034**) has superior buffering capacity in the pH range 7.2–7.4, but is relatively expensive and can be toxic to some cell types at higher concentrations. HEPES (**Cat. No. H4034**) buffered cultures do not require a controlled gaseous atmosphere.

Most commercial culture media include phenol red (**Cat. Nos. P3532/P0290**) as a pH indicator so that the pH status of the medium is constantly indicated by the color. Usually the culture medium should be changed/replenished if the color turns yellow (acidic) or purple (alkaline).

Carbohydrates

The main source of energy is derived from carbohydrates generally in the form of sugars. The major sugars used are glucose and galactose; however, some media contain maltose or fructose. The concentration of sugar varies from basal media containing 1 g/L to 4.5 g/L in some more complex media. Media containing the higher concentration of sugars are able to support the growth of a wider range of cell types.

Vitamins

Serum is an important source of vitamins in cell culture. However, many media are also enriched with vitamins making them consistently more suitable for a wider range of cell lines. Vitamins are precursors for numerous co-factors. Many vitamins, especially B group vitamins, are necessary for cell growth and proliferation and for some lines the presence of B_{12} is essential. Some media also have increased levels of vitamins A and E. The vitamins commonly used in media include riboflavin, thiamine, and biotin.

Proteins and Peptides

These are particularly important in serum-free media. The most common proteins and peptides include albumin, transferrin, fibronectin, and fetuin and are used to replace those normally present through the addition of serum to the medium.

Fatty Acids and Lipids

Like proteins and peptides these are important in serum-free media since they are normally present in serum, e.g., cholesterol and steroids essential for specialized cells.

Trace Elements

These include trace elements such as zinc, copper, selenium, and tricarboxylic acid intermediates. Selenium is a detoxifier and helps remove oxygen free radicals.

While all media may be made from the basic ingredients, this is time consuming and may predispose to contamination. For convenience most media are available as ready mixed powders or as 10× and 1× liquid media. All commonly used media are listed in the Cell Culture Manual. If powder or 10× media are purchased, it is essential that the water used to reconstitute the powder or dilute the concentrated liquid is free from mineral, organic, and microbial contaminants. It must also be pyrogen-free (**Cat. No. W3500**, Water, tissue culture grade). In most cases water prepared by reverse osmosis and resin cartridge purification with a final resistance of 16-18 MΩ is suitable. Once prepared the media should be filter sterilized before use. Obviously purchasing 1× liquid media direct from Sigma eliminates the need for this.

Serum

Serum is a complex mix of albumins, growth factors, and growth inhibitors and is probably one of the most important components of cell culture medium. The most commonly used serum is fetal bovine serum. Other types of serum are available, including newborn calf serum and horse serum. The quality, type and concentration of serum can all affect the growth of cells, and it is therefore important to screen batches of serum for their ability to support the growth of cells. In addition, there are other tests that may be used to aid the selection of a batch of serum including cloning efficiency, plating efficiency, and the preservation of cell characteristics.

Serum is also able to increase the buffering capacity of cultures, which can be important for slow growing cells or where the seeding density is low (e.g., cell cloning experiments). It also helps to protect against mechanical damage, which may occur in stirred cultures or while using a cell scraper. A further advantage of serum is the wide range cell types with which it can be used, despite the varying requirements of different cultures in terms of growth factors. In addition, serum is able to bind and neutralize toxins. However, serum is subject to batch-batch variation that makes

standardization of production protocols difficult. There is also a risk of contamination associated with the use of serum. These risks can be minimized by obtaining serum from a reputable source, such as Sigma-Aldrich, since suppliers of large quantities of serum perform a battery of quality control tests and supply a certificate of analysis with the serum. In particular serum is screened for the presence of bovine viral diarrhea virus (BVDV) and mycoplasma. Heat inactivation of serum (incubation at 56 °C for 30 minutes) can help to reduce the risk of contamination, since some viruses are inactivated by this process. However, the routine use of heat inactivated serum is not an absolute requirement for cell culture. The use of serum also has a cost implication, not only in terms of medium formulation, but also in downstream processing. A 10% FBS supplement contributes approximately 4.8 mg of protein per milliliter of culture fluid, which complicates downstream processing procedures.

Guidelines for Serum Use

Fetal bovine serum (FBS) has been used to prepare a number of biologicals and has an excellent record of safety. The recognition of Bovine spongiform encephalopathy (BSE) in 1986 and its subsequent spread into continental Europe alongside the announcement of the probable link between BSE and a new variant of Creutzfeldt Jacob disease in humans, stimulated an increased concern about safe sourcing of all bovine materials. In 1993 the Food and Drug Administration (FDA) "recommended against the use of bovine derived materials from cattle which have resided in, or originated from countries where BSE has been diagnosed. The current EU (European Union) guidelines on viral safety focus on sourcing, testing, and paying particular attention to the potential risk of cross contamination during slaughtering or collection of the starting tissue. As far as BSE is concerned, the EU guidelines on minimizing the risk of BSE transmission via medicinal products, CPMP/BWP/877/96, recommends the main measures to be implemented in order to establish the safety of bovine material versus the BSE risk. Again, similarly the focus is on geographical origin, the age of the animals, the breeding and slaughtering conditions, the tissue to be used, and the conditions of its processing.

Alternative Culture Systems

Cell Culture Scale-up Systems

Most tissue culture is performed on a small scale where relatively small numbers of cells are required for experiments. At this scale cells are usually grown in T flasks ranging from 25 cm^2 to 225 cm^2. Typical cell yields from a T-175 flask range from 1×10^7 for an attached line to 1×10^8 for a suspension line. However, exact yields will vary depending on the cell line. It is not practical to produce much larger quantities of cells using standard T flasks, due to the amount of time required for repeated passaging of the cells, demand on incubator space, and cost.

When considering scaling up a cell culture process there are a whole range of parameters to consider which will need to be developed and optimized if scale-up is to be successful. These include problems associated with nutrient depletion, gaseous exchange, particularly oxygen depletion, and the build up of toxic by-products such as ammonia and lactic acid. Optimizing such a process for quantities beyond 1 L volumes is best left to expert process development scientists.

However, there are many commercially available systems that attempt to provide a "half-way house" solution to scale-up, which do not necessarily require expert process development services. A selected list of some of the systems available, along with a brief summary of their potential yields, advantages and disadvantages is provided in **Table 2**.

A word of caution — although the systems listed in **Table 2** are often described as off-the-shelf solutions to scale-up they are not universally applicable to all cell types and often require a period for the user to adapt to the system as well as the cells.

Table 2. Solutions to scale-up — without attempting to adapt cells or the process

Technology	Suspension	Attached	Max Vol (mL)	Max S/A (cm^2)	Max cells (susp)	Max cells (att)	Advantages	Disadvantages
T Flask	•	•	159	225	1.5×10^8	$\sim 10^7$	Cheap, disposable, no cleaning/ sterilizatin required.	Small scale. Multiples required for larger batches.
Multi-layer Flask	•	•	150	525	1.5×10^8	3×10^7	Cheap, disposable, no cleaning/ sterilizatin required.	Difficult to harvest attached cells. Multiples required for larger batches.
Multi-layer Culture Chambers	N/A	•	8,000	25,280	N/A	1.5×10^{10}	Disposable – Single batch manufacture.	Require additional equipment (vessels, etc. which may require cleaning). Difficult to harvest cells.
Roller Bottles	•	•	1,000	1,700	1×10^9	1×10^8	Cheap, disposable, no cleaning/ sterilizatin required. Versatile. Automated systems available.	Require "decks" to turn. Multiples required for larger batches. Automation very costly.
Expanded Roller Bottles	N/A	•	(1,000)	3,400	N/A	2×10^8	As above	As above (no advantage for suspension cells)
Shake Flasks	•	N/A	1,000	N/A	1×10^9	N/A	Some disposables available.	Suspension Only*. Glass vessels to be cleaned & sterilzied. Requires Shaker incubator.
Spinner Flasks	•	N/A	up to 36 L	N/A	1×10^9 3.6×10^{10}	N/A	Some disposables available.	Suspension Only*. Glass vessels to be cleaned & sterilzied. Stirrer-base + incubator.

Roller Bottle Culture

This is the method most commonly used for initial scale-up of attached cells (also known as anchorage dependent cell lines). Roller bottles are cylindrical vessels that revolve slowly (between 5 and 60 revolutions per hour), which bathe the cells that are attached to the inner surface with medium. Roller bottles are available typically with surface areas of 490–1,750 cm^2. The size of some of the roller bottles presents problems since they are difficult to handle in the confined space of a microbiological safety cabinet. Recently, roller bottles with expanded inner surfaces have become available, which has made handling large surface area bottles more manageable, but repeated manipulations and subculture with roller bottles should be avoided if possible. A further problem with roller bottles is with the attachment of cells, since some cells lines do not attach evenly. This is a particular problem with epithelial cells. This may be partially overcome by optimizing the speed of rotation, generally by decreasing the speed, during the period of attachment for cells with low attachment efficiency.

Spinner Flask Culture

This is the method of choice for suspension lines including hybridomas and attached lines that have been adapted to growth in suspension e.g., HeLa S3. Spinner flasks are either plastic or glass bottles with a central magnetic stirrer shaft and side arms for the addition and removal of cells and medium, and gassing with CO_2 enriched air. Inoculated spinner flasks are placed on a stirrer and incubated under the culture conditions appropriate for the cell line. Cultures should be stirred at 100–250 revolutions per minute. Spinner flask systems designed to handle culture volumes of 125 mL–36 L are available from Sigma.

Other Scale-up Options

The next stage of scale up for both suspension and attached cell lines is the bioreactor, used for large culture volumes (in the range 100–10,000 liters). For suspension cell lines, the cells are kept in suspension by either a propeller in the base of the chamber vessel or by air bubbling through the culture vessel. However, both of these methods of agitation give rise to mechanical stresses. A further problem with suspension lines is that the density obtained is relatively low, in the order of 2×10^6 cells/mL.

For attached cell lines the cell densities obtained are increased by the addition of microcarrier beads. These small beads are 30–100 mm in diameter and can be made of dextran, cellulose, gelatin, glass, or silica, and increase the surface area available for cell attachment considerably. The range of microcarriers available means that it is possible to grow most cell types in this system.

A recent advance has been the development of porous micro-carriers, which has increased the surface area available for cell attachment by a further 10–100 fold. The surface area on 2 g of beads is equivalent to 15 small roller bottles.

Cell Culture Protocols

Given below are a few of the essential "do's and don'ts" of cell culture. Some of these are mandatory, e.g., use of personal protective equipment (PPE). Many of them are common sense and apply to all laboratory areas. However, some of them are specific to cell culture.

The Do's

1. Use personal protective equipment, (laboratory coat/gown, gloves and eye protection) at all times. In addition, thermally insulated gloves, full-face visor, and splash-proof apron should be worn when handling liquid nitrogen.
2. Always use disposable caps to cover hair.
3. Wear dedicated PPE for tissue culture facility and keep separate from PPE worn in the general laboratory environment. The use of different colored gowns or laboratory coats makes this easier to enforce.
4. Keep all work surfaces free of clutter.
5. Correctly label reagents, including flasks, media, and ampules, with contents and date of preparation.
6. Only handle one cell line at a time. This common sense point will reduce the possibility of cross contamination by mislabeling etc. It will also reduce the spread of bacteria and mycoplasma by the generation of aerosols across numerous opened media bottles and flasks in a cabinet.
7. Clean the work surfaces with a suitable disinfectant (e.g., 70% ethanol) between operations and allow a minimum of 15 minutes between handling different cell lines.
8. Wherever possible maintain separate bottles of media for each cell line in cultivation.
9. Examine cultures and media daily for evidence of gross bacterial or fungal contamination. This includes media that has been purchased commercially.
10. Quality Control all media and reagents prior to use.
11. Keep cardboard packaging to a minimum in all cell culture areas.
12. Ensure that incubators, cabinet, centrifuges, and microscopes are cleaned and serviced at regular intervals.
13. Test cells for mycoplasma on a regular basis.

The Don'ts

1. Do not continuously use antibiotics in culture medium as this may inevitably lead to the appearance of antibiotic resistant strains and may render a cell line useless for commercial purposes.
2. Don't allow waste to accumulate, particularly within the microbiological safety cabinet or in the incubators.
3. Don't have too many people in the lab at any one time.
4. Don't handle cells from unauthenticated sources in the main cell culture suite. They should be handled in quarantine until quality control checks are complete.
5. Avoid keeping cell lines continually in culture without returning to frozen stock.
6. Avoid cell cultures becoming fully confluent. Always sub-culture at 70-80% confluency or as advised on ECACC's cell culture data sheet.
7. Do not allow media to go out of date. Shelf life is only 6 weeks at 2–8 °C once glutamine and serum is added.
8. Avoid water baths becoming dirty by using Sigma Clean (**Catalog No. S5525**).
9. Don't allow essential equipment to become out of calibration. Ensure microbiological safety cabinets are tested regularly.

Cell Culture Basics section is a joint publication of Sigma-Aldrich and ECACC.

Cryopreservation of Animal Cells

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Introduction

Maintaining healthy, growing cell cultures is a demanding task made more difficult by the ever-present risk of their loss through accidents or contamination. In addition, actively growing cell cultures are not static but, like all populations of microorganisms, subject to age-related or environmentally-induced changes, which can result in their ongoing evolution and potential loss.

These problems are reduced by using cryogenic preservation to stop biological time for cell cultures, effectively putting them into true suspended animation. This concept, long a favorite ploy of science fiction writers and movie producers, has been a reality since the important discovery by Polge, Smith, and Parkes[11] in 1949 that glycerol prevents injury to cells caused by freezing. Many cook book-style protocols are now available for freezing cells and these procedures usually perform well.[3,6,13-16] It is essential; however, when problems arise or protocol adaptations and improvements must be made, that the underlying concepts on which they are based are well understood. This guide examines both the basic theoretical concepts and practical aspects necessary for successfully freezing animal cells and managing a cell repository.

Advantages of Freezing Cell Cultures

Once successfully frozen and stored, cell cultures require little time and effort for their maintenance. The only real cost is the expense of maintaining an ultracold (–130 °C or lower) mechanical freezer or liquid nitrogen supply. This limited expense compares very favorably with the time, effort, and substantial cost of the media and supplies necessary for maintaining actively growing cultures, or for the cost of obtaining a new culture from a repository. Frozen cultures provide an important backup supply for replenishing occasional losses due to contamination or accidents and provide the assurance of a homogeneous culture supply. Cellular changes or alterations occur in all actively growing populations. These changes often result in the loss of important characteristics during evolution of the cultures, thereby, introducing unwanted variables into long-term experiments. Cryogenically preserved cultures apparently do not undergo any detectable changes once they are stored below –130 °C.[1,8] Therefore, the biological effects of *in vitro* cellular aging and evolution may be minimized by frequently returning to frozen stock cultures, allowing ongoing long-term culture experiments to be successfully completed without these unwanted variables. Frozen cultures also provide a valuable baseline against which future experimentally induced changes may be compared or measured.

General Events During Cell Freezing

To understand why freezing protocols work, it is necessary to examine both the intracellular and extracellular events occurring in animal cell cultures during the freezing process.[2,4,8] Initial cooling from room temperature to 0 °C slows cellular metabolism, rapidly disrupting active transport and ionic pumping. Usually this disruption does not result in cellular damage if the culture medium is osmotically balanced. As cooling continues (0 °C to –20 °C) ice crystals begin to form in the extracellular environment, which increases the solute concentration of the culture medium. As a result, water begins to move out of the cells and into the partially frozen extracellular medium, beginning the process of cellular dehydration and shrinkage.

When the cooling process is rapid, intracellular ice crystals form before complete cellular dehydration has occurred. These ice crystals disrupt cellular organelles and membranes and lead to cell death during the recovery (thawing) process.

When the cooling process is slow, free intracellular water is osmotically pulled from the cells resulting in complete cellular dehydration and shrinkage. This can also cause cellular death, but there is little agreement on the mechanisms involved. The physical stresses of cellular shrinking may cause some damage resulting in irreparable membrane loss and cytoskeletal and organelle disruption. Damage may also be caused by the high concentrations of solutes in the remaining unfrozen extracellular medium (essentially a brine solution). These solutes attack cells both externally and internally, resulting in membrane damage, pH shifts, and general protein denaturation.

However, when the cooling rate is slow enough to prevent intracellular ice formation, but fast enough to avoid serious dehydration effects, cells may be able to survive the freezing and thawing process. This survival zone or window is readily observed in many bacteria and other prokaryotes, but for most eukaryotic cells it is nonexistent or very difficult to find without using cryoprotective agents. These agents have little effect on the damage caused by fast freezing (intracellular ice crystal formation), but rather prevent or lessen the damage caused by slow freezing (dehydration and shrinkage).[8]

The final storage temperature is also critical for successful cryopreservation. To completely stop biological time, storage temperatures must be maintained below –130 °C, the glass transition point below which liquid water does not exist and diffusion is insignificant. While many cell cultures are successfully stored at –70 °C to –90 °C for months or even years, biological time is not stopped, only slowed, and cellular damage or changes will accumulate.

Storage in liquid nitrogen at –196 °C effectively prevents all thermally driven chemical reactions. Only photo-physical effects caused by background ionizing radiation still operate at this temperature. Thousands of years are estimated to be necessary before background radiation will have a noticeable effect on cryopreserved cultures.[2,8]

Practical Aspects of Cell Freezing

Under the best of circumstances the process of freezing remains stressful to all cell cultures. It is important that everything possible be done to minimize these stresses on the cultures in order to maximize their subsequent recovery and survival. The following suggestions and recommendations are designed to augment the protocols referred to earlier.

I. Cell Selection

First ensure that the cells are in their best possible condition. Select cultures near the end of log phase growth (approximately 90% confluent) and change their medium 24 hours prior to harvesting. Carefully examine the culture for signs of microbial contamination. Facilitate this by growing cultures in antibiotic-free medium for several passages prior to testing. This allows time for any hidden, resistant contaminants (present in very low numbers) to reach a higher, more easily detected level. Samples of these cultures are then examined microscopically and tested by direct culture for the presence of bacteria, yeasts, fungi, and mycoplasmas. Mycoplasmas present a special problem since they can be found in cultures at very high concentrations (up to 10^8 organisms per milliliter of medium) without any visible effects or turbidity. As a result, as many as 20% of all animal cell cultures are contaminated by these ubiquitous, but unseen organisms. Although special efforts are required to detect mycoplasmas, the serious consequences of their presence makes testing frozen culture stocks absolutely essential.[9,12]

Check for both the identity of the cultures and the presence of any expected special characteristics. Monitor cell identities by karyology and isoenzyme analysis, ensuring that they are, at the very least, the correct species.[10]

II. Cell Harvesting

Start with the standard harvesting procedure generally recommended for the culture and be as gentle as possible. Remove all dissociating agents by washing or inactivation (especially important when using serum-free medium). Centrifugation, when absolutely necessary, should only be hard enough to obtain a soft pellet; 100 × *g* for 5 to 6 minutes is usually sufficient. To ensure uniformity of the final frozen stock, pool the contents of all harvested culture vessels. This also makes it much easier to perform essential quality control testing for microbial contamination and culture identity.

Count and then dilute or concentrate the harvested cell suspension to twice the desired final concentration, which is usually 4×10^6 viable cells per milliliter. An equal volume of medium containing the cryoprotective agent at twice its final concentration will be added later to achieve the desired inoculum. Keep the cells chilled to slow their metabolism and prevent cell clumping. Avoid alkaline pH shifts by gassing with CO_2 when necessary.

III. Cryoprotection

As mentioned earlier, cryoprotective agents are necessary to minimize or prevent the damage associated with slow freezing. The mechanisms providing this protection, although not completely understood, appear to work primarily by altering the physical conditions of both the ice and solutions immediately surrounding (external to) the cells. Permeation of the cells by cryoprotectants does not appear to be necessary for their proper functions.[4] Remember, protection against fast freezing damage (internal ice formation) is not provided by these agents, but rather by careful control of the freezing rate. A wide variety of chemicals provide adequate cryoprotection, including methyl acetamide, methyl alcohol, ethylene glycol, and polyvinyl pyrrolidone. However, dimethylsulfoxide (DMSO) and glycerol are the most convenient and widely used. Many of these agents, although providing excellent cryoprotection, have toxic side effects on cultures making their use difficult.[7]

DMSO is most often used at a final concentration of 5 to 15% (v/v). Always use reagent or other high purity grades that have been tested for suitability. Sterilize by filtration through a 0.2 mm nylon membrane in a polypropylene or stainless steel housing and store in small quantities (5 mL). **CAUTION**: Take special care to avoid contact with solutions containing DMSO. It is a very powerful polar solvent capable of rapidly penetrating intact skin and carrying in with it harmful contaminants such as carcinogens or toxins. Some cell lines are adversely affected by prolonged contact with DMSO. This can be reduced or eliminated by adding the DMSO to the cell suspension at 4 °C and removing it immediately upon thawing. If this does not help, lower the concentration or try glycerol or another cryoprotectant.

Glycerol is generally used at a final concentration of between 5 and 20% (v/v). Sterilize by autoclaving for 15 minutes in small volumes (5 mL) and refrigerate in the dark. Although less toxic to cells than DMSO, glycerol frequently causes osmotic problems, especially after thawing. Always add it at room temperature or above and remove slowly by dilution.

High serum concentrations may also help cells survive freezing. Replacing standard media-cryoprotectant mixtures with 95% serum and 5% DMSO may be superior for some overly sensitive cell lines, especially hybridomas.

Add cryoprotective agents to culture medium (without cells) immediately prior to use to obtain twice the desired final concentration (2×). Mix this 2× solution with an equal volume of the harvested cell suspension (also 2×) to obtain the inoculum for freezing. This method is less stressful for cells, especially when using DMSO as the cryoprotectant.

IV. Storage Vessels

After the cryoprotective solution is mixed with the cell suspension, the resulting inoculum is added in small aliquots (usually 1 to 2 mL) to each storage vessel. Due to the extremely low temperatures encountered during cryogenic storage, not all vessel materials or designs are suitable or safe. Many materials become very brittle at these temperatures; vessels made from them may shatter or crack during storage or thawing. Carefully check the vessel manufacturers' recommendations on proper selection and use.

Also important is selecting the sealing system or cap design used to maintain the integrity of the vessel, especially for storage in liquid nitrogen. If these vessels leak during storage (as many do) they will slowly fill with liquid nitrogen. When they are eventually returned to room temperature, the liquid nitrogen quickly vaporizes causing a rapid pressure buildup. The vessels may then violently blow off their caps or explode to vent the pressure and release their contents into the atmosphere. This is a very dangerous situation, especially if the vessels contained pathogenic organisms or potentially toxic or harmful substances. Storage above liquid nitrogen to reduce these potential hazards is strongly recommended in such situations.

Two types of vessels are commonly used for cryogenic storage: heat-sealable glass ampules and plastic (usually polypropylene) screw-capped vials. Both are available in a variety of sizes (1 to 5 mL capacity) although the smaller sizes are preferred for cryogenic storage.

Because of sealing and labeling problems, glass ampules are no longer widely used in cell culture laboratories. Invisible pinhole leaks may occur in vials during the sealing process; if these are later stored submerged in liquid nitrogen, they may explode when removed for thawing. Pinholes can usually be detected before freezing by immersing sealed ampules for 30 minutes in a chilled solution of 70% ethanol containing 1% methylene blue. This solution will rapidly penetrate and stain any leaky ampules; after rinsing with water, defective ampules are then easily detected and discarded. Due to their greater safety and convenience, plastic vials have largely replaced glass ampules for cryogenic storage. The wide variety of styles and special features like printed marking areas and colored caps for easier identification also add to their popularity. Several cap styles are available, some with an internally threaded stopper, and others with externally threaded designs which help minimize contamination.

V. Labeling and Recordkeeping

Providing for long-term location and identification of frozen cultures is the most frequently overlooked area of cryogenic storage. A cryogenic cell repository is expected to outlast the laboratory workers who contribute to it, but poorly maintained or missing inventory records, and improperly or illegibly labeled vials and ampules may prevent this, especially after the people responsible have gone.

Labels must contain enough information to locate the appropriate records; usually the culture's identity, date frozen, and initials of the person responsible are sufficient. Most plastic vials have printed marking spots or areas for easy labeling. On vials and ampules without marking spots, use cloth labels with special adhesives formulated for cryogenic conditions.

Special ceramic-based inks are available for labeling glass ampules. These are applied prior to filling and then baked onto the glass, usually during dry heat sterilization. Permanent marking spots can be applied on glass ampules with white nail polish. A laboratory marking pen is then used to write on the spot once it has dried.

No matter which labeling method is chosen, use special care to check its permanency under cryogenic conditions. Some marking spots, inks, and labels may flake off or fade during long-term storage; a trial run of at least several weeks is recommended.

Fully detail in the records the culture's storage conditions, including all of the following information: culture identity, passage or population doubling level, date frozen, freezing medium and method used, number of cells per vial, total number of vials initially frozen, and the number remaining, their locations, their expected viability and results of all quality control tests performed (sterility, mycoplasma, species, karyotype, etc.). Additional culture information, especially their origin, history, growth parameters, special characteristics, and applications, is also helpful and should be included whenever possible.

