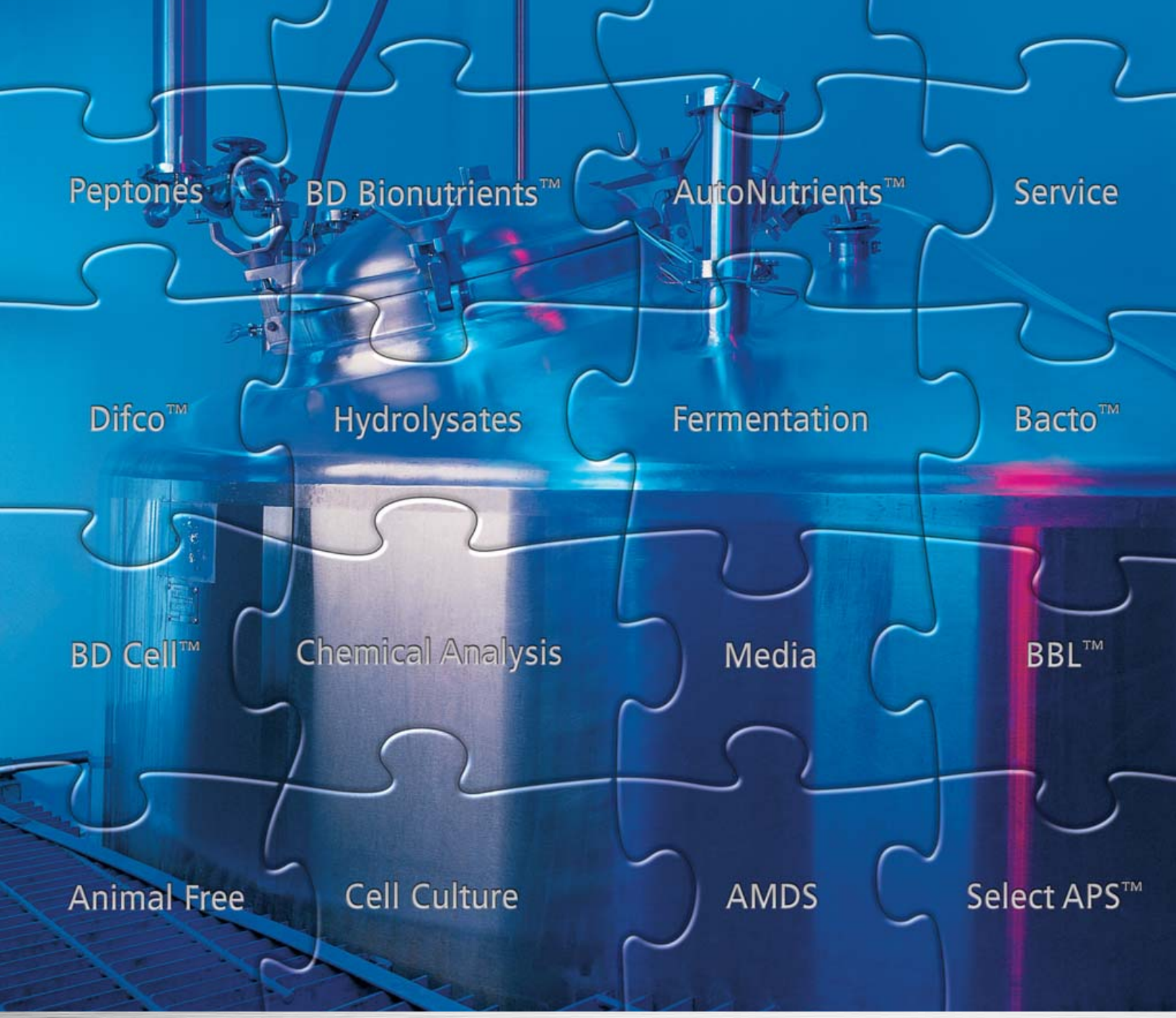


BD Bionutrients™ Technical Manual

Advanced Bioprocessing

Third Edition Revised

BD

Helping all people
live healthy lives

Table of Contents

Introduction

The BD Bionutrients™ Technical Manual has been provided to assist you in your selection of BD products for use in cell culture and microbial fermentation production, from Research and Development to the final finished product.

It is our commitment to innovation and product consistency that makes BD (Becton, Dickinson and Company) such a strong global supplier and partner. In over 142 countries worldwide, BD offers a full line of bionutrients and media, for the biotechnology, pharmaceutical, animal and human vaccine, and bioremediation markets. BD offers products for both cell culture and microbial fermentation production, as well as applications for industrial research, QA/QC and environmental monitoring.

Capability

Our commitment to the cell culture and fermentation media market is exemplified in our wide range of capabilities:

- **Largest media manufacturing plant in the world**—In 1999, as part of the Difco Laboratories acquisition, BD opened a manufacturing plant in Sparks, Maryland, which is our Center of Manufacturing Excellence. With over 101,000 sq. ft. of production capacity, the center is a state-of-the-art modern facility that manufactures dehydrated culture media, prepared culture media, bionutrient ingredients, stains, and other products for microbiological and cell culture use worldwide.
- **Full line of meat peptones**—Building on the reputation of the Difco meat peptones, BD continues manufacturing operations in Detroit as a source for the high quality Difco™ and Bacto™ brand products. This commitment to tradition carries on the quality and performance established under the Difco name, long recognized throughout the industry for superior quality. BD continues investing in Research and Development for peptone products, which continually expands our understanding of their application in cell culture and microbial fermentation.
- **Expanding line of animal-free products**—As early as 1998, BD started offering animal-free products to the fermentation industry, introducing its Select APS™ (Alternative Protein Source) Super Broth, Select APS LB Broth Base, and Select Soytone. BD continues to leverage its expertise in creating high performing animal-free products to meet evolving customer needs in the cell culture and fermentation industry.
- **Custom Media Program**—BD has available a custom media program to meet individual customer requirements. The program offers three levels of customization, from special

Today, BD offers the following Animal-Free products

- BBL™ Phytone™ Peptone
- Difco™ Select Phytone™ UF
- Difco™ Select Soytone

- Bacto™ Malt Extract

- Bacto™ TC Yeastolate
- Difco™ TC Yeastolate, UF

- Bacto™ Yeast Extract
- BBL™ Yeast Extract
- Bacto™ Yeast Extract, Technical
- Difco™ Yeast Extract UF

- Select APS™ LB Broth Base
- Select APS™ Super Broth

- BD Cell™ MAb Medium Animal Component Free
- BD Cell™ MAb Medium Quantum Yield

- Difco™ M9 Minimal Salts, 53
- Difco™ Yeast Nitrogen Base
- Difco™ Yeast Nitrogen Base w/o Amino Acids
- Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

packaging and QC testing requirements to full formula optimization services. It is our goal to service our customers with the highest level of technical support and manufacturing flexibility.

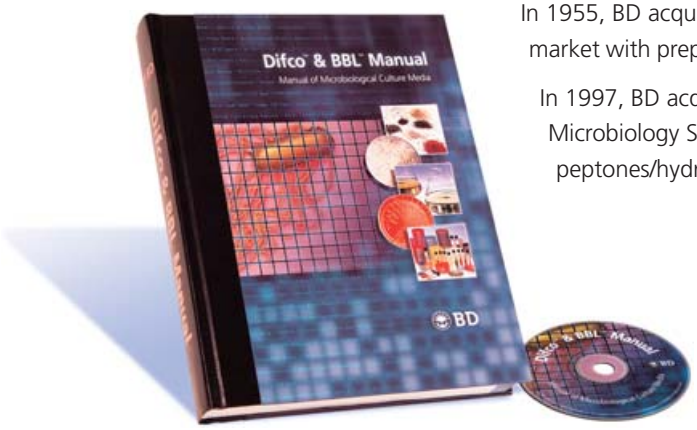
- **Media Optimization Program**—At the highest level of technical interaction, the AutoNutrient™ Media Design Service (AMDS) allows customers to benefit from our high throughput capabilities. By accessing BD expertise in media development, customers can take advantage of our experience in yield enhancement and media formulation scale-up.
- **Dedicated animal-free equipment and environment**—With rising concerns over bovine spongiform encephalopathies and transmissible spongiform encephalopathies (BSE/TSE), BD has met the challenge by dedicating process equipment to the production of animal-free products. Specific orders can be produced in this animal-free environment.

History

Beginning in 1895, Difco Laboratories produced high quality enzymes, dehydrated tissues and glandular products to aid in the digestion process. Meat tissue and other protein digests were developed to stimulate growth of bacteria and fungi. Extensive research led to the development of Difco Bacto Peptone, which was introduced in 1914. The Bacto brand continues to set the standard for premium quality for peptones.

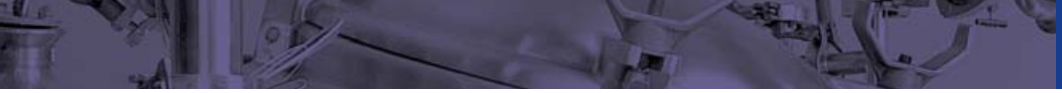
Building on this knowledge base, Difco continued to develop more peptones to add to the Bacto line of products. Bacto Proteose Peptone, Bacto Proteose Peptone No. 2, and Bacto Proteose Peptone No. 3 were created from the accumulated information that no single peptone was the most suitable nitrogen source for growing fastidious bacteria and supplementing cell culture. Today, many cell culture procedures, in addition to microbial cultures, call for the addition of a peptone to enhance yield.

In 1935, the Baltimore Biological Laboratory (BBL) was founded by Theodore J. Carski and Dr. Einar Leifson, employees of the Johns Hopkins Hospital. The laboratory undertook a study of the preparation of peptones. The acronym "BBL" was often used and became the brand name for products offered by the company. New formulations were added, resulting in the development of a full line of culture media. Many of these media utilize peptones of known derivation, such as Trypticase™ Peptone, a pancreatic digest of casein, and Phytone™ Peptone, a papaic digest of soybean meal.



In 1955, BD acquired BBL and used its expertise to continually advance the clinical market with prepared media and diagnostic tools.

In 1997, BD acquired Difco Laboratories and merged Difco Laboratories and BBL Microbiology Systems into one BD division that provides customers with media, peptones/hydrolysates and extracts.



Combined Strengths Build the Largest Breadth of Line

Today, BD offers a broad range of products for cell culture, fermentation and microbiology with a combined total of over 170 years of culture media experience. With products from the Bacto™, Difco™ and BBL™ lines, BD offers peptones/hydrolysates manufactured from meat, animal tissue, collagen, gelatin, casein and from animal-free materials. The combined BD brands provide quality products with lot-to-lot consistency, backed up by BD service, support, and custom programs to address customer requirements. All this, combined with our proactive responses to BSE/TSE concerns makes BD stand out as the best choice for fermentation and cell culture ingredients.

BD brand yeast extracts are produced from primary grown (baker's) yeast and provide lot-to-lot consistency that out-performs brewer's yeast, as well as competitive products. Due to increasing concerns over infectious agents that may be present in animal-based peptones, BD continues to expand its line of non-animal peptones.

Product Quality

Regulatory Compliance—BD plants are ISO 9001 and 13485 Certified, and regularly inspected by the FDA to conform with our cGMP manufacturing practices. We also offer the biotherapeutic industry comprehensive programs in documenting raw material origin, manufacturing change control and Drug Master Files (DMF) for key products.

In our effort to reduce BSE/TSE issues, BD sources raw materials from known BSE-free countries. Raw materials are tested upon receipt to assure that they meet BD incoming specifications. The final products are tested prior to release to assure quality and consistency. After final release, the products are packaged and retention samples are held for stability studies and any additional testing required at a later date.

Certificates of Analysis and Certificates of Origin for each product contain information required for traceability of raw materials included in each product. These certificates are available from the BD web site at **www.bdregdocs.com**.

Service

BD maintains inventory in our BD Distribution Centers in Maryland, USA and Temse, Belgium. With multiple manufacturing locations, BD is prepared to provide products to support customers' needs.

Please contact your local BD representative if you have a need for a product that you do not find in this BD Bionutrients™ Technical Manual or visit our website at **www.bd.com/ds**.

About This Manual

This manual provides insight into using BD Bionutrients for both cell culture and fermentation applications. Every section of the 3rd edition has been updated to provide you with the most relevant information on BD Bionutrients, including the following new features:

- BSE/TSE Risk Mitigation—A new section highlighting the BD response to your need for limiting risk of TSEs.
- Cell Culture Applications—A new essay on bionutrients media and process optimization for cell culture featuring new proliferation and production data.
- Fermentation Applications—A newly-revised section featuring media design and peptone selection for fermentation.
- Animal-Free Peptones and Yeast Extracts—This expanded section now includes Bacto™ Yeast Extract, Technical.
- Casein and Whey Peptones—This expanded section now features Difco™ Casein Digest and Bacto™ TC Lactalbumin Hydrolysate.
- BD Bionutrients™ Media—This new section features three BD Cell™ MAb Medium products: Quantum Yield, Animal Component Free, and Serum Free. Also included are Select APS™ LB Broth Base and Super Broth.

The 3rd edition of the *BD Bionutrients™ Technical Manual* is organized into the following sections:

- Animal-Free Peptones and Yeast Extracts
- Meat Peptones
- Casein and Whey Peptones
- BD Bionutrients™ Media

Individual product descriptions include applications, physical characteristics, and product availability, and are supplemented with new molecular weight distribution charts and performance data. Peptone product sections include chemical analysis and amino acid data. A complete listing of regulatory services is provided and an alphabetical listing of products appears in the back of the manual.

Thank you for your past and continued business.

BSE / TSE Risk Mitigation

History of BSE

Bovine spongiform encephalopathy (BSE), a transmissible spongiform encephalopathy (TSE), was first identified in late 1986, in the United Kingdom. The number of new cases continued to escalate in the UK until reaching a peak in early 1993. By early 1996, there was scientific evidence that BSE had crossed the species barrier to humans, as new variant CJD (Creutzfeldt-Jakob disease) was identified in the UK. However, BSE and vCJD did not stay confined to the UK. Both have expanded their reach to many countries around the world, even though a ruminant-to-ruminant feed ban has dramatically reduced the number of new cases of BSE worldwide. Although there is greater control of the causes of these diseases, neither has been eliminated, and both are fatal and universally feared.

BD Response

Since 1991, BD has continuously worked to limit the BSE risk of our products. Our primary approach has been to focus on controlling the sourcing of the materials we use in the manufacture of our products. Initially, this was done by identifying the countries of animal origin for the bovine-sourced materials, and requiring these to come from BSE-free countries. Later, we expanded this initiative to include identifying the origin and related animal safety and traceability information of all of our animal-sourced materials. Based upon the animal safety and traceability information we obtain from our suppliers, we have developed stringent specifications for our animal-sourced and animal-free materials. BD maintains this information in sophisticated systems and databases, and routinely provides animal origin information for our finished products by way of our Certificates of Analysis. This approach has been and continues to be based upon a close working relationship with our suppliers, and is consistent with what the FDA requires of medical device manufacturers.

Beginning in 1998, BD began focusing on animal-free products in order to meet the growing needs of the biopharm industry. Although the FDA continued to request that only BSE/TSE minimal to no risk materials be used in the manufacture of products of animal origin, the industry was clearly moving away from using BSE/TSE-relevant species wherever possible, and toward animal-free materials. In mid-2003, BD commissioned an animal-free production suite, which has recently been expanded to meet demand. As the demand for animal-free products grows, BD will continue to expand manufacturing capacity to meet the needs of the biopharmaceutical industry.

Hydrolysis to Hydrolysate

Proteins are molecules essential to the structure and function of all living organisms. They are made up of chains of amino acids linked by peptide bonds and folded in a variety of complex structures. Proteins must be broken down into amino acids and peptides by hydrolysis, using strong acids, bases or proteolytic enzymes, in order to provide nutrients in forms that cells may easily utilize. Protein hydrolysates, also called peptones, are the result of the hydrolysis process on protein material.

For over a century, peptones have been used as the main nutritive components in culture media. BD Dehydrated Culture Media (DCM) formulations for diagnostic and fermentation applications are designed around peptones supplying the carbon, nitrogen, minerals and growth factors needed to support microbial growth. Mammalian cell culture media formulations also rely on peptones as supplements and serum replacements. Peptones contain peptides and amino acids, minerals, and other micronutrients that may serve as growth factors and aids to mammalian cell metabolic processes, thereby promoting cell proliferation and production.