Make special efforts to keep all records up to date and to ensure everyone in the facility is properly using them. Use pre-printed forms to make the information recording process easier and more likely to be completed. Keep updated, duplicate copies of all critical records in a safe place removed from the laboratory area to guard against their accidental loss or destruction. This is especially important if a computer-based recordkeeping system is used; a current backup copy should always be maintained in addition to the information stored in the computer.

VI. Cooling Rate

The cooling rate used to freeze cultures must be just slow enough to allow the cells time to dehydrate, but fast enough to prevent excessive dehydration damage. A cooling rate of –1 °C to –3 °C per minute is satisfactory for most animal cell cultures. Larger cells, or cells having less permeable membranes may require a slower freezing rate since their dehydration will take longer.

The best way to control cooling rates is using electronic programmable freezing units. Although expensive, they allow precise control of the freezing process, give very uniform and reproducible results, and can freeze large numbers of vials or ampules. Most units are available with chart recorders for a permanent record of the cooling process.

There are a variety of mechanical freezing units that provide adequate control of the cooling rate and are relatively inexpensive. Some units use racks designed to hold vials at predetermined depths in the neck of a liquid nitrogen freezer. The cooling rate is dependent on the total number of vials and the depth at which the rack is placed. Another design uses an alcohol filled metal or plastic canister containing a rack with a capacity of up to 24 vials. The filled canister is placed in an ultracold mechanical freezer where the alcohol acts as a bath to achieve more uniform heat transfer and cooling. After freezing 4 to 5 hours, the vials are removed from the canister and transferred to their final storage locations.

Insulated cardboard or polystyrene foam boxes are commonly used as freezing chambers in ultracold freezers. These homemade devices work well with many cell lines, but do not always give controlled, reproducible, or uniform cooling. As a result, there may be serious differences in viability among the vials upon thawing. This homemade approach is not recommended for valuable or irreplaceable cultures.

No matter which cooling method is used, transfer from the cooling chamber or device to the final storage location must be done quickly to avoid warming of the vials. Use an insulated container filled with dry ice or liquid nitrogen as a transfer vessel to ensure that the cells remain below –70 °C.

VII. Cryogenic Storage

Frequently check nitrogen levels in freezers; a schedule should be established and strictly adhered to. Nitrogen evaporation is dependent on both the degree of use and the static holding time of the freezer. Sudden, unexplained increases in the evaporation rate may signal damage to the insulation or other problems with the freezer and must be carefully investigated. Avoid frost or ice buildup around freezer openings; this increases the nitrogen evaporation rate and can cause elevated temperatures in the upper portion of vapor phase freezers. Audible alarm systems for detecting low liquid nitrogen levels are available to provide additional safeguards; however, they provide a false sense of security if not monitored 24 hours a day.

VIII. Thawing

CAUTION: Always use appropriate safety equipment when removing vials and ampules from liquid or vapor phase nitrogen freezers. A full face shield, heavy gloves and lab coat are strongly recommended for protection against exploding vials or ampules.

Remove the vial or ampule from its storage location and carefully check both the label and storage record to ensure that it is the correct culture. Place the vessel in warm water, agitating gently until completely thawed. Rapid thawing (60 to 90 seconds at 37 °C) provides the best recovery for most cell cultures; it reduces or prevents the formation of damaging ice crystals within cells during rehydration.

IX. Recovery

Since some cryoprotective agents may damage cells upon prolonged exposure, remove the agents as quickly and gently as possible. Several approaches are used depending on both the cryoprotective agents and characteristics of the cells.

Most cells recover normally if they have the cryoprotective agent removed by a medium change within 6 to 8 hours of thawing. Transfer the contents of the ampule or vial to a T-75 flask or other suitable vessel containing 15 to 20 mL of culture medium and incubate normally. As soon as a majority of the cells have attached, remove the medium containing the now diluted cryoprotective agent and replace with fresh medium.

For cells that are sensitive to cryoprotective agents, removing the old medium is easily accomplished by gentle centrifugation. Transfer the contents of the vial or ampule to a 15 mL centrifuge tube containing 10 mL of fresh medium and spin for 5 minutes at 100 × *g*. Discard the supernatant containing the cryoprotectant and resuspend the cell pellet in fresh medium. Then transfer the cell suspension to a suitable culture vessel and incubate normally.

When glycerol is used as the cryoprotectant, the sudden addition of a large volume of fresh medium to the thawed cell suspension can cause osmotic shock, damaging or destroying the cells. Use several stepwise dilutions with an equal volume of warm medium every 10 minutes before further processing to give the cells time to readjust their osmotic equilibrium.

X. Problem Solving Suggestions

Viability problems associated with cryogenic storage are usually noticed soon after cultures are thawed and plated. There are four major areas where problems occur:

1. During harvesting and processing of the cells. Problems may be caused by excessive exposure of the cells to dissociating agents; using a cryoprotective agent that is toxic; or allowing high density cell suspensions to remain too long at room temperature or at a pH that is too basic.
2. During the cooling (freezing) process. Excessive cell damage and reduced culture viability often result from using a cooling rate that is too fast or too slow, or when the cooling process is temporarily interrupted. Not using a suitable cryoprotective agent at an appropriate concentration will also result in viability problems.
3. During cyrogenic storage. Culture viability is often reduced when vials are allowed to warm up during transfer to the freezer, or if the repository temperature is not consistently maintained at appropriate cryogenic temperatures.
4. During thawing and recovery. Problems arise when the thawing process is too slow or the cryoprotectants are improperly removed (see above).

These viability problems can often be corrected by using the following technique to identify the stage in the freezing process where the problem originates. Harvest enough cells to prepare at least four vials. Then remove a sample of cell suspension, equivalent in cell number to that which will be placed into the vials, and immediately place it into a culture vessel with an appropriate amount of medium and incubate. This culture will be used as a control to compare with the cultures set up in the remaining steps.

Next, add the cryoprotective agent to the remaining cells and divide among three vials. Place one vial at 4 °C for one hour. Then remove the cells from the vial, process as though they had just been thawed from the freezer, and plate in medium as above. This culture will be compared with the control culture to determine if there are any problems associated with the cryoprotective agent.

Meanwhile, process the remaining vials through the slow cooling process as usual. One vial is then immediately thawed and processed as above. This culture will be compared with the control culture to determine if there are any problems associated with the slow cooling process.

The remaining vial is then transferred to the cryogenic freezer and stored overnight before being thawed and processed as above. This culture will be compared with the control culture to determine if there are any problems associated with the cryogenic storage conditions. If additional vials of cells are available, several different recovery techniques should be used to determine if the recovery technique is the source of the problem.

By comparing all of the cultures to the original culture, it should then be possible to determine at which stage of the freezing process the problem occurred. Once this is known, the information presented in this guide and its references should be enough to eliminate the problem.

XI. Managing a Cell Repository

The effort and expenses of managing a repository should be kept in line with the value of the cultures stored within it. This value is determined by answering two questions: How much time, money, and effort is already invested in these stored cell cultures? And, what are the consequences of losing them? Cultures that are easily replaced through other labs or commercial sources may not require special efforts, but unique cultures, such as hybridomas and other genetically engineered cells, are irreplaceable and require that special efforts be made to ensure their safety. The answers to these questions will help determine just how extensive and thorough your efforts should be.

Next, identify the potential problem areas that can cause the loss of these cultures. Some of these areas, such as vessel selection, recordkeeping, labeling, freezer monitoring, storage conditions, and quality issues (contamination and species identity), have already been discussed in this guide. Decide what steps are necessary to eliminate or minimize these problems. Split irreplaceable or extremely valuable cultures among several freezers, with at least one freezer in a separate location to protect against fire or other natural disasters. Colleagues in other labs or buildings may be able to provide good backup storage, especially if a reciprocal arrangement is made for them.

One final step remains; plan ahead for emergencies! One of the most serious and unexpected emergencies is the failure of a cryogenic freezer. Careful monitoring of the liquid nitrogen level or charting the temperature may give an early warning that failure is occurring, but middle of the night failures can and do happen. Have plans prepared in advance to deal with freezer failure and other problems. If these involve a colleague's equipment, get permission and make all necessary arrangements in advance – late night phone calls are usually not appreciated.

For additional questions please call Sigma-Aldrich Technical Service or visit the Corning Life Sciences Web site at: **corning.com/lifesciences**.

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Cryogenic Preservation and Storage of Animal Cells

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Introduction

Cryogenic preservation (storage below –100 °C) of cell cultures is widely used to maintain backups or reserves of cells without the associated effort and expense of feeding and caring for them. The success of the freezing process depends on four critical areas:

1. Proper handling and gentle harvesting of the cultures
2. Correct use of the cryoprotective agent
3. A controlled rate of freezing
4. Storage under proper cryogenic conditions

The last three points are discussed in more detail below.

A wide variety of chemicals provide adequate cryoprotection. However, dimethyl sulfoxide (DMSO, **Cat. No. D2650**) and glycerol (**Cat. No. G2025**) are the most convenient and widely used. DMSO is most often used at a final concentration of 5 to15% (v/v). Some cell lines are adversely affected by prolonged contact with DMSO. This can be reduced or eliminated by adding the DMSO to the cell suspension at 4 °C and removing it immediately upon thawing. If this does not help, lower the concentration or try glycerol. Glycerol is generally used at a final concentration of between 5 and 20% (v/v). Although less toxic to cells than DMSO, glycerol can cause osmotic problems, especially after thawing. Always add it at room temperature or above, and remove slowly by dilution. High serum concentrations may also help cells survive freezing. Replacing standard medium-cryoprotectant mixtures with 95% serum and 5% DMSO may be superior for some overly sensitive cell lines, especially hybridomas.

The cooling rate used to freeze cultures must be just slow enough to allow the cells time to dehydrate, but fast enough to prevent excessive dehydration damage. A cooling rate of –1 °C to –3 °C per minute is satisfactory for most animal cell cultures. Larger cells or cells having less permeable membranes may require a slower freezing rate since their dehydration will take longer.

The best way to control cooling rates is by using electronic programmable freezing units. Although expensive, they allow precise control of the freezing process, give very uniform and reproducible results, and can freeze large numbers of vials. Most units are available with chart recorders for a permanent record of the cooling process. There are a variety of mechanical freezing units that provide adequate control of the cooling rate and are relatively inexpensive. Some systems use racks designed to hold vials at predetermined depths in the neck of a liquid nitrogen freezer. The cooling rate is dependent on the total number of vials and the depth at which the rack is placed. Another approach uses an alcohol-filled container designed to slowly freeze vials placed in the system. The filled container is placed in an ultracold mechanical freezer where the alcohol acts as a bath to achieve more uniform heat transfer and cooling. After freezing overnight, the vials are removed from the canister and transferred to their final storage locations.

Only freezers capable of continually maintaining temperature below –130 °C should be considered for long-term cryogenic storage. Although most liquid nitrogen cooled freezers and some specially designed mechanical freezers meet this requirement, most cell culture laboratories prefer liquid nitrogen freezers. The final choice is often based on the availability of a reliable supply of liquid nitrogen, the storage capacity required and the size of the budget. Liquid nitrogen freezers permit storage either in the vapor phase above the liquid at temperature between –140 °C and –180 °C, or submerged in the liquid at a temperature below –196 °C. Using vapor phase storage greatly reduces the possibility of leaky vials or ampules exploding during removal.

The following procedure can be easily adapted to a wide variety of cell lines. For additional information and references refer to the **General Guide for Cryogenically Storing Animal Cell Cultures** on the Corning Life Sciences Technical Information Web site at **corning.com/lifesciences**.

Supplies

Nonsterile

1. Pipetting aids
2. Disposal tray or bucket for discarding used pipettes
3. Bottle of 70% alcohol for wiping down work area
4. Paper towels
5. Marking pen
6. Controlled rate freezer. There are a variety of mechanical freezing units that provide adequate control of the cooling rate and are relatively inexpensive.
7. Inverted phase contrast microscope
8. Ice bucket
9. Liquid waste container
10. Hemacytometer or electronic cell counter
11. Laminar flow hood

Sterile

1. Cell culture medium appropriate for the cell line being frozen
2. Healthy, near confluent cell culture in T-75 flask
3. 1, 5, 10, and 25 mL pipettes (**Cat. Nos. Z371580, Z371602, Z371610,** and **Z371629**)
4. 15 mL screw cap centrifuge tubes (**Cat. No. C8046**)
5. Phosphate Buffered Saline: Calcium- and Magnesium-free, DPBS (**Cat. No. D8537**). Unlike Hanks' and Earle's buffered saline solutions, the calcium and magnesium is removed because these cations play a role in cell-to-cell attachment.
6. Trypsin solution or other dissociating agent in Calcium/Magnesium-free phosphate-buffered saline (CMF-PBS). Trypsin concentration should be optimized so as to remove the cells as quickly as possible, but with a minimum of stress or damage, see cell dissociation section for trypsin solutions.
7. 2 mL Cryogenic vials (**Cat. Nos. Z713597, Z713554,** or **Z713740**)
8. Cryoprotective medium – complete culture medium containing 10% dimethyl sulfoxide (DMSO). Always use reagent or other high purity grades that have been tested for cell culture suitability. Sterilize DMSO by filtration through a 0.2 µm nylon membrane (**Cat. No. Z711705**) in a polypropylene or stainless steel housing and store in small quantities (5 mL).
9. 0.04% Trypan blue solution (**Cat. No. T8154**) for viability staining

Procedure

Examination

Prior to freezing, the cells should be maintained in an actively growing state to insure maximum health and a good recovery. Ideally, the culture medium should be changed the previous day. Using an inverted microscope, quickly check the general appearance of the culture. Look for signs of microbial contamination. It is also important to examine the culture with the unaided eye to look for small fungal colonies that may be floating at the medium-air interface and thus not visible through the microscope. It is best if the cultures are maintained antibiotic-free for at least one week prior to freezing to help uncover any cryptic (hidden) culture contaminants.

Cell Harvesting and Freezing

Treat the cells gently during harvesting since it is very difficult for cells damaged during harvesting to survive the additional damage that occurs during the freezing and thawing processes. You should be able to obtain up to 1.5×10^7 cells from a near confluent T-75 flask (depending on cell type and degree of confluency). This should be enough cells to set up at least several vials at 2×10^6 cells/vial.

1. Using a sterile pipette, remove and discard the old culture medium.
2. For a T-75 flask, rinse the cell monolayer with 5 mL of calcium- and magnesium-free phosphate buffered saline, **Cat. No. D8537** to remove all traces of fetal bovine serum.
3. Add 4 to 5 mL of the trypsin solution to the flask and allow cells to incubate for at least one minute. (Prewarming of the enzyme solution will decrease the exposure period.) Withdraw about 3 mL of the trypsin solution and allow the cells to round up and loosen.
4. Check the progress of the enzyme treatment every few minutes on an inverted phase contrast microscope. Once all of the cells have rounded up, gently tap the flask to detach them from the plastic surface. Then add 5 mL of growth medium to the cell suspension and, using the same pipette, vigorously wash any remaining cells from the bottom of the culture vessel.
5. Collect the suspended cells in a 15 mL centrifuge tube and place on ice. Take a sample for counting and then spin at $100 \times g$ for 5 minutes to obtain a cell pellet. While the cells are spinning, do a viable cell count (with the trypan blue solution) and calculate the number of cells/ml and the total cell number.
6. Remove the supernatant from the centrifuged cells and resuspend the cell pellet in enough of the cryoprotective medium containing 10% DMSO (DMSO is most often used at a final concentration of 5 to 15%) to give a final cell concentration of 1 to 2×10^6 cells/mL. Although not directly toxic, DMSO is a very powerful solvent and is able to rapidly penetrate intact skin (leaving a fishy or garlicky taste in your mouth). As a result, there is a **POTENTIAL HAZARD** associated with using this compound. It is very important to avoid contact with DMSO and dispose of any wastes containing DMSO properly.
7. Label the appropriate number of cryogenic vials with the cell line and the date. Then add 1.5 to 1.8 mL of the DMSO containing cell suspension to each of the vials and seal.
8. Place the vials in the controlled rate freezer overnight. After 24 hours, the cells should be transferred to a liquid nitrogen freezer for permanent storage.
9. Record the appropriate information about the cells in your cell repository records. Fully detail in these records the culture's storage conditions, including all of the following information: culture identity, passage or population doubling level, date frozen, freezing medium and method used, number of cells per vial, total number of vials initially frozen and the number remaining, their locations, their expected viability and results of all quality control tests performed (sterility, mycoplasma, species, karyotype, etc.). Additional culture information, especially its origin, history, growth parameters, special characteristics and applications, is also helpful and should be included whenever possible.

Cell Thawing and Recovery

1. Using appropriate safety equipment, remove the vial from its storage location and carefully check both the label and storage record to ensure that it is the correct culture. Place the vessel in warm water, agitating gently until completely thawed. Rapid thawing (60 to 90 seconds at 37 °C) provides the best recovery for most cell cultures; it reduces or prevents the formation of damaging ice crystals within cells during rehydration.
2. Since some cryoprotective agents may damage cells upon prolonged exposure, remove the agents as quickly and gently as possible. Several approaches are used depending on both the cryoprotective agents and characteristics of the cells:
   a) Most cells recover normally if they have the cryoprotective agent removed by a medium change within 6 to 8 hours of thawing. Transfer the contents of the ampule or vial to a T-75 flask or other suitable vessel containing 15 to 20 mL of culture medium and incubate normally. As soon as a majority of the cells have attached (usually 3 to 4 hours), remove the medium containing the now diluted cryoprotective agent and replace with fresh medium.
   b) For cells that are sensitive to cryoprotective agents, removing the old medium is easily accomplished by gentle centrifugation. Transfer the contents of the vial or ampule to a 15 mL centrifuge tube containing 10 mL of fresh medium and spin for 5 minutes at $100 \times g$. Discard the supernatant containing the cryoprotective agent and resuspend the cell pellet in fresh medium. Then transfer the cell suspension to a suitable culture vessel and incubate normally.

Additional Notes

Be prepared for emergencies. Learning that your liquid nitrogen freezer has failed without warning (destroying the cultures contained within) is a terrible, but all too common event. Frequently check nitrogen levels in freezers; a schedule should be established and strictly adhered to. Audible alarm systems for detecting low liquid nitrogen levels are available to provide additional safeguards. However, they provide a false sense of security if not monitored 24 hours a day. Valuable or irreplaceable cultures should be stored in at least two separate facilities. ATCC and ECACC provide a safe deposit service for this purpose.

Precautions

A. Take special care to avoid contact with solutions containing DMSO. It is a very powerful polar solvent capable of rapidly penetrating intact skin and carrying in with it harmful contaminants such as carcinogens or toxins.

B. Corning strongly recommends that cryogenic vials always be stored in the vapor phase above the liquid nitrogen to reduce the possibility of the vials filling with liquid nitrogen during extended storage. Vials filled with liquid nitrogen may explode violently upon removal from the freezer.

C. Always use appropriate safety equipment when removing vials and ampules from liquid or vapor phase nitrogen freezers. A full-face shield, heavy gloves, and lab coat are strongly recommended for protection against exploding vials or ampules.

For additional questions please call Sigma-Aldrich Technical Service or visit the Corning Life Sciences Web site at **corning.com/lifesciences**.

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Cell Freezing Media (Cat. Nos. C6039, C6164 and C6295)

Product Description

Cell Freezing Media are complete ready-to-use reagents which are designed to protect and preserve cells during frozen storage. **Cat. Nos. C6039** and **C6164** contain Minimum Essential Medium (MEM) supplemented with a mixture of fetal bovine serum and calf serum and contain 10% glycerol or DMSO. **Cat. No. C6295** is a serum-free preparation prepared by Sigma according to the original published formula of Charity Waymouth.[1] It contains 8.7% DMSO in MEM supplemented with methylcellulose.

Product Use

Cell Freezing Media may be used in standard freezing protocols. The following protocol may be used:

1. Thaw the Cell Freezing Medium and hold it on wet ice.
2. Remove adherent cells with trypsin or other appropriate means. For optimal results cells should be in the log phase of growth.
3. Gently pellet (10 minutes at 250 × *g,* 2–8 °C) the cells by centrifugation and remove as much of the growth medium as possible.
4. Suspend the cells in freezing medium at 10^6 to 10^7 cells/mL. Myelomas or hybridomas may require a higher cell density.
5. Aliquot cells into freezing vials, holding them on wet ice until freezing begins (within 5 minutes).
6. Freeze cells according to standard protocols. Store below –70 to –196 °C.

Thawing of frozen cells may be done as follows:

1. Remove cells from frozen storage and quickly thaw in a 37 °C waterbath.
2. Dilute 1 mL of cell suspension with 10 ml of complete growth medium.
3. Gently mix and pellet cells by gentle centrifugation.
4. Suspend cells in complete growth medium and plate in appropriate vessels.

Product Storage

Cell Freezing Media should be stored at –20 °C. After thawing, these products may be stored at 2–8 °C for up to 5 days. We do not recommend storing reagents in a frost-free freezer as temperature cycling may cause deterioration of the products.

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Corning® Cell Culture Surfaces

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Introduction

For over eighty years Corning has been developing products and surfaces for cell culture. Corning currently offers five polystyrene-based surfaces for growing cells including the most recent technology revolution, the patented Corning® CellBIND® surface (U.S. Patent 6,617,152): This guide will focus on the five polystyrene surfaces (**Table 1**) that are used for cell culture. Cell attachment and spreading onto the surface of a culture vessel is critical for the growth of anchorage-dependent cells. Cells attach and grow well on glass; furthermore, glass is clear and allows direct microscopic observation of attached cells. As a result, Pyrex® glass was the material of choice for cell culture applications until the 1960s when plastic culture vessels became available.

Table 1. Corning cell culture surfaces

Corning Surface	Binding Interaction	Sample Properties
Untreated polystyrene	Hydrophobic	Significantly reduces the attachment of most cells
Ultra Low Attachment coated polystyrene	Hydrophilic and nonionic	Hydrogel layer prevents the attachment of almost all cells
Tissue culture treated polystyrene	Hydrophilic and ionic	Allows cell attachment and binding to polystyrene
Corning® CellBIND® modified polystyrene	Hydrophilic and ionic (negatively charged)	Improves cell attachment and binding to polystyrene
Poly-D-lysine coated polystyrene	Hydrophilic and ionic (positively charged)	Improves cell attachment and binding to polystyrene

Most of these early plastic vessels were made from polystyrene, a long carbon chain polymer with benzene rings attached to every other carbon. Polystyrene was chosen because it has excellent optical clarity, is easy to mold, and is relatively inexpensive. However, it also has one significant drawback — it is a very hydrophobic (nonwettable) polymer to which cells have difficulty attaching. Fortunately, the surface of polystyrene can be easily modified by a variety of chemical (sulfuric acid) and physical (corona discharge, gas-plasma, or irradiation) methods (Hudis, 1974; Amstein and Hartman, 1975; Curtis et al., 1983; Ramsey et. al., 1984). Using these methods, hydroxyl, ketone, aldehyde, carboxyl, and amine groups can readily be grafted onto the polymer. These groups modify the surface characteristics changing the uncharged hydrophobic surface into a more ionic hydrophilic surface. Polystyrene can also be modified through chemical reactions to allow the covalent attachment of a variety of reactive groups that can be used for the subsequent covalent immobilization of biomolecules. For additional information, please check the References.

Untreated Polystyrene Surface

Natural, unmodified polystyrene surfaces are hydrophobic and only bind cells and biomolecules through passive hydrophobic interactions. Corning offers untreated polystyrene culture dishes and microplates for growing cells in stationary suspension or other applications where reduced cell attachment is desired. However, these untreated vessels are sterilized by low dose gamma irradiation, which slightly increases the wettability of the surface (Onyiriuka, et al., 1991). Since some transformed cell lines (CHO-K1, for example) and macrophages will attach and grow on these hydrophobic surfaces, Corning also offers an **Ultra Low Attachment Surface** for use in situations where cell attachment must be kept to an absolute minimum.

Sigma and Corning offer untreated cell culture dishes and culture tubes.

Ultra Low Attachment Coated Polystyrene Surface

The Corning Ultra Low Attachment surface is a covalently bound hydrogel layer that is hydrophilic and neutrally charged. Since proteins and other biomolecules passively adsorb to polystyrene surfaces through either hydrophobic or ionic interactions, this hydrogel surface naturally inhibits nonspecific immobilization via these forces, thus inhibiting subsequent cell attachment. This surface is very stable, noncytotoxic, biologically inert, and nondegradable.

This Ultra Low Attachment surface has been shown to successfully inhibit attachment of anchorage dependent MDCK, VERO, and C6 cells grown for a period of time equal to that necessary to obtain confluent cell growth on the control surface (standard tissue culture treated polystyrene). This surface has also been shown to inhibit the attachment and activation of macrophages and neutrophils (Shen and Horbett, 2001).

Ultra Low Attachment culture vessels are useful for:

- Studying tissue-specific functions of certain cancer cells (i.e., MCF-7 breast cancer cells)
- Studying activation and inactivation mechanisms of macrophages and other phagocytic cells
- Procedures requiring the reduction of eukaryotic and microbial cell attachment
- Preventing stem cells from attachment-mediated differentiation
- Preventing anchorage-dependent cells from dividing
- Selectively culturing tumor or virally transformed cells as unattached colonies (substitute for soft agar assays)
- Reducing binding of attachment and serum proteins to the substrate

Sigma and Corning offer the Ultra Low Attachment surfaces on dishes and microplates.

Standard Tissue Culture Treated Polystyrene Surface

Standard Corning® polystyrene cell culture vessels are surface modified using either corona discharge (flasks, dishes and microplates) or gas-plasma (roller bottles and culture tubes). These processes generate highly energetic oxygen ions which graft onto the surface polystyrene chains so that the surface becomes hydrophilic and negatively charged when placed in medium (Hudis, 1974; Amstein and Hartman, 1975; Ramsey et. al., 1984).

This standard Corning® cell culture surface:

- Has been successfully used to grow cells for 30 years
- Works with most attachment-dependent cell lines
- Is very economical

Sigma and Corning offer the standard tissue culture treated surface on flasks, dishes, multiple well plates, CellSTACK® Chambers, roller bottles and culture tubes.

Corning® CellBIND® Modified Polystyrene Surface

The Corning® CellBIND® culture surface, the first novel cell culture surface treatment in over 20 years, is designed to improve cell attachment under difficult conditions, such as reduced-serum or serum-free medium, resulting in higher cell yields. It is also useful for growing "difficult" cells such as primary cultures or transfected cells over expressing proteins. Developed by Corning scientists, this patented technology (U.S. Patent 6,617,152) uses a novel microwave plasma process for treating the culture surface. This process improves cell surface more than traditional plasma or corona discharge treatments, rendering it more hydrophilic (wettable) and increasing the stability of the surface.

Unlike biological coatings, the Corning® CellBIND® surface is a nonbiological surface that requires no special handling or storage. Because the polymer is treated, rather than coated, the surface is more consistent and stable. This enhanced cell performance has already led to a major biotechnology company choosing Corning® roller bottles with the Corning® CellBIND® surface for producing a new FDA approved protein therapeutic.

Corning® CellBIND® surface benefits:

- Gives more consistent and even cell attachment for difficult to attach cell lines, especially transfected cells
- Quickly adapts cells to reduced serum or serum-free conditions
- Reduces premature cell detachment from confluent cultures especially in roller bottles and during cell-based assays
- May eliminate the need for tedious, time-consuming, expensive, and low stability biological coatings
- Stable at room temperature, requires no refrigeration or special handling

Corning® CellBIND® surface applications:

- Primary cultures — may eliminate the need for expensive biological coatings for attachment
- Reduced or serum-free medium — reduced serum proteins decrease attachment
- Roller bottle cultures — constant rotation makes attachment difficult and cells often peel off
- Transfected cells — often attach poorly, especially when over-expressing proteins
- Cell-based assays — washing steps remove cells from wells leading to higher CVs

Corning offers the Corning® CellBIND® surfaces on flasks, multiple well plates, CellSTACK® Chambers and roller bottles.

Poly-D-Lysine Coated Surface

Some assays and procedures require enhanced binding of cells to polystyrene. Corning® poly-D-lysine (PDL) microplates are coated with PDL (molecular weight range of 70 to 150 kDa) by a proprietary method. This synthetic polymeric coating creates a uniform net positive charge on the plastic surface which, for some cell types, can enhance cell attachment, growth, and differentiation, especially in serum-free and low serum conditions. PDL surfaces often improve attachment and growth of primary neurons, glial cells, neuroblastomas, and a variety of transfected cell lines, including HEK-293.

Sigma and Corning offer poly-D-lysine coated 96 and 384 well microplates.

For additional questions please call Sigma-Aldrich Technical Service or visit the Corning Life Sciences Web site at **corning.com/lifesciences**.

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Subculturing Monolayer Cell Cultures

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Introduction

Most animal cell lines and primary cultures grow as a single thickness cell layer or sheet attached to a plastic or glass substrate. Once the available substrate surface is covered by cells (a *confluent* culture), growth slows and then ceases. Thus, in order to keep the cells healthy and actively growing, it is necessary to subculture them at regular intervals. Usually, this subcultivation process involves breaking the bonds or cellular 'glue' that attaches the cells to the substrate and to each other by using proteolytic enzymes such as trypsin, Dispase, or collagenase. Occasionally, these enzymes or dissociating agents are combined with divalent cation chelators such as EDTA (binds calcium and magnesium ions). The loosened cells are then removed from the culture vessel, counted, diluted, and subdivided into new vessels. Cells then reattach, begin to grow, and divide, and, after a suitable incubation period (depending on the initial inoculum size, growth conditions, and cell line), again reach saturation or confluency. At this point, the subcultivation cycle can be repeated.

The following protocol covers the basic techniques that are suitable for subculturing many cell lines.

Supplies

Nonsterile

1. Pipetting aids
2. Disposal tray or bucket for pipettes
3. Bottle of 70% alcohol for wiping down work area
4. Paper towels
5. Marking pen
6. Inverted phase contrast microscope
7. Ice bucket
8. Liquid waste container
9. Hemacytometer (**Cat. No. Z359629**)
10. 37 °C incubator

Sterile

1. Flask of actively growing cells that are 80 to 90% confluent
2. Cell culture medium. This should contain all of the additives (fetal bovine serum, glutamine etc.) required by the cell line.
3. Calcium- and magnesium-free phosphate-buffered saline (DPBS) (10 mL). This simple salt solution is used to maintain proper pH and osmotic balance while the cells are being washed to remove protease inhibitors that are found in most animal sera.
4. 0.1% Trypsin solution. Trypsin is normally used in concentrations ranging from 0.05% to 0.25%. Working concentrations are usually determined by using the lowest trypsin concentration that can remove the cells from the substrate and give a single cell suspension in a relatively short time (5 to 10 minutes). Trypsin solutions are often supplemented with other enzymes (collagenase) or chelating agents (EDTA) to improve its performance. (See Cell Dissociation section for Trypsin product selection)
5. 15 mL disposable screw cap centrifuge tubes (**Cat. No. C8046** or **Z711551**)
6. Appropriate culture vessels
7. 1, 5, 10, and 25 mL pipettes (**Cat. Nos. Z371580, Z371602, Z371610** and **Z371629**)
8. Sterile 0.04% Trypan Blue Solution (**Cat. No. T8154**) for viability staining
9. 20 µL pipettor (**Cat. No. Z714100**)
10. Pipette tips
11. Laminar flow hood

Application Note: A phosphate buffered saline is used for both rinsing and the trypsin solution since it maintains a physiological pH without requiring a closed system (required by buffers based on Hanks' saline) or gassing with carbon dioxide (required by buffers based on Earle's saline). Calcium and magnesium are omitted because these play a role in cell attachment.

Procedure

1. Examination

It is important to examine your cultures daily and always prior to subcultivation. Using an inverted phase contrast microscope (100 to 200×), quickly check the general appearance of your culture. Look for signs of microbial contamination. Many cells round up during mitosis, forming very refractile (bright) spheres that may float free of the surface when the culture is disturbed. Dead cells often round up and become detached, but are usually not bright or refractile.

Do not forget to examine the culture vessel with the unaided eye to look for small fungal colonies that may be floating at the media-air interface (especially near the vessel neck) and thus not visible through the microscope.

2. Cell Harvesting

This step removes the cells from the plastic substrate and breaks cell-to-cell bonds as gently as possible. When using enzymatic dissociation: a) the old medium is removed and discarded; b) the cell monolayer is gently rinsed; c) the enzyme solution is added and the culture incubated until the cells are released. There are many variations of this protocol; the following is a commonly used approach.

a) Using a sterile pipette, remove and discard the culture medium. All materials and solutions exposed to cells must be disposed of properly. Medium can be left in the pipettes if they are placed in disinfectant.

b) For a T-75 flask, wash the cell monolayer by adding 5 to 10 mL of DPBS without calcium and magnesium to the flask and then slowly rock it back and forth to remove all traces of fetal bovine serum. Remove and discard the wash solution. Failure to remove traces of fetal bovine serum is frequently responsible for failure of the trypsin solution to remove the cells from the vessel. Proportionally reduce or increase the volumes used in this protocol for smaller or larger culture vessels.
Helpful Hint: Two washes and/or rinsing with trypsin can be used for more difficult to remove cells.

c) Add 5 mL of the trypsin solution to the flask and place the flask back in an incubator at 37 °C to increase the activity of the enzyme solution. (Prewarming of the enzyme solution to 37 °C will decrease the required exposure period.)

d) Check the progress of the enzyme treatment every few minutes with an inverted phase contrast microscope. Once most of the cells have rounded up, gently tap the side of the flask to detach them from the plastic surface. Then add 5 mL of growth medium to the cell suspension and, using a 10 mL pipette, vigorously wash any remaining cells from the bottom of the culture vessel. At this point a quick check on the inverted microscope should show that the cell suspension consists of at least 95% single cells. If this is not the case, more vigorous pipetting may be necessary.
Helpful Hint: For difficult to break up cell clumps, try holding the pipette tip tight against and perpendicular to the side of the flask and then forcibly expel its contents. This will create a strong shearing force that should break up cell clumps.

e) Collect the suspended cells in a 15 mL centrifuge tube and place on ice. Some dissociating agents should be removed at this point by centrifugation to prevent carry over which can cause poor cell attachment or toxicity. However, the trypsin in the cell suspension will be inactivated by the serum and does not absolutely need to be removed. If removal is desired, spin the cell suspension at 100 × *g* for 5 minutes. Then remove the trypsin-containing medium and replace with fresh medium.
Helpful Hint: Storing cells on ice will slow cell metabolism. This will improve cell viability and reduce cell clumping.

3. Cell Counting

To determine growth rates or set up cultures at known concentrations it is necessary to count the cell suspension. Hemacytometers or electronic cell counting devices can be used. The hemacytometer has the added advantages of being both less expensive and allowing cell viability determinations to be made during counting.

a) Vortex the cell suspension and remove a 0.5 mL sample and place in a tube for counting. To this add 1 mL of the vital stain trypan blue (0.04%). Mix well by vortexing, withdraw a 20 µL sample with a wide tip pipettor and carefully load a clean hemacytometer. (Do not overfill!)

b) Do a viable cell count and calculate the number of viable cells/mL and the total cell number.
Helpful Hint: Frequently, instead of counting the cells in the suspension, the suspension is split among a number of culture vessels. For example, a 1:2 split would divide the cell suspension of one vessel into two new vessels of equivalent surface area. This is a quick and easy method for the routine maintenance of cell lines.

4. Plating

After making the appropriate dilutions, add the correct amount of cells to each culture vessel. Then add fresh medium to bring the culture vessel to its recommended working volume (See **Table 1**). Be sure to label all vessels accurately; write on the sides of flasks and around the outer edge of the dish tops so as not to interfere with microscopic observation.

Corning recommends using 0.2 to 0.3 mL of medium for every square centimeter of growth area.

Table 1. Typical cell yields and recommended medium volumes for Corning® flasks and dishes

Corning Products	Average Cell Yield*	Recommended Medium Volume (mL)	Maximum Working Volume (mL)**
Corning® Flasks			
25 cm^2	2.5×10^6	5–7.5	10
75 cm^2	7.5×10^6	15–22.5	60
150 cm^2	1.5×10^7	30–45	210
162 cm^2	1.6×10^7	32–48	175
175 cm^2	1.75×10^7	35–52.5	250
225 cm^2	2.25×10^7	45–67.5	370
Corning® Dishes			
35 mm	8.0×10^5	1.6–2.4	N/A
60 mm	2.1×10^6	4.2–6.3	N/A
100 mm	5.5×10^6	10–15	N/A
150 mm	1.48×10^7	30–45	N/A
245 mm (square)	5.0×10^7	100–150	N/A

* Assumes an average yield of 1×10^5 cells/cm^2 from a 100% confluent culture. Yields from many cell types can be lower or higher than this.

** Maximum working volume is the amount a flask can hold in the horizontal position when filled to the neck.

5. Incubation

Most mammalian cell cultures do best at a temperature between 35 °C and 37 °C. In addition to maintaining constant temperature, some incubators also maintain high humidity levels and CO_2 concentrations. The high humidity cuts down evaporation losses in open systems such as petri dishes and microplates that would otherwise result in hypertonic culture medium and stressed cells. The elevated CO_2 concentrations (usually 5% to 10%, depending on bicarbonate concentrations in the medium) help maintain the proper pH (7.4 ± 0.2) when used with the correct bicarbonate buffer system. In order for this type of buffer system to work it is necessary to allow gas exchange by using unsealed dishes and plates or flasks with gas permeable (vented) caps.

a) Leave caps on flasks slightly loosened (or use vented caps on the flasks for extra protection against spillage and contamination) and place on a shelf in a 37 °C, humidified CO_2 incubator.

b) Examine cultures daily and change medium as needed.

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Understanding Cell Culture Contaminants

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Introduction

No cell culture problem is as universal as that of culture loss due to contamination. All cell culture laboratories and cell culture workers have experienced it. Culture contaminants may be biological or chemical, seen or unseen, destructive or seemingly benign, but in all cases they adversely affect both the use of your cell cultures and the quality of your research. Contamination problems can be divided into three classes:

- Minor annoyances — when up to several plates or flasks are occasionally lost to contamination
- Serious problems — when contamination frequency increases or entire experiments or cell cultures are lost
- Major catastrophes — contaminants are discovered that call into doubt the validity of your past or current work

Some consequences of contamination:

- Loss of time, money, and effort
- Adverse effects on the cultures
- Inaccurate or erroneous experimental results
- Loss of valuable products
- Personal embarrassment

The most obvious consequence of cell culture contamination is the loss of your time, money (for cells, culture vessels, media and sera), and effort spent developing cultures and setting up experiments. However, the less obvious consequences are often more serious. First there are the adverse effects on cultures suffering from undetected chemical or biological contaminants. These hidden (cryptic) contaminants can achieve high densities altering the growth and characteristics of the cultures. Worse yet are the potentially inaccurate or erroneous results obtained by unknowingly working with these cryptically contaminated cultures. Products, such as vaccines, drugs, or monoclonal antibodies, manufactured by these cultures will probably be useless. For some researchers the most serious consequence of contamination is suffering the embarrassment and damage to their reputation that results when they notify collaborators or journals that their experimental results are faulty and must be retracted due to contaminants in their cultures.

Preventing all cell culture contamination has long been the dream of many researchers, but it is an impractical, if not impossible, dream. Contamination cannot be totally eliminated, but it can be managed to reduce both its frequency of occurrence and the seriousness of its consequences. The goal of this bulletin is to review the nature of cell culture contamination and the problems it causes, and then to explore some of the key concepts and practical strategies for managing contamination to prevent the loss of valuable cultures and experiments.

What Are the Major Cell Culture Contaminants?

A cell culture contaminant can be defined as some element in the culture system that is undesirable because of its possible adverse effects on either the system or its use. These elements can be divided into two main categories: chemical contaminants and biological contaminants.

Chemical Contamination

Chemical contamination is best described as the presence of any nonliving substance that results in undesirable effects on the culture system. To define further is difficult; even essential nutrients become toxic at high enough concentrations. Nor is toxicity the only concern since hormones and other growth factors found in serum can cause changes that, while not necessarily harmful to cultures, may be unwanted by researchers using the system. (Reviewed in references 1–3.)

Types and sources of potential chemical contaminants:

- Metal ions, endotoxins, and other impurities in media, sera, and water
- Plasticizers in plastic tubing and storage bottles
- Free radicals generated in media by the photoactivation of tryptophan, riboflavin, or HEPES exposed to fluorescent light
- Deposits on glassware, pipettes, instruments etc., left by disinfectants or detergents, antiscaling compounds in autoclave water, residues from aluminum foil or paper
- Residues from germicides or pesticides used to disinfect incubators, equipment, and labs
- Impurities in gases used in CO_2 incubators

Media

The majority of chemical contaminants are found in cell culture media and come either from the reagents and water used to make them, or the additives, such as sera, used to supplement them. Reagents should always be of the highest quality and purity and must be properly stored to prevent deterioration. Ideally, they should be either certified for cell culture use by their manufacturer or evaluated by the researcher before use. Mistakes in media preparation protocols, reading reagent bottle labels, or weighing reagents are other common sources of chemical contamination.

Sera

Sera used in media have long been a source of both biological and chemical contaminants. Due to cell culture-based screening programs currently used by good sera manufacturers, it is unusual to find a lot of fetal bovine sera that is toxic to a majority of cell cultures. However, it is common to find substantial variations in the growth promoting abilities of different lots of sera for particular cell culture systems, especially for cultures that have specialized or differentiated characteristics. Uncontrollable lot-to-lot variation in hormone and growth factor concentrations makes this problem inevitable; careful testing of sera before purchase or switching to serum-free media can avoid these problems.

Remember also that serum proteins have the ability to bind substantial quantities of chemical contaminants, especially heavy metals, that may have entered the culture system from other sources, rendering them less toxic. As a result, switching from serum-containing medium to a serum-free system can unmask these toxic chemical contaminants, exposing the cells to their adverse effects.

Water

The water used for making media and washing glassware is a frequent source of chemical contamination and requires special care to ensure its quality. Traditionally, double or triple glass distillation was considered to be the best source of high quality water for cell culture media and solutions. Newer purification systems combining reverse osmosis, ion exchange and ultrafiltration are capable of removing trace metals, dissolved organic compounds, and endotoxins, and are increasingly popular. However, these systems must be properly maintained and serviced to ensure continued water quality. Because of its aggressive solvent characteristics, highly purified water can leach potentially toxic metal ions from glassware or metal pipes, and plasticizers from plastic storage vessels or tubing. These contaminants can then end up in media or deposited on storage vessels and pipettes during washing and rinsing. Water used to generate steam in autoclaves may contain additives to reduce scale buildup in pipes; these potentially toxic additives can also end up on glassware.

Endotoxins

Endotoxins, the lipopolysaccharide-containing by-products of Gram-negative bacteria, are another source of chemical contaminants in cell culture systems. Endotoxins are commonly found in water, sera, and some culture additives (especially those manufactured using microbial fermentation) and can be readily quantified using the Limulus Amebocyte Lysate assay (LAL). These highly biologically reactive molecules have major influences *in vivo* on humoral and cellular systems. Studies of endotoxins using *in vitro* systems have shown that they may affect the growth or performance of cultures and are a significant source of experimental variability. (Reviewed in references 6 and 39.) Furthermore, since the use of cell culture produced therapeutics, such as hybridomas and vaccines, are compromised by high endotoxin levels, efforts must be made to keep endotoxin levels in culture systems as low as possible.

In the past, sera have been a major source of endotoxins in cell cultures. As improved endotoxin assays (LAL) led to an increased awareness of the potential cell culture problems associated with endotoxins, most manufacturers have significantly reduced levels in sera by handling the raw products under aseptic conditions. Poorly maintained water systems, especially systems using ion exchange resins, can harbor significant levels of endotoxin-producing bacteria and may need to be tested if endotoxin problems are suspected or discovered in the cultures.

Storage Vessels

Media stored in glass or plastic bottles that have previously contained solutions of heavy metals or organic compounds, such as electron microscopy stains, solvents, and pesticides, can be another source of contamination. The contaminants can be adsorbed onto the surface of the bottle or its cap (or absorbed into the bottle if plastic) during storage of the original solution. If during the washing process they are only partially removed, then once in contact with culture media they may slowly leach back into solution. Residues from chemicals used to disinfect glassware, detergents used in washing, or some aluminum foils and wrapping papers for autoclaving or dry heat sterilization can also leave potentially toxic deposits on pipettes, storage bottles and instruments.

Fluorescent Lights

An important but often overlooked source of chemical contamination results from the exposure of media containing HEPES (N-[2-hydroxylethyl] piperazine-N'-[2-ethanesulfonic acid]) – an organic buffer commonly used to supplement bicarbonate-based buffers), riboflavin, or tryptophan to normal fluorescent lighting. These media components can be photoactivated producing hydrogen peroxide and free radicals that are toxic to cells; the longer the exposure the greater the toxicity.[4,5] Short-term exposure of media to room or hood lighting when feeding cultures is usually not a significant problem; but leaving media on lab benches for extended periods, storing media in walk-in cold rooms with the lights on, or using refrigerators with glass doors where fluorescent light exposure is more extensive, will lead to a gradual deterioration in the quality of the media.

Incubators

The incubator, often considered a major source of biological contamination, can also be a source of chemical contamination. The gas mixtures (usually containing carbon dioxide to help regulate media pH) perfused through some incubators may contain toxic impurities, especially oils or other gases such as carbon monoxide, that may have been previously used in the same storage cylinder or tank. This problem is very rare in medical grade gases, but more common in the less expensive industrial grade gas mixtures. Care must also be taken when installing new cylinders to make sure the correct gas cylinder is used. Other potential chemical contaminants are the toxic, volatile residues left behind after cleaning and disinfecting incubators. Disinfectant odors should not be detectable in a freshly cleaned incubator when it is placed back into use.

Keep in mind that chemical contaminants tend to be additive in cell culture; small amounts contributed from several different sources that are individually nontoxic, when combined together in medium, may end up overloading the detoxification capabilities of the cell culture resulting in toxicity-induced stress effects or even culture loss.

Biological Contamination

Biological contaminants can be subdivided into two groups based on the difficulty of detecting them in cultures:

- Those that are usually easy to detect — bacteria, molds, and yeast
- Those that are more difficult to detect, and as a result potentially more serious culture problems — viruses, protozoa, insects, mycoplasmas, and other cell lines

For a comprehensive review, see references 7 and 8.

Ultimately, it is the length of time that a culture contaminant escapes detection that will determine the extent of damage it creates in a laboratory or research project.

Bacteria, Molds, and Yeasts

Bacteria, molds, and yeasts are found virtually everywhere and are able to quickly colonize and flourish in the rich and relatively undefended environment provided by cell cultures. Because of their size and fast growth rates, these microbes are the most commonly encountered cell culture contaminants. In the absence of antibiotics, microbes can usually be readily detected in a culture within a few days of becoming contaminated, either by direct microscopic observation, or by the effects they have on the culture (pH shifts, turbidity, and cell destruction). However, when antibiotics are routinely used in culture, resistant organisms may develop into slow growing, low level infections that are very difficult to detect by direct visual observation. Similar detection problems can occur with naturally slow growing organisms or very small or intracellular bacteria that are difficult to see during routine microscopic culture observation. These cryptic contaminants may persist indefinitely in cultures causing subtle, but significant alterations in their behavior. By the time these cryptic contaminants are discovered, many experiments and cultures may have been compromised.

Viruses

Due to their extremely small size, viruses are the most difficult cell culture contaminants to detect in culture, requiring methods that are impractical for most research laboratories. Their small size also makes them very difficult to remove from media, sera, and other solutions of biological origin. However, most viruses have stringent requirements for their original host species' cellular machinery (may also be tissue specific) which greatly limits their ability to infect cell cultures from other species. Thus, although viruses may be more common in cell cultures than many researchers realize, they are usually not a serious problem unless they have cytopathic or other adverse effects on the cultures. (Reviewed in references 7 and 40.)

Since cytopathic viruses usually destroy the cultures they infect, they tend to be self-limiting. Thus, when cultures self-destruct for no apparent reason and no evidence of common biological contaminants can be found, cryptic viruses are often blamed. They are perfect culprits, unseen and undetectable; guilty without direct evidence. This is unfortunate, since the real cause of this culture destruction may be something else, possibly mycoplasma or a chemical contaminant, and as a result will go undetected to become a more serious problem.

A major concern of using virally infected cell cultures is not their effects on the cultures, but rather the potential health hazards they pose for laboratory personnel. Special safety precautions should always be used when working with tissues or cells from humans or other primates to avoid possible transmission of viral infection (HIV, hepatitis B, Epstein-Barr, simian herpes B virus, among others) from the cell cultures to laboratory personnel.[9] Contact your safety office for additional assistance if in doubt as to appropriate procedures for working with potentially hazardous tissues, cultures, or viruses.

Protozoa

Both parasitic and free-living, single-celled protozoa, such as amoebas, have occasionally been identified as cell culture contaminants. Usually of soil origin, amoebas can form spores and are readily isolated from the air, occasionally from tissues, as well as throat and nose swabs of laboratory personnel. They can cause cytopathic effects resembling viral damage and completely destroy a culture within ten days. Because of their slow growth and morphological similarities to cultured cells, amoebas are somewhat difficult to detect in culture, unless already suspected as contaminants.[7] Fortunately, reported cases of this class of contaminants are rare, but it is important to be alert to the possibility of their occurrence.

Invertebrates

Insects and arachnids commonly found in laboratory areas, especially flies, ants, cockroaches, and mites, can both be culture contaminants as well as important sources of microbial contamination. Warm rooms are common sites of infestation. By wandering in and out of culture vessels and sterile supplies as they search for food or shelter, they can randomly spread a variety of microbial contaminants. Occasionally they are detected by the trail of "foot prints" (microbial colonies) they leave behind on agar plates, but usually they don't leave any visible signs of their visit other than random microbial contamination. Mites can be a serious problem in plant cell culture facilities, especially those doing large scale plant propagation. Although bacteria, molds, and yeast may sometimes appear to 'jump' from culture to culture, these multilegged contaminants really can. While not nearly as common as other culture contaminants, it is important to be alert to the presence of these invertebrates in culture areas.