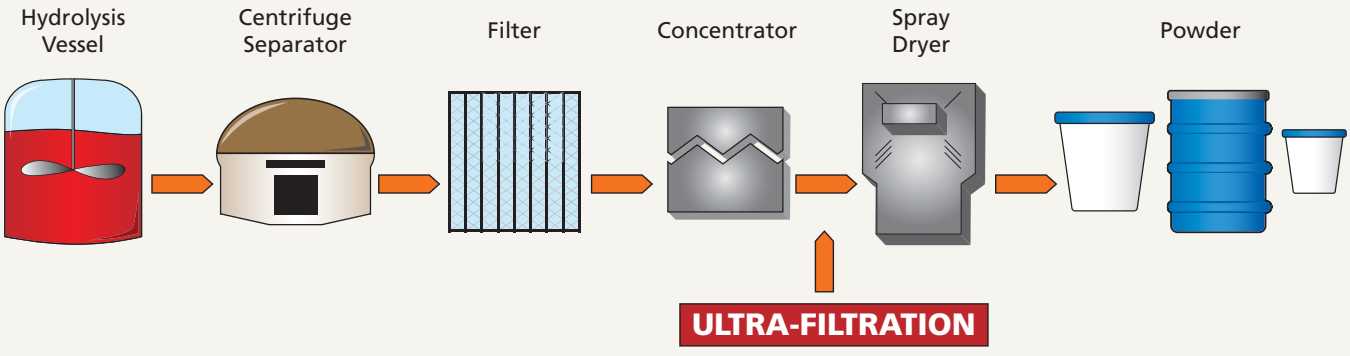
The unique characteristics of each BD peptone product depends on the quality and source of the protein starting material, the quality and source of the enzyme, and the method of hydrolysis used to make the peptone. The starting materials for peptones vary from animal to vegetable. Protein sources include meat, casein and whey (milk proteins), gelatin, soybean, yeast and grains. Enzyme sources include animal organs (pancreatin and pepsin), papaya (papain), fig (ficin), pineapple (bromelain) and microbes.[1] BD offers many different peptones representing numerous combinations of protein and enzyme sources in order to satisfy a wide variety of nutritional and regulatory needs.

Protein Hydrolysis

Acid hydrolysis is a harsh process, usually carried out at high temperature, which attacks all peptide bonds in the protein substrate, destroying some of the individual amino acids liberated. Tryptophan is usually totally lost in an acid hydrolysis. Cystine, serine and threonine

Figure 1

Basic Steps of Peptone Manufacture



are partially broken down and asparagine and glutamine are converted to their acidic forms. Vitamins are mostly destroyed by acid hydrolysis. Salt may be formed during neutralization of an acid hydrolysis, resulting in a product with high salt content.

Proteolytic enzymes hydrolyze proteins more gently than acids, do not require high temperature and usually target specific peptide bonds. The material that results from a proteolytic digestion is a mixture of amino acids and polypeptides of varying lengths. The enzyme pepsin will cut an amino acid chain where there is a phenylalanine or leucine bond. Papain will cut the chain adjacent to arginine, lysine and phenylalanine, and pancreatin shows activity at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds.[2]

Microbial proteases, proteolytic enzymes secreted by microorganisms, are becoming more widely used in peptone production. Proteases from bacterial, algal, fungal and yeast sources cover a wide variety of enzyme activities, can be produced in large scale, and usually require only simple purification.[3]

The hydrolysis process used for preparing yeast extract products is different from the process for protein hydrolysis described above. Yeast extracts are autolysates; yeast cell hydrolysis is performed by enzymes from within the yeast organism itself.

Peptone Manufacture

Most peptones are manufactured similarly, with steps for hydrolysis and downstream processing. Figure 1 shows the basic steps of peptone manufacture: hydrolysis/digestion, centrifugation, filtration, concentration and drying. Protein and demineralized water are combined to form a thick suspension of protein material in large-capacity digestion vessels, which are stirred continuously throughout the hydrolysis process. The protein suspension is then adjusted to the optimal pH for the specific enzyme chosen for the hydrolysis. For example, pepsin is most effective at pH 2.0 and trypsin shows maximum activity at pH 8.5.[1] Enzyme is added when the pH and temperature are optimal. The amount of enzyme necessary, time for digestion, and control of pH and temperature are dependent on the desired degree of hydrolysis.

Products by Category

Animal Free Peptones:

- Bacto™ Malt Extract
- BBL™ Phytone™ Peptone
- Difco™ Select Phytone™ UF (Ultra Filtered)
- Difco™ Select Soytone
- Bacto™ TC Yeastolate
- Difco™ TC Yeastolate, UF
- Bacto™ Yeast Extract
- BBL™ Yeast Extract
- Bacto™ Yeast Extract, Technical
- Difco™ Yeast Extract, UF

Meat Peptones:

- BBL™ Beef Extract, Powder
- Bacto™ Beef Extract, Desiccated
- BBL™ Gelysate™ Peptone
- Bacto™ Neopeptone
- Bacto™ Peptone
- BBL™ Polypeptone™ Peptone
- Bacto™ Proteose Peptone
- BiTek™ Proteose Peptone
- Bacto™ Proteose Peptone No. 2
- Bacto™ Proteose Peptone No. 3
- BiTek™ Proteose Peptone No. 3
- Bacto™ Proteose Peptone No. 4
- BBL™ Thiotone™ E Peptone
- Bacto™ Tryptose

Casein and Whey Peptones:

- BBL™ Acidicase™ Peptone
- BBL™ Biosate™ Peptone
- Bacto™ Casamino Acids
- Bacto™ Casamino Acids, Technical
- Difco™ Casein Digest
- Bacto™ Casitone
- BBL™ Trypticase™ Peptone
- Bacto™ Tryptone
- BiTek™ Tryptone
- Bacto™ TC Lactalbumin Hydrolysate

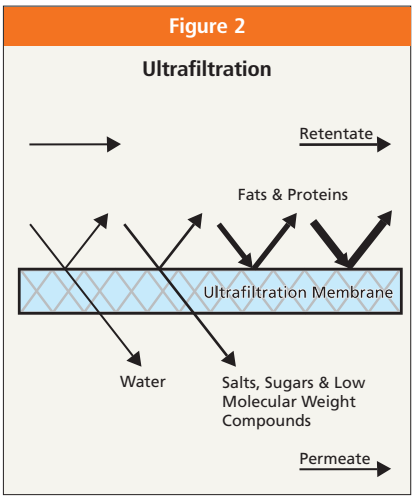
BD Bionutrients™ Media Products:

- BD Cell™ MAb Medium, Animal Component Free
- BD Cell™ MAb Medium, Quantum Yield
- BD Cell™ MAb Medium, Serum Free
- BBL™ Select APS™ LB Broth Base
- Difco™ Select APS™ Super Broth
- Difco™ M9 Minimal Salts, 53
- Difco™ Yeast Nitrogen Base
- Difco™ Yeast Nitrogen Base w/o Amino Acids
- Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Chemically Defined Media Products:

- BD Cell™ MAb Medium, Quantum Yield
- Difco™ M9 Minimal Salts, 53
- Difco™ Yeast Nitrogen Base
- Difco™ Yeast Nitrogen Base w/o Amino Acids
- Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Figure 2



Once the predetermined degree of protein digestion is achieved, the enzymatic activity must be halted; the suspension is either heated to inactivate enzymes or neutralized to inactivate acids or bases. The protein slurry is then centrifuged and/or filtered to remove insoluble materials and to clarify and concentrate the product. Vacuum-evaporation may be used for rapid concentration. This peptone syrup, which contains approximately 67% solids, may then undergo further processing for pH adjustment, pasteurization, and/or filtration. The final drying step of the process further concentrates the peptone by spray-drying or by pan-drying in vacuum ovens, which readies the material for packaging.

Yeast extract manufacture is an exception because it is an autolysate. Baker's yeast, *Saccharomyces cerevisiae*, is usually grown to a high cell density in a molasses-based medium optimized for the particular yeast strain. The batch culture is exposed to a controlled temperature or osmotic shock that causes the yeast to die without inactivating the yeast's endogenous enzymes and begins the autolysis, where the yeast's own digestive enzymes are responsible for breaking down the yeast protein. Once autolysis is halted, insoluble material is separated out by centrifugation and several filtration steps.[4] The final filtration product may then be concentrated and drum or spray dried.

Ultrafiltration

Ultrafiltration (UF) is a membrane filtration process used to separate or concentrate constituents of protein solutions based on molecular weight. BD offers several peptone and yeast extract products that are ultrafiltered using a 10k Dalton Molecular Weight Cut Off (MWCO) membrane. The result of using the 10k Da MWCO is a retentate containing molecules over 10k Da MW, which may include fats, larger MW polypeptides and proteins, and a permeate that contains salts, sugars, peptides, smaller polypeptides and other compounds of less than 10k Da MW. (see Figure 2)

In peptone manufacture, ultrafiltration is used to create a product that is low in endotoxin, the toxin-containing lipopolysaccharide part of the cell wall shed from viable gram-negative bacteria and released when gram-negative bacteria die. Endotoxins will cause illness in humans, so they are considered contaminants that must be avoided or minimized in the preparation of pharmaceutical products. The ultrafiltration step takes place before drying in the peptone manufacturing process.

References

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.
2. Dixon and Webb. 1979. Enzymes, 3rd ed. Longman Group Limited, London.
3. Cowan. 1991. Industrial enzymes. *In* Moses and Cape (ed.), Biotechnology, the science and the business. Harwood Academic Publishers GmbH, Chur, Switzerland.
4. Sommer. 1996. Yeast extract: production, properties and components. 9th International Symposium on Yeasts, Sydney, Australia.

Cell Culture Applications

Introduction

In the biopharmaceutical industry, the requirement for high-performance animal-free processes has prompted a greater focus on media and process optimization.[1] Serum supplementation has traditionally been used to compensate for base media deficiencies. However, the regulatory issues and high costs associated with using serum have led to a widespread effort to find animal free or chemically defined alternatives. While chemically defined media are ideal, they often do not meet the expected production goals. Through additional work it has been observed that peptone supplementation, when appropriately applied, can exceed the performance of serum while meeting stringent regulatory requirements. Additionally, downstream processing requirements are greatly reduced due to the lack of contaminating serum proteins, thereby reducing processing time and costs (Figure 1). In order to achieve this type of success, a complete process optimization must occur where the base media, peptone supplementation, and feed strategy are empirically determined through the use of a methodical optimization strategy.[2]

Base Medium Selection

Base medium selection is critical to the optimization process, so it is necessary to identify a medium prior to initiating any peptone supplementation. In general, complete formulations designed for biopharmaceutical production perform the best with peptone supplementation; so, deficient traditional media can be excluded from the media screen. Figure 2 shows the superior performance of a hybridoma cell line cultured in an optimized formulation compared to the inferior performance of DMEM. The antibody yields were further increased in both base media

Analysis of Peptones for Cell Culture

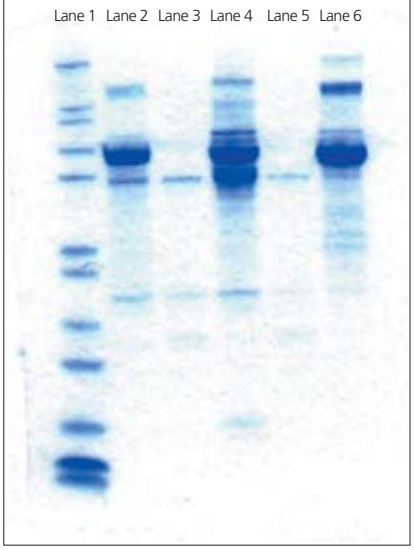
Product Name	Osmolality (µOsm)*	Hypoxanthine (µg/g)*	Thymidine (µg/g)*
Phytone™ Peptone	51	<2	<10
Select Phytone™ UF	52	<2	<10
Proteose Peptone No. 3, Bacto™	53	233	74
Select Soytone	48	18	<10
TC Lactalbumin Hydrolysate	48	7	9
TC Yeastolate UF	64	32	<10
TC Yeastolate, Bacto™	59	31	<10
Tryptone, Bacto™	51	1412	577
Yeast Extract, Bacto™	60	24	<10
Yeast Extract, UF	61	39	<10

* Values derived from an average of three lots

Peptones for Cell Culture

Product	Substrate	Applications
Phytone™ Peptone	Soy	Excellent for growth promotion and protein production, as well as a good, animal-free alternative to serum.
Select Phytone™ UF	Soy	An ultrafiltered version of Phytone with an endotoxin high limit of 500EU/g.
Proteose Peptone No. 3	Porcine	Excellent for growth promotion and protein production, as well as a good alternative to serum.
Select Soytone	Soy	Excellent for growth promotion and protein production.
Bacto™ Tryptose	Meat	Excellent serum-free supplement for human diploid fibroblasts.
TC Lactalbumin	Milk	Excellent for amino acid supplementation.
TC Yeastolate	Yeast	Good for growth promotion, especially with insect cells.
TC Yeastolate UF	Yeast	An ultrafiltered version of TC Yeastolate with an endotoxin high limit of 500EU/g.
Bacto™ Yeast Extract	Yeast	Good for growth promotion, especially with insect cells.
Yeast Extract UF	Yeast	An ultrafiltered Yeast Extract with an endotoxin high limit of 150EU/g.

Figure 1



Lane 1: 2.5–200 kDA Marker
Lane 2: BSA Supplementation
Lane 3: Peptone Supplementation
Lane 4: 10% FBS Supplementation
Lane 5: Peptone Supplementation
Lane 6: BSA Supplementation

when peptones were added, but yields in the optimized medium were greater than 2-fold higher compared to the DMEM conditions.

The ideal base medium is one that requires minimal to no cellular adaptation. Also, it must demonstrate the ability to maintain viable cell numbers for an extended period of time while achieving an acceptable level of production. Although there are many good commercially available media, none are designed to meet the specific requirements of a particular cell line. Base medium optimization is an effective method for identifying a cell specific formulation.[3] The most effective base media optimizations identify the optimal concentration for each component in the context of the other components. To ensure a successful optimization, all supplements required by the cells should be added to all of the media at each optimization step.

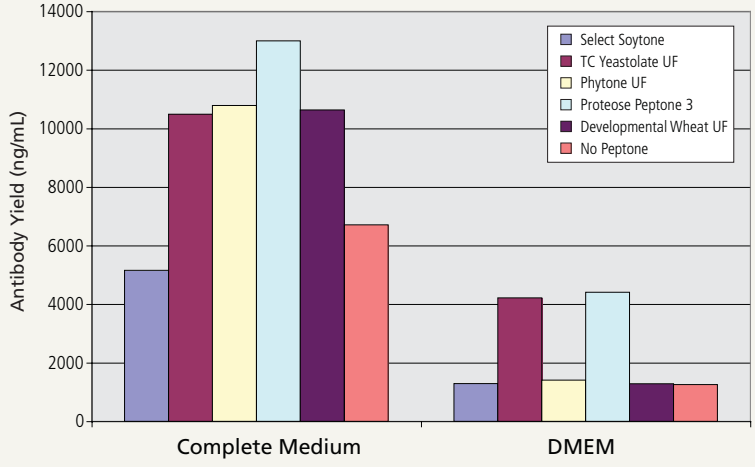
Peptone Selection

The benefits of peptone supplementation in cell culture applications have been well documented for many years. Due to the complex composition of peptones, they provide a wide range of benefits to the cells. In some cases, peptides of various lengths have resulted in increased cell performance.[4] Others have benefited from free amino acids and other low molecular weight nutrients.[5] Since the nutritional requirements for each cell line are different, it is important to identify a peptone that will meet the unique requirements of a particular cell line. Figure 3 shows the different antibody yields achieved with a CHO line tested with different peptones supplemented at 5 g/L in the same complete medium. The wide range of antibody yields demonstrates the diversity of cell requirements and the importance of selecting the right peptone.

With the number of peptones that are available, it is critical to evaluate a wide variety of products. Since every peptone is different, multiple peptones produced using the same base material should be included. In order to run the most effective peptone screen, the list of potential peptones should be narrowed to include only the best candidates. This can be accomplished with an understanding of the specific regulatory and process requirements.

Figure 2

Antibody Yields of Various Peptones at 3 g/L Hybridoma Cell Line in Different Base Media



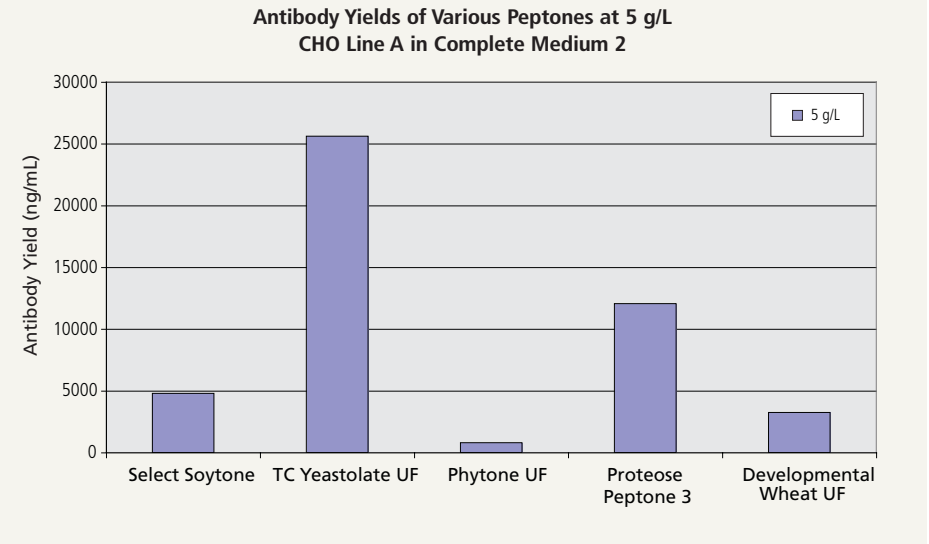
While animal-derived peptones have shown good performance, they should be excluded from the screen if there is an animal-free requirement. Endotoxin levels are usually a concern, so the screen should be limited to ultra-filtered (UF) peptones. Also, if the cell line utilizes the Glutamate Synthetase (GS) expression system, wheat peptones might be excluded from the screen due to their glutamine rich composition. Regulatory requirements and release criteria should be considered as these factors vary between manufacturers.