Mycoplasmas

Mycoplasmas were first detected in cell cultures by Robinson and coworkers in 1956. They were attempting to study the effects of PPLO (pleuropneumonialike organisms – the original name for mycoplasma) on HeLa cells when they discovered that the control HeLa cultures were already contaminated by PPLO.[10] In addition, they discovered that the other cell lines currently in use in their laboratory were also infected with mycoplasma, a common characteristic of mycoplasma contamination. Based on mycoplasma testing done by the FDA, ATCC, and two major cell culture testing companies, at least 11 to 15% of the cell cultures in the United States are currently infected by mycoplasmas. Since many of these cultures were from laboratories that test routinely for mycoplasma, the actual rates are probably higher in the many laboratories that do not test at all.[11-13] In Europe, mycoplasma contamination levels were found to be even higher: over 25% of 1949 cell cultures from the Netherlands and 37% of 327 cultures from former Czechoslovakia were positive.[14] The Czechoslovakia study had an interesting, but typical finding: 100% of the cultures from labs without mycoplasma testing programs were contaminated, but only 2% of the cultures from labs that tested regularly. Other countries may be worse: 65% of the cultures in Argentina and 80% in Japan were reported to be contaminated by mycoplasma in other studies.[11]

Unfortunately, mycoplasmas are not relatively benign culture contaminants, but have the ability to alter their host culture's cell function, growth, metabolism, morphology, attachment, membranes, virus propagation and yield, interferon induction and yield, cause chromosomal aberrations and damage, and cytopathic effects including plaque formation.[12] Thus, the validity of any research done using these unknowingly infected cultures is questionable at best. (See references 11, 12, and 15–18 for good overviews of this very serious mycoplasma contamination problem.)

What gives mycoplasmas this ability to readily infect so many cultures? Three basic characteristics: a) these simple, bacteria-like microbes are the smallest self-replicating organism known (0.3 to 0.8 µm in diameter), b) they lack a cell wall, and c) they are fastidious in their growth requirements. Their small size and lack of a cell wall allow mycoplasmas to grow to very high densities in cell culture (10^7 to 10^9 colony forming units/mL are common) often without any visible signs of contamination — no turbidity, pH changes, or even cytopathic effects. Even careful microscopic observation of live cell cultures cannot detect their presence. These same two characteristics also make mycoplasmas, like viruses, very difficult to completely remove from sera by membrane filtration. In addition, their fastidious growth requirements (unfortunately, easily provided for by cell cultures) make them very difficult to grow and detect using standard microbiological cultivation methods. Thus, these three simple character-

istics, combined with their ability to alter virtually every cellular function and parameter, make mycoplasmas the most serious, widespread, and devastating culture contaminants.

Mycoplasmas have been described as the "crabgrass" of cell cultures, but this is too benign a description for what are the most significant and widespread cell culture contaminants in the world. Unfortunately, even with the advances in detection methods (discussed in detail later) mycoplasma infection rates have not changed noticeably since they were first detected in cell cultures. Aggressive management against mycoplasma contamination must be the central focus for any cell culture laboratory contamination or quality control program.[16]

Cross-Contamination by Other Cell Cultures

With the advent of improved karyotyping methods in the late 1950s, it soon became apparent that some cell lines were cross-contaminated by cells of other species.[7] In 1967, isoenzyme analysis was used to show that 20 commonly used human cell lines were intraspecies contaminated by HeLa cells.[19,20] Contaminated is actually a misnomer since in fact 100% of the original cells had been replaced by the HeLa contaminant. Unfortunately, the scientific community was slow to respond to this very serious problem. Tests done at one research center on 246 cell lines over an 18 month period prior to 1976 showed that nearly 30% were incorrectly designated: 14% were the wrong species and 25% of the human cell lines were HeLa cells.[21] A 1981 survey of cultures showed over 60 cell lines that were actually HeLa cells, 16 other human cell lines contaminated by non-HeLa human cell lines, and 12 cases of interspecies contamination. Nor is the problem limited to contamination by HeLa cells. The advent of DNA analysis has shown that cells from a variety of sources have contaminated many other cell lines.[42]

The seriousness of cross-contamination, while not as common as microbial contamination, cannot be overstated. The validity of experimental results from cultures having inter- or intraspecies contamination is, at the very least, questionable. Furthermore, their use can lead to the embarrassment of having to retract published results. Whenever the invading cell is better adapted to the culture conditions and thus faster growing than the original cells, it will almost always completely replace them.

Because of the outward physical similarities of different cell lines and the wide morphological variations that can be caused by the culture environment, it is impossible to rely only on microscopic observation to screen for cross-contamination of cultures. Simple accidents are one of the most common means by which other cell lines gain entry into cultures and will be discussed separately in the next section.

Remember, the seriousness of any culture contaminant is usually directly proportional to the difficulty of detecting it; those that go undetected the longest have the most serious consequences. Cultures containing nonlethal (but not harmless), cryptic chemical or biological contaminants are sometimes used in research for months or even years before being uncovered; during this time the quality and validity of all research done with those cultures is compromised, as is the reputation of the researchers using them.

What Are the Sources of Biological Contaminants?

To reduce the frequency of biological contamination, it is important to know not only the nature and identity of the contaminants, but also where they come from and how they gain entry into cultures. This section will detail some of the most common sources of biological contaminants.[3]

Nonsterile Supplies, Media, and Solutions

Unintentional use of nonsterile supplies, media, or solutions during routine cell culture procedures is a major source of biological contaminants. These products may be contaminated as a result of improper sterilization or storage, or may become contaminated during use.

Glassware, including storage bottles and pipettes, is usually sterilized by autoclaving or dry heat sterilization. Serious contamination outbreaks are frequently traced to improper maintenance or operation of sterilization autoclaves and ovens. Packing too much into an autoclave or dry heat oven will cause uneven heating, resulting in pockets of nonsterile supplies. Using too short a sterilization cycle, especially for autoclaving volumes of liquids greater than 500 ml per vessel or solutions containing solids or viscous materials, such as agar or starches, is a common mistake. The size, mass, nature, and volume of the materials to be sterilized must always be considered and the cycle time appropriately adjusted to achieve sterility.[23] Then, once achieved, sterility must be maintained by properly storing the supplies and solutions in a dust- and insect-free area to prevent recontamination. Care must also be taken to avoid condensation on bottles of solutions stored in refrigerators and cold rooms. Of course, good aseptic technique is also required to maintain the sterility of properly sterilized supplies and solutions once they are in use.

Plastic disposable cell culture vessels, pipettes, centrifuge tubes, etc. are usually sterilized by their manufacturer using a high intensity gamma or electron beam radiation source after they are sealed in their packaging. This is a very reliable process; however, care must be taken when opening and resealing the packaging to avoid contaminating the products within.

Most media, sera, and other animal-derived biologicals are not heat sterilizable and require membrane filtration (sometimes radiation is also used) to remove biological contaminants. Products filter sterilized in your laboratory should always be tested for sterility before use (discussed in detail later); commercially produced sterile products are tested by the manufacturer before being sold. While filtration through 0.2 µm membranes is very effective in removing most biological contaminants, it cannot guarantee the complete removal of viruses and mycoplasmas, especially in sera.[16,18,24]

In an excellent review of the rates and sources of mycoplasma contamination,[25] Barile and coworkers reported that 104 out of 395 lots (26%) of commercial fetal bovine sera tested were contaminated by mycoplasma. They concluded in the early 1970s that animal sera were among the major sources of cell culture contamination by mycoplasma.

Many sera manufacturers responded to this problem over the next decade by improving both filtration and testing procedures; they currently use serial filtration through at least three filter membranes rated at 0.1 µm or smaller to remove mycoplasmas. This approach has been very successful at reducing the problem of mycoplasma in sera and other animal-derived products.[16] While these products are no longer a major source of mycoplasma contamination, they must still be considered as potential sources to be evaluated whenever mycoplasmas are detected in cultures.

Airborne Particles and Aerosols

In most laboratories, the greatest sources of microbial contamination are airborne particles and aerosols generated during culture manipulations. The microbial laden particles are relatively large (generally 4 to 28 µm in diameter) and settle at a rate of approximately one foot per minute in still air. As a result, the air in a sealed, draft-free room or laboratory (no people, open windows or doors, air handling units, air conditioners, etc.) is virtually free of biological contaminants. However as soon as people enter the room, particles that have settled out will be easily resuspended. In addition certain equipment and activities can generate large amounts of microbial laden particulates and aerosols: pipetting devices, vacuum pumps and aspirators, centrifuges, blenders, sonicators, and heat sources such as radiators, ovens, refrigerators and freezers. Animal care facilities and the animals they house are especially serious particle and aerosol generators, and should always be kept as far from the culture area as possible.

McGarrity used a cell culture that was intentionally infected with mycoplasma as a model to study how mycoplasmas are spread in a laminar flow hood during routine subculturing procedures.[26] (This reference is especially recommended for a better understanding of how mycoplasma can be spread in a lab.) Following trypsinization of the infected culture in a laminar flow hood, live mycoplasma were isolated from the technician, the outside of the flask, a hemocytometer, the pipettor, and the outside of the pipette discard pan. Live mycoplasma could even be successfully recovered from the surface of the laminar flow hood four to six days later! A clean culture, that was subcultured once a week in the same hood following the work with the contaminated cells, tested positive for mycoplasma after only 6 weeks. It is easy to understand from this study how the entry of a single mycoplasma infected culture into a laboratory can quickly lead to the infection of all the other cultures in the laboratory. This explains the frequent finding that if one culture in a laboratory is mycoplasma contaminated then usually most if not all of the other cultures will be as well. Currently, the major source of mycoplasma contamination is infected cultures acquired from other research laboratories or commercial suppliers.

Another major source of particulates and aerosols are laboratory personnel. Street clothes and dirty lab coats are dust magnets. Placing a dust-laden sleeve into a laminar flow hood generates a cloud of dust particles that can easily fall into and contaminate cultures during routine processing. Talking and sneezing can generate significant amounts of aerosols that have been shown to contain mycoplasma.[26] Mouth pipetting is both a source of mycoplasma contamination and a hazard to personnel and must not be permitted under any circumstances. Dry, flaky skin is another source of contamination laden particles; this common condition is aggravated by the frequent hand washing required in the laboratory; even the lotions designed to moisten dry skin have occasionally been found to be contaminated. Some laboratory personnel shed yeast-containing particles for several days following bread making or beer brewing at home. Attempts by these individuals at cell culturing during this period have routinely ended in failure due to yeast contamination.

Incubators, especially those maintained at high humidity levels, can be a significant source of biological contamination in the laboratory. Dirty water reservoirs, and shelves or culture vessels soiled by spilled media, allow the growth of spore-generating fungi. The fans used in many incubators to circulate the air and prevent temperature stratification can then spread these spores and other particulates. Some incubators humidify incoming gases by bubbling them through the water reservoirs at the bottom of the incubator; the aerosols generated by this will quickly spread any contaminants in the water.

While laminar hoods and incubators are the major sites where biological contamination occurs, transporting cultures between these two sites also provides opportunities for contamination. Most cell culture laboratories try very hard to keep their incubators and laminar flow areas clean, but sometimes they overlook the potential sources of contamination found in less clean laboratory areas transversed going from one location to the other. Rooms containing open windows, air conditioners, microbiology and molecular biology work areas, and the other major particle generators discussed above, add to the potential hazards of moving cultures around the laboratory. This problem increases both with the distance traveled and when the culture vessels are unsealed.

Swimming, Growing, and Crawling into Cultures

Unsealed culture plates and dishes, as well as flasks with loose caps to allow gas exchange, provide another common way for contaminants to enter cultures. It is very easy for the space between the top and bottom sidewalls of a dish, or a flask and its cap to become wet by capillary action with medium or condensation. This thin film of liquid then provides a liquid bridge or highway for microorganisms to either swim or grow into the culture vessel. Even without any detectable film, fungi, as well as other microorganisms, can grow on the outside of culture vessels.

Accidents

Accidents are often overlooked as a significant source of cell culture problems. An accident is defined as "an undesirable or unfortunate happening, unintentionally caused and usually resulting in harm, injury, damage or loss" (Webster's Encyclopedic Unabridged Dictionary, 1989). Cell culture-related accidents are one of the leading causes of cross-contamination by other cell cultures. The following actual cases demonstrate how relatively simple accidents can result in serious cross-contamination problems:

- A technician retrieved a vial labeled WI-38 from a liquid nitrogen freezer thinking it contained the widely used diploid human cell line. Once in culture, it was immediately discovered to be a plant cell line derived from a common strain of tobacco called Wisconsin 38, also designated WI-38.
- Two separate research laboratories, both attempting to develop cell lines from primary cultures, shared a walk-in incubator. One lab used the acronyms HL-1, HL-2, etc. to identify the primary cultures they derived from human lung. The other lab worked with cultures derived from human liver, but they too (unknowingly) used the identical coding system. It wasn't long before a culture mix up occurred between the two laboratories.

Fortunately, both of the above accidental cross-contamination cases, although serious, were caught before they caused catastrophic problems. But how many times have similar accidents occurred and not been caught? Based on continuing reports in the literature[7,8,19-22] many researchers have not been lucky enough to identify their mistakes.

The information presented above is designed to provide you with an increased awareness and understanding of the nature of biological and chemical contamination, and its serious consequences. The remaining sections will cover some basic ideas, techniques and strategies for actively detecting and combating cell culture contamination in your own laboratory.

How Can Cell Culture Contamination Be Controlled?

Cell cultures can be managed to reduce both the frequency and seriousness of culture-related problems, especially contamination. Lack of basic culture management procedures, especially in larger laboratories, frequently leads to long-term problems, making contamination more likely for everyone. One solution is to actively manage your cultures to reduce problems and if necessary set up a program for use in your laboratory.[27,28] This program should be designed to meet the needs of your specific working conditions and be based on the nature of your past cell culture problems; it can be very simple and informal, or more structured if required.

The first step in managing cultures is to determine the extent and nature of the culture losses in your lab. Everyone in the laboratory should keep an accurate record for a month or more of all problems, no matter how minor or insignificant, that result in the loss of any cultures. These problems may not only be contamination related, but can also be from other causes such as incubator or equipment failures. Next, review the problems as a group to determine their nature, seriousness and frequency. The group's findings may be surprising: what were thought to be individual and minor random occurrences of contamination often turn out to have a pattern and be more extensive than any individual realized. This problem sharing is often a painful process, but remember the goal is not to place blame, but to appreciate the extent and nature of the problems confronting the laboratory. A critical part of this process is understanding the seriousness and actual costs of culture loss; placing a dollar value on these losses is often required before the full extent of the losses can be appreciated. It is very important for everyone in the laboratory to know the answers to the following questions:

1. How much time, money and effort have been invested in your cultures and experiments?
2. What are the consequences of their loss?
3. How expensive or difficult will it be to replace them?

Once the nature and consequences of the problems in the laboratory are better understood, the need for a management system, if necessary, can be determined. Basic problem solving tools[2] can be used to help identify the source of problems; changes to minimize or prevent the problems from reoccurring can then be implemented.

The following suggestions, concepts and strategies, combined with basic management techniques, can be used to reduce and control contamination. These may require modification to fit your own needs and situation.

Use Good Aseptic Techniques

Aseptic technique is designed to provide a barrier between microorganisms in the environment, and your cultures and sterile supplies, yet permit you to work with them. There are many successful techniques for achieving and maintaining aseptic cell cultures; ultimately, your technique is "good" if it routinely protects both you and your cultures from contamination. Teaching aseptic technique is beyond the scope of this guide; the goal here is to review some of its basic tenets and present some suggestions for improving it. The reader is referred to Freshney[3] for a basic introduction to this very important area.

Table 1. Steps for Reducing Contamination Problems

• Use good aseptic techniques
• Reduce accidents
• Keep the laboratory clean
• Routinely monitor for contamination
• Use frozen cell repository strategically
• Use antibiotics sparingly if at all

The first step in developing sound, rational aseptic techniques is a solid understanding of both the nature and potential sources of biological contamination. This is reviewed in the beginning of this bulletin and covered in many of the references.

The second step, based on the nature of your work, is to determine the level of risk or danger to yourself and other laboratory personnel and then design your culture techniques accordingly. This is especially true when working with cultures that are virally contaminated or derived from human and other primate sources. Ensure that all laboratory personnel have been trained in the safe handling and disposal of any potentially hazardous cultures and materials; refer to your facility's safety office for any necessary assistance or guidance.[9]

Next, based on the potential costs and consequences if the cultures are lost, determine how rigorous your technique must be, and what degree of redundancy if any, is required. Very valuable or irreplaceable cultures can be carried by two or more workers using media from different sources and separate incubators to reduce the chance of their simultaneous loss.[27,28] Evaluate whether workers need to be gloved, gowned and masked to reduce the potential for contamination.

The nature of your working environment and any problems it may present must also be considered in choosing appropriate aseptic techniques. Certified laminar flow hoods and safety cabinets are recommended for use whenever possible. Some of the aseptic techniques taught in introductory microbiology classes for use on the open bench, such as flaming, while popular, are not appropriate or necessary in laminar flow hoods.[16] Hood manufacturers recommend against the use of Bunsen burners and other sources of flames in hoods; they disrupt the moving curtain of filtered air and the resulting turbulence can increase the probability of contamination by microbial laden aerosols and particles generated during routine culture manipulation.

The following suggestions are recommended to reduce the probability of contamination:

- Make it more difficult for microorganisms to gain entry by using sealed culture vessels whenever possible, especially for long-term cultures. The multiple well plates can be sealed with labeling tape or placed in sealable bags, 35 and 60 mm dishes can be placed inside 150 or 245 mm dishes. Use vented cap flasks whenever possible. These have hydrophobic filter membranes that allow sterile gas exchange, but prevent the passage of microorganisms or liquids.
- Avoid pouring media from cell culture flasks or sterile bottles by using 50 or 100 mL pipettes to transfer larger volumes. Using a disposable aspirator tube and vacuum pump is an economical way to quickly and safely remove medium from cultures. A drop of medium remaining on the vessel's threads after pouring can form a liquid bridge when the cap is replaced providing a means of entry for bacteria, yeasts, and molds. If pouring cannot be avoided, carefully remove any traces of media from the neck of the vessel with a sterile gauze or alcohol pad.
- Always carry unsealed cultures in trays or boxes to minimize contact with airborne contaminants. Square 245 mm dishes are excellent carriers for 384 and 96 well plates as well as for 35 mm and 60 mm dishes.
- Do not use the hood as a storage area. Storing unnecessary boxes, bottles, cans etc. in the hood, besides adding to the bioburden, disrupts the airflow patterns.
- Never mouth pipette. Besides the risk of injury to laboratory personnel, mouth pipetting has been implicated as the likely source of human mycoplasma species (*M. orale* and *M. salivarium*) often found in cell cultures.[15]
- Use clean lab coats or other protective clothing to protect against shedding contaminants from skin or clothes. Their use should be restricted to the cell culture area to avoid exposure to dirt and dust from other areas.
- Work with only one cell line at a time in the hood, and always use separate bottles of media, solutions, etc. for each cell line to avoid possible cross-contamination. Use disinfectant to wipe down the hood's work surfaces between cell lines.
- Use antibiotic-free media for all routine culture work; this is a very important concept and will be discussed in detail below.
- Whenever possible, package sterile solutions, such as trypsin, L-glutamine and antibiotics, in small volumes (i.e., stored in 15 mL tubes) to reduce the number of times each tube must be entered and thus reduce the probability of contamination.
- Leave laminar flow hoods running 24 hours a day. Only turn them off when they will not be used for extended periods.

Reduce Opportunities for Accidents

Accidents usually involve people, and reducing them must take into consideration both human nature and stress. Based on personal experience, accidents are far more likely on:

a) Friday afternoons,
b) the day before a vacation begins,
c) with new employees, or
d) when people are stressed, overworked, or rushed.

The following suggestions can help reduce the confusion and misunderstanding that cause many accidents to happen in the laboratory.

- Be very careful when labeling solutions, cultures, etc. Always clearly indicate if solutions or other supplies have been sterilized. Reduce misunderstandings in crowded or busy labs by using a color coding system: assign each worker their own color for labeling tape and marking pen inks.
- Be very careful with the use and choice of acronyms. Everyone in the laboratory should understand and agree to their meaning.
- Whenever possible use standardized recordkeeping forms; this simplifies their use and makes it more likely that good records will be kept.
- Use written protocols and formulation sheets when preparing media and solutions, listing the reagents used, lot numbers, weights, volumes, pH and any special treatments that were done. These will both reduce the potential for errors as well as provide a valuable aid in tracking down the cause of problems.

Clean Up the Work Area and Surrounding Environment

Reducing the amount of airborne particulates and aerosols in the laboratory, especially around the incubator and the laminar flow hood, will reduce the amount of contamination. Routinely wipe floors and work surfaces to keep down dust. Incubators, especially those that maintain high humidity levels, require periodic cleaning and disinfecting. Often overlooked but important sources of contaminants are the water baths used to thaw sera and warm media. Dirty water baths not only coat bottles with a layer of heavily contaminated water right before they are placed under the hood, but the water dripping from bottles generates heavily contaminated aerosols which can end up on lab coats and hands. Water baths should be emptied and cleaned on a regular basis, well before odor or visible turbidity develops. Pipette disposal trays and buckets, and other waste containers provide a source of potentially heavily contaminated materials in close proximity to the laminar flow hood and are a potential mycoplasma source.[26] Waste containers should be emptied daily and the wastes disposed of safely. Autoclaving of any wastes that have been in contact with cells is recommended.

The cooling coils on refrigerators and freezers are a major source of microbial laden airborne particulates that are often overlooked in otherwise very clean laboratories. These should be vacuumed at least yearly; besides removing a significant source of contamination, regular vacuuming will extend the life of the cooling units and allow them to run more efficiently.

Some laboratories may also need to consider a pest management program to reduce the presence of mice, ants, cockroaches, and other multilegged creatures that can be sources of contamination. Potted plants, although attractive, can provide a home for these creatures and should not be kept in the culture vicinity. Care must be taken when using pesticides as part of a pest management program to prevent accidentally chemically contaminating the cultures in the laboratory.

Sterility Testing

The best strategy for reducing contamination is to be proactive by routinely monitoring supplies, media and solutions, work areas and, most importantly, cell cultures for contaminants before they are used in critical applications and experiments. The key to developing a realistic contamination monitoring program is to keep it as simple as possible so that people use it, yet ensure that it can get the job done. Unfortunately there are no easy solutions: no single microbiological medium can detect all types of biological contaminants, and practical testing methods often miss low levels of contaminants. The process of detection is made even more difficult by the presence of antibiotics. The techniques and concepts presented below offer some practical approaches for monitoring contamination that can be readily adapted to meet the needs of most cell culture laboratories.

All autoclaves and dry heat ovens used to sterilize glassware, solutions, and other supplies must be regularly maintained, and personnel properly trained in their loading and operation. Thermometers and chart recorders should be tested and calibrated periodically to ensure their accuracy. Inexpensive (when compared to the cost of a single autoclave failure) autoclave thermometers, spore test strips and capsules, or other testing devices can be placed inside autoclaves or into bottles of solutions or other packaged supplies during every run, or as necessary, to ensure proper loading and operation.

Samples of all in-house filter-sterilized solutions should be tested for sterility each time they are prepared and the solutions not used until testing is complete. Standard microbiological testing methods for bacteria, yeasts, and fungi usually require placing samples for testing into several different broths (trypticase soy, thioglycolate and Sabouraud broths, for example) and semisolid media (brain-heart infusion, blood agar), and then incubating them at both 30 °C and 37 °C for at least two weeks.[29]

Cell culture media, especially unopened bottles of media that are outdated or no longer used in the lab (as long as they do not contain any antibiotics) can provide a very rich, readily available and useful substitute for standard microbiological media. A small amount of serum (3 to 5% — again outdated or unwanted sera can be used) should be added to promote growth. The medium can be dispensed in 10 mL amounts into sterile 16 mm × 125 mm glass or plastic screw cap culture tubes or clear 15 mL plastic centrifuge tubes and be stored at 4 °C until needed. The sterility of either filtered solutions or cultures and products suspected of being contaminated can be routinely and easily checked by placing a small sample into each of two tubes and incubating one at 30 °C and the other at 37 °C for at least two weeks.

This sterility test media substitute is also very useful for evaluating the amount or source of particulate contamination in an area, near a piece of equipment or by a technique. Hoods, and especially incubators, are frequently blamed by laboratory personnel as the source of their contamination problems as in: "my cultures keep getting contaminated because something is wrong with the hood" (or incubator). Until these areas are screened and eliminated as the source of the problem, the real problem, often simply aseptic technique, can not be dealt with effectively. These suspected problem areas can be screened by dispensing the test medium into 96 well culture plates or 100 mm culture dishes (use agar-gelled media for the dishes). The vessels are then opened (with unopened vessels as controls) for 30 to 60 minutes at several locations within the test site prior to being sealed and incubated. Cultures can be initially checked for contamination after two to three days although slow growing contaminants may take two weeks or longer to appear. The rate of contamination (number of colonies or contaminated wells/vessel or unit area/unit time) can then be calculated and analyzed. Besides giving an accurate level of the bioburden in that area, microscopic observation of the contaminants in the liquid test media also allows their morphological comparison with the microorganisms found causing problems in the cell cultures. Past experience with this approach has shown it is a very useful tool when teaching aseptic technique as it clearly demonstrates that the air in a room, or even inside a humidified incubator is usually not a major source of contamination in a well maintained laboratory. It is also a useful tool in tracking down mysterious contamination outbreaks.

Detecting Mycoplasma in Cultures

No monitoring program is complete unless it can effectively detect contaminated cultures, especially those infected by mycoplasma. Unfortunately mycoplasma detection is not simple, and because of this, and a lack of awareness, many cell culture users simply don't bother to test. (As many as 50%, see survey results presented in **Table 2**.) As a result, it is estimated that at least 15% of all cell cultures in the United States are contaminated with mycoplasma. Because of these outrageously high levels of contamination and the proven ease with which mycoplasmas can be spread from contaminated cultures,[26] it is very important to quarantine all cultures coming into the laboratory until they have been tested for mycoplasma. This is especially true of gifts of cell lines from other labs; often these "gifts" end up infecting your cultures.

There are two basic testing methods for mycoplasma: direct culture in media, or indirect tests that measure specific characteristics of mycoplasma.[16] Direct culture is the most effective and sensitive method for detecting mycoplasma, but it is also the most difficult and time consuming. It requires several carefully tested liquid and semisolid media and controlled environmental conditions (See reference 30 for detailed protocols), and must be run with live mycoplasma controls. Additionally, although direct culture is the most sensitive method, it is the slowest (requiring up to 28 days) and it may not reliably detect some fastidious strains of mycoplasma, making it less than 100% effective. Budget permitting, direct culture testing is best contracted to an outside testing facility for two reasons: first, given the ease with which mycoplasma can spread in the laboratory, bringing live mycoplasma into a cell culture facility for the required controls is not recommended; second to do it well, direct testing requires a serious effort and commitment of resources better spent in doing cell culture. These tests are commercially available at a reasonable cost from several cell culture testing companies.

There are a wide variety of indirect test methods available for mycoplasma detection, including PCR-based kits, DNA fluorochrome staining, autoradiography, ELISA, immunofluorescence and specific biochemical assays. These tests are faster than direct culture, all are commercially available in kit form, and they can detect the fastidious, difficult to cultivate strains that are occasionally missed by direct culture. However they lack the sensitivity of direct culture, requiring much higher levels of contamination for detection. As a result, they have more frequent false negatives than direct culture methods, potentially leaving researchers who rely solely on a single indirect test with a false sense of security (Reviewed in references 11, 12, and 18).

The most widely used and recommended indirect test is DNA fluorochrome staining. (See reference 31 or the Corning Web site for detailed protocols.) This easy and relatively fast procedure stains DNA using a fluorescent dye. When stained and fixed cells are examined under a UV microscope equipped with the proper filter package, DNA fluoresces brightly. Not only will this test detect mycoplasma, but as an added benefit it will also detect any other microbial contaminants. This staining method can be combined with an indicator cell line to increase its sensitivity.

Interpreting results is not always easy, especially with hybridoma cultures; suitable positive and negative control slides should always be used to help interpret staining results. These positive and negative mycoplasma control slides are commercially available; since they have already been fixed, they are safe to use in the laboratory.

The best overall testing approach is a combination of both methods: direct culture can provide very high sensitivity while DNA fluorochrome staining can detect any fastidious mycoplasma that the direct culture misses. Both the FDA and USDA requires this approach for cell culture derived products, such as monoclonal antibodies, vaccines and drugs, and the cells required to produce them. If resources do not permit the combined approach, then the DNA fluorochrome staining procedure using an indicator cell line, combined with one other indirect test method should provide a minimum level of security.

Detecting Other Biological Contaminants in Cultures

The traditional microbiological media described earlier for testing the sterility of solutions can be adapted for testing cultures for bacteria, yeasts, and fungi.[29] However, the direct culture tests and the indirect DNA fluorochrome test for mycoplasma, although not designed for this purpose, will also detect most bacteria, yeasts, and fungi, including intracellular forms, reducing the need for the traditional tests. Special culture procedures are also available for detecting suspected protozoan contaminants in culture (Details can be found in reference 32).

There are several other important quality control tests that should be used to both identify and characterize the cell cultures used in your research. Besides the serious and widespread problem of cross-contamination by other cell lines described earlier, cells are also continually evolving in culture: important characteristics can be lost, mutations can occur, or chromosomes can undergo rearrangements or changes in number. Monitoring these changes is important because altered cell cultures can have a significant impact on the reproducibility of your research (Reviewed in reference 33). The following characterization methods are recommended for monitoring cell cultures; refer to the cited references for details.

Most laboratories should incorporate at least one of these methods as part of their monitoring program:

- Karyotyping, a relatively simple method used to determine the modal chromosome number and presence of any unique marker chromosomes.[34]
- Electrophoresis and isoenzyme analysis to generate a protein 'fingerprint' that can be used to determine species or for future comparisons.[33]
- Immunological or biochemical techniques to detect markers that are unique to the tissue, cell line, or the species from which it is derived.[33]
- DNA fingerprinting, a relatively new technique but one that is becoming increasingly useful, can be used to detect both intra- and interspecies contamination.[35]

The results from these characterization tests can serve as an important baseline against which any future changes can be compared.

Recommendations for a Testing Program

The cell culture testing program you choose should be the best you can afford, as it is the cornerstone of your research. An inadequate program (or worse, no program at all) provides a false sense of security and can eventually lead to failure compromising the validity of your research. The following steps are recommended for setting up a sound, yet practical culture monitoring program:

- Test all current in-house cell lines using the methods described above to ensure they are free from mycoplasma and other microbial contaminants, and to check their identity. Then incorporate these tested cultures into your cell repository and rely only on them for all future experiments.
- Quarantine and then test all incoming cell lines and any cultures currently stored in your cell repository that were not tested when they were frozen.
- Test all cell lines that are in continuous use at least every three to four months and any time they behave suspiciously. Better yet, save time, money and effort by periodically discarding these cultures and replacing them with cultures from your tested cell repository. (This strategy will be discussed in detail later in the section on using a cell repository.)
- New lots of sera should be evaluated for any critical applications before widespread use. The simplest test method is to use the new serum in an indicator cell culture for several weeks and then test the culture for mycoplasma contamination using DNA staining or other suitable test.

Detecting Chemical Contaminants

Determining that a chemical contaminant is the cause of a cell culture problem is usually much more difficult than with biological contaminants because it is so hard to detect. Often the first signs that something is wrong are widespread alterations in the growth, behavior, or morphology of the cultures in the laboratory; however, it may take weeks before these changes are noticed. Once noticed, the cause is frequently misconstrued to be of biological origin; only after extensive and unsuccessful testing for the usual microbial suspects does attention focus on the possibility it might be a chemical contaminant.

Begin the problem solving process by identifying all changes that have occurred in the lab in the weeks prior to the problem being noticed, especially in equipment, solutions, media and supplies, that may be related to the problem. Good recordkeeping is essential for this process to be successful. Bring together laboratory personnel to brainstorm for all of the possible causes and then select the best possibilities for evaluation. Simple comparison experiments can then be done to eliminate each possibility as the source of the problem; media, solutions, sera, and other products to use as controls in the testing can be obtained from other labs or sources. The best way to avoid chemical contamination is to test all new lots of reagents, media and especially sera, and test the water purity at least yearly using the most sensitive culture assay available.

Strategic Use of a Frozen Cell Repository

A cryogenic cell repository is commonly used in laboratories to reduce the need to carry large numbers of cultures and to provide replacements for cultures lost to contamination or accidents. Freezing cultures also stops biological time for them, preventing them from acquiring the altered characteristics that can normally occur in actively growing cells as a result of environmental or age related changes. However, a cell repository is only a reliable resource if the cultures it contains have been properly tested, labeled, and stored. (Reviewed in reference 36.)

Equally important, a cell repository can also be used strategically to convert continuously carried cultures into a series of short-term cultures, thereby greatly reducing both the amount of quality control testing required and potential problems from cryptic contaminants. When cultures are continuously carried for long periods in the laboratory they should be tested for contaminants at least every three to four months (more often for critical applications). If they are not tested regularly, then when a cryptic contaminant, such as a mycoplasma or another cell line, is finally uncovered, it is impossible to determine how long it has been in the culture and how much research has been invalidated by its presence. In addition, if the contaminant is mycoplasma, it is likely to have spread by then to other cultures. However, regular testing, although very important to ensure the integrity of your cultures, can require considerable effort, especially in laboratories using multiple cell lines. Rather than test cultures several times a year, it is easier to simply discard them every three months replacing them from the repository with cultures from the same lot or batch that have been previously tested to ensure their integrity.

Tested stocks should be set up in the cell repository for each culture that is routinely used in your laboratory. The cultures should be grown for at least two weeks in antibiotic-free media, then thoroughly tested to check their viability, ensure they are free of contamination, and confirm their identity and presence of any important characteristics. Testing should be done both immediately before and after freezing; however, if you don't mind assuming some added risk, testing can be left until after freezing. The freezer stock should always be prepared from pooled cultures and contain enough vials, assuming a consumption rate of five vials per year (or higher based on your experience), to last the planned lifetime of any research projects involving them. A better alternative may be to first develop a seed or master stock (10 to 20 vials is usually sufficient, depending on your envisioned needs), and then from that develop a working stock (approximately 20 vials). When the original working stock is depleted, it is replaced by using a vial from the seed stock to develop a new working stock. Assuming a consumption rate of five vials per year, each working stock will be good for four years, with the seed stock lasting for 40 to 80 years. Hopefully, this will be long enough to finish a research project! This approach reduces the amount of routine testing to practical levels since only newly introduced cultures will require testing. Equally important, discarding cultures after growing them for three months also destroys any undiscovered biological contaminants that may have gained access to the cultures, limiting both their damage to the integrity of the research and their spread to other cultures.

Strategic Use of Antibiotics

When used intelligently, antibiotics are a useful tool in cell culture, but they can be very dangerous when overused or used incorrectly. Experienced cell culture users have recommended for many years that antibiotics never be used routinely in culture media.[3,7,12,17,18,26,27] In a major study, Barile found that 72% of cultures grown continuously in antibiotics were contaminated by mycoplasma, but only 7% grown without antibiotics were contaminated, a ten-fold difference.[37] Similar results are common: workers who routinely and continuously use antibiotics in their media tend to have higher contamination problems, including mycoplasma, than workers who don't. Over reliance on antibiotics leads to poor aseptic technique. It also leads to increased antibiotic resistance among common culture contaminants. In an ongoing study[41] of the antibiotic sensitivity of culture-derived mycoplasmas, 80% were resistant to gentamycin, 98% to erythromycin, and 73% to kanamycin, all commonly used antibiotics widely claimed to be effective against mycoplasmas. Mycoplasmas also showed resistance to the antibiotics recommended and sold specifically for cleaning up mycoplasma infected cultures: 15% were resistant to ciprofloxacin, 28% to lincomycin, and 21% to tylosin.

Why does the routine use of antibiotics lead to higher rates of mycoplasma contamination? Everyone generates and sheds a relatively constant flow of particles, consisting of fibers, aerosols, and droplets, as they work in the laboratory. These particles can have a mixture of bacteria, yeast, fungi, and even mycoplasmas bound to them. If one of these contamination-laden particles enters an antibiotic-free culture, the chances are that at least one of the contaminants will produce a highly visible infection within 24 to 48 hours. As a result the contaminant is quickly detected and the culture discarded. It is very unlikely that particles shed by laboratory personnel would ever consist of just difficult to detect contaminants, such as mycoplasmas, that could enter cultures and not cause visible signs of contamination. However, if the culture contains antibiotics, there is a chance that the antibiotics will prevent the growth of the usually more easily detected contaminants, but allow mycoplasma or other cryptic contaminants to grow undetected. As a result, instead of being discarded, the cryptically infected culture remains in use, is utilized in experiments, and becomes a potential source of serious contamination for the other cultures in the laboratory.

Antibiotics should never be used as a substitute for good aseptic technique; however, they can be used strategically to reduce the loss of critical experiments and cultures. The key is to use them only for short term applications: for the first week or two of primary cultures, during the initial production stages of hybridomas, for experiments in general where the cultures will be terminated in the end. Whatever their use, the antibiotics ultimately chosen should be proven effective, noncytotoxic, and stable.[37]

Curing Contaminated Cultures

Autoclaving is the preferred method for dealing with contaminated cultures — it always works and is guaranteed to keep the infection from spreading to other cultures. However, occasionally contamination will be found in a valuable culture that cannot be replaced and attempts will be made to save it. This is a task that should not be undertaken lightly as it usually entails considerable effort and frequently turns out to be unsuccessful. In addition, cultures can lose important characteristics as a result of the clean up procedure. If the contaminant is a fungus or yeast, success is unlikely since antifungal agents, such as amphotericin B (Fungizone) and

Nystatin, will not kill these organisms, but only prevent their growth. Many bacterial culture contaminants come from human or animal sources and are likely to have developed resistance to most commonly used cell culture antibiotics.

Most clean up attempts, however, are usually made against mycoplasma infected cultures. Treating with antibiotics is the most widely used approach, but as discussed earlier, cell culture mycoplasma strains are often resistant to some of the antibiotics specifically recommended for cleaning up mycoplasma infected cultures. Furthermore, the more attempts made at cleaning up contaminated cultures with these antibiotics the more likely resistant mycoplasma strains will develop. Other approaches, usually combining the use of antibiotics with specific antisera or other chemical treatments, can be used as well. (Reviewed in references 11, 16, and 37.) However, none of these methods are 100% successful and clean up should only be tried as a last resort. A word of caution: often these treatments reduce the level of contamination below that which can be detected by indirect methods such as DNA staining or PCR. As a result, clean up attempts often appear successful for the first month or more following treatment because the low level of surviving mycoplasmas can escape detection. But eventually the few remaining undetected mycoplasmas recover leading to more serious problems. There are several commercially available mycoplasma clean up services for contaminated cultures, it is relatively expensive but usually successful.[17]

Table 2. Contamination Survey Results[a]

A. Do you consider microbial contamination (bacteria, yeast, fungi, mycoplasmas) of your cultures to currently be a problem?	
50%	Yes, minor
8%	Yes, serious
33%	No
9%	Not sure
B. How often is it a problem?	
67%	1–5 times/year
20%	6–10 times/year
12%	More than 10 times/year
C. Have you ever encountered mycoplasma contamination in any of your cultures?	
9%	Yes, once
14%	Yes, several times
33%	Never
44%	Maybe, not sure
D. Do you currently test your cultures for mycoplasma?	
50%	No
32%	Yes, occasionally
18%	Yes, an average of 4 times/year
E. Do you use antibiotics in your culture medium?	
65%	Yes, usually
7%	Yes, short-term only
17%	Occasionally
11%	Never

[a]Combined summary of three surveys (130 respondents) conducted at Corning seminars in Baltimore, Boston and St. Louis in 1990.

A Final Warning

In the United States alone, losses due to cell culture contamination, especially by mycoplasma, cost cell culture users millions of dollars annually; this is money that could otherwise be used for additional research. Unfortunately this serious problem does not appear to be getting any better. As shown by the survey results in **Table 2**, contamination is a problem for most cell culture workers. At least 23% of respondents have experienced mycoplasma contamination of their cultures, but an additional 44% suspected mycoplasma contamination but were not sure. The reason for their uncertainty is clarified by the response to the next question: 50% of all respondents do not test for mycoplasma, as a result they are unaware of the status of their cultures. The answer to the last question points out one important reason for widespread contamination problems — the over use of antibiotics. With 65% of respondents using antibiotics on a regular basis, the continued frequent occurrence of cryptic contaminants, especially mycoplasmas, is likely.

Because of the very serious nature of mycoplasma contamination and its widespread distribution, it is important to summarize the major sources of mycoplasma contamination and review the basic steps for preventing it from happening in your laboratory. Currently, the number one source of mycoplasma contamination is other infected cell lines; it is essential to quarantine all cultures brought into the laboratory until they have been screened for mycoplasma contamination, and to use only tested cultures in research. The second common source is the cell culturist; good aseptic technique combined with the strategic use of a tested cell repository and limited use of antibiotics will greatly reduce the opportunities for contamination via this route. The last important source of mycoplasma is sera and other biologicals that are sterilized by filtration; buy only from sources that have a good reputation and that use currently acceptable filtration (0.1 µm or smaller) and testing procedures.

Cell culture contamination will never be totally eliminated, but through a better understanding of the nature of contamination and the implementation of some basic concepts it can be better controlled and its damage greatly reduced. The information in this bulletin has been compiled to provide you with the foundation upon which you can build a contamination management program designed to fit your own needs. For additional assistance in these areas, please visit **corning.com/lifesciences**, or call Corning Incorporated Technical Information Center at 1.800.492.1110. International customers please call 978.635.2200.

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Endotoxin in Cell Culture

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Introduction

Contamination of cell cultures has long been a serious problem for researchers as well as for manufacturers producing cell-based parenteral (for injection) drug products. In the past, most efforts for avoiding contamination-induced culture losses have focused on biological contaminants: bacteria, mycoplasmas, yeasts, fungi, and even other cell lines.[19] However, for companies producing cell culture-based products, such as vaccines and injectable drugs, endotoxin, a chemical contaminant produced by some bacteria, has also been a major concern. The presence of endotoxin in products for injection can result in pyrogenic responses ranging from fever and chills, to irreversible and fatal septic shock. There is increasing evidence that endotoxins can also create a variety of problems for researchers using cell cultures. Our goals are to review some of the current information on endotoxin: its characteristics, potential sources, and especially its effects on cultured cells; and to offer some suggestions on avoiding endotoxin-induced culture problems.

What is Endotoxin?

Endotoxin is a complex lipopolysaccharide (LPS) which is a major component of the outer membrane of most Gram-negative bacteria. A single *Escherichia coli* contains about 2 million LPS molecules per cell. Bacteria shed endotoxin into their environment in small amounts when they are actively growing, and in large amounts when they die. LPS consists of a very hydrophobic lipid group (lipid A) covalently bound to a long complex polysaccharide tail. Lipid A usually consists of two phosphorylated sugars (glucosamines) which are each coupled with several fatty acids. Lipid A anchors LPS to the bacterial membrane and is responsible for most of its biological effects.

The long hydrophilic polysaccharide tail consists of two domains: a conserved core consisting of a small group of sugars and a much larger and more variable O-specific polysaccharide region (O-antigen). The core contains heptoses (7 carbon sugars) plus two units of an eight carbon sugar (Kdo: 3-deoxy-D-manno-octulosonic acid) unique to Gram-negative bacteria that links lipid A to the O-antigen. This region varies both within species and between species, consisting of 20 to 40 repeating units of three to eight sugars each. The O-antigen is responsible for the specific antigenic response in humans that provides immunity to Gram-negative infections. (Reviewed in 12, 13, 16, and 24). Endotoxins are amphipathic molecules characterized by a net negative charge in solution, high heat stability, and a tendency to form very large aggregates (1,000,000 Da or more depending on pH, salt concentration, surfactants, etc.) in aqueous solution. Because of their hydrophobicity, they tend to have strong affinities for other hydrophobic materials, especially some plastic products used in the laboratory.[18,24]

Detection and Measurement

The first method for detecting endotoxin contamination was the rabbit pyrogen test developed in the 1940s for screening water and solutions used when injecting humans. This test is based on the ability of endotoxin to cause fever (an endotoxin-induced pyrogenic response) when injected into rabbits. Although very successful in reducing pyrogenic episodes, the rabbit test is expensive, time consuming, and not very quantitative. In the 1970s, an *in vitro* assay was developed based on observations that the lysate from horseshoe crab *(Limulus polyphemus)* amebocytes would clot in the presence of very low levels of endotoxin. This Limulus amebocyte lysate (LAL) gel clot assay is very sensitive, detecting down to 0.03 Endotoxin Units (EU)/mL. One EU equals approximately 0.1 to 0.2 ng endotoxin/mL of solution depending on the reference standard used. Endotoxin Units, rather than units of weight, were developed by the U.S. Food and Drug Administration (FDA) for testing comparisons because the potency of an endotoxin for causing pyrogenic effects depends on a variety of factors: polysaccharide chain length, aggregation, solubility in biological fluids, bacterial source, associated substances, etc.[4] In addition to the gel clot assay, more sensitive LAL kinetic turbidimetric or chromogenic assays have now been developed that can detect down to 0.001 EU/mL.

Sources of Endotoxin in Cell Culture Water

High purity water is essential in any cell culture laboratory, not only for preparing media and solutions, but also for glassware washing. Traditional glass distillation is very effective in removing endotoxin if the equipment is maintained and used properly. Because of the endotoxin's size, reverse osmosis is also very effective. Both methods are recognized by the U.S. Pharmacopeia (USP) for preparing sterile water for human injection.[24,26] Water prepared by passing it through ion exchange resin and activated carbon columns coupled with a final ultrafiltration treatment may also be satisfactory.

Poorly maintained water systems, especially systems using ion exchange resins, can harbor significant levels of endotoxin-producing bacteria and should be tested if endotoxin problems are suspected or discovered in cultures. Equally important are the storage conditions used for the water after it has been purified. Bacteria are often found growing in glass or plastic water storage containers and associated tubing, and can quickly raise endotoxin levels in the stored water to unacceptable levels. When in doubt about the quality of the water from a purification system, a simple LAL assay should be done to check endotoxin levels. If the water is found to be a source of endotoxin and the problem cannot be fixed, nonpyrogenic water for injection (WFI) can be purchased and used for preparing media and other critical solutions.

Sera

In the past, sera, especially fetal bovine sera (FBS), have been a major source of endotoxin in cell culture. But as improved endotoxin tests (LAL assay) led to an increased awareness of the endotoxin levels in sera, manufacturers were able to significantly reduce these levels by handling the raw products under more aseptic conditions. In the early 1980s, Case Gould[2] found endotoxin levels as low as 0.006 ng/mL and as high as 800 ng/mL when screening FBS from ten sera manufacturers. Out of a total of 111 lots screened, 86 (77%) had less than 1 ng/mL of endotoxin. Most sera manufacturers currently offer premium or high quality, low endotoxin FBS (less than 1 ng/ml); this is usually more expensive than their standard quality FBS and may not be necessary for many cell lines.

Media and Additives

Currently, most commercially prepared media are tested for endotoxin and certified to contain less than 0.1 ng/mL of endotoxin. For laboratory prepared media, the endotoxin level will be determined primarily by the endotoxin level of the water used to dissolve the other components. However, reagents added to the medium after filtration, even though sterile, can also be a source of endotoxin. In a test of five different batches of commercially prepared bovine serum albumin (5 mg/mL), Dumoulin et al.[5] found endotoxin levels ranged from a low of 0.1 ng/mL to a high of 12 ng/mL. Case Gould reported in her survey of media additives[2] that erythropoietin had endotoxin levels ranging from 80 to 2,000 ng/mL; some 1 M amino acid solutions had endotoxin levels as high as 50 ng/mL. These amino acids may have been produced by bacterial fermentation. Whenever possible, ask manufacturers to certify the endotoxin levels of their products; when in doubt test the media endotoxin levels both before and after adding any new components.

Potential Sources of Endotoxin in Cell Culture:

- Water used for glassware washing or making media and solutions
- Commercially obtained media and sera
- Media components and additives
- Laboratory glassware and plasticware
- Glassware

Endotoxins can adhere strongly to laboratory glassware and may be difficult to remove completely during washing. While standard laboratory autoclaving procedures will sterilize glassware, they have little if any effect on endotoxin levels due to endotoxin's high heat stability. Subjecting glassware to 250 °C for more than 30 minutes or 180 °C for three hours is recommended to destroy any contaminating endotoxin.[24] This has the added benefit of sterilizing the glassware.

Plasticware

The high temperatures used to melt plastic resins during the molding of plastic laboratory products will usually destroy any contaminating endotoxins. However, significant levels of both biological and chemical contamination can occur after molding during the routine handling associated with assembly and packaging. Unfortunately, while the sterilization process (electron beam or gamma irradiation) will destroy microbial contaminants, endotoxin will be left largely intact. Roslansky et al.[18] showed that endotoxin levels in 50 mL sterile polypropylene centrifuge tubes varied widely among four manufacturers, ranging from a low of 0.007 EU/tube to a high of 15.0 EU/tube. To eliminate this potential problem Corning Incorporated, Life Sciences, certifies that all of their cell culture flasks, dishes, and plates; centrifuge tubes; filters; storage bottles and serological pipets are nonpyrogenic, having less than 0.5 EU/mL is shown using an LAL kinetic chromogenic assay.