Once the appropriate peptones have been identified, an experimental design should be created in which each peptone is evaluated in a pre-selected base medium at a wide range of concentrations. Culture performance will be different for each cell line, peptone, and base medium combination, making it critical that a number of concentrations are evaluated for each peptone.[6] All of these factors were studied to demonstrate the impact each of these factors has on the production process. Figures 4 and 5 show the performance difference when a CHO line is evaluated with two different peptones in the same base medium. A CHO line and a hybridoma line were tested with the same peptone and base medium combination (Figures 6 and 7). Finally, a CHO line was evaluated in two different base media with the same peptone (Figures 8 and 9). In each situation, the production and proliferation profiles were different when one factor in the study was changed. An effective peptone screen should consider all of these factors to ensure the appropriate peptone and concentration are identified.

Blends of peptones should also be considered, as synergistic effects have been observed in some processes when multiple peptones were used.[7] In Figure 10, a CHO line was evaluated with a blend of peptones, an individual peptone, and without any peptone. For this cell line and base medium combination, the peptone blend resulted in a significantly higher antibody yield compared to the other two conditions.

Significantly higher antibody yields can be achieved with the identification of a peptone that meets the specific requirements of the cell line. Selection of the peptone or blend of peptones should be based upon both the proliferation and production data since these two parameters do not always correlate in a production process.

Figure 3



Process Optimization

The improved performance obtained through the identification of a new base medium and peptone supplementation will be further enhanced when coupled with an effective feed strategy.[8] Using spent media analysis to understand the cell's nutritional requirements makes it possible to design a peptone feed strategy that greatly enhances the process performance. In some cases a chemically defined feed may be utilized. However, in many situations a peptone based feed can result in substantial increases in cell proliferation and production.

Determining how to apply the peptone is essential to achieving the increased performance. While some processes require that peptone is present from the beginning of the run, others perform best when the peptone is added as a feed later in the process (Figure 11). In some cases, optimal performance is achieved when the process begins with one peptone then another is added as a feed later in the production run (Figure 12).

Having a thorough understanding of the production process is critical for maintaining consistent results. There are many potential sources of variability and each needs to be identified and controlled. Sources of variability include base media between manufacturers or lots, key components added at the beginning or as a feed supplement, and the use of generic manufacturing processes that are suboptimal for a particular cell line. Peptones are also cited as a

Figure 4

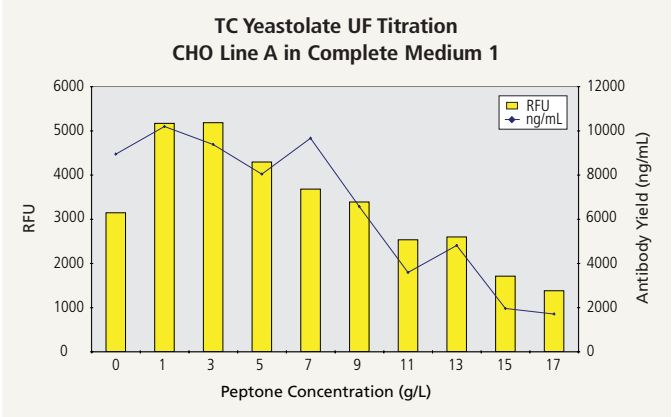


Figure 5

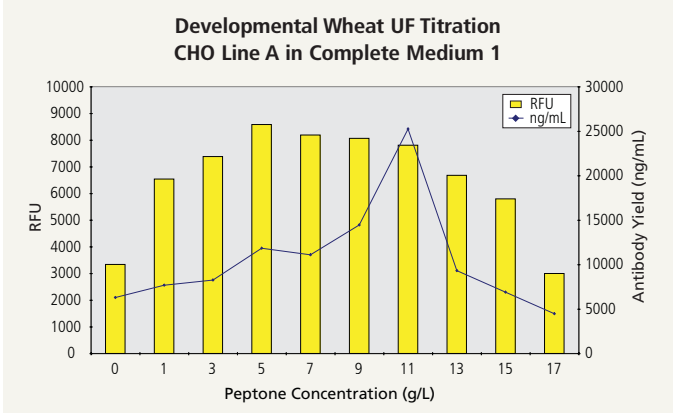


Figure 6

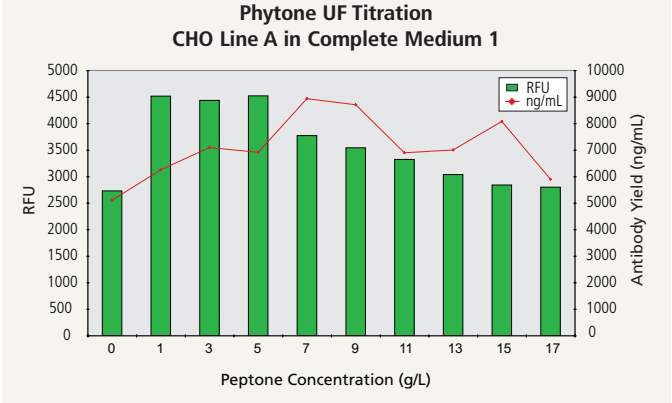
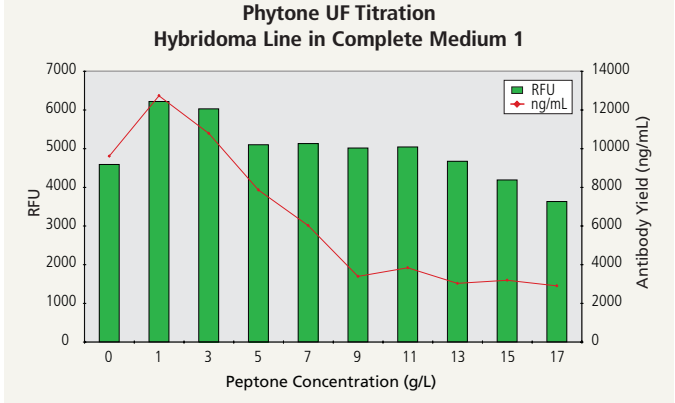


Figure 7



potential source of variability. Peptones designed for the biopharmaceutical industry are manufactured and released to strict specifications. In general, the variability that is observed when these peptones are used is the result of their use in a poorly understood biopharmaceutical production process. Through a comprehensive analysis of the spent media from multiple production runs using multiple peptone lots, key component concentrations can be identified and maintained at the appropriate levels. Once the process is properly controlled, the expected results will be consistently achieved.

BD AutoNutrient™ Media Design Service (AMDS)

Performing thorough optimizations can require significant time and resources, so the decision is often made to eliminate many potentially critical design points. By truncating the design, the desired production goal could be missed. To address this need and ensure the identification of the optimal media formulation that meets production goals, BD offers the AutoNutrient™ Media Design Service (AMDS). The BD team of dedicated, experienced scientists works with each customer in a highly collaborative process to develop a media formulation that satisfies the requirements. Through the AMDS program, BD offers a library of 45 diverse, chemically defined media, as well as a number of peptones designed specifically for the biopharmaceutical industry. Proprietary DOEs are used to optimize a base medium specifically for the cell line or

Figure 8

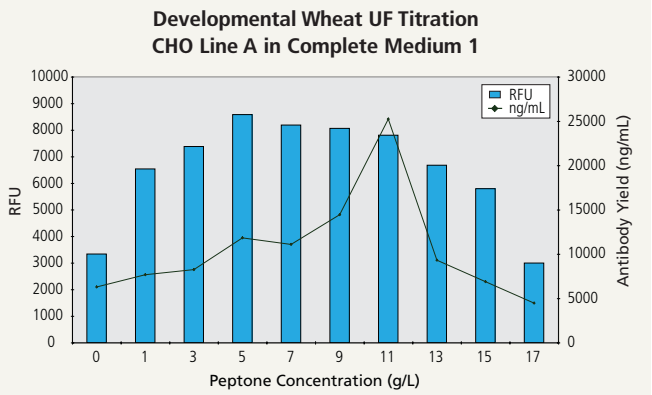


Figure 9

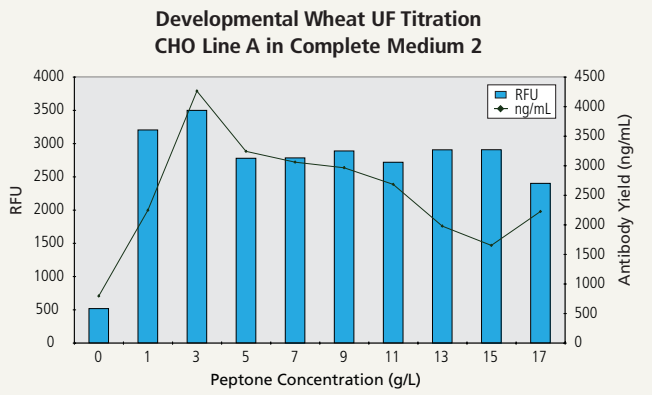


Figure 10

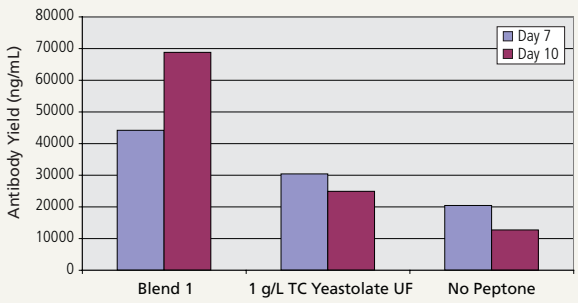


Figure 11

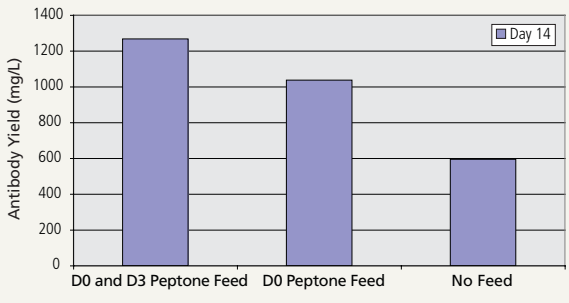
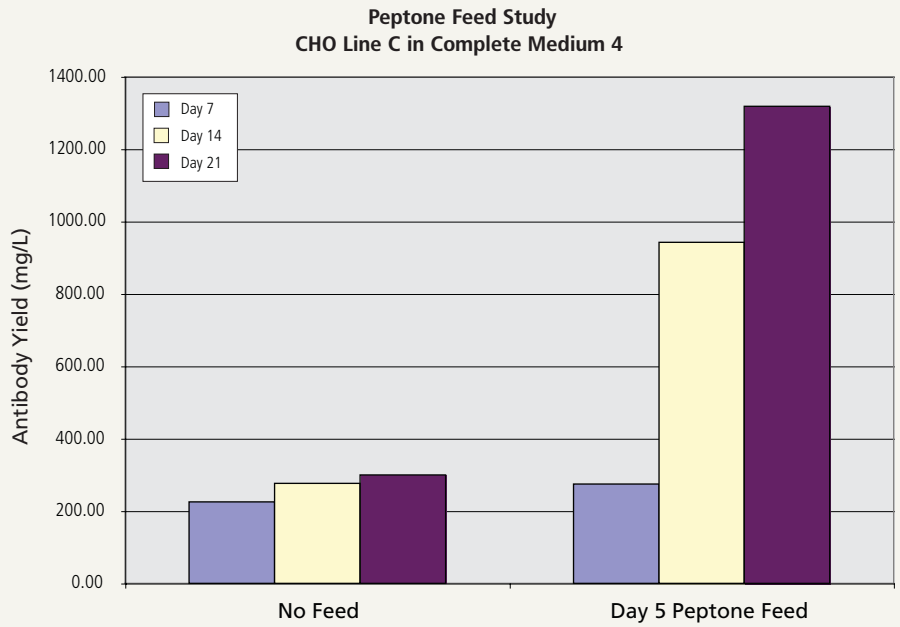


Figure 12



rapidly identify the appropriate peptone supplementation. Spent media analysis is quickly performed using the vast analytical capability of BD. AMDS partners with the customer from initial screens through final scale up to ensure an optimized process at each step.

References

1. Jerums and Yang. 2005. Optimization of cell culture media. *In* Scott (ed.) Culture media, a growing concern in biotechnology. BioProcess International. *3*(3):38-44.
2. Burteau, Verhoeye, Mols, Ballez, Agathos and Schneider. 2003. Fortification of a protein-free cell culture medium with plant peptones improves cultivation and productivity of an interferon-g-producing CHO cell line. *In Vitro* Cell Dev. Biol. Anim. *39*(7): 291-296.
3. Hammett, Kuchibhatla, Hunt, Holdread and Brooks. 2005. Developing chemically defined media through DOE: complete optimization with increased protein production in less than 8 months. Presented at ESACT 2005.
4. Franek, Eckschlager and Katinger. Enhancement of monoclonal antibody production by lysine-containing peptides. Biotechnol. Prog. *19*(1):169-174.
5. Heidemann, Zhang, Qi, Rule, Rozales, Park, Chuppa, Ray, Michaels, Konstantinov and Naveh. 2000. The use of peptones as medium additives for the production of a recombinant therapeutic protein in high density perfusion cultures of mammalian cells. Cytotechnology. *32*(2):157-167.
6. Holdread, Hunt, Oshunwusi and Brooks. 2004. Advances in media optimization: approaches that increase expression while reducing development time. Presented at Cell Engineering Meeting, 2004.
7. Kuchibhatla, Hunt; Holdread and Brooks. 2004. A rapid and effective screening process of animal component free hydrolysates to increase cell performance. Presented at IBC, September 2004.
8. Holdread, Kim, Oshunwusi, Kuchibhatla, Hunt and Brooks. 2005. Rapid media and process optimization using DOE: a case study. Presented at ESACT 2005.

Fermentation Applications

Defined vs. Complex Media

Fermentation media formulations are of two types: defined and complex. Defined media are made by the addition of chemically-defined ingredients to water for injection (WFI) or distilled water. Complex media are made with peptone digests or extracts of plant or animal origin (see "Hydrolysis to Hydrolysate").[1] The advantages of chemically-defined media are greater reproducibility, cleaner downstream processing steps and simplicity in the analysis of the end product. The disadvantages are lower yields and greater expense, especially if the list of media components include growth factors and vitamins.[2] The advantages of complex media are that they are relatively inexpensive, they support a wide variety of growth from a large group of microorganisms, they promote growth of the more fastidious organisms that will not grow on a chemically-defined medium, they stimulate toxin production and they routinely produce higher yields than many defined media. The disadvantages of complex media are that the downstream processing may be more difficult and reproducibility can sometimes be compromised.