Endotoxin Effects on *In Vitro* Cell Growth and Function

In 1984, Case Gould published a review on the effects of endotoxins on cell cultures.[2] Among the effects documented were the stimulation of leukocyte cultures to produce tissue factors, the activation of murine macrophages, and the inhibition of murine erythroid colony formation by very low levels (less than 1 ng/mL) of endotoxin. Since then, many more papers have been published that have reported on the effects of endotoxins on *in vitro* cell functions and growth. While a comprehensive literature survey is beyond the scope of this bulletin, the following examples will give an overview of some of the varied and significant effects endotoxins can have on cells in culture.

Macrophages and mononuclear phagocytes are known to produce and release a variety of cytokines, including tumor necrosis factor (TNF) and interleukins, in response to endotoxin stimulation both *in vivo* and *in vitro*. These cytokines mediate the harmful effects of the endotoxins *in vivo* leading to endotoxemia, which can result in septic shock and multiple system organ failure (Reviewed in 12). Morris et al.[11] found that endotoxin levels as low as 0.5 ng/mL could significantly increase the production of interleukin-6 in equine peritoneal macrophages after only a six hour exposure. Mattern et al.[9] showed that 100 ng/mL endotoxin could stimulate the proliferation of human T cells and their production of lymphokines, but this stimulation required direct physical contact with previously endotoxin exposed monocytes. Sibley et al.[20] reported that 10 to 200 ng/mL endotoxin stimulated a murine B cell tumor cell line (70Z/3) to complete the production of IgM which was then expressed on the cell surface.

Endotoxin has been shown *in vivo* to impair blood vessel vasoconstrictor responses that can eventually lead to circulatory collapse. It has also been shown to have a variety of *in vitro* effects on blood vessel derived cultures. Organ cultures of rat aortic rings increased their release of TNF and IL-1 and exhibited impaired contractility after exposure to as little as 1 ng/mL endotoxin.[10] This showed endotoxin affected both the outer endothelial cell layer (cytokine release) as well as the inner smooth muscle layer (impaired contractility) in these aortic rings. Colburn et al.[3] reported that endotoxin (10 ng/mL) increased the intracellular production of heparan sulfate proteoglycan in a rabbit aortic endothelial cell line, while its extracellular level in the matrix was depleted. Tao and McKenna[23] demonstrated that 10 ng/mL endotoxin could induce contractile dysfunction in cultured rat heart myocytes by increasing nitric oxide synthase activity. Porcine aortic endothelial cells were shown by Buchman et al.[1] to undergo apoptosis (programmed cell death) as the result of exposure to 25 ng/mL endotoxin coupled with exposure to a nonlethal heat shock or sodium arsenite treatment. Sugiura et al.[22] showed that higher levels of endotoxin (100 ng/mL) stimulated the release of endothelin, a very powerful vasoconstrictor, from transformed bovine aortic endothelial cultures. Clearly these papers demonstrate that blood vessel derived cells are very sensitive to endotoxins *in vitro*.

What about endotoxin effects on nonblood-derived cells? Wille et al.[25] showed that very high levels of endotoxin (5,000 to 25,000 ng/mL) — endotoxin levels far higher than would be expected under worse case conditions in culture — could alter the cloning efficiency of human urethral epithelial cells in serum-free medium. These endotoxins were from a variety of bacteria: some raised the cloning efficiency, some lowered it, and others had no observed effect. This suggests that the highly diverse polysaccharide component of endotoxin may contribute in some way to its effects. Epstein et al.[6] reported that endotoxin levels as high as 20 ng/mL had no detectable effects on the growth of seven cell lines, including the widely used WI-38, 3T3, and CHO cell lines. They did, however, find that 10 to 20 ng/mL endotoxin altered the production of a recombinant protein by CHO cells.

Other interesting endotoxin effects on cell growth are described in papers[7,21] from two different *in vitro* fertilization clinics that reported endotoxin levels of 0.1 to 1 ng/mL reduced the rate of successful pregnancies. In one case,[21] the source of the endotoxin was traced to ultrapure water that had been stored for a short time in a plastic container and then polished in a carbon cartridge, both of which contained endotoxin. However, another laboratory[5] reported that endotoxin levels as high as 500 µg/mL had no effect on human sperm viability and it took 50 µg/mL endotoxin to affect fertilization and subsequent *in vitro* development of mouse embryos.

Based on the above evidence, it is clear that endotoxins do not affect all cultured cells equally. Some cell cultures, perhaps lacking appropriate endotoxin receptors, may only be sensitive to very high levels of endotoxin — levels not likely to be found in cell culture systems by accident. Cell lines that have been grown in culture for many years (CHO, 3T3, WI-38, HeLa, etc.) may have been naturally selected for resistance to endotoxin by long-term, inadvertent exposure to high levels of endotoxins that could often be found in media, sera, and culture additives before endotoxin testing became widely used.

Possible Mechanisms for These Effects

Much is still unknown about the mechanisms through which endotoxins interact with cell cultures (Reviewed in 12, 13, 14, and 15). A 60 kDa glycoprotein, LPS Binding Protein (LBP), has been found in serum that binds LPS through its lipid A domain and greatly enhances its ability to interact with cells of the immune system. This enhancement appears to require a specific 55 kDa glycoprotein receptor, CD14, on the cell surface. This membrane anchored receptor is found on a variety of immune cells and, when bound with the LPS-LBP, starts a chain of events resulting in the cytokine mRNA production and the subsequent release of a variety of cytokines by the cell.

Exactly how the intracellular signaling occurs is not fully understood. A paper by Han et al.[8] showed that binding of LPS to CD14 triggered a tyrosine kinase phosphorylation cascade involving a protein, p38, that has been shown to be a MAP kinase. Perhaps CD14 transfers the LPS-LBP complex to a yet undiscovered transmembrane receptor to effect the internal cell signaling.

LPS may also interact with cells by other means, since some cells lacking CD14 receptors have been shown to be affected by LPS. LPS may enter the cell by unknown receptors, endocytosis, or by directly entering the cell membrane.[15] Risco et al.[17] demonstrated that LPS could bind to cytoskeleton microtubules, and at high concentrations, inhibit microtubule polymerization. This binding could explain one possible mechanism for some of the cytotoxic effects of LPS that do not appear to involve cytokine activation.

Conclusions

While endotoxin may not be a problem for all cell culture users, it clearly is for some. Endotoxin contamination of cell-based products is a major concern to the pharmaceutical industry, since endotoxin in vaccines and other cell-based parenteral drug products render them unfit for use. Endotoxin has also been shown in many cell culture experiments to affect cell growth and function as well as being a source of significant variability.[2] This is especially true when using cells known to be sensitive to the low endotoxin levels that are commonly found in cell culture systems.

Therefore, unless you are sure endotoxin has no effect on your cultures and is not a potential source of variability in your experiments, you should reduce the possibility of endotoxin-related problems by taking some basic precautionary steps. The first step is to use cell culture media, sera, and plasticware that are certified either by their manufacturers or through in-house testing to be nonpyrogenic. The second step is to test the source of the water used for making solutions and for washing glassware and plasticware to ensure the water is not a source of endotoxin (Reviewed in 4). As an extra precaution, the culture medium can be tested for endotoxin after any additional components have been added.

For additional questions please call Sigma-Aldrich Technical Service or visit the Corning Life Sciences Web site at corning.com/lifesciences.

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Cell Culture Troubleshooting

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Introduction

Although very popular among life science researchers, cell culture can be a very difficult tool to work with in the laboratory. Unlike other common laboratory tools such as electrophoresis or chromatography, cell culture makes dynamic, ongoing use of living organisms. These living cultures often respond to our mistakes not only by the erratic behavior common to other laboratory tools, but by dying — the total, irreplaceable destruction of the tool itself.

As one of the major suppliers of vessels used in cell culture, Corning Life Sciences often receives calls from anxious customers who are experiencing growth or attachment problems with their cell lines. Usually the customer, searching for a cause (and solution) to their problems, suspects that a change or mistake in the manufacturing process is responsible, or that perhaps the special surface treatment process used on most cell culture vessels was not properly done. Culture media and sera producers also receive similar calls from customers trying to determine who is responsible for the erratic behavior or loss of their cultures.

Because of the complex nature of cell culture, identifying the underlying causes of culture behavior problems is often a difficult, time-consuming task. Erratic culture behavior can take many forms; unusual growth patterns or inconsistent, spotty, and uneven cell attachment are the most common problems. Gradual or abrupt changes in growth rate or unexplainable experimental results are also experienced occasionally. In cell culture, any sudden change is suspect and a potential problem and, therefore, to be avoided.

Corning Life Sciences has spent many years helping customers deal with these problems. By using some of the information gained from these customer contacts, Corning has produced this guide to help identify and solve some of the common and uncommon causes of cell culture problems. The focus will be limited to three common problem areas: technique, incubators, and media. In addition to the information provided in this guide, it is strongly suggested that you refer to the articles listed in the references or on the Corning Life Sciences Web site (**corning.com/lifesciences**) for additional help and recommendations.

Surface Treatment Process

The initial and most common suspects for cell culture problems are usually either the culture vessels or the medium being used. Problems associated with culture medium will be dealt with in a later section. Much of the suspicion surrounding plastic cell culture vessels is due to the mystery or lack of understanding concerning the special treatment process used to modify the surface of the plastic. Virgin polystyrene, the resin used to manufacture most cell culture vessels, is hydrophobic in its untreated state. Protein attachment factors do not bind well to this natural surface resulting in poor cell adhesion and growth. Because of this, either a corona discharge or plasma treatment is used under carefully controlled conditions during the manufacturing process to insert oxygen atoms (in the form of carboxyl groups) into the backbone of the polystyrene chain (Ramsey et al, 1984; Amstein and Hartman, 1975; Hudis, 1974). This alteration of the plastic polymer (not a peelable coating) results in a hydrophilic surface with a net negative charge that creates a surface suitable for cell attachment and growth. The culture vessels are then sterilized and thoroughly evaluated by quality control tests to insure they received the proper degree of treatment.

Since this modified surface is not visibly different from the untreated surface, there is no easy way, short of growing cells, for customers to check the adequacy of the treatment process. As a result, many customers assume cell attachment and growth problems are caused by mistakes made during manufacturing. It is very important that the plastic culture vessel's status as the suspected cause of the problem be resolved as quickly as possible so the real cause can be identified and eliminated. Usually, the first step is comparing the performance of the suspected vessel against the same product from a different production lot, or against similar products from another manufacturer. If a difference is found or the results are not clear, then it is time to contact the product manufacturer for assistance. Once the vessel is eliminated as the problem's cause, the customer can then continue to look elsewhere for a solution. The following examples will help identify some common problems and causes often incorrectly associated with poor surface treatment of plastic vessels and, where possible, will offer some solutions that can be used to eliminate them.

Problems Related to Technique

The greatest opportunity for cell culture problems occurs during the day-to-day activities of feeding and maintaining the cultures. Culture loss due to contamination is one of the most frequent and more serious of these problems. However, many other problems, while less serious and not as noticeable as contamination, still adversely affect the cultures and experiments. Often, the first sign that something is wrong occurs when the cells are microscopically examined and uneven or unusual patterns of cell attachment or growth are observed. Identifying the problem's cause is the first and usually most difficult step, followed by finding the right solution. Sometimes transient growth problems occur and then disappear without ever identifying a cause. Please note that many of these growth problems are not readily observed during routine microscopic observation of live cultures. The occurrence and extent of these problems are best observed when sample cultures are first fixed (2.5% glutaraldehyde or 70% ethanol) and stained (1% crystal violet stain) prior to observation.

Growth Problems in Flasks, Dishes, and Plates

Spotting

Clear areas, often resembling single or small clusters of viral plaques, occur along the sides or in the middle of the vessels. This usually results when the initial cell inoculum contains foam or bubbles. Although they appear to float on top of the medium, bubbles also push below the surface, displacing the cell-containing medium and reducing the cells available for attaching. Should the bottom of the bubble contact the bottom of the vessel, it will prevent cells from attaching in that area. Bubbles only need to stay intact for a short period to have this effect. Bubbles that occur during refeeding of cultures, but after cells have attached, may cut off the cells from the fresh medium. This will result in cell necrosis under those areas, if the bubbles last long enough. Bubbles can usually be avoided by careful attention to mixing and pipetting techniques.

Uneven Growth

Inadequate mixing of cell inoculum with medium during addition to vessels, especially dishes, can result in uneven distribution, attachment and growth of cells. Experience or simple experimentation will generally result in effective ways of mixing medium thoroughly without creating bubbles or foam.

Uneven growth can also occur as a result of the shear forces generated by medium sweeping across cell monolayers during medium changes or while moving cultures between the laminar flow hood and the incubator (Tchao, 1996). This effect is often more pronounced in serum-free cultures.

Static Electricity

Static electrical charges that build up on plastic vessels can also adversely affect cell attachment. This problem occurs more frequently when the relative humidity is very low during the winter (or year round in some laboratory locations). Wiping the outside of vessels with a clean damp towel, increasing the room humidity, or using commercially available antistatic devices may eliminate or reduce this problem. Extra care should be used to avoid rubbing the vessels against the packaging when opening them (especially roller bottles) as this can increase the static charge.

Meniscus Rings

When too small a volume of cell inoculum is used, a pattern of heavier growth will appear along the sides of flasks or as a ring or halo in dishes. (This halo effect is often more pronounced in multiple well plates.) This pattern occurs because the meniscus along the sides of the vessel is deeper and contains more cell inoculum and medium per unit surface area than does the thinner film of inoculum toward the center of the vessel. A similar effect is observed when too little medium is used in refeeding cultures. As a rule of thumb, 0.2 to 0.3 milliliters of medium should be used for every square centimeter of growth surface.

Mycoplasma

Although the problem of cell culture contamination is beyond the scope of this guide, it is important to draw attention to another potential and widespread source of mysterious cell attachment and growth problems. For more detailed information on the problem of cell culture contamination, refer to Lincoln and Gabridge, 1998; Rottem and Barile, 1993; McGarrity, 1982; McGarrity, 1976. Due to the very high densities they can achieve in cell culture (up to 10^8/ml), mycoplasmas (unlike other contaminants such as bacteria and fungi) cause serious adverse effects on cell cultures without clouding the medium or being observed under the microscope. Mycoplasmas often grow attached to the cell membrane; as a result, a single cell may have several hundred mycoplasma on its membrane which greatly affects the ability to attach and grow. An ongoing mycoplasma screening program is an essential requirement for all cell culture labs working with cell lines (Lincoln and Gabridge, 1998; Masover and Buck, 1983; McGarrity et al, 1985; McGarrity, 1982). Without such a program, mycoplasma contamination, along with the associated problems, is likely to occur at some point.

Table 1. Recommended Medium Volumes for Corning® Dishes and Flasks*

Corning Plastic Culture Flasks (area)	Recommended Medium Volumes
25 cm^2	5–7.5 mL
75 cm^2	15–22.5 mL
150 cm^2	30–45 mL
162 cm^2	32–48 mL
175 cm^2	35–52.5 mL
225 cm^2	45–67.5 mL
35 mm	1.6–2.4 mL
60 mm	4.2–6.3 mL
100 mm	11–16.5 mL
150 mm	30–45 mL
245 mm (square)	100–150 mL

*Based on using 0.2 to 0.3 mL medium per cm^2 of growth area.

Growth Problems in Roller Bottles

The constant movement of the medium across the surface of the bottle, as slow as it appears, can make it more difficult for cells to attach and grow in roller bottles compared to stationary vessels such as flasks and dishes. The constant motion of the medium can also lead to a more stressful cell environment than is found in stationary culture systems. Consequently, any technique-related issues that reduce the attachment ability of cells is magnified and clearly stands out (Freshney, 1994). Please note than many of these growth problems are not readily observed during routine microscopic observation of live cultures. The occurrence and extent of these problems are best observed when sample cultures are fixed and stained prior to analysis.

Uneven Cell Attachment and Clumping

One of the most frequently encountered problems using roller bottles is difficulty getting the cells to attach and form an even monolayer in the bottle. Rotating bottles at inappropriate speeds is a common cause of attachment problems. If the bottles are rotated too quickly for cells to easily attach, areas of heavy cell growth often appear as circular bands towards both ends of the bottles. This is because the medium flow is slightly slower at the ends than in the middle of the bottles. Rotating bottles too fast may also result in large clumps of cells. This results from the tendency of cells to form clumps since they find it easier to adhere to each other than to the surface of the roller bottle.

Eventually these clumps become large enough to attach to the bottle surface. A recommended starting speed for initiating roller bottle cultures is 0.5 to 1.0 revolutions per minute (rpm) to start. However, if cells have difficulty attaching, slower speeds (0.1 to 0.4 rpm) should be used until the cells are attached.

Cell damage during subculturing, or incomplete inactivation or removal of dissociating enzymes can also make it more difficult for cells to attach and may result in banding or clumping. The protein-based cell receptors used to initiate cell attachment become damaged by the dissociating procedures and must be replaced before the cells can reattach. Poorly regulated incubator temperatures (temperatures that are too high or too low) will also make it more difficult for cells to evenly attach to roller bottles. If the bottles are initially rotated too slowly, or if they slip or stop turning even for

a short time during the initial cell attachment period, uneven longitudinal bands of cell growth may appear. Cleaning the rollers on the roller apparatus should alleviate slipping bottles. If necessary, rubber bands can be placed around the ends of the bottles to improve traction.

Bands of heavy growth at just one end of the bottle are often the result of the roller apparatus not being level, causing an increased amount of medium and cells at the end of the bottle that is lower. Furthermore, the longer it takes the cells to attach, the more time there is for them to gradually roll down the side of the bottle to the lower end before attaching. Standing a bottle on end for too long after initially seeding it with cells can have a similar effect.

Clear Bands

Occasionally, clear circular bands will occur on roller bottles where the cells appear to have been swept away. While small pieces of rolling debris or large cell clumps may cause this to occur, one of the most common causes is the short-term presence of bubbles in the initial cell inoculum. These bubbles, when in contact with the sides of the slowly rotating bottle, can act as miniature plows, scraping off the cells as they begin to attach. Avoid bubble formation by carefully pouring medium down the sides of the bottles, or by pipetting it directly into the bottom of the bottles. Cell suspensions used for inoculating roller bottles should be carefully prepared to ensure they are bubble-free.

Streaking

Condensation (essentially pure water) falling onto exposed cells can cause some unusual patterns and events. This problem usually occurs in roller bottles that have been removed from an incubator and are standing upright at cooler room temperatures awaiting processing. Due to temperature differences, water vapor will condense on the inside of the cap. The resulting droplets may then coalesce and run down the sides of the bottle across the cells that are now only covered by a very thin film of medium. These cells will then undergo a strong osmotic shock. If they have formed a confluent monolayer, they may tear or pull apart from each other along the path the water takes, creating a visible dagger-like streak. Cells that have not reached confluency may round up and float off into the medium, leaving behind a long clear streak devoid of cells.

Peeling

Heavily confluent cell monolayers (especially fibroblasts) will occasionally start to peel away from the surface of the roller bottle. This also occurs in flasks, dishes, and microplates. This results, not from surface treatment failure, but from the formation of a flexible sheet of tightly interconnected cells and cell-manufactured extracellular matrix. Over time, mechanical stresses can develop in the cell sheet from cellular movements and contractions that may then cause the cell sheet to tear or pull away from the roller bottle. Physical damage from pipetting directly onto the cell sheet, tearing it with the end of the pipette, or other manipulations to the cell sheet may also initiate cell sheet peeling.

Problems Related to Incubators

Cells spend nearly all of their existence in incubators, yet these units may not always provide the stable, consistent environment cells require. Besides the obvious function of maintaining temperature, incubators used in open culture systems also control humidity, the gaseous environment around the cells, and indirectly, the pH of the culture medium. Ideally these parameters should be constant and not a source of experimental variation. Unfortunately, variation does occur and can be a major problem if not recognized and eliminated (Freshney, 1987).

Temperature

Temperature differences within the incubator, even though small, can create problems even when the differences are only a few tenths of a degree. Constant opening and closing of poorly insulated incubator doors can result in significant temperature reductions, usually localized toward the front of the incubator. Often this effect is first noticed when heavy condensation forms on vessels located near the front of the incubator. As a result of slightly cooler temperatures, these vessels may have considerably slower growth rates than their neighbors to the rear as well as being more prone to fungal contaminants from the condensate. One solution is to set aside a separate incubator, with reduced traffic in and out, for all critical experiments, thus minimizing temperature fluctuations. Where this is not practical, those areas within the incubator that have the least temperature fluctuation (usually towards the rear) should be utilized for critical work.

Usually temperature effects are difficult to visualize and are less likely to be recognized. Cells will display a distinctive growth pattern in the monolayer formation. Areas of a plate where high temperature fluctuates greatly will have few, if any, cells present. Examples of this pattern can be observed in situations where incubators are frequently accessed resulting in a lower temperature across areas of the plate most exposed. This effect is most pronounced during the first few hours following inoculation.

Conversely, there are also characteristic growth patterns with temperature increases in environments where temperature is above optimal. Cell growth will be sparse or absent in areas where the surface temperature and surrounding medium are too high. This uneven growth due to temperature increase may be noted when temperatures are increased by less than one half of a degree.

Stacking vessels together can also result in vessel-to-vessel differences in temperature and growth rate. The vessel on the bottom of the stack, which is in contact with the metal shelf, warms up fastest when initially placed in the incubator. The vessel on the top is likely to cool faster, while a vessel in the middle is more insulated from any temperature fluctuations. It is very important to consider these positional effects when designing experiments where growth rates will be evaluated. Using spacers or empty "dummy" vessels to avoid direct contact with perforated shelves, and not stacking vessels, although more difficult, may help avoid these problems.

Evaporation

Evaporation induced changes in the osmolality of the culture medium can affect both the cell growth rate and, occasionally, the patterns of growth. Evaporation effects are easily observed in 96 well plates where the outer peripheral wells (especially the four corner wells) often show a marked reduction in media volume over time. While tighter fitting lids can reduce this effect, it cannot be eliminated. Visible evaporation effects in other types of culture vessels, although present, are much harder to detect.

Evaporation losses depend on the type of culture vessel used (flasks, dishes, multiple well plates, etc.), their location in the incubator, and frequency of entry into the incubator. The humidification system, positioning of circulating fans, amount of insulation, and general airflow patterns will all help determine local evaporation levels. Evaporation can be minimized by keeping water reservoirs full and humidifying incoming gases (usually carbon dioxide) using a gas washing cylinder (**Corning Cat. No. 31770-500EC** for example) filled with purified water in-line with any gases being fed into the incubator. The following method can be used to create a 'map' for an incubator showing both the expected evaporation levels and those areas to be avoided for experiments requiring long term incubation.

Procedure for determining evaporation levels in different sections of an incubator:

1. Determine the total number of dishes that will be needed. How many shelves and dishes per shelf will be tested? At least 3 shelves (top, middle, and bottom) with 9 to 16 dishes per shelf (in a 3 × 3 or 4 × 4 matrix) are recommended for each test.
2. Consecutively number the bottoms of all dishes to be used. Then accurately pipette the appropriate amount of medium (4 to 5 mL for a 60 mm dish) into each dish, and record the weight (without the cover in place). Water can be substituted for medium without affecting accuracy, thus eliminating some expense and the problem of contamination. Cover the dish and place in the appropriate position in the incubator. Make sure that the position of each dish is recorded so that the map can later be accurately constructed.
3. Incubate for the desired time period. This is usually the length of your average experiment. It is preferable during this period to continue normal use of the incubator to better reflect actual expected evaporation levels. If not, normal use should be simulated by periodically opening the incubator.
4. Remove the dishes in small groups from the incubator and quickly weigh each without the covers in place. Any cover condensation represents water lost from the medium and should never be counted in the weighing process. The difference between the initial dish plus medium or water weight (in grams) and final weight, after evaporation losses, for each dish divided by the volume of water in milliliters initially added (× 100) will very closely approximate the total evaporation loss for the dish expressed as a percent.

$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Volume of water added (in mL)}} \times 100 = \text{\% Evaporation (in grams)}$$

5. Construct the map showing evaporation levels on the different incubator shelves. Any 'hot spots' will clearly stand out; their cause can then be determined and corrected or the area can be avoided in the future. Evaporation levels higher than 10 to 15% may have adverse effects on cultures. Wide differences in evaporation levels in different locations within the incubator will cause considerable variation in experimental results as well.

Vibration

Incubator vibration is responsible for some of the most bizarre growth patterns that occur in culture vessels. Its effect primarily occurs on cells when they are trying to initially attach to the surface following inoculation of the vessel. In dishes, vibration will push cells to the edges or middle of the vessel or will sometimes form concentric rings of cells.

Finding the source of the vibration is difficult. It may be as simple as a loose fan motor within the incubator, but is far more likely to be caused by a more remote source. Heavy foot traffic, air handling units, compressors, laminar flow hoods, centrifuges, refrigerators and other motorized appliances should be kept as far from incubators as possible.

Incubators should be placed on heavy, sturdy tables or benches that are not shared with any vibrating equipment. Positioning them on a floor directly over major structural supports will reduce the effects of natural building vibration. Spaces along well-supported outside walls, if located away from heavy traffic areas both inside and out, will often have less vibration than the central spaces. If the incubators are not fully supported and carefully leveled, then vibration effects can be enhanced. Setting up experiments at the end of the day may help eliminate some of the vibration caused by people-associated activities.