Media Design

The role of the medium is to provide essential nutrients that can be utilized and integrated into the dividing cells of the fermentation. A properly designed medium should contain carbon, nitrogen and energy sources, as well as, essential growth factors (vitamins and trace minerals).[3] Selection of medium components can have an impact on growth, function, even the genetic stability of cells *in vitro*. Some components may perform multiple roles, e.g. peptones act as both a carbon source as well as a nitrogen source. Many peptones also provide buffering capacity for the media. It is simpler to design a medium for rapid initial growth than for maximum product accumulation especially in the case of secondary metabolites. It is essential to understand the dynamics of your organism's product production. Phosphate levels and the presence/absence of iron are often cited as affecting secondary metabolite production.[4]

Selecting a Peptone

Successful media development is a multifaceted process. In order to comprehensively cover all the variables with the least time and effort it is usual to employ statistical methods.[5] When developing a new formulation, care should be taken in choosing the peptones for the new formulation. Individual experimentation with a variety of peptones is suggested to select the optimum peptone or combination of peptones. Figures 1 and 2 demonstrate such a preliminary screen for multiple peptones and two different organisms. Each peptone was prepared as 1% solution with 0.4% glucose and buffering salts. Growth testing was performed using the Labsystems Bioscreen C Kinetic Optical Density Reader. Review of the growth support curves for Proteose Peptone No. 3 illustrates poor growth support (Figure 1), while Figure 2 shows good growth support. Based on these results, one would likely create a formulation consisting of TC Yeastolate and Proteose Peptone No. 3 to support the growth of *Enterococcus faecalis*. Another important consideration for media formulation development pertains to recent influx of

Figure 1

***S. cerevisiae* Growth on Peptones**

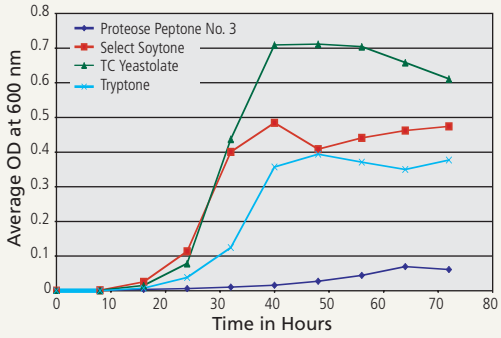


Figure 2

***E. faecalis* Growth on Different Peptones**

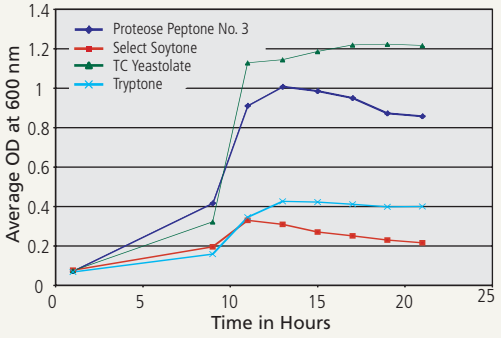
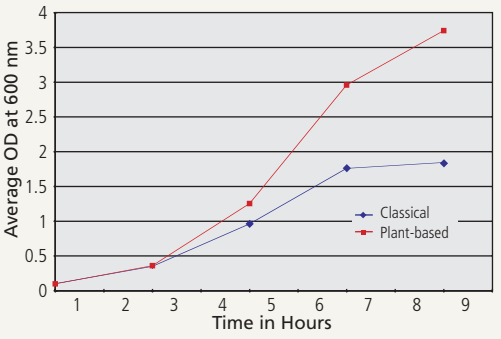


Figure 3

Comparison of *E. coli* Growth on Select APS LB Broth vs. Classical LB Broth



E. coli was grown at 35°C and 250 rpms in baffled shake flasks.

Figure 4

Comparison of *E. faecalis* Growth on Traditional Media vs. Plant-based Media

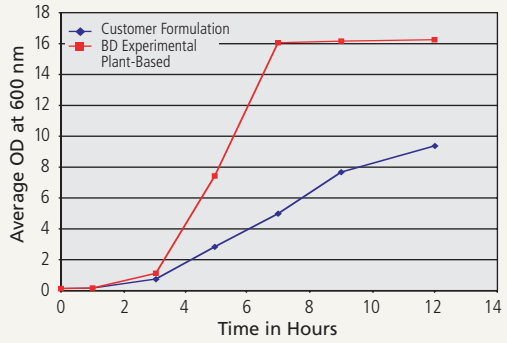


Figure 5

Growth of DH5a *E. coli* with Plasmid on Various Peptones

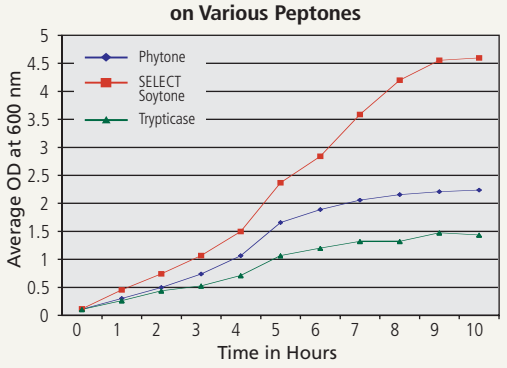
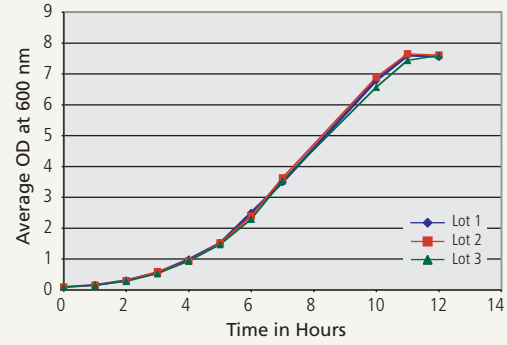


Figure 6

Three Lot Comparison of Select APS Super Broth



ultrafiltered peptones in the market. When switching a formulation from peptones to ultrafiltered peptones, a titration could be necessary to determine the optimum amount of ultrafiltered peptone necessary in the new formulation. For example, 5 g/L may have worked in the original formula but 5.5 or 6 g/L may be needed in the new ultrafiltered formulation.

Moving to Animal-Free Media Components

With the continuing emergence of new confirmed cases of BSE/TSE, a prime directive for the development of new fermentation products has been to either source raw materials from a country free from BSEs or reformulate the media using animal-free components.[6]

BD began offering animal free alternatives to classical media formulations in 1997. In 1998, we introduced our line of Select APS™ (alternative protein source) products. In the case of the LB Broth reformulation, the performance of the test organism, *E. coli* DH5a was enhanced (Figure 3). The experiment was conducted in a shake incubator set at 250 rpm and 35°C.

Another example of enhanced performance is demonstrated in Figure 4. *E. faecalis* was grown side-by-side in New Brunswick BioFlo 3 fermentors. In this case growth enhancement, as measured by the mass or OD reading, doubled when the medium formulation was changed to all animal-free components.

Figure 5 shows growth curves for two of the five different soy peptones available from BD. It also demonstrates the differing responses an organism may have to different peptones made with the same starting materials. For an *E. coli* with a plasmid, the Select Soytone provides better growth support than Trypticase™ Peptone. In this experiment the peptones were in 2% solutions with some buffering salts. The purpose of the experiment was to observe what type of growth support the individual peptones contributed to a multi-component medium.

Figure 6 demonstrates the rigorous quality control testing these media undergo. Three lots of Select APS™ Super Broth were growth tested using an *E. coli* strain containing a plasmid. The three growth curves are nearly identical in their growth support. Product consistency is demonstrated through the quality control testing of the final product.

References

1. Demain and Solomon. 1986. Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, D.C.
2. Cote. 1999. Media composition, microbial, laboratory scale. *In* Flicker and Drew (ed.), Encyclopedia of bio process technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.
3. Moat and Foster. 1995. Microbial physiology, 3rd ed. Wiley-Liss, New York.
4. Corbett. 1985. Design, preparation and sterilization of fermentation media. *In* Moo-Young (ed.), Comprehensive biotechnology, vol. 1. Pergamon, Oxford.
5. Haaland. 1989. Experimental design in biotechnology. Marcel Dekker, New York.
6. Baron, Safar, Groth, DeArmond and Prusiner. 2001. Prions. *In* Block (ed.), Disinfection, sterilization, and preservation, 5th ed. Lippincott Williams & Wilkins, Baltimore.

Animal-Free Peptones and Yeast Extracts

Traditionally, microbial and mammalian cell lines have been propagated in complex media containing animal components. Prion disease (BSE and CJD) outbreaks have caused media formulators to eliminate animal-sourced components in their new media formulations.[1] In response to this trend, BD has developed a broad offering of soy peptones, yeast extracts and animal-free media.

In addition to the more common animal-free components such as soy and yeast, many new plant-based products are being tested in the market. Wheat, pea and potato have become candidates of experimental interest. These plant-based materials offer a new supplementation source with potentially strong performance characteristics.

Soy peptone, one of the first successful plant-based peptones to be optimized into cell culture, is processed in several different ways to provide various nutrient mixes. Yeast Extracts and Tissue Culture (TC) Yeastolates offer a different range of nutritional choices to enhance production in both bacterial fermentation and (as a supplement) in cell culture.

Soy Peptones

The BD Difco™ Soy Peptones are all enzymatic digests of soy flour. Soy contains several heat labile protease inhibitors.[2] The most common way of eliminating these factors is to heat or toast the defatted soy beans in a processing plant under controlled conditions. Soy flour, the principle substrate in a soy peptone, is rich in high-quality protein, carbohydrates, calcium and B vitamins.[3] The enzymes used in the digestion of soy flour are typically from animal-free sources or from microorganisms that have been grown in animal-free media.

Yeast Products

Yeast extract is defined in the USP as "a water-soluble, peptone-like derivative of yeast cells (*Saccharomyces*)."[4] Yeast extract is readily available in the U.S. as a spray-dried powder. In Europe, pharmaceutical companies use it as a liquid or paste, as well as in the powdered form.

Yeast extract is used by the health food industry as an inexpensive source of vitamins, and has long been recognized as a major source of B-complex vitamins. Yeast extract, as a substrate in a media formulation, supplies not only vitamins, but also proteins, carbohydrates and some micronutrients.

There are many kinds of yeast extract. The two principle sources of yeast extract are "brewer's" yeast and "baker's" yeast. Brewer's yeast is a by-product from the brewing industry. It requires de-bittering (removal of hop resins) before it is suitable for fermentation use.[5] A wide variety of strains and growth processes have been used in the manufacture of brewer's yeast, thus precluding any consistency of the final product.

Baker's yeast (*Saccharomyces cerevisiae*) is defined as a primary yeast because the yeast is grown for the specific purpose of being used as a substrate in a bioprocess or as a food product/flavoring. Manufacture of baker's yeast is a reproducible and controlled process. The yeast organism is grown on a molasses-based medium optimized for the specific yeast.[6] Commercial yeast fermentations are always fed-batch type fermentations lasting from 12-20 hours.[7] Commercial baker's yeast manufacturers have found that the more highly aerated a culture, the higher the final product yield.[7]

The process of manufacturing baker's yeast extract is unique compared to the manufacture of peptones. Yeast extract is an autolysate; i.e. cell hydrolysis is performed by the endogenous

enzymes of the *Saccharomyces* organism. Autolysis is usually begun by either a controlled temperature shock or, for the food industry, an osmotic shock, which causes the yeast cells to expire. The temperature shock is not high enough to inactivate the proteases of the yeast cell, which proceed to degrade the cell. Autolysis can proceed from 10 to 60 hours. After autolysis, soluble material is separated from the insoluble material by means of centrifugation and several filtration steps.[7] The final filtration product is concentrated and then spray dried, or can be left in the concentrated paste form, which contains approximately 60-80% solids.

Temperature, pH, addition of other enzymes, type of medium substrate for the growth of the *Saccharomyces* and duration of autolysis are all variables that create the large variety of yeast extracts available.

References

1. Collinge. 2001. Prion diseases of humans and animals: their causes and molecular basis. Annu. Rev. Neurosci. *24*:519-50.
2. Kunitz. 1945. Crystallization of a trypsin inhibitor from soybeans. Science. *101*:668-9.
3. Human Nutrition Service, U.S. Department of Agriculture. 1986. Composition of foods: legume and legume products. Agriculture handbook, No. 8-16, revised. U.S. Department of Agriculture, Washington, D.C.
4. United States Pharmacopeial Convention, Inc. 2004. The United States pharmacopeia 27/The national formulary 22–2004. United States Pharmacopeial Convention, Inc., Rockville, Md.
5. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.
6. Sommer. 1996. Yeast extract: production, properties and components. 9th International Symposium on Yeasts, Sydney, Australia. http://www.ohly.de/sommer.htm.
7. Reed and Nagodawithana. 1991. Yeast technology, 2nd ed. Van Nostrand Reinhold, New York.

Bacto™ Malt Extract

Availability

Product Description	Cat. No.	Qty.
Bacto™ Malt Extract	218630	500 g
Bacto™ Malt Extract	218610	10 kg

Product Description

Bacto™ Malt Extract is the water-soluble portion of malted barley. The extraction process breaks down the polysaccharides into simple sugars. After the malting process is complete, the extract is prepared from the malted barley by cracking the grain in a mill and then extracting the grain with a warm liquor. The resulting "wort" is filtered and evaporated or dried under vacuum.[1,2]

Potential Applications

Bacto Malt Extract is used in the culture of yeasts and molds. Bacto Malt Extract is very high in carbohydrate content.[3] This product is suitable for the culture of yeasts and molds because of the high concentration of reduced sugars, especially the maltoses. Malt extract in the agar form is recommended for the detection and isolation of yeasts and molds from dairy products and foods and as a medium for stock culture maintenance.

Physical Characteristics

Bacto Malt Extract is a medium tan, free-flowing, homogeneous powder.

References

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.
2. How malt is made, Briess Malting Company. 2 Dec. 2002. http://www.briess.com/HomebrewNew/hbhow.htm
3. Cote. 1999. *In* Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.

BBL™ Phytone™ Peptone
Difco™ Select Phytone™ UF
Difco™ Select Soytone
Bacto™ Soytone

Product Description

All the Difco™ and BBL™ brand soy peptones are enzymatic digests of soybean meal/flour. They are recommended for use in media for the cultivation of a wide variety of organisms, including fungi. The soybean protein in these peptones contains naturally occurring high concentrations of vitamins and carbohydrates.

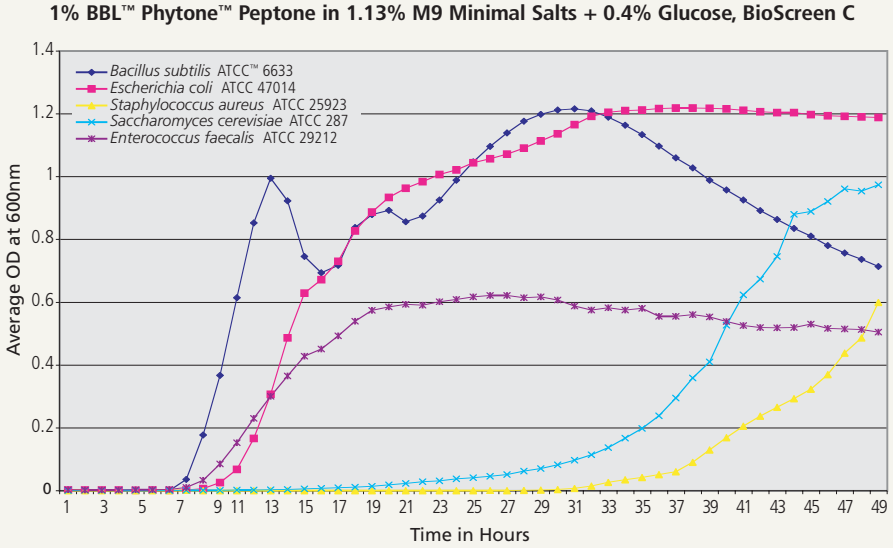
Potential Applications

BD offers a diverse choice of soy peptones. The individual characteristics of each peptone are the result of processing methods engineered to consistently deliver these characteristics from batch to batch. The nutritional requirements of microorganisms and cell lines vary according to each individual strain. While some organisms or cell lines may prefer short chain or free amino acids, others benefit from longer chain amino acids. While the typical analysis profiles for each peptone in this manual can help direct the end-user to the correct peptone match, it is recommended that end-users supplement the typical analytical information with evaluations in their own individual growth models.

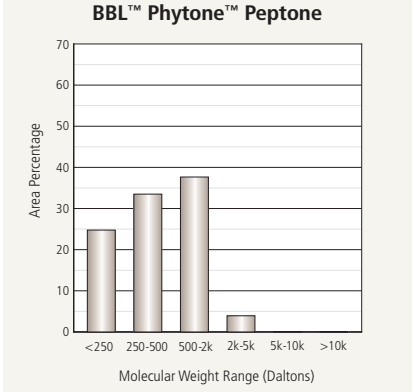
Select Phytone™ UF is an ultrafiltered peptone that was developed specifically for the tissue culture market. Its nitrogen content combined with the naturally occurring vitamins has demonstrated remarkable growth support with monoclonal antibodies and protein expression. It has an endotoxin level of less than or equal to 500 EU/g.

BD offers three other soy peptone products suitable for a variety of bacterial cultures.

Growth Curve



Molecular Weight



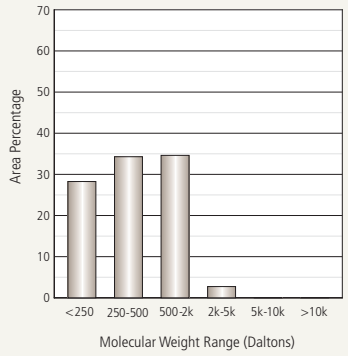
Phytone Peptone is an animal-free soy peptone. Phytone Peptone retains the high vitamin and high carbohydrate content of the soy plant tissue. It is an excellent plant peptone for the cultivation of fungi and fastidious types of bacteria, such as members of the *Clostridium* and *Neisseria* genera.[1] It has been used in cell culture applications due to its high carbohydrate content.

Select Soytone demonstrates excellent growth support for *Escherichia coli*. Select Soytone is also used in Select APS™ Super Broth. Subtle differences in the digestion process give Select Soytone improved performance in cell culture.