Problems Related to Culture Media

As mentioned earlier, both culture medium and culture vessels are prime suspects whenever mysterious cell growth or attachment problems occur without obvious causes. Unless heavily contaminated, good culture medium is not visibly different in appearance from defective culture medium. The only good way to determine medium quality is to attempt to grow cells with it; this is the basic quality control procedure used by most media manufacturers and the only good method for homemade media as well.

Cell cultures respond to deficient or toxic media in different ways depending upon both the nature and the degree of the problem. These responses can range from minor changes in growth rate or cell attachment to the total destruction of the culture. Determining if the medium is responsible for a problem is relatively easy; simply test the suspected batch against a sample proven effective. Determining why the medium is defective is extremely difficult due to the numerous reagents and complex steps involved. Therefore, time and energy are much better spent preventing media problems than trying to find and fix them later; management by prevention is the key to successful media production. The following sections will discuss some of the common and uncommon problem areas for making and using culture media (Jakoby and Pastan, 1979).

Formulation Errors

The first step is deciding which medium is best for your applications. Usually recommendations from scientific literature or colleagues are good starting points. Unfortunately, there is much confusion in this area from the many formulations using the same or similar acronyms. The current catalog of one major media manufacturer offers 17 different formulations of 1× liquid media collectively called MEM for Eagle's Minimal Essential Medium. This does not include six formulations for α-MEM. The same catalog lists 22 additional liquid media formulations for Dulbecco's Modified Eagle Medium (DMEM) that have very different formulations from Eagle's MEM. It should be no surprise that confusion and mistakes are frequently made when selecting or ordering media.

Formulations printed in scientific journals also contain occasional errors or intentional changes that add to the confusion (Burke and Croxall, 1983). Special care and effort must be taken to ensure that the medium you make or buy is what was recommended and that you know its contents.

When making media from scratch, the formulations used should be carefully checked with at least two reputable sources. The most obvious media problems arise from mistakes made during media preparation. A master formulation sheet and preparation protocol, including any special instructions or precautions, should be prepared for all media and solutions, and then used by everyone in the lab. Preparation log sheets listing all reagents used, their lot numbers, weight or volumes (both desired and delivered), pH, date, preparers, and storage conditions, are essential to insure consistency of product, reduce the potential for errors and track down mistakes if they occur.

Reagent Quality

The next major source of problems is the medium ingredients including the water, reagents, and any special supplements such as sera and antibiotics. The highest quality water available should always be used. Special care must be taken to remove all trace metals, dissolved organics, and endotoxins. Endotoxins can have a variety of effects on cultured cells and are often found in water purified by systems that use ion-exchange resins. Purified water should not be stored for long periods before use. Water quality should be periodically checked using sensitive growth assays at clonal densities against known standards (Freshney, 1994).

It is wise to purchase chemicals of the highest purity available to avoid problems associated with trace contaminants. This is especially important for serum-free media where the effects of trace contaminants are not masked by sera. Once purchased, the optimal storage conditions for the chemicals must be carefully maintained to prevent any breakdown in quality. The same lot of chemicals should be used each time medium is made; when a lot is replaced, the new medium should be immediately compared against a previous batch to insure the replacement medium is satisfactory.

Buffers

After mixing, the medium is then buffered by the addition of sodium bicarbonate and adjusted to the proper pH. It is also helpful to check the osmolality at this point; mistakes in adding reagents can be uncovered as a result of finding unexpected deviations in osmolality measurements.

Many growth problems result when customers do not supply the CO_2 levels required by the bicarbonate-based buffering system of the medium they are using. This results in poor pH control and will adversely affect cell attachment and growth. Usually the higher the level of sodium bicarbonate, the higher the level of CO_2 required for optimal buffering capacity.

The most common system is a medium buffered with Earle's Balanced Salts containing 2.2 g/L of sodium bicarbonate. This system is designed for use in open culture vessels (dishes, microplates, or flasks with loose caps) that allow gas exchange with a humidified and enriched CO_2 environment (usually 5%). The second system is a medium buffered with Hanks' Balanced Salts containing only 0.35 g/L of sodium bicarbonate. This buffering system is designed for use in a sealed or gas-tight system and is not suitable for dishes and microplates.

Often, the above bicarbonate-based buffer systems are supplemented with the addition of HEPES, a widely used organic buffer. The use of this organic buffer can lead to additional problems upon exposure of the medium to fluorescent light.

Fluorescent Light-induced Toxicity

The deleterious effect of fluorescent light on culture media may be the single most overlooked source of chemically induced cytotoxicity. It is very important to store media and cells growing in culture vessels in the dark away from sources of fluorescent light that will interact with light sensitive media components (riboflavin, tryptophan, and HEPES). These interactions result in the production of hydrogen peroxide and free radicals that are directly toxic to cells. This well-documented problem is often ignored when there are cell growth issues (Wang, 1976, Wang and Nixon, 1978). Since the toxic effects of improperly stored media slowly increase with time, this problem is particularly difficult to identify. Besides direct cytotoxicity, other light-induced damaging effects include genetic damage (increase in mutation rates and chromosomal aberrations).

Filtration

Sterile filtration is usually the last step prior to the addition of any sterile supplements. There are two potential problems that may occur: first, interfering substances may be washed into the medium from the membrane or, second, valuable medium components may be lost by binding to the filter membrane. While most membranes contain usually harmless trace levels of leachable (extractable) substances, some membranes, especially some cellulose-based membranes, contain wetting agents that, at higher concentrations, may affect cell growth. These agents can be easily and safely removed from filters prior to use by running a small amount of warm high purity water through the filter unit. Some membrane materials, such as cellulose nitrate or nylon, may bind specific medium components, especially peptides and proteins used as growth or attachment factors (Brock, 1983). Testing should be performed to ensure these important supplements or factors are not being lost due to filtration.

Sera

The sera used as media additives have long been a source of problems in cell culture. These problems include the high cost of serum, its variable quality and performance, and its potential as a source of mycoplasmas, endotoxins, and other contaminants. While many advances have been made in the use of serum-free and reduced serum media, many cell culturists still use sera in their media. When practical, it is still a good idea to pretest several lots of serum before choosing one for purchase. The most sensitive assay possible that reflects the expected use of the serum should be used.

Problem-solving Suggestions

This guide has attempted to identify and solve some of the basic causes of cell culture problems. Many other problems can and will occur. Below are some recommended steps that can be used to help identify cell culture problems and find their causes:

1. Clearly identify and define the problem. It may require additional testing to repeat or duplicate the problem. It helps to make this a team effort, utilizing everyone in the laboratory whose knowledge or experience might contribute any helpful information.
2. It often helps to break up complex problems into smaller pieces that can be handled and understood more easily.
3. Organize all known facts surrounding the problem. Be specific, look for cause and effect relationships and then discard all facts that clearly do not apply and work with the rest. Don't overlook the obvious. A problem well stated is a problem half solved.
4. Once there is a clear understanding of the problem, begin looking for the cause. Try to avoid the urge to fix problems by changing everything; it may worsen the situation or mask the original problem.
5. Brainstorm and search for all the possible causes. Identify all changes that have occurred in the lab, in the cultures, in media, solutions, etc. that may relate to the problem. Good record keeping is essential for this step. Then select the best possibilities and begin to evaluate if they are actually contributing to the problem. Be creative! This may require some testing and experimentation.
6. Determine and implement the best long-term solution, not only to fix the problem but also to minimize or prevent any chance of a recurrence of the problem. This will take very careful planning.

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Use of Corning® Cloning Cylinders for Harvesting Cell Colonies

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Introduction

Several approaches are available to isolate and develop a population of cells derived from the descendants of a single cell. The choice of method depends upon the type of cell to be isolated and the manner in which the clonal cultures were established. One of the simplest procedures is to encircle a colony with a small cloning cylinder and to harvest the cells within the cylinder in the same way as would be done in a flask or plate. These cylinders (glass, porcelain, or stainless steel) are sealed to the plate with sterile silicone high vacuum grease to prevent leakage. The main advantage of this method is that the cells being picked have already demonstrated their ability to undergo cell division. The disadvantage is that there is no guarantee that the colony originated from only one cell.

Materials

Sterile Supplies

1. Medium forceps
2. Canister of short Pasteur pipettes or 200 µL micropipettor with tips
3. 1 mL pipettes (**Cat. No. Z371580**)
4. 6 well microplates (**Cat. No. M9530**) or T-25 Flasks (**Cat. No. Z363278**)
5. 60 mm glass Petri dish containing 10 cloning cylinders (**Cat. No. C1059**)
6. 60 mm glass Petri dish (**Cat. No. Z139734**) containing Dow Corning® 976V silicone high vacuum grease (sterilize by placing a small amount in the bottom of a glass Petri dish and autoclaving under standard conditions)

Medium and Solutions

1. Calcium- and magnesium-free phosphate buffered saline (**Cat. No. D8537**).
2. 0.25% Trypsin solution (**Cat. No. T4424**).
3. Growth medium appropriate for cells being picked
4. 100 mm dish containing 20 to 40 colonies suitable for picking

Procedure

1. Examine the culture dish containing well-isolated clones with an inverted or dissecting microscope. By manipulation of the intensity and angle of illumination it is possible to visualize the living colonies with a dissecting microscope; however, an inverted phase contrast microscope with a 4x or 10x objective is better.
2. Once satisfactory colonies have been located, draw a circle around them on the bottom of the dish with a marking pen. Select colonies that are of average size (very large colonies probably started from a clump of cells) and well isolated from other colonies.
3. Remove and discard the growth medium. Rinse the plate twice with CMF-PBS to remove any floating cells.
4. Using sterile medium forceps, pick up a cloning cylinder. Gently press the flat bottom of the cylinder into the smooth silicone grease and remove with a sudden vertical motion. If done properly, this will give even distribution of grease on the bottom of the cylinder. Set the cylinder over a colony. Gently press the cylinder down evenly with the forceps. Uneven pressure will cause the grease seal to leak. Be very careful not to slide the cylinder across the colony. This will smear the silicone grease over the cells and prevent the trypsin from contacting them.
5. Verify positioning of the cylinder over the colony with the microscope. Make sure there are no other colonies within the sealed area in the cylinder.
6. Add about 0.2 mL of the 0.25% trypsin to the cloning cylinder.
7. Incubate the dish at 36.5 °C for 5 minutes. Then examine cells under the microscope every two to three minutes until the cells have begun to round up and come off the dish bottom. Add a few drops of growth medium to the cylinder and gently aspirate the cells with a Pasteur pipette or micropipettor.
8. Transfer the cells to a suitable culture vessel and add the appropriate volume of medium. For large colonies, a T-25 flask or well in a 6 well plate can be used. For smaller colonies a well from a 24 or 12 well plate should be used. Diluting the cells too much will often result in slow or no growth. It may be necessary to rinse the cylinder with additional medium to remove cells left after the initial transfer.
9. Incubate cells as usual.

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Acknowledgments

This protocol evolved from protocols developed for cell culture training courses at the former W. Alton Jones Cell Science Center in Lake Placid, New York; Manhattan College, New York City; and the University of Connecticut, Storrs, Connecticut. I would like to thank all of my colleagues and students who, over the years, have contributed ideas and suggestions to its development.

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Fibroblast Elimination Protocols

Complement Mediated Cellular Cytoxicity

We have tested using anti fibroblast surface protein (**Cat. No. F4771**) to remove human fibroblasts from a mixed culture. This should also work with other antibodies. This protocol utilizes a rabbit complement (**Cat. No. S7764**).

Complement Mediated Cytotoxicity Assay for Fibroblasts

Materials

1. Cultured Fibroblasts
2. DMEM (Dulbecco's Modified Eagle's Medium)
3. Trypsin EDTA solution: 0.25% trypsin, 0.02% EDTA in saline
4. FBS-heat inactivated
5. DMEM + 10% FBS: DMEM enriched with 4 mM L-glutamine, antibiotics and 10% FBS
6. Complement: Rabbit complement S7764. Determine the working dilution of the complement by the appropriate assay, such that it will give the desired cytolytic activity in the presence of antibody and less than 5% cytotoxic effect in the absence of antibody or with non relevant antibody (approximately 1:3–1:20 final dilution)
7. Trypan Blue solution: 1% Trypan Blue in PBS
8. Ice-cold absolute ethanol (96%)
9. Paper Towels
10. Flat bottom 96 well microplate, suitable for the growth of anchorage dependent cells

Procedure

NOTE: To avoid non-specific damage to cells, prewarm (37 °C) the DMEM + 10% FBS prior to use.

1. Prepare a single cell suspension of fibroblasts, using the Trypsin EDTA solution, and seed fibroblasts in the microplate at 10^3 cells/well, in DMEM + 10% FBS, one day prior to assay.
2. On the day of the assay, discard medium and blot plate on paper towel.
3. Dilute antibody in DMEM + 10% FBS. Add 50 µL to the appropriate wells. Use non-relevant antibody as a negative control.
4. Incubate for 1 hour at 37 °C.
5. Add 10 µL of the appropriate dilution of complement, mix and incubate for 2 to 3 hours at 37 °C.
6. Wash twice with DMEM + 10% FBS. Discard supernatant and blot on paper towel.
7. Add ice-cold absolute ethanol, 100 µL/well for 5 minutes. Discard ethanol and blot on paper towel. Leave to dry 1 minute at room temperature.
8. Add 100 µL of Trypan Blue solution. Leave for 2 to 3 minutes, discard supernatant and wash gently with tap water. Blot on paper towel and dry in room temperature. Do not over dry.
9. Using the inverted microscope, determine the number of fields needed to be inspected to count at lest 100 intact cells in the negative control wells (usually 5 fields at 10 × 10 magnification),

For each well, count the number of intact cells in the same number of fields and calculate the relative percentage of unaffected cells (compared to the negative control).

Mitomycin C Treatment

Cell functions that require cellular division are abolished by treatment with mitomycin C (**Cat. No. M4287**), which causes DNA crosslinking. This can be used to treat cells for feeder layers, for mixed lymphocyte reactions and for removal of fibroblasts from mixed cell cultures. Mitomycin is used at 25 µg/mL to treat cells at 1–6 × 10^7 cells/mL. Cells are treated for 25 minutes at 37 °C.[1]

BrdU Treatment

BrdU (**Cat. Nos. B5002, B9285**) is incorporated into the DNA of dividing cells as an analog of thymidine and when activated by UV light, breaks down causing DNA damage and subsequent cell death. This is a method of selective killing of dividing cells. A growing cell culture is pulsed with BrdU at approximately 3 µg/mL (10 µM) to a growing culture for 24 hours in the dark. After the pulse, the cultures are exposed to intense UV light for 20 to 30 minutes using tubes located 4–6 inches above the culture dishes. Wash cells free of excess BrdU and resuspend in fresh culture media and return to culture.[2]

Also see the following reference using G418 (**Cat. No. G5013**). We have not tested this method. Cell cultures are treated with G418 for 2 days.[3]

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ITS, SITE, SPIT, SPITE, Fatty Acid-Albumin Supplements

Introduction

Most cells will not survive or exhibit optimal phenotypic properties for any length of time when cultured in basal medium alone. They require supplementation with additional growth and survival factors, such as hormones, transport proteins, trace elements, or ECM factors. Traditionally, serum has been the supplement of choice to provide these factors. However, many investigators prefer to work in a serum-free culture environment to avoid the variability and contaminants that can be introduced by serum.

Serum-free formulations that substitute a purified form of the factors normally supplied by serum are suitable for many *in vitro* growth and differentiation studies. These factors include insulin, transferrin, selenium, pyruvate, and ethanolamine. Addition of other components varies greatly, depending on the cell type being studied and the basal medium employed.

Role of Components

INSULIN is a polypeptide hormone that promotes the uptake of glucose and amino acids and may owe observed mitogenic effect to this property.

TRANSFERRIN is an iron-transport protein. Iron is an essential trace element, but can be toxic in the free form. To nourish cells in culture, it is supplied bound to transferrin in serum.

SELENIUM is an essential trace element normally provided by serum.

SODIUM PYRUVATE has been shown to be beneficial as an additional energy source in some instances.

ETHANOLAMINE is a highly polar alcohol that plays a significant role in the proliferation of hybridoma cells and frequently is added to supplements used for culturing these cells.

Medium Supplements

Nutritional studies indicate that the supplement components described are utilized by most mammalian cells. They enhance cell proliferation and decrease the serum requirement of many cell types. When the following supplements are used with 2 to 4% serum, proliferation is reported to be similar to medium supplemented with 10% serum.

ITS is a mixture of bovine insulin, human transferrin (substantially iron-free), and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g., MEM, RPMI-1640) and complex media (e.g., Ham's F-12, DME/F-12, MEM) *with* sodium pyruvate.

SITE is a mixture of bovine insulin, human transferrin (substantially iron-free), sodium selenite, and ethanolamine. It is a general cell supplement designed for use in non-complex media (e.g., MEM, RPMI-1640) and complex media (e.g., Ham's F-12, DME/F-12, MEM) *with* sodium pyruvate.

SPIT is a mixture of bovine insulin, human transferrin (substantially iron-free), sodium selenite, and sodium pyruvate. It is designed for cell cultures in which media *without* sodium pyruvate are used.

SPITE is a mixture of bovine insulin, human transferrin (substantially iron-free), sodium selenite, sodium pyruvate, and ethanolamine. It is designed for cell cultures in which media *without* sodium pyruvate are used.

FATTY ACID-ALBUMIN complexes have been employed as alternative sources of lipids in the development of serum-free media. Fatty acids bind to serum proteins in high proportions. Such proteins may release beneficial fatty acids and bind those that are inhibitory. Oleic acid bound to BSA has been shown to be beneficial to the growth of a variety of cell types (e.g., BHK, hybridoma). Similar observations have been made regarding linoleic acid, a precursor of prostaglandins. A mixture of polyunsaturated and monosaturated fatty acids (i.e., linoleic acid and oleic acid) used as a medium supplement has been reported to exhibit a synergistic effect.

Medium Supplement Formulation Table

Final Conc. [1×]	ITS I1884	SPIT S5791	SPITE S5666	SITE S4920	ITS I3146	ITS +1 I2521	ITS +3 I2771	SITE +3 S5295	FAC +LO L9655	FAC +L L9530	FAC +0 O300
insulin mg/L	5	10	10	10	10	10	10	10	—	—	—
transferrin mg/L	5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	—	—	—
selenium mg/L	5	5	5	5	5	5	5	5	—	—	—
pyruvate mg/L	—	110	110	—	—	—	—	—	—	—	—
ethanolamine mg/L	—	—	2	2	—	—	—	2	—	—	—
BSA mg/L	—	—	—	—	—	0.5	0.5	0.5	1.0	1.0	1.0
linoleic acid mg/mL	—	—	—	—	—	4.7	4.7	4.7	9.4	9.4	—
oleic acid mg/mL	—	—	—	—	—	—	4.7	4.7	9.4	—	9.4

NOTE: These products are sold at [100×] concentration. The concentration in the vial is [100×] value shown in the table.

Additional Product Information

Dimethyl Sulfoxide (DMSO) (Cat. No. D2650)

Product Description

Human and animal cell lines grown in culture are generally stored frozen. Freezing protects the cell line from changes due to genetic drift and minimizes the risk of contamination. Liquid nitrogen used in conjunction with a cryoprotective agent such as DMSO has become a widely used method for preserving cells. Without the presence of a cryoprotective agent, freezing is lethal to most mammalian cells. Damage is caused by mechanical injury by ice crystals, concentration of electrolytes, dehydration, pH changes, and denaturization of proteins. These lethal effects are minimized by adding a cryoprotective agent which lowers the freezing point and allows for a slower cooling rate.

DMSO has also been used in some fusion protocols in which fusion is difficult to achieve.

Recommendations for Cell Freezing

DMSO is supplied as a sterile liquid. It may be used as a cryoprotective agent in conjunction with complete media for preservation of cell lines at –70 °C or below. To prepare a solution for freezing cells:

1. Prepare freezing medium containing culture medium with 10–20% serum and 5–10% DMSO.
2. Remove adherent cells with trypsin or other appropriate means. (For optimal results cells should be in log phase of growth.)
3. Gently pellet the cells by centrifugation (10 minutes at 250 × *g*, 4 °C) and remove culture medium.
4. Resuspend the cells in the freezing medium at 10^6–10^7 cells/mL.
5. Aliquot into freezing vials.
6. Freeze cells according to standard freezing protocols. Store at –70 °C or below.

Recommendations for Fusion

1. Prepare 40–50% PEG solution.
2. Add 10% DMSO.
3. Follow normal fusion protocols.

Product Storage

Store at room temperature.

Methotrexate (Cat. No. M8407)

Product Description

Dihydrofolate analog which acts as an inhibitor of eukaryotic dihydrofolate reductase.

Recommendations for Selection

Used as a selective agent to kill non-transformed cell by inhibition of endogenous dihydrofolate reductase. Cells transformed with a plasmid carrying the bacterial dihydrofolate reductase gene are rescued from the action of methotrexate. Recommended working concentration is 0.4 µM. Please refer to the literature for specific applications.

Ouabain (Cat. No. O5754)

Product Description

Glycoside which has been shown to inhibit membrane associated Na^+/K^+ ATPase.

Recommendations for Selection

Use in the selection of hetero-hybridomas of human/mouse origin. Human cell lines normally die in the presence of 10^{-7} M ouabain, whereas rodent cell lines are resistant up to 10^{-3} M ouabain. Unfused human cells can be selected against with 10^{-5} M (10 µM) ouabain.

Polyethylene Glycol [PEG] (Cat. Nos. P2906, P7181, P7306, and P7777)

Product Description

Polyethylene glycol (PEG) has been found to promote cell-cell membrane fusion by acting as a lipolytic agent in which the cellular membranes are sufficiently solubilized to form a fusion product. PEG has been used successfully with many different cell fusions, some of which include spleen-myeloma and plant protoplasts.

PEG as a fusing agent is offered in two different molecular weights. Determining which molecular weight to use will depend upon several factors such as cell types to be fused, difficulty in achieving a fusion product, use of dimethyl sulfoxide (DMSO) and temperature of the fusion.

Recommendations (Cat. Nos. P2906, P7777)

PEG is supplied as a sterile, waxy substance that does not lend itself readily to forming aqueous solutions at room temperature. To prepare a solution for cell fusion:

1. Melt the PEG at 45 °C or higher in a waterbath. **Cat. No. P2906** will require autoclaving at 121 °C for 5 minutes to melt.
2. Dilute to 30–50% using sterile, pre-warmed serum-free medium with phenol red (e.g., for a 50% solution add 5 mL to the 5 *g* vial of PEG).
3. The pH of the solution should be slightly alkaline (pink, not orange or purple). Adjustment of the pH with sterile NaOH may be required.

Recommendations (Cat. Nos. P7306, P7181)

These solutions are ready-to-use in a normal fusion protocol requiring 50% PEG. If a less concentrated solution is desired, dilute with sterile DPBS without calcium (**Cat. No. D5773**). Some precipitation may appear after being exposed to cooler temperatures. This is normal and the precipitation should disappear as the solution warms.

Product Storage (Cat. Nos. P2906, P7777)

Solid: Room Temperature

Solution: Below 0 °C. Prolonged storage or repeated freezing and thawing is not recommended.

Product Storage (Cat. Nos. P7306, P7181)

Store ampules at 2–8 °C. Solution may be frozen if desired, but should first be aliquotted to avoid repeated freeze/thaw cycles.

Red Blood Cell Lysing Buffer (Cat. No. R7757)

Product Description

Red Blood Cell Lysing Buffer has been developed for use in hybridoma protocols to remove red blood cells from mouse splenocyte suspensions before fusion. It is also useful in systems where it may be desirable to remove red blood cells from cell suspensions, such as whole blood. Red Blood Cell Lysing Buffer is supplied as a sterile solution and contains 0.83% ammonium chloride in 0.01 M Tris buffer.

Recommendations

1. Add 1 mL of buffer to the cell pellet (cell pellet = 1 spleen or 1–2 × 10^8 million cells).
2. Gently mix for 1 minute.
3. Dilute the buffer with 15–20 mL of medium or salt solution.
4. Centrifuge at 250–500 × *g* for 7 minutes and decant the supernatant.
5. Cells may be diluted and prepared for counting or fusion.

NOTE: If lysis is incomplete, steps 1–4 may be repeated.

Product Storage

Red Blood Cell Lysing Buffer is stable at room temperature.

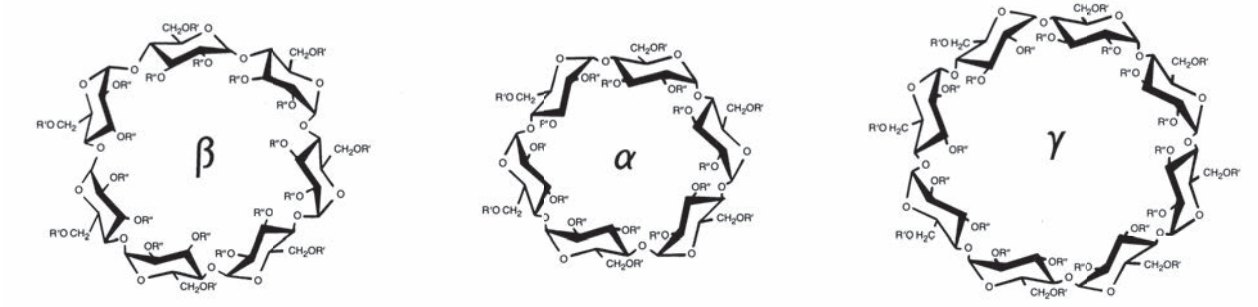
Water Soluble Complexes

Cyclodextrins—What Are They?

Many metabolically important compounds, such as fat-soluble vitamins and hormones, have very low solubilities in aqueous solutions. Various approaches have been taken to utilize these compounds in tissue and cell culture applications. Two most frequently used approaches are: 1) pre-dissolving the compounds in organic solvents and 2) using "carrier" molecules to facilitate the dissolution of these compounds. One such class of "carrier" molecules is the **cyclodextrins** or cycloamyloses.

Cyclodextrins are cyclic oligosaccharides consisting of 6, 7, or 8 glucopyranose units, usually referred to as α-, β-, or γ-cyclodextrins, respectively. These naturally occurring compounds have relatively rigid doughnut-shaped structures, and have attracted interest as possible natural complexing agents. The unique structures of these compounds owe their stability to intramolecular hydrogen bonding between the C2- and C3-hydroxyl groups of neighboring glucopyranose units. The molecule takes on the shape of a torus with the C2- and C3-hydroxyls located around the larger opening and the more reactive C6-hydroxyl aligned around the smaller opening. The arrangement of C6-hydroxyls opposite the hydrogen bonded C2- and C3-hydroxyls forces the oxygen bonds into close proximity within the cavity, leading to an electron rich, hydrophobic interior. The size of this hydrophobic cavity is a function of the number of glucopyranose units forming the cyclodextrin.

The solubility of natural cyclodextrins is very poor and initially this prevented cyclodextrins from becoming effective complexing agents. In the late 1960s, it was discovered that chemical substitutions at the 2,3, and 6 hydroxyl sites would greatly increase solubility. The degree of chemical substitution, as well as the nature of the groups used for substitution, determine the final maximum concentration of cyclodextrin in an aqueous medium. Most chemically modified cyclodextrins are able to achieve a 50% (w/v) concentration in water.



Cavity size is the major determinant as to which cyclodextrin is used in complexation. "Fit" is critical to achieving good incorporation of cyclodextrins. Six-glucopyranose unit compounds or α-cyclodextrins have small cavities which are not capable of accepting many molecules. Eight-glucopyranose unit compounds or γ-cyclodextrins have much larger cavities than many molecules to be incorporated and cyclodextrin hydrophobic charges can't effectively interact to facilitate complexation. The cavity diameter of β-cyclodextrins or 7-glucopyranose unit compounds is well-suited for use with molecules the size of hormones, vitamins, and many compounds frequently used in tissue and cell culture applications. For this reason, β-cyclodextrin is most commonly used as a complexing agent.

Hydrophobic molecules are incorporated into the cavity of cyclodextrins by displacing water. This reaction is favored by the repulsion of the molecule by water. This effectively encapsulates the molecule of interest within the cyclodextrin, rendering the molecule water soluble. When the water soluble complex is diluted in a much larger volume of aqueous solvent, the process is reversed, thereby releasing the molecule of interest into the solution.

Sigma's product line of water-soluble complexes includes host cyclodextrins and cyclodextrin-encapsulated forms of many components commonly used in tissue and cell culture applications. For product listings, see the **Reagents** section of this catalog.

References

1. The Source. Water-Soluble Complexes . . . Part 1: Cyclodextrins–What are they? Vol. **7**, No. 3, 1991.
2. The Source. Water-Soluble Complexes . . . Part 2: Cyclodextrins and Cell Culture. Vol. **8,** No. 1, 1992.

Cell Culture Protocols and Technical Articles

For additional cell culture protocols and technical articles, please visit the Corning Technical Web site at **corning.com/lifesciences**. The following are some of the technical articles that can be found there.

Introduction to Animal Cell Culture

This 10-page Corning Technical Guide is designed to serve as a basic introduction to animal cell culture. It is appropriate for both laboratory workers who are using this tool for the first time or for those who interact with cell culture researchers and who want a better understanding of the key concepts and terminology in this interesting and rapidly growing field. Topics covered include:

- What is animal cell and tissue culture?
- How are cell cultures obtained?
- What are cultured cells like?
- What are the problems faced by cultured cells?
- How to decide if cultured cells are "happy"?
- What is cell culture used for?

Identifying and Correcting Common Cell Growth and Attachment Problems

This Corning Technical Guide reviews some of the common and not so common cell growth and attachment problems that are often very difficult to identify and eliminate. Topics covered include:

- Identifying the causes of unusual growth patterns due to technique or incubator problems.
- Problems with culture media, including HEPES and fluorescent light-induced problems.
- Applying cell culture problem solving strategies.

General Guide for Cryogenically Storing Animal Cell Cultures

This 8-page Corning Technical Guide examines both the theoretical and practical aspects of cryogenic preservation and reviews key strategies for managing a cell repository. Special attention will be given to:

- Understanding and controlling the freezing process
- Selecting cryogenic storage systems
- Recordkeeping
- Quality control procedures to minimize culture loss

Endotoxins and Cell Culture

This 8-page Corning Technical Guide discusses endotoxins and their effects on cell cultures. Special attention will be given to:

- What are endotoxins?
- Sources of endotoxins in cell culture
- Endotoxin effect on *in vitro* cell growth and function

Helpful Hints to Manage Edge Effects of Cultured Cells for High Throughput Screening

This technical note is a compendium of techniques, collected from Corning Cell Culture facilities and customers, to reduce the occurrence of irregular patterns of cell adhesion or "edge effect" in microplates.

Analysis of CHO Cell Requirements for Assay Miniaturization in High Throughput Screening

This technical note analyzes the culture conditions for optimal growth of Chinese hamster ovary (CHO) cells for assay miniaturization in high-throughput screening.

Mycoplasma Detection Using DNA Staining

This 3-page protocol provides a detailed procedure for using Hoechst stain #33258 to stain the DNA in cell monolayers for detecting mycoplasmas or other prokaryotic organisms.

*For more information, see the **Mycoplasma Detection and Elimination** section starting on page 235.*

Roller Bottles Selection and Use Guide

This 9-page guide describes the characteristics of all the glass and plastic Corning® roller bottles and offers tips on solving cell growth and attachment problems that can occur in roller bottles.

Transwell® Permeable Supports Selection and Use Guide

Selecting the right Transwell inserts for your research application does not have to be difficult. This 11-page guide describes all of the importance physical and performance characteristics of the Corning Transwell permeable support products and offers tips on using them.

Transwell and Snapwell™ Bibliography

This bibliography lists several hundred references, organized by research application, citing the use of Corning Transwell permeable supports in cell culture research.

For additional questions please call Sigma-Aldrich Technical Support or visit the Corning Life Sciences Web site at corning.com/lifesciences.

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Glossary of Cell Culture Terms

Acclimatization

Adaptation of an organism to a new environment. In cell culture, this process may involve adjusting to a new temperature, carbon dioxide concentration, etc. Becoming accustomed to such changes may include physiological changes which better enable an organism to utilize nutrient sources and environmental conditions.

Adaptation

Cellular adjustment from a change in environment; often from serum-supplemented conditions to serum-free conditions.

Adventitious Viral Agents (AVA)

Adventitious viral agents, as defined by 9CFR, include mycoplasma, bacteria and fungi as well as agents that cause hemadsorption in specified cell lines.

Anaerobic Growth

Growth that occurs in the absence of air or oxygen. Some anaerobic organisms are killed by brief exposure to oxygen, whereas oxygen may just retard or stop the growth of other anaerobic organisms.

Anchorage Dependence

Cells or cultures which will survive and maintain function only when attached to an inert substrate; the use of this term does not imply that these cells are normal or that they are neoplastically transformed.

Animal-component Free (ACF)

A term used to describe cell culture media which does not contain animal-derived components such as galactose or cod liver oil, but may still contain recombinant proteins or undefined components.

Animal-protein Free (APF)

A term used to describe cell culture media which does not contain animal proteins, but may still contain animal-derived components.

Antibody

A protein that is secreted into the blood or other bodily fluids in response to an antigenic stimulus or a foreign substance such as a bacterium, virus, parasite or a transplanted organ. Also known as immunoglobulins, antibodies are infection-fighting molecules that bind specifically to antigens in order to neutralize and help destroy foreign microorganisms or toxins.

Antigen

A substance that, when introduced into the body, induces an immune response. Each antigen may contain more than one site capable of binding to a particular antibody. An immunogen can cause the production of a number of antibodies with different specificities. Antigenicity is the capacity of a substance to function as an antigen, and therefore to trigger an immune response.

Apoptosis

A programmed series of events that lead to cell death. Also known as caspase-mediated cell death, where proteases mediate apoptosis by controlling the number of cells able to survive.

AQIS (Australian Quarantine and Inspection Service)

AQIS is an operating group within the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF). It provides quarantine inspection for the arrival of international passengers, cargo, mail, animals and plants or their products into Australia, and inspection and certification for a range of animal and plant products exported from Australia.

Bacteriophage

A virus that infects bacteria. Bacteriophage can have a protein "tail" attached to the capsid which is used to infect the host bacteria.

Baculovirus

A virus gene that is frequently used for insect cell infection. This system enables the production of proteins with polysaccharides which are attached via DNA clones. This is a unique and beneficial event because these polysaccharides (sugar chains) are not produced when cloned DNA makes protein using bacteria.

Batch Culture

A closed system of microorganisms that is cultured to a maximum density in a bioreactor, harvested and processed as a batch. The cell inoculum is grown with specific nutrients and other predetermined environmental conditions. A batch culture is the opposite of a continuous culture.

Bioreactor

A vessel used for bioprocessing to facilitate the creation of biological products. Bioreactor systems control the temperature, aeration, pH and other controlled conditions of this changing system.

Biotechnology

The development of products using a biological process. Production may be carried out by using cells in culture or by using natural substances (such as enzymes) from organisms. Some biotechnology products include antibiotics, insulin, interferon, recombinant DNA and the products of several other techniques.

Bovine Spongiform Encephalopathy (BSE)

BSE is the bovine variant of TSE. The disease has a long incubation period of four to five years and it is fatal for cattle within weeks to months of its onset. The nature of the BSE agent is still being debated. Strong evidence currently available supports the theory that the agent is composed largely, if not entirely, of a self-replicating protein, referred to as a prion. It is transmitted through the consumption of BSE-contaminated meat and bone meal supplements in cattle feed.

Cell Density

The total number of cells in culture, usually indicated in the density of viable and non-viable cells.

Cell Line

A culture of a particular type of cell, reproduced indefinitely. When cells from the first culture are used to make subsequent cultures, a cell line is established. Immortal cell lines can replicate indefinitely, as a result of genetic or other manipulations. The term "cell line" implies that resulting cultures consist of generations of the cells originally present in the primary culture.

Glossary of Cell Culture Terms: Cell Transformation

Cell Transformation

A permanent alteration of the cell phenotype presumed to occur via an irreversible genetic change which may be spontaneous or induced by chemical or viral means or by exposure to radiation. Transformation is often characterized (but not defined) by the emergence of an established cell line from a primary explant, an alteration of typical morphology, loss of contact inhibition, abnormal karyotype, changes in viral susceptibility, changes in antigenic and neoplastic properties and the loss of anchorage dependence.

Chemically Defined (CD)

Term used to describe a product in which every component's chemical and physical properties are known, including the component's molecular weight and structure. Chemically defined items include defined animal components, defined proteins and other defined components such as insulin, amino acids, salts and vitamins. Defined components do not include hydrolysates, BSA, transferrin and serum.

Chemically Defined Medium

A nutrient medium for culturing cells in which the chemical structure of each component is known. Distinct from other serum-free formulations where other incompletely characterized constituents may be used to replace serum.

Clone

A population of genetically identical cells derived from a single parent cell by mitosis. A Chinese Hamster Ovary (CHO) clone is one such example.

Cloning Efficiency

The percentage of cells plated (seeded/inoculated) that form a clone.

Cloning Vectors

Methods of transferring desired genes into organisms that will be used to express them. Cloning vectors are used to make recombinant organisms.

Code of Federal Regulations (CFR)

The codification of the general and permanent rules published in the Federal Register by the executive departments and agencies of the Federal Government of the United States. It is divided into 50 titles that represent broad areas subject to Federal regulation which include, but are not limited to, the manufacture of pharmaceutical drugs and medical devices. Each volume of the CFR is updated once each calendar year and is issued on a quarterly basis.

Confluent

A cultured population which occupies all available growth surface of a vessel.

Contact Inhibition

Inhibition of cell membrane motility when cells are in complete contact with adjacent cells, as in a confluent culture. Often precedes cessation of cell proliferation.

Continuous Cell Line

A population of cells which can be propagated for an indefinite number of passages.

Cryopreservation

A technique used to freeze cells or other biological materials at temperatures low enough to maintain genetic stability and make the material metabolically inert. Cryopreservation includes maintenance in freezers (–80 °C) or in liquid nitrogen (–196 °C).

Current Good Manufacturing Practices (cGMP)

Regulations governing standards of design, operation, practice and sanitization set forth by the Federal Government of the United States that outline general requirements that manufacturers of medical devices must follow to assure the highest quality attainable.

Cytopathic

Causing disease symptoms in a cell.

Cytotoxic

Able to produce a toxic effect on cells or cause cell death.

DHFR (Dihydrofolate Reductase)

Dihydrofolate Reductase is an enzyme involved in the biosynthesis of folic acid coenzymes that transfers hydrogen from NADP to dihydrofolate, yielding tetrahydrofolic acid, an essential vitamin cofactor in purine, thymidine and methionine synthesis. The DHFR-Selection System utilizes cells that are DHFR-deficient as part of a specific selection pathway.

Differentiation

The process by which cells or tissues undergo a change toward a more specialized form or function, especially during embryonic development. Differentiated cells maintain, in culture, the specialized structure and function typical of the cell type *in vivo*.

Drug Master File (DMF)

A submission to the Food and Drug Administration (FDA) that may be used to provide confidential detailed information about facilities, processes or articles used in the manufacturing, processing, packaging and storing of one or more human drugs. The submission of a DMF is not required by law or FDA regulation. The information contained in the DMF may be used to support an Investigational New Drug Application (IND), a New Drug Application (NDA), an Abbreviated New Drug Application (ADNA), another DMF, an Export Application or amendments and supplements to any of these. DMFs are generally created to allow a party other than the holder to reference material without disclosing to that party the contents of the file.

Efficacy

The ability of a substance such as a therapeutic protein to produce a desired clinical effect. The efficacy of a substance is measured by its strength and ability to elicit a response.

Endogenous

Growing or developing from a cell or organism, or arising from causes within the organism. Chinese Hamster Ovary (CHO) cells have endogenous levels of L-glutamine.

Endotoxin

A contaminant found in cell culture products that comes from the cell wall of bacteria and can negatively affect the growth of cells in culture. In the form of a fat/sugar complex (lipopolysaccharide) this poison forms a part of the cell wall of some types of bacteria. It is released only when the cell is ruptured and can cause septic shock and tissue damage. Pharmaceuticals are routinely tested for endotoxins.

Expression

The process by which a gene's coded information is converted into the structures present and operating in the cell. Expression includes the production of a protein by a gene inserted into a new organism.

Fermentor

A device used to grow bacteria or yeasts in liquid culture. A fermentor is used for fermentation, while a "bioreactor" in its truest sense is for use with cell culture.

Formulation

The list of ingredients that constitute a product or products, also referred to as the list of components.

Gamma Irradiation

The process of treating material with gamma radiation, providing greater assurance that low levels of microorganisms will be inactivated or reduced and the risks associated with animal-derived components minimized. It is a procedure typically used in the biotech industry to ensure the inactivation of low levels of animal-derived contaminants.

Gene Therapy

Introducing new genes into the DNA of a cell in order to replace, manipulate or correct nonfunctional or disfunctional genes.

Generation Time

The interval from one point in the cell division cyle to the same point in the cycle one division later. Distinct from doubling time or population time.

Good Laboratory Practices (GLP)

A set of rules and criteria for a quality system developed to promote the quality and validity of data generated in order to facilitate their recognition for purposes of assessment and other uses relating to the protection of human health and the environment.

Good Manufacturing Practices (GMP)

Practices required by FDA regulations. Quality systems for FDA-regulated products, including biologics, are known as Good Manufacturing Practices, or GMPs. Manufacturers establish and follow these quality system guidelines to help ensure that products consistently meet applicable requirements and specifications. GMP facilities operate under the guidelines of the CFR, or Code of Federal Regulations.

Growth Curve

A semi-log plot of cell number versus time on a scale which demonstrates the proliferation of cells in culture. Usually divided into lag phase, before growth is initiated; logarithmic phase, the period of exponential growth; plateau or stationary phase, where a stable cell count is achieved and the culture stops growing at a high cell density; and death phase, during which nutrients are depleted and cell number decreases.

Heat Inactivation

Heating serum at 56 °C for 30 minutes to deactivate complement proteins which may interfere with certain procedures such as immunological assays.

Host

A cell or organism infected with a virus, plasmid or other form of foreign DNA and used for growth and the production of cloned substances.

Host-vector System

The combination of DNA-receiving cells (hosts) and DNA-transporting substances (vectors) used for introducing foreign DNA into a cell.

Hybridoma

The result of fusing an antibody-producing lymphocyte and a myeloma cell into a single mononucleate cell. Subculturing results in a hybrid cell line. A hybridoma cell is specialized for continually producing specific antibodies.

Hydrolysate

The product of hydrolysis. In cell culture medium, hydrolysates are an undefined source of amino acids, peptides, some lipids and trace amounts of low molecular weight substances. Its use in mammalian and insect cell culture media significantly improves cell growth and culture longevity of most cell lines.

Immunoassay

A laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample.

Immunoglobulin

Any of a group of large glycoproteins, secreted by plasma cells that function as antibodies in the immune response by binding with specific antigens. There are five classes: IgA, IgD, IgE, IgG and IgM.

International Organization For Standardization (ISO)

The source of ISO 9000 and the more than 14,000 international standards for business, government and society; a network of national standards institutes from 148 countries working in partnership with international organizations, governments, industry, business and consumer representatives.

in vitro

A biological or biochemical process occurring in an artificial environment outside of a living organism, such as in a laboratory setting.

in vivo

A biological or biochemical process occurring within a living organism or taking place in a cell.

Lyophilization

The process of isolating a solid substance from solution by freezing the solution and evaporating the ice under vacuum. Also known as freeze drying, this process can be used in purification.

Microcarrier

A microscopic particle (often a 200 mm polymer bead) that supports cell attachment and growth in suspension culture. The use of microcarriers in a bioreactor provides an increased surface area for anchorage-dependent cells, and therefore assists in growth and proliferation of a culture.

Molecular Weight

The sum of the atomic weights of all of the component atoms in a molecule.

Monoclonal Antibody (MAb)

An antibody produced by a cloned cell derived from a single lymphocyte, typically from a hybridoma. The hybridoma cell that produces the antibody of choice is usually cloned or reproduced to large numbers for larger-scale production of MAbs. The antibodies produced in this method are highly specific and they recognize and target only a single antigen.

Glossary of Cell Culture Terms: Monolayer

Monolayer

A single layer of uniformly oriented anchorage-dependent cells growing on a substrate.

Mutant

A cell that varies phenotypically from the original population due to a modified or added gene. A mutation is a change in the genetic material of a cell.

Mycoplasma

The name given to the genus of the smallest and simplest form of bacteria. Mycoplasma are self-replicating and have no cell walls. These prokaryotes are spherical to filamentous in shape and can cause diseases in humans, animals and plants. They are recognized as a contaminant of cell cultures. Once they have contaminated a cell line, they can easily spread to other cell lines in the laboratory. Mycoplasma testing of cell culture media and the individual components of the media is paramount in avoiding infection of one's cell lines.

Myeloma

A tumor derived from myeloid cells used in monoclonal antibody technology to form hybridomas.

Organic Compound

A compound containing carbon.

Osmolality

The number of dissolved particles per kilogram of solution, commonly reported as mOsm/kg by freeze-point depression. This differs from osmolarity, which is the number of dissolved particles per liter of solution.

Peptide

A compound consisting of two or more amino acids, joined together by a "peptide bond." Peptides are combined to form proteins.

Perfusion Culture

A culture of microorganisms maintained under conditions with a constant nutrient supply. Continuous cultures are grown steadily for an extended period of time, which is the opposite of a batch culture.

Plating Efficiency

The percentage of cells seeded in culture that successfully give rise to colonies.

Polyclonal Antibody

An antibody produced by a number of different lymphocytes as part of the normal immune response.

Polypeptide

A peptide which is composed of more than two amino acids.

Population Density

The number of cells per unit area or per unit volume in a culture vessel. Also called cell density. This information is usually reported in the number of viable cells vs. the total number of cells.

Population Doubling Time

The amount of time it takes for a population to double in size.

Primary Culture

A culture initiated from an explant of cells, tissues or organs taken from an organism. A culture is a primary culture until it is subcultured for the first time, at which point it becomes a cell line.

Prion

An infectious protein similar to a virus but having no detectable nucleic acid. Prions are the agents responsible for several degenerative diseases of the nervous system.

Protein

Molecule composed of one or more polypeptide chains, each possessing a characteristic amino acid sequence and molecular weight.

Protein-free (PF)

Media formulations that are considered protein-free do not contains proteins, either human, animal or recombinant. Such formulations may contain animal-derived components, as well as hydrolysates.

Seeding Density

The amount of cells that are introduced into a system ("seeded") in order to provide enough cells to grow in culture. Different cell lines have different optimal seeding densities which help promote growth and ensure the viability of the culture.

Seeding Efficiency

The percentage of inoculated cells which attach to the surface of the culture vessel within a specified time.

Selective Medium

A nutrient material capable of supporting the growth of specific organisms while inhibiting the growth of others.

Serum-free Medium (SFM)

A medium which does not require serum supplementation, but may contain other proteins, growth factors, animal-derived components and hydrolysates.

Spent Media

Liquid cell culture media that has been in culture and is depleted of its vital nutrients. Spent media is generally poured off of attached cultures or those that have been centrifuged in order to re-feed the culture with fresh media. Spent media can be analyzed to evaluate the rate or level at which cells utilize nutrients in media. Spent media is also referred to as "conditioned media."

Sterile

The state of non-contamination by viable or non-viable microorganisms or the use of techniques to ensure the prevention of contamination.

Sterile Filtration

Membrane filtering of product used to reduce the amount of contaminant particles in a solution, usually liquid medium, serum or other cell culture reagent.

Subculture

The process of transferring or transplanting a cell population from one culture vessel to another. This usually involves "splitting" or dividing the cell culture by dilution. Synonymous with the term "passage." The passage number is the number of times a population of cells has been subcultured or passaged.

Substrate

A reactant (protein, carbohydrate or fatty acid) that when bound to an enzyme's active site generates a product with a lower activation energy.

Surfactant

A substance that lowers the surface tension of water or reduces the shear forces applied to cells in suspension. A common surfactant used in cell culture is Pluronic® F68.

Suspension Culture

A culture in which cells have been adapted to grow and divide without being attached to a substrate.

Transfection

The transfer of genetic material from one cell to another by artificial means. Transfection implies the transfer of less than the whole nucleus of the donor cell and is usually achieved by using isolated chromosomes, DNA or cloned genes. One such method used is electroportation, where an electric current is applied to a living surface, such as the skin or the plasma membrane of a cell, in order to open pores or channels through which something (as a drug or DNA) may pass.

Transmissible Spongiform Encephalopathy (TSE)

TSE is a family of diseases in humans and animals that are characterized by spongy degeneration of the brain, with severe and fatal neurological signs and symptoms.

Vaccine

A preparation that consists of killed or weakened disease-causing organisms, or parts of such organisms, administered for the prevention amelioration or treatment of infectious diseases. Vaccines include the formation of antibodies or immunity against the pathogen administered.

Virus

An ultra-microscopic microorganism, parasitic within living cells, which can cause disease in humans. It consists of a strand of nucleic acid (DNA or RNA) that contains genetic instructions concerning viral reproduction that is enveloped by a protein coat. Cellular rupture (and death) liberates the newly formed viruses.

Wild Type

An organism as it occurs most frequently in nature, before any sort of mutation or genetic engineering.

Validate your miRNA target.

Simplify validation of your miRNA gene target with the MISSION® 3'UTR Lenti GoClone™ collection from Sigma® Life Science.

Biovalidate.

MISSION 3'UTR Lenti GoClones are a lenti-viral based functional and quantitative tool for validation of gene targets regulated by microRNA. Sigma Life Science and SwitchGear Genomics™, have partnered to offer you a comprehensive collection of lentiviral constructs, each with a unique human 3'UTR cloned downstream of an optimized *Renilla* luciferase.

Start validating your microRNA gene targets
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