Bacto™ Soytone was found to be effective in the recovery of stressed *E. coli*.[2] It was found that Bacto Soytone with the addition of 7 vitamins replaced yeast extract as an economical

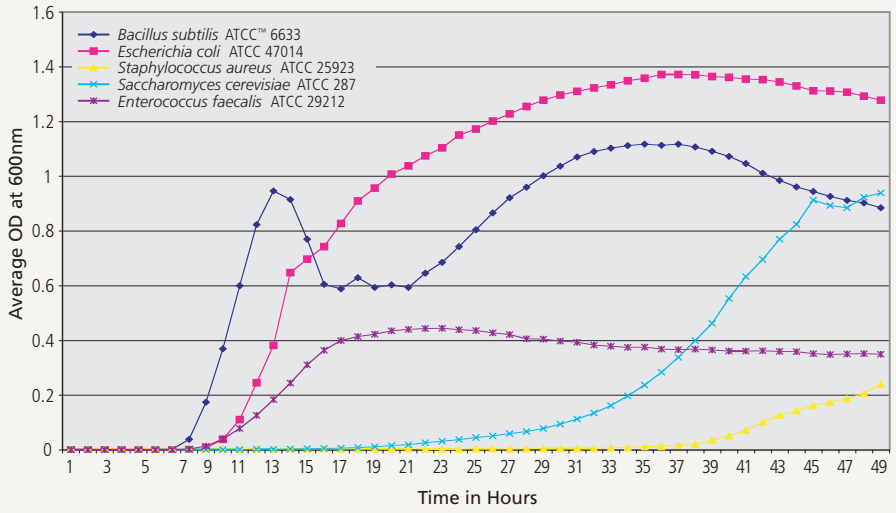
Molecular Weight

Difco™ Select Phytone™ UF



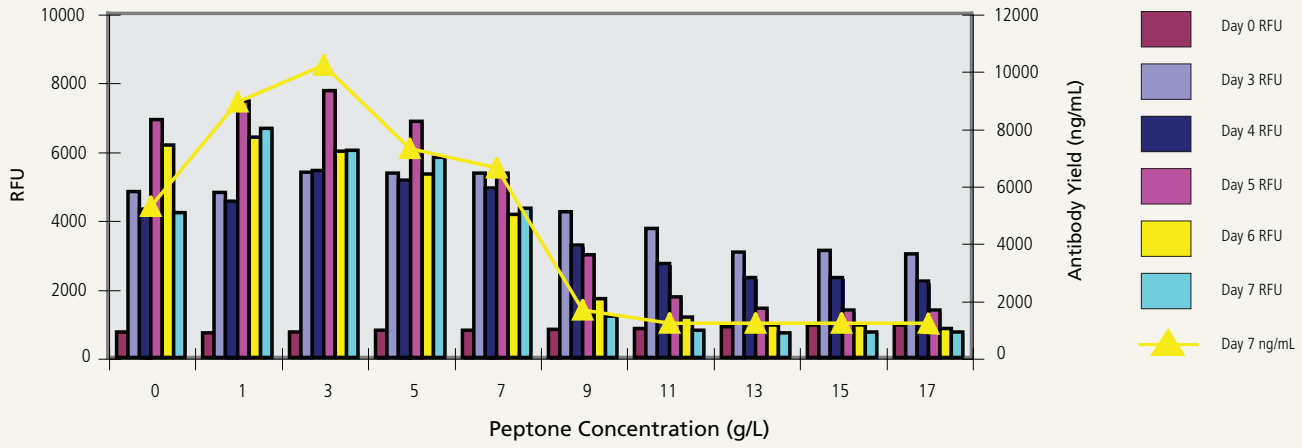
Growth Curve

1% Difco™ Select Phytone UF in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Titration

Phytone™ UF Titration
Hybridoma Proliferation and Production Data



alternative for the production of lactic acid by *Lactobacillus rhamnosus*.[3] It should be noted that Bacto Soytone utilizes an animal based enzyme in the digestion of the soy flour.

Physical Characteristics

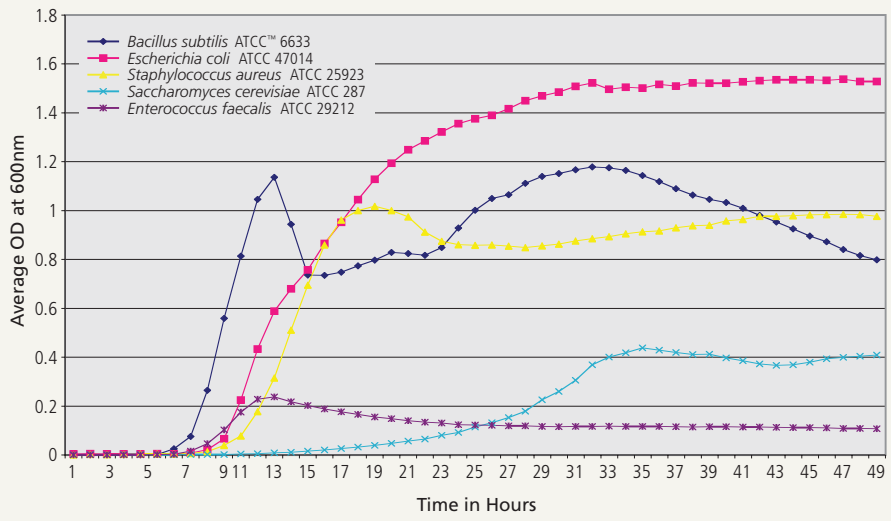
BBL™ Phytone™ Peptone is a light tan, free-flowing, homogeneous powder.

Difco™ Select Phytone™ UF is a light tan, free-flowing, homogeneous powder.

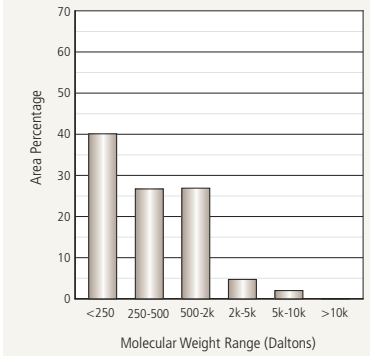
Difco™ Select Soytone is a tan, free-flowing, homogeneous powder.

Bacto™ Soytone is a light to medium tan, free-flowing, homogeneous powder.

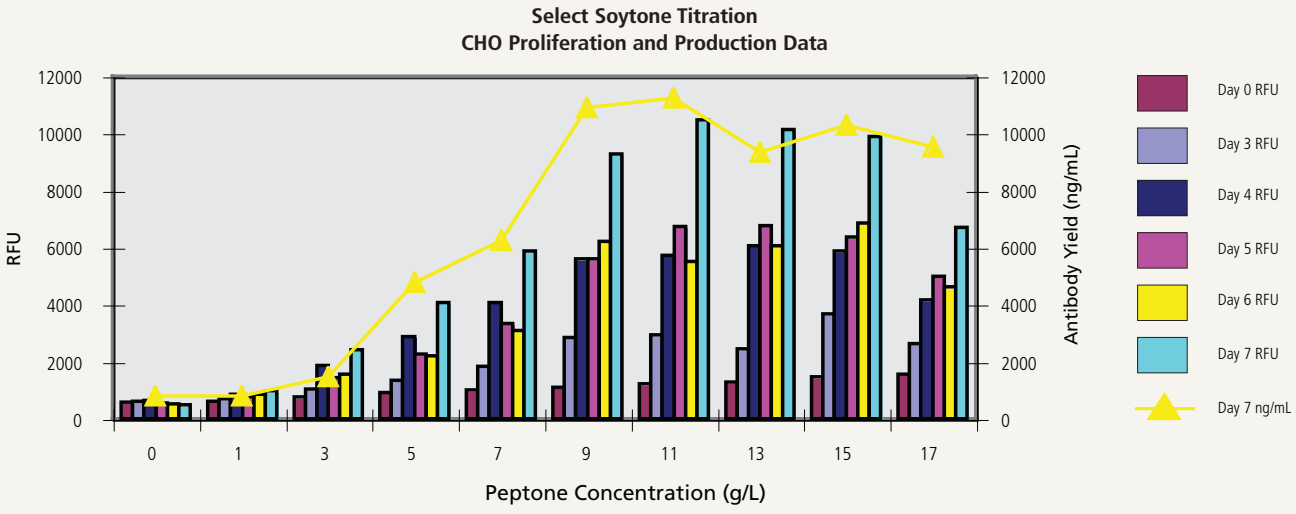
Growth Curve



Molecular Weight

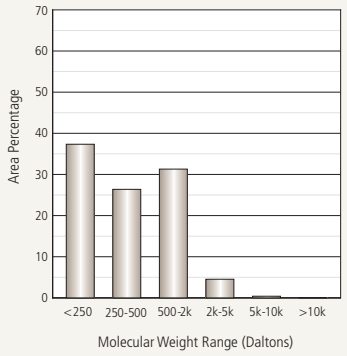


Titration



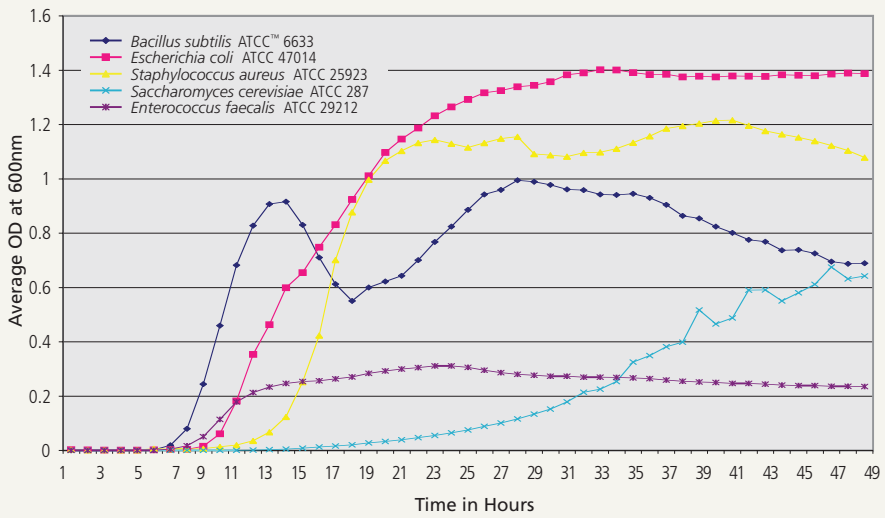
Molecular Weight

Bacto™ Soytone



Growth Curve

1% Bacto Soytone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Availability

Product Description	Cat. No.	Qty.
BBL™ Phytone™ Peptone	211906	454 g
BBL™ Phytone™ Peptone	298147	5 lb (2.3 kg)
BBL™ Phytone™ Peptone	292450	10 kg
Difco™ Select Phytone™ UF	210931	500 g
Difco™ Select Phytone™ UF	210936	10 kg
Difco™ Select Soytone	212488	500 g
Difco™ Select Soytone	212489	10 kg
Bacto™ Soytone	243620	500 g
Bacto™ Soytone	243610	10 kg

References

1. Power (ed.). 1988. Manual of BBL™ products and laboratory procedures, 6th ed. Becton Dickinson Microbiology Systems, Cockeysville, Md.
2. Chou and Cheng. 2000. Recovery of low-temperature stressed *E. coli* O157:H7 and its susceptibility to crystal violet, bile salt, sodium chloride and ethanol. Int. J. Food Microbiol. *61*:127-136.
3. Kwon, Lee, Lee, Chang, Keun and Chang. 2000. Production of lactic acid by *Lactobacillus rhamnosus* with vitamin-supplemented soybean hydrolysate. Enzyme Microb. Technol. *26*:209-215.

Bacto™ TC Yeastolate
Difco™ TC Yeastolate, UF

Product Description

TC Yeastolate products are animal-free and water-soluble portions of autolyzed yeast or *Saccharomyces cerevisiae*. TC Yeastolate is a mixture of peptides, amino acids, carbohydrates, simple and complex as well as vitamins. TC Yeastolate, UF has been ultrafiltered at a 10,000 MWCO (Molecular Weight Cut-Off). It has an endotoxin value of less than 500 EU/g.

Potential Applications

TC Yeastolate products are intended as nutritional supplements for bacterial, insect and mammalian cell culture. TC Yeastolate has been used in insect cell nutrition. TC Yeastolate was found to be a very versatile supplement to enhance growth and production characteristics of Sf9 and High Five™ cells.[1-5]

Physical Characteristics

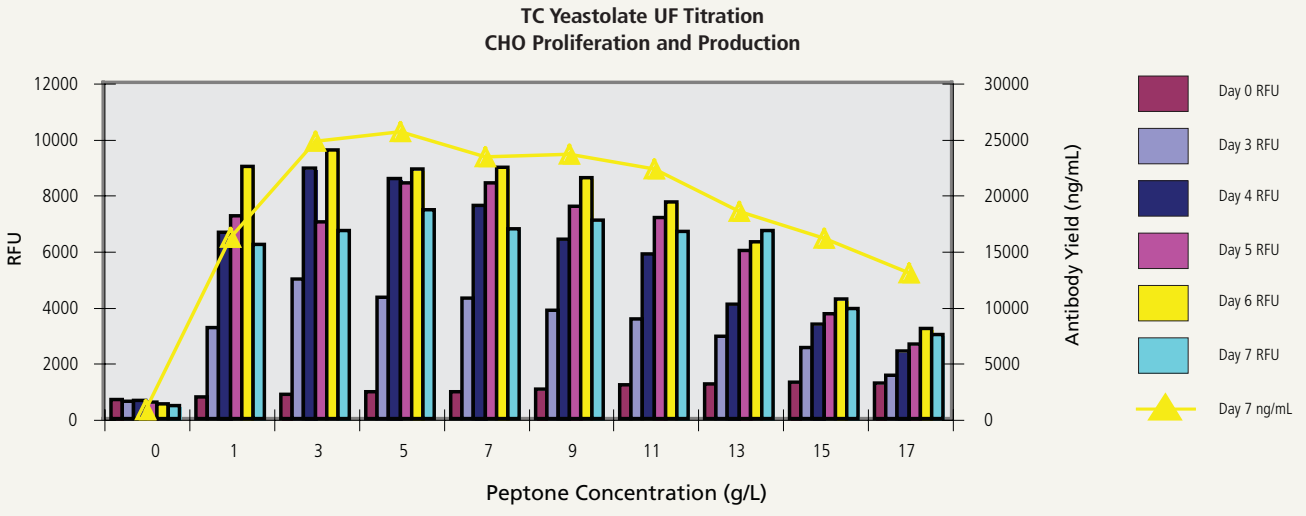
Bacto™ TC Yeastolate is a beige free-flowing, homogeneous, spray-dried powder.

Difco™ TC Yeastolate, UF is a free-flowing, homogeneous, spray-dried powder.

Availability

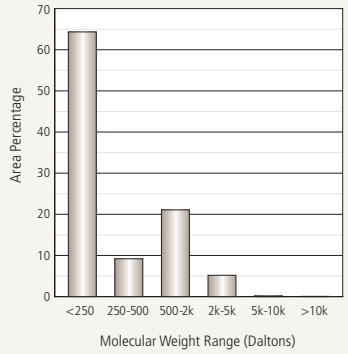
Product Description	Cat. No.	Qty.
Bacto™ TC Yeastolate	255772	100 g
Bacto™ TC Yeastolate	255771	10 kg
Bacto™ TC Yeastolate	292731	25 kg
Difco™ TC Yeastolate, UF	292804	500 g
Difco™ TC Yeastolate, UF	292805	10 kg

Titration



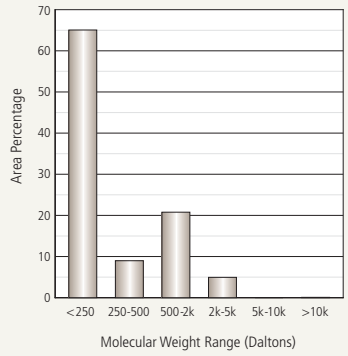
Molecular Weight

Bacto™ TC Yeastolate



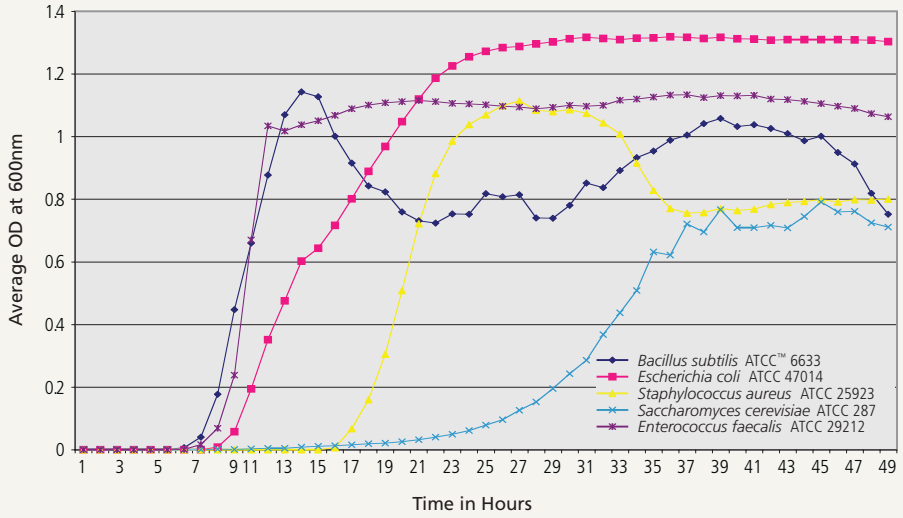
Molecular Weight

Difco™ TC Yeastolate, UF



Growth Curve

1% Bacto TC Yeastolate in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



References

1. Chan, Greenfield and Reid. 1998. Optimising fed-batch production of recombinant proteins using the baculovirus expression vector system. Biotechnol. Bioeng *59*:178-188.
2. Nguyen, Jarnagin, Williams, Chan and Barnett. 1993. Fed-batch culture of insect cells: a method to increase the yield of recombinant human nerve growth factor (rhNGF) in the baculovirus expression system. J Biotechnol. *31*:205-217.
3. Ikonomou, Bastin, Schneider, Agathos. 2001. Design of efficient medium for insect cell growth and recombinant protein production. *In Vitro* Cell Dev. Biol. Anim. *37*:549-559.
4. Bedard, Kamen, Tom and Maassie. 1994. Maximization of recombinant protein yield in the insect cell/baculovirus system by one-time addition of nutrients to high-density batch cultures. Cytotechnology *15*:129-138.
5. Donaldson and Shuler. 1998. Low-cost serum-free medium for the BTI-TN5B1-4 insect cell line. Biotechnology Prog. *14*:573-579.

Bacto™ Yeast Extract
BBL™ Yeast Extract
Bacto™ Yeast Extract, Technical
Difco™ Yeast Extract, UF

Product Description

BD Yeast Extracts are concentrates of the water soluble portion of *Saccharomyces cerevisiae* cells that have been autolyzed. BD Yeast Extracts are derived from primary grown baker's yeast. Yeast extract is an animal-free product and is used extensively for many animal-free formulations for bacterial, fungal, mammalian and insect cell culture. Yeast Extract will provide essential water soluble vitamins, amino acids, peptides and carbohydrates to any medium formulation.

Potential Applications

BD Yeast Extracts are animal-free products suitable for use as multi-functional nutritional supplements in cell culture, microbial fermentation and insect cell culture applications.

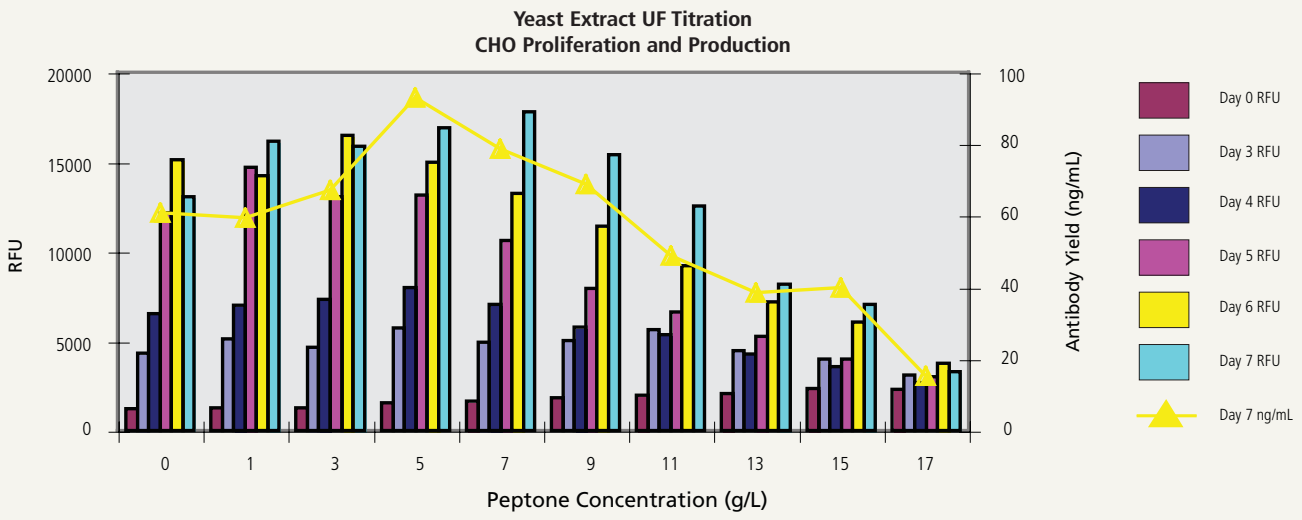
Bacto™ Yeast Extract is one of the most complete and versatile fermentation bionutrients available. It is an important ingredient for the microbiological assay of vitamins. Yeast extract is also of value in the assay of antibiotics. B factor, a growth substance necessary for the production of rifampin in a *Nocardia* sp., can be isolated from yeast extract.[1]

Difco™ Yeast Extract, UF is ultrafiltered and specifically designed for tissue culture applications. With its low endotoxin level and high content of naturally occurring B vitamins, it is an ideal substitute for fetal bovine serum. It has an endotoxin level of less than or equal to 150 EU/g.

Yeast Extract was developed to provide a product for the biotechnology/pharmaceutical market with acceptable clarity and growth promoting characteristics. Media formulations containing yeast extract are specified in standard methods for various applications.[2-8]

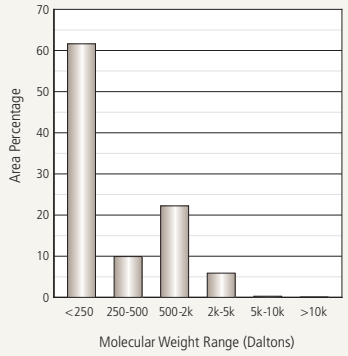
Bacto™ Yeast Extract, Technical, was developed to provide products priced for the biotechnology market with acceptable clarity and growth promoting characteristics.

Titration



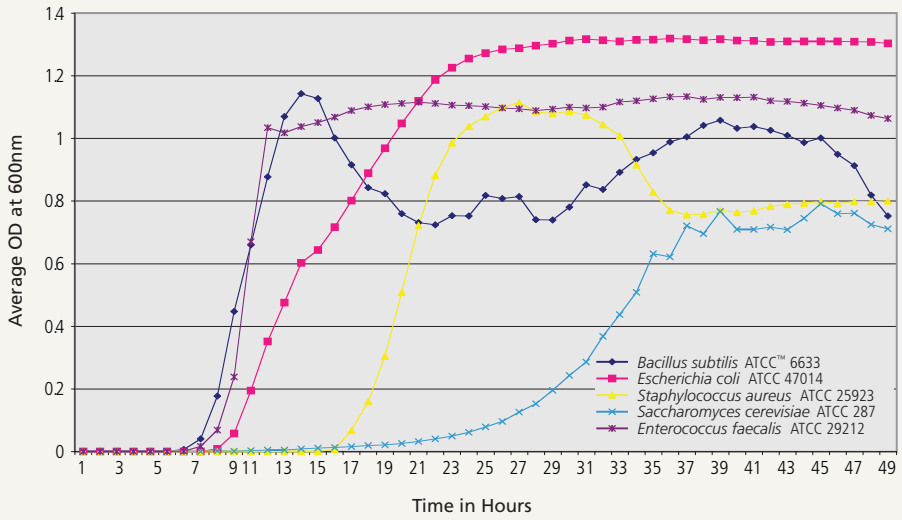
Molecular Weight

Bacto™ Yeast Extract



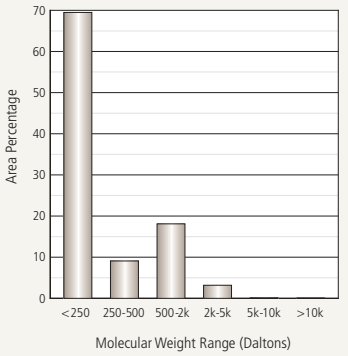
Growth Curve

1% Bacto™ Yeast Extract in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



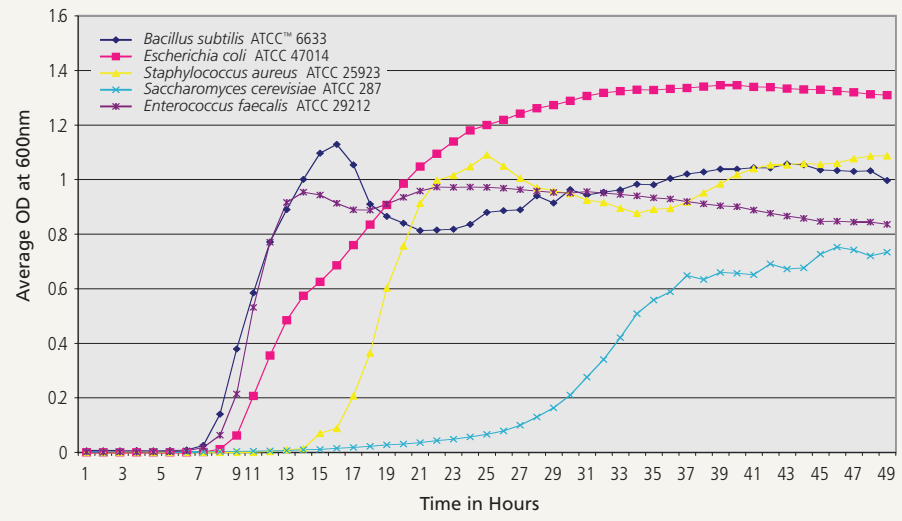
Molecular Weight

BBL™ Yeast Extract



Growth Curve

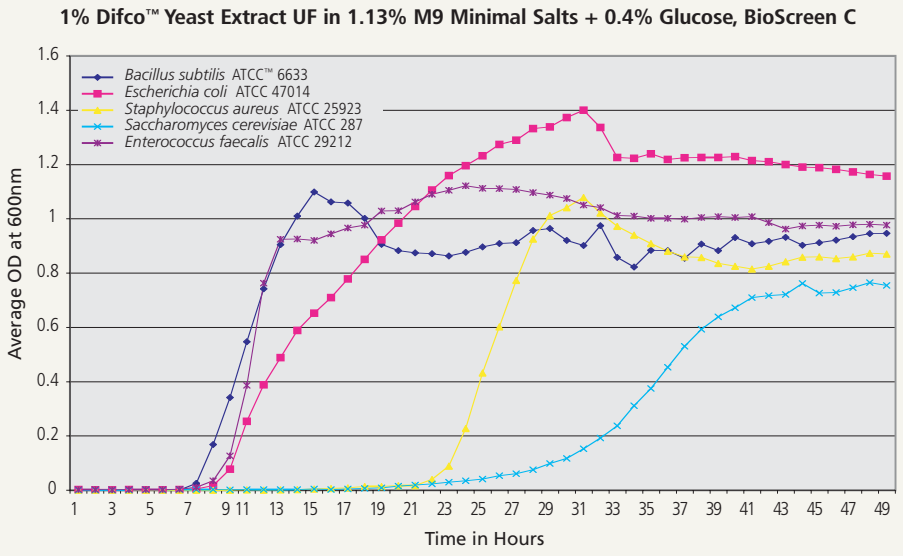
1% BBL™ Yeast Extract 211929 in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



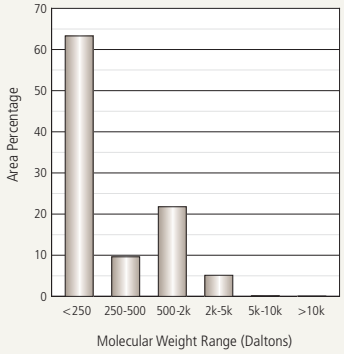
Physical Characteristics

BD Yeast Extracts are light to medium beige, free-flowing, homogeneous, spray-dried powders.

Growth Curve



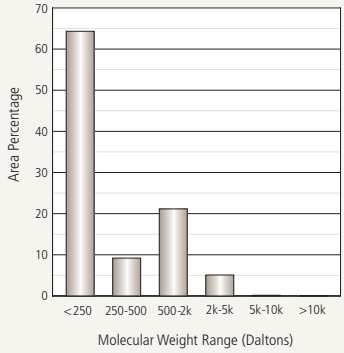
Molecular Weight



Availability

Product Description	Cat. No.	Qty.
Bacto™ Yeast Extract	212750	500 g
Bacto™ Yeast Extract	212720	2 kg
Bacto™ Yeast Extract	212730	10 kg
Bacto™ Yeast Extract	212710	50 kg
BBL™ Yeast Extract	211929	454 g
BBL™ Yeast Extract	211930	5 lb (2.3 kg)
BBL™ Yeast Extract	211931	25 lb (11.3 kg)
Bacto™ Yeast Extract, Technical	288620	500 g
Bacto™ Yeast Extract, Technical	288610	10 kg
Difco™ Yeast Extract, UF	210929	500 g
Difco™ Yeast Extract, UF	210934	10 kg

Molecular Weight



References

1. Kawauchi, Asahi, Satoh, Uozumi and Beppu. 1984. J. Antibiot. *37*:1587.
2. Horowitz (ed.) 2005. Official methods of analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, Md.
3. U.S. Food and Drug Administration. 1998. Bacteriological analytical manual, 8th ed., rev A. AOAC International. Gaithersburg, Md.
4. Downes and Ito (ed.) 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. U.S. Environmental Protection Agency (USEPA). 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington D.C.
6. Wehr (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
7. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
8. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA, Washington, D.C.

Animal-Free Peptones and Yeast Extracts

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Total Carbohydrate (mg/g)	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (1% Solution)	Calcium (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)
Malt Extract, Bacto™	0.3	0.3	0.97	1037.4	0.3	3.1	0.2	5.2	111	5.8	130	603	713	0.07	0.07	0.08	0.1	0.1	0.0	0.1	0.0	0.0
Phytone™ Peptone, BBL™	9.0	2.4	0.27	392.9	12.4	1.5	4.0	7.1	1001	89.8	2435	31547	34037	0.76	0.67	0.64	0.3	2.6	0.6	2.1	0.1	0.3
Select Phytone UF, Difco™	9.4	2.6	0.28	394.2	12.5	4.9	4.0	7.0	900	59.5	1700	21200	36100	0.76	0.58	0.71	0.3	3.1	0.8	2.4	0.2	0.2
Select Soytone, Difco™	9.2	3.7	0.40	336.2	10.7	3.5	0.0	7.0	250	61.0	1749	29787	31087	0.07	2.65	1.03	0.5	3.6	0.4	2.1	0.4	0.2
Soytone, Bacto	9.4	3.1	0.33	292.5	12.0	4.6	0.2	7.2	550	68.2	1610	22200	34040	0.17	2.33	0.82	0.4	2.5	2.1	2.8	0.3	0.2
TC Yeastolate, Bacto	10.7	6.0	0.56	143.0	11.7	2.2	0.6	7.0	228	73.7	250	50850	8190	0.30	0.49	2.63	4.6	4.6	1.7	2.4	1.2	1.8
TC Yeastolate UF, Difco	10.6	6.5	0.61	124.2	13.3	2.1	1.0	7.0	247	52.5	267	60940	3716	0.52	0.89	2.46	5.5	5.7	1.9	3.2	1.3	2.1
Yeast Extract, Bacto	10.9	6.0	0.55	163.3	11.2	3.1	0.1	6.7	130	55.3	750	31950	4900	0.38	0.09	3.27	4.4	5.6	1.4	2.6	1.0	1.6
Yeast Extract, BBL	11.4	6.9	0.60	67.6	13.1	1.0	0.2	7.0	230	62.1	799	58013	1003	0.07	0.65	3.73	5.7	6.2	2.0	3.0	1.0	2.2
Yeast Extract, Technical, Bacto	11.1	6.0	0.54	132.1	10.0	5.0	0.0	6.9	320	32.3	400	51030	760	0.12	0.55	1.10	3.3	3.6	2.5	3.4	1.4	1.5
Yeast Extract, UF, Difco	10.7	6.0	0.56	108.2	18.2	0.7	0.0	7.0	191	57.9	558	59240	1244	0.13	1.02	2.70	4.8	5.4	1.5	2.6	1.2	1.7

LEGEND

* = Partially destroyed during hydrolysis
0.0 = Below limit of detection
■ = Free Amino Acids
■ = Total Amino Acids

For analytical methods, see Methods of Detection

Typical Analyses Table

Aspartic Acid (% Total)	Cystine (% Free)	Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)	Methionine (% Total) *	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)*	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
0.1	0.0	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
3.9	0.4	0.3	5.9	0.0	0.2	1.5	0.3	0.8	0.2	1.3	0.8	2.3	1.2	2.4	0.2	0.2	0.2	1.4	0.1	1.8	0.2	0.5	0.1	0.5	0.0	0.2	0.8	0.1	1.5
4.7	0.5	0.4	6.5	0.0	0.2	1.8	0.1	0.9	0.2	1.6	0.9	2.7	1.5	2.8	0.2	0.3	0.3	1.6	0.1	1.9	0.3	0.6	0.1	0.6	0.1	0.3	1.0	0.1	1.7
6.2	0.5	0.7	6.9	0.2	0.1	2.2	0.5	1.3	0.9	2.6	2.2	3.9	2.6	3.4	0.4	1.0	1.3	2.4	0.2	2.6	0.3	1.2	0.5	1.0	0.1	0.9	2.0	1.0	2.8
5.5	0.4	0.4	8.9	0.1	0.2	2.1	0.2	1.1	0.6	2.8	1.7	4.3	1.9	2.9	0.3	0.5	1.2	3.1	0.2	2.0	0.3	1.4	0.2	1.1	0.2	1.3	1.3	0.4	2.7
4.8	0.2	6.6	8.7	0.3	1.3	2.7	0.5	1.1	2.1	3.6	3.5	4.9	2.3	4.2	0.8	0.8	2.3	3.3	0.9	1.8	1.5	1.4	1.3	1.4	0.6	0.8	0.9	2.4	3.7
6.4	0.2	7.3	11.0	0.2	1.5	3.0	0.6	1.5	2.1	3.2	3.0	4.0	2.5	5.1	0.8	0.9	2.2	2.9	1.1	2.0	1.9	2.1	1.5	1.7	0.9	0.1	0.9	2.7	4.0
5.3	0.2	6.6	9.4	0.2	1.0	3.0	0.4	1.3	1.8	3.0	3.0	4.1	1.9	4.6	0.6	0.8	2.0	2.6	0.8	2.0	1.3	1.6	1.1	1.6	0.5	0.8	1.2	2.2	3.5
5.9	0.2	7.3	11.0	0.1	1.6	3.3	0.3	1.4	2.5	4.7	4.0	6.2	2.7	4.9	0.9	1.1	2.7	4.4	1.3	2.3	1.3	1.9	1.7	1.8	0.7	0.9	1.2	3.0	4.8
4.8	0.9	5.7	8.5	0.6	1.1	2.2	0.6	1.0	1.9	2.3	3.5	3.7	2.0	3.8	1.1	1.0	2.1	2.2	0.8	1.7	1.9	2.3	1.0	1.8	2.2	0.8	1.0	2.5	3.0
5.4	0.2	6.8	10.0	0.3	1.3	2.9	0.6	1.2	1.8	3.8	2.8	4.7	2.2	4.6	0.7	0.8	2.1	3.6	0.9	1.9	1.6	1.7	1.3	1.6	0.5	0.5	0.8	2.4	4.1

Meat Peptones

Meat peptones are proteins from animal sources that have been hydrolyzed, or broken down into amino acids and peptides, to provide nitrogen for microorganisms. Meat peptones can be tailored to specific nutritive needs of microorganisms by controlling the quality and origin of the protein, the quality and source of the enzyme used to digest the protein, and the methods used for hydrolysis, concentration and drying the peptone. Peptone manufacture methods are discussed in the section titled Hydrolysis to Hydrolysate.

Sources of animal protein include meat from muscle tissue or offal (waste parts, entrails) and gelatin. Muscular tissue and offal are utilized fresh, frozen or dried. Gelatin is extracted by boiling collagen, the fibrous protein found in connective tissue, bone and cartilage.

A variety of proteolytic enzymes, or proteases, may be used to accomplish enzymatic hydrolysis of animal protein. Pepsin and trypsin are widely used for animal peptone manufacture. Pepsin is isolated from porcine or other animal stomach. Trypsin, along with chymotrypsin, carboxypeptidase A, carboxypeptidase B, and elastase, are enzymes isolated from animal pancreas.

BBL™ Beef Extract Powder
Bacto™ Beef Extract, Desiccated

Product Description

Beef Extract is derived from infusion of beef and provides an undefined source of nutrients. Beef Extract is not exposed to the harsh treatment used for protein hydrolysis, so it can provide some of the nutrients lost during peptone manufacture.[1] Beef Extract is a mixture of peptides and amino acids, nucleotide fractions, organic acids, minerals and some vitamins. "Its function can therefore be described as complementing the nutritive properties of peptone by contributing minerals, phosphates, energy sources and those essential factors missing from peptone."[2] Beef Extract Powder is a meat extract dried to powder form. Bacto™ Beef Extract, Desiccated, is the dried form of Beef Extract paste.

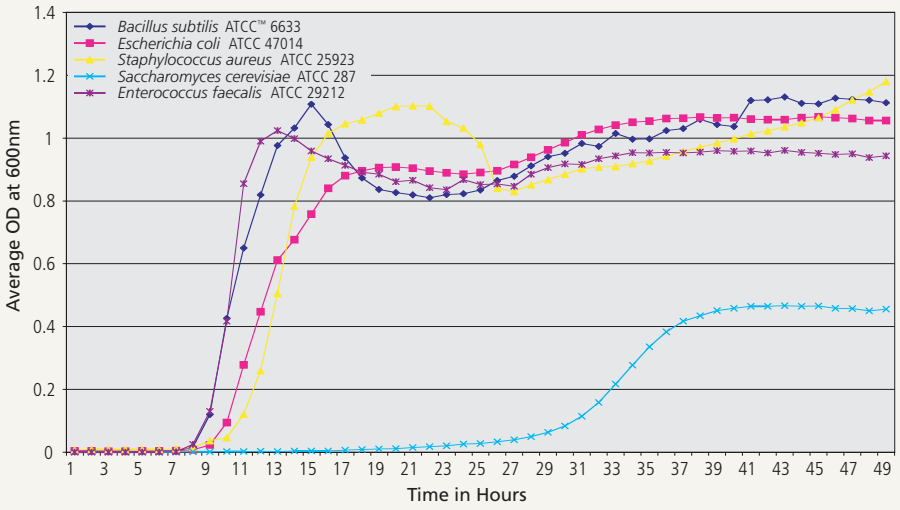
Potential Applications

Beef Extract is intended to replace aqueous infusion of meat in microbiological culture media. Beef Extract is frequently used at a concentration of 0.3 to 1.0% in culture media, although concentrations may vary depending on the nutritional requirements for the medium formulation.

Beef Extract was used in media for early studies of non-sporulating anaerobes of the intestinal tract and as a stock broth in the study of nutritional needs of streptococci. Prokofeva et al.[3] used Beef Extract for growing thermoacidophilic organisms newly isolated from hot springs in Kamchatka, Russia. Kataoka and Tokiwa[4] used Beef Extract as a nitrogen source in studies of mannose production by *Clostridium tertium* strains isolated from soil and methanogenic sludge. In addition, Beef Extract is a nutritive ingredient in many classical culture media, including Antibiotic Assay media described in *The United States Pharmacopeia*,[5] and several media recommended for standard methods applications.[6-8]

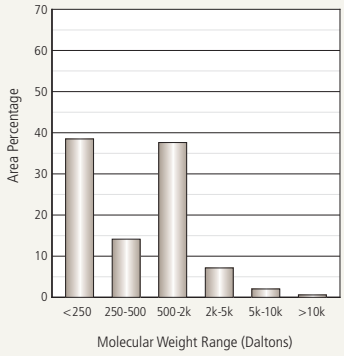
Growth Curve

1% BBL™ Beef Extract Powder 212303 in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



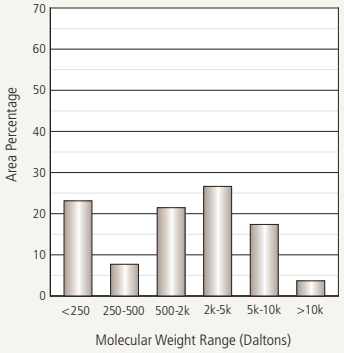
Molecular Weight

BBL™ Beef Extract Powder



Molecular Weight

Bacto™ Beef Extract, Desiccated



Physical Characteristics

BBL™ Beef Extract Powder is a light to medium, cream to tan, free-flowing, homogeneous powder.

Bacto™ Beef Extract, Desiccated is a medium to dark brown, crystalline powder.

Availability

Product Description	Cat. No.	Qty.
BBL™ Beef Extract Powder	212303	500 g
Bacto™ Beef Extract, Desiccated	211520	500 g

References

1. Cote. 1999. Media composition, microbial, laboratory scale. *In* Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.
2. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.
3. Prokofeva, Miroshnichenko, Kostrikina, Chernyh, Kuznetsov, Tourova and Bonch-Osmolovskaya. 2000. *Acidilobus aceticus* gen. nov., sp. nov., a novel anaerobic thermoacidophilic archaeon from continental hot vents in Kamchatka. Int. J. Syst. Evol. Microbiol. *50*: Pt 6:2001-2008.
4. Kataoka and Tokiwa. 1998. Isolation and characterization of an active mannanase-producing anaerobic bacterium, *Clostridium tertium* KT-5A, from lotus soil. J. Appl. Microbiol. *84*:357-367.
5. United States Pharmacopeial Convention. 2006. The United States pharmacopeia 29/The national formulary 24—2006. United States Pharmacopeial Convention, Inc., Rockville, Md.
6. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. 1998. Bacteriological analytical manual, 8th ed., rev. A. AOAC International, Gaithersburg, Md.
8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

BBL™ Gelysate™ Peptone

Product Description

BBL™ Gelysate™ Peptone is a pancreatic digest of gelatin. Gelatin is extracted from collagen, which is the fibrous protein in bone, cartilage and connective tissue. Gelatin hydrolysate is high in proline residues.[1] Gelysate Peptone is deficient in carbohydrates and is characterized by low cystine, methionine and tryptophan content.

Potential Applications

Gelysate Peptone may be used for cultures requiring low carbohydrates, cystine, and tryptophan levels in cell culture and bacterial fermentation.

Physical Characteristics

BBL™ Gelysate™ Peptone is a tan, fine, free-flowing, homogeneous powder.

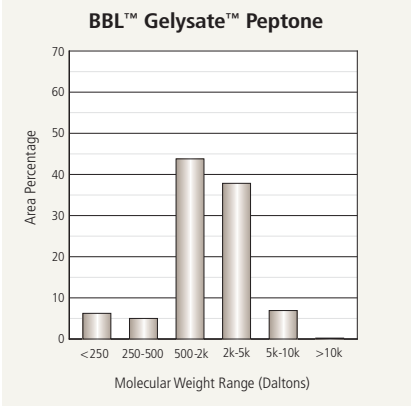
Availability

Product Description	Cat. No.	Qty.
BBL™ Gelysate™ Peptone	211870	454 g

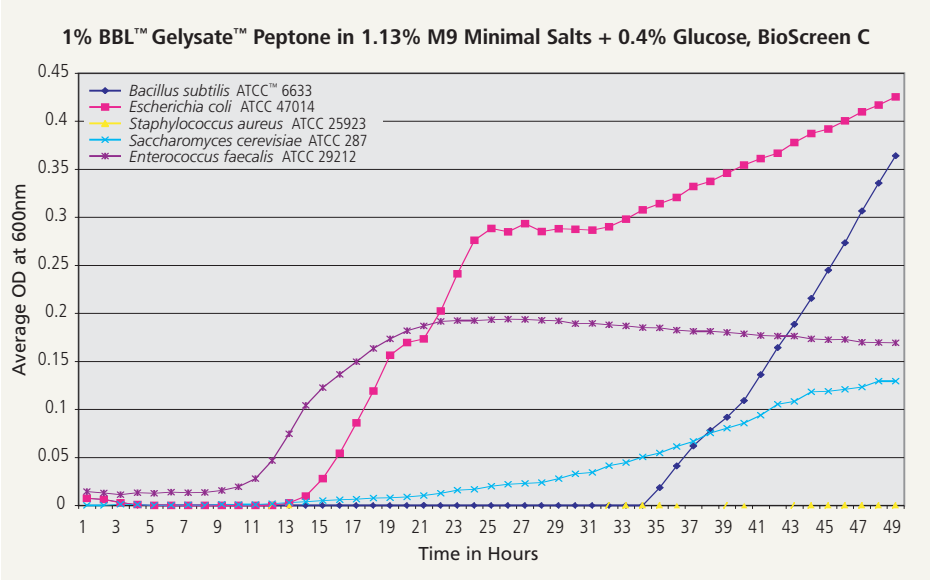
Reference

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.

Molecular Weight



Growth Curve



Bacto™ Neopeptone

Product Description

Bacto™ Neopeptone is an enzymatic digest of protein. Neopeptone contains a wide variety of peptide sizes in combination with vitamins, nucleotides and minerals.

Potential Applications

Neopeptone is recommended for use in media for detection of fungi.[1] Apodaca and McKerrow[2] used Neopeptone for the cultivation of *Trichophyton rubrum* for study of its proteolytic activity. Neopeptone has been cited as a component of culture media used for cultivation of human pathogens, notably, *Bordetella pertussis* and group A streptococci.

Neopeptone has also been reported to provide nutrients for support of spirochetes and protozoa. Wyss et al.[3] used Neopeptone as a component of a medium for cultivation of *Treponema maltophilum* sp. nov., a fastidious oral anaerobe. Ifediba and Vanderberg[4] reported that Neopeptone, in addition to calf serum, was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Cushion and Ebbets[5] utilized Neopeptone in their investigations of various media for cultivating *Pneumocystis carinii* without feeder cells; optimal replication of *P. carinii* separated from host fungi cells was observed in media with Neopeptone and N-acetylglucosamine at low pH.

Physical Characteristics

Bacto™ Neopeptone appears as tan, free-flowing, granules.

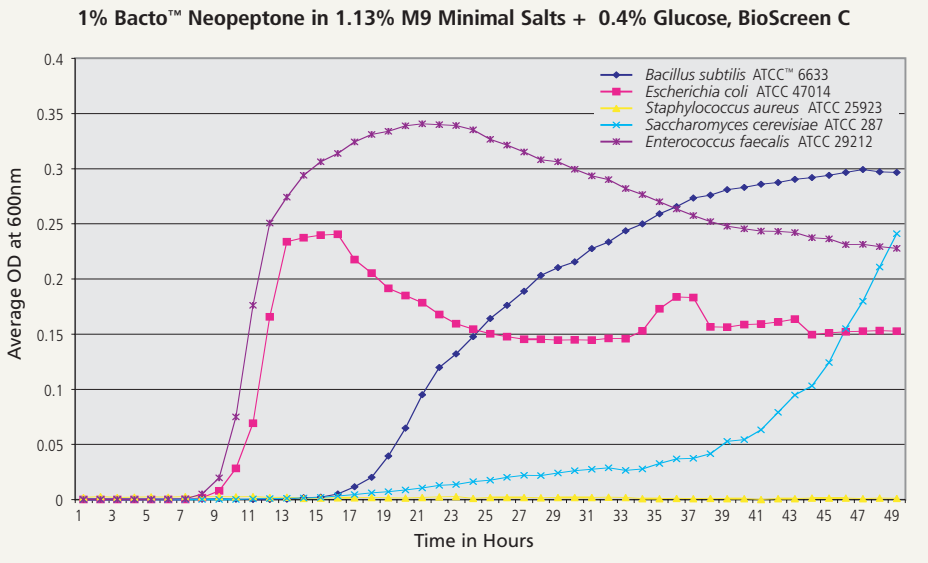
References

1. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed., 9-131-137. American Public Health Association, Washington, D.C.
2. Apodaca and McKerrow. 1990. Expression of proteolytic activity by cultures of *Trichophyton rubrum*. J. Med. Vet. Mycol. *28*:159-171.
3. Wyss, Choi, Schupbach, Guggenheim and Gobel. 1996. *Treponema maltophilum* sp. nov., a small oral spirochete isolated from human periodontal lesions. Int. J. Syst. Bacteriol. *46*:745-752.
4. Ifediba and Vanderberg. 1980. Peptones and calf serum as a replacement for human serum in the cultivation of *Plasmodium falciparum*. J. Parasitol. *66*:236-239.
5. Cushion and Ebbets. 1990. Growth and metabolism of *Pneumocystis carinii* in axenic culture. J. Clin. Microbiol. *28*:1385-1394

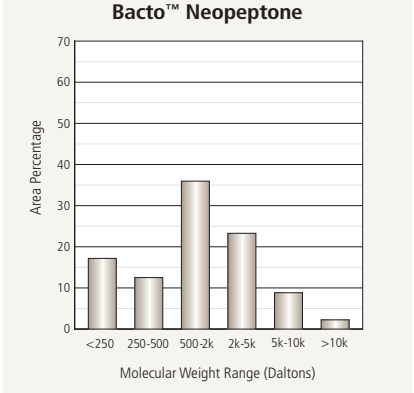
Availability

Product Description	Cat. No.	Qty.
Bacto™ Neopeptone	211681	500 g
Bacto™ Neopeptone	211680	10 kg

Growth Curve

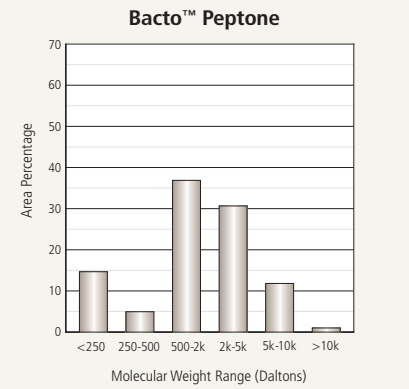


Molecular Weight



Bacto™ Peptone

Molecular Weight



Product Description

Bacto™ Peptone is an enzymatic digest of animal protein. Bacto Peptone was first introduced in 1914 and became the standard Peptone for the preparation of bacteriological culture media. The nutritive value of Bacto Peptone is largely dependent on the amino acid content that supplies essential nitrogen. Bacto Peptone contains only a negligible quantity of proteoses and more complex constituents.

Potential Applications

Bacto Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi. For example, Iwanaga et al.[1] utilized Bacto Peptone for production of cholera toxin by *Vibrio cholerae* O1 El Tor. Benkerroum et al.[2] reported using Bacto Peptone in a selective medium developed for isolating *Leuconostoc* spp. from food samples. Bacto Peptone was used in a culture medium for two anaerobic, extremely thermophilic Archaea, *Thermococcus celer* and *Pyrococcus woesei*, by Blamey et al.[3]

Bacto Peptone has also been utilized as a nitrogen source in cell culture media formulations. Taylor et al.[4] used Bacto Peptone to supplement serum-free medium for several mammalian cell lines and reported the solubility of Bacto Peptone as very good at 10 g/100 mL water. Sakoda and Fukusho[5] also utilized Bacto Peptone in serum-free culture for maintaining porcine kidney epithelial cells. Bacto Peptone is also useful as a supplement in cell culture with serum.

Researchers uncovered estrogenic activity associated with Bacto Peptone when including the peptone in medium for culture of yeast; the estrone contained in Bacto Peptone was converted to estrodiol by *Saccharomyces cerevisiae*. These findings suggest that adding estrogens to a medium containing Bacto Peptone for studies of estrodiol production by yeast may confound results.[6,7]

Physical Characteristics

Bacto™ Peptone is a tan, free-flowing, homogeneous powder.

Availability

Product Description	Cat. No.	Qty.
Bacto™ Peptone	211677	500 g
Bacto™ Peptone	211820	2 kg
Bacto™ Peptone	211830	10 kg

References

1. Iwanaga, Yamamoto, Higa, Ichinose, Nakasone and Tanabe. 1986. Culture conditions for stimulating cholera toxin production by *Vibrio cholerae* O1 El Tor. Microbiol. Immunol. *30*:1075-1083.
2. Benkerroum, Misbah, Sandine and Elaraki. 1993. Development and use of a selective medium for isolation of *Leuconostoc* spp. from vegetables and dairy products. Appl. Environ. Microbiol. *59*:607-609.
3. Blamey, Chiong, Lopez and Smith. 1999. Optimization of the growth conditions of the extremely thermophilic microorganisms *Thermococcus celer* and *Pyrococcus woesei*. J. Microbiol. Methods *38*:169-175.
4. Taylor, Dworkin, Pumper and Evans. 1972. Biological efficacy of several commercially available peptones for mammalian cells in culture. Exp. Cell Res. *74*:275-279.
5. Sakoda and Fukusho. 1998. Establishment and characterization of a porcine kidney cell line, FS-L3, which forms unique multicellular domes in serum-free culture. *In Vitro* Cell. Dev. Biol. Anim. *34*:53-57.
6. Feldman and Krishnan. 1995. Estrogens in unexpected places: possible implications for researchers and consumers. Environ. Health Perspect. 103 Suppl *7*:129-133.
7. Miller, Bottema, Stathis, Tokes and Feldman. 1986. Unexpected presence of estrogens in culture medium supplements: subsequent metabolism by the yeast *Saccharomyces cerevisiae*. Endocrinology *119*:1362-1369.

BBL™ Polypeptone™ Peptone

Product Description

BBL™ Polypeptone™ Peptone is a mixture of peptones made up of equal parts of pancreatic digest of casein and peptic digest of animal tissue. Polypeptone Peptone includes the high content of amino acids and small polypeptides characteristic of pancreatic digest of casein and the larger polypeptides characteristic of peptic digest of animal tissue.

Availability

Product Description	Cat. No.	Qty.
Polypeptone™ Peptone	211910	454 g
Polypeptone™ Peptone	297108	10 kg

Potential Applications

Researchers have found Polypeptone Peptone to meet nutritional requirements of various bacteria, fungi and mammalian cells, where a single source of casein or meat peptones has been unsatisfactory. Polypeptone Peptone has been utilized in culture media for the production of trypsin inhibitor by *Cephalosporium* sp.;[1] the production of bacterial cellulose by *Acetobacter* sp. A9;[2] production of succinic acid from whey by *Anaerobiospirillum succiniciproducens*;[3] mass production of luciferase-bacterial magnetic particles by recombinant *Magnetospirillum magneticum* AMB-1;[4] and the production of a novel tumor-killing factor by human macrophage-monocyte hybridomas.[5]

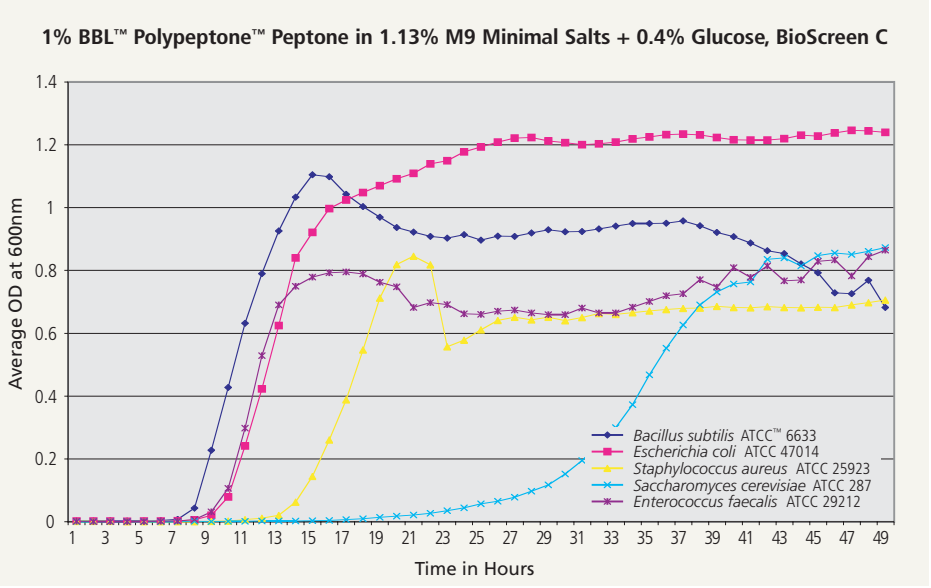
Physical Characteristics

BBL™ Polypeptone™ Peptone is a yellow to tan, fine, free-flowing, homogeneous powder.

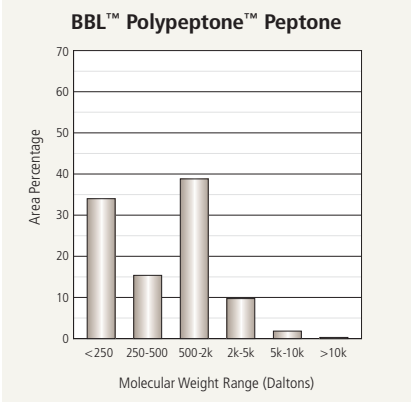
References

1. Tsuchiya and Kimura. 1978. Production of trypsin inhibitor by a *Cephalosporium* sp. Appl. Environ. Microbiol. *35*:631-635.
2. Son, Heo, Kim and Lee. 2001. Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. Biotechnol. Appl. Biochem. *33*(Pt 1):1-5.
3. Lee, Lee, Kwon, Lee and Chang. 2000. Batch and continuous cultivation of *Anaerobiospirillum succiniciproducens* for the production of succinic acid from whey. Appl. Microbiol. Biotechnol. *54*:23-27.
4. Yang, Takeyama, Tanaka and Matsunaga. 2001. Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant *Magnetospirillum magneticum* AMB-1. Enzyme Microbiol. Technol. *29*:13-19.
5. Taniyama, Yoshida and Furuta. 1988. Demonstration of a novel tumor-killing factor secreted from human macrophage-monocyt hybridomas. J. Immunol. *141*:4061-4066.

Growth Curve



Molecular Weight



Bacto™ Proteose Peptone
BiTek™ Proteose Peptone
Bacto™ Proteose Peptone No. 2
Bacto™ Proteose Peptone No. 3
BiTek™ Proteose Peptone No. 3
Bacto™ Proteose Peptone No. 4

Product Description

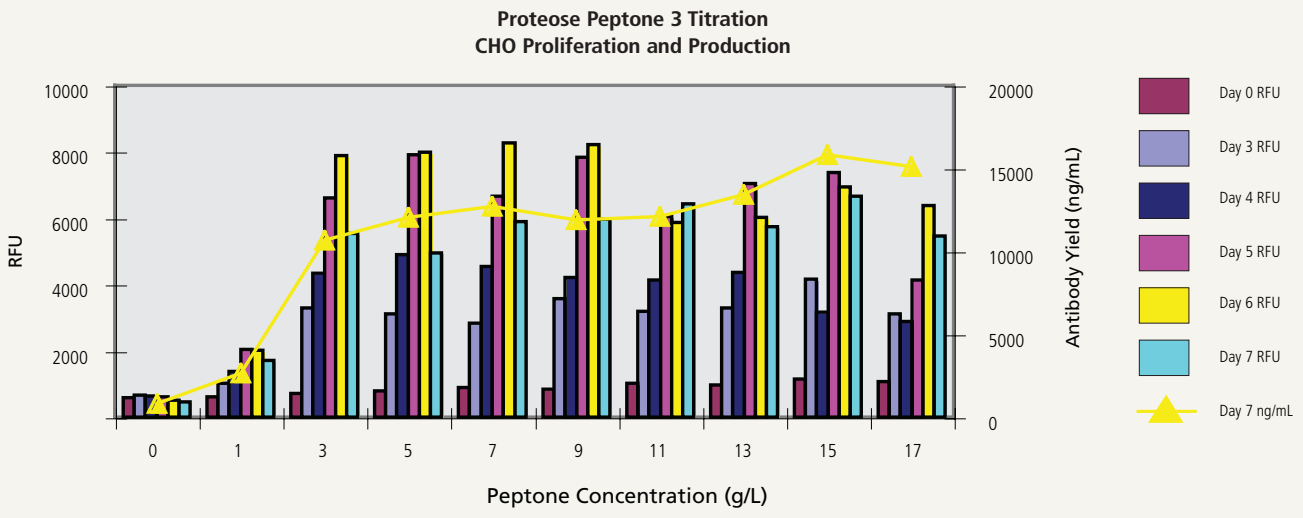
The Bacto™ Proteose Peptones are enzymatic digests of protein. Studies of peptic digests of animal tissue prepared under varying digestion parameters led to the development of Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3. Data accumulated during these studies demonstrated that no one peptone is the most suitable nitrogen source for every microbiological application. Bacto Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone.

BiTek™ Proteose Peptone and BiTek Proteose Peptone No. 3 are enzymatic digests of protein, developed to offer alternatives to the Bacto Proteose Peptones for scale-up to production applications.

Potential Applications

Bacto Proteose Peptone is used in preparing microbiological culture media and in producing bacterial toxins. Bacto Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency from cultures of *Corynebacterium diphtheriae*. Studies support the use of Proteose Peptone for production of diphtheria toxin, toxin-antitoxin mixtures and toxoid.[1,2] Proteose Peptone is also valuable in the production of other bacterial toxins: *Clostridium botulinum* toxin;[3] toxin from *Clostridium perfringens*;[4] toxin of hemolytic streptococci;[5] pneumococcus toxin;[6] and toxin from *Salmonella pullorum* (*Salmonella cholerasuis* subsp. *cholerasuis*).[7]

Titration



Many factors account for the suitability of Proteose Peptone for the culture of fastidious pathogens, including the nitrogen components, buffering range and the high content of proteoses. These elements create an environment beneficial to the maintenance of virulence and the elaboration of bacterial by-products, thus stock cultures are well preserved on media containing Bacto Proteose Peptone.

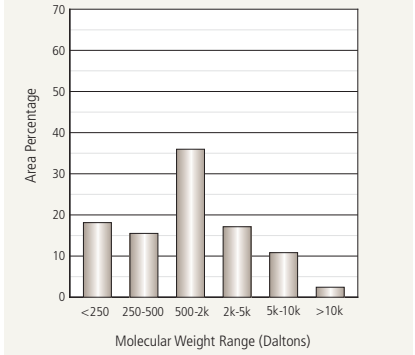
Bacto™ Proteose Peptone may be used in culture medium for a variety of applications, including production of substances from the culture of bacteria, fungi and mammalian cells. Proteose Peptone has been utilized in a medium for producing glycosidases from *Bacteroides fragilis*,[8] and to stimulate amyloglucosidase production by *Aspergillus* sp.[9] It has been used to cultivate halophilic bacteria isolated from soil in Egypt for production of polymers.[10] Jan et al.[11] reported that Proteose Peptone as supplementation to defined medium resulted in significant increases in cell number and specific monoclonal antibody production in batch culture system. Proteose Peptone has also been used to provide nutrients for axenic culture of amoeba.[12]

BiTek™ Proteose Peptone was developed to provide an alternative product to Bacto Peptone with growth characteristics similar to Bacto Proteose Peptone.

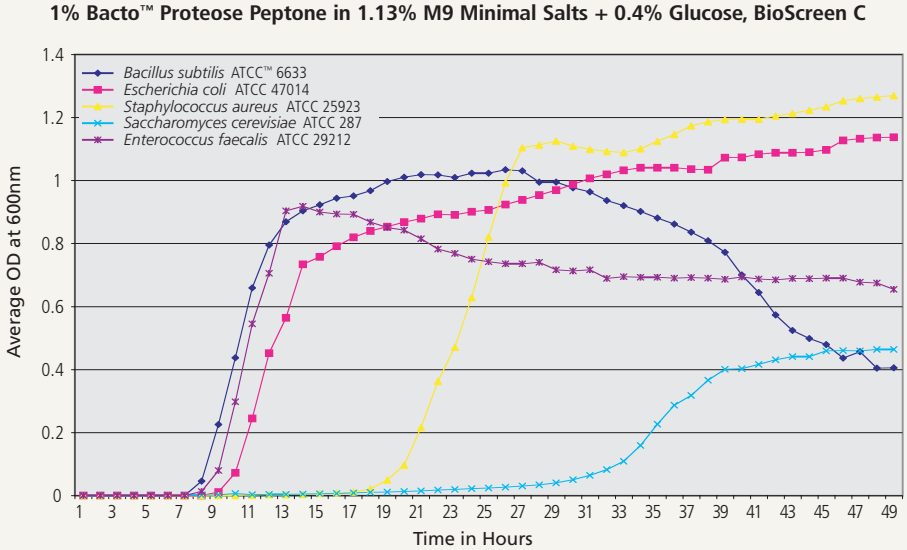
Bacto™ Proteose Peptone No. 2 is used in preparing microbiological culture media. It was originally developed for use in media for the production of diphtheria toxin. Bunney and Thomas[13] reported good yield of diphtheria toxin with Proteose Peptone No. 2 in a simple peptone-sugar-sodium acetate medium.

Bacto™ Proteose Peptone No. 3 is used in preparing microbiological culture media. It is a modification of Proteose Peptone adapted for use in the preparation of chocolate agar for propagation of *Neisseria* species and chocolate tellurite agar for *Corynebacterium diphtheriae*. While investigating the nutritional values of the Proteose Peptones, Difco Laboratories found that Proteose Peptone No. 3 provides superior nutrition for fastidious microorganisms. It supports growth of streptococci, staphylococci, pneumococci, gonococci and other organisms that require a highly nutritious substrate. For example, Ifediba and Vanderberg[14]

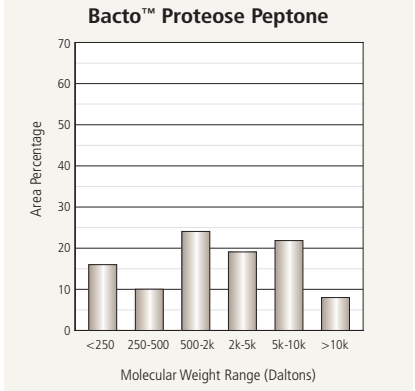
Molecular Weight



Growth Curve

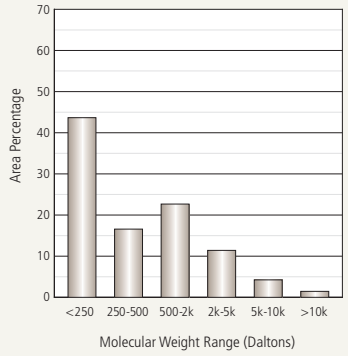


Molecular Weight



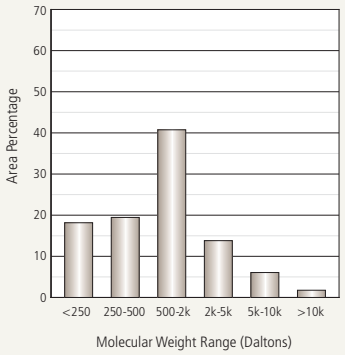
Molecular Weight

Bacto™ Proteose Peptone No. 2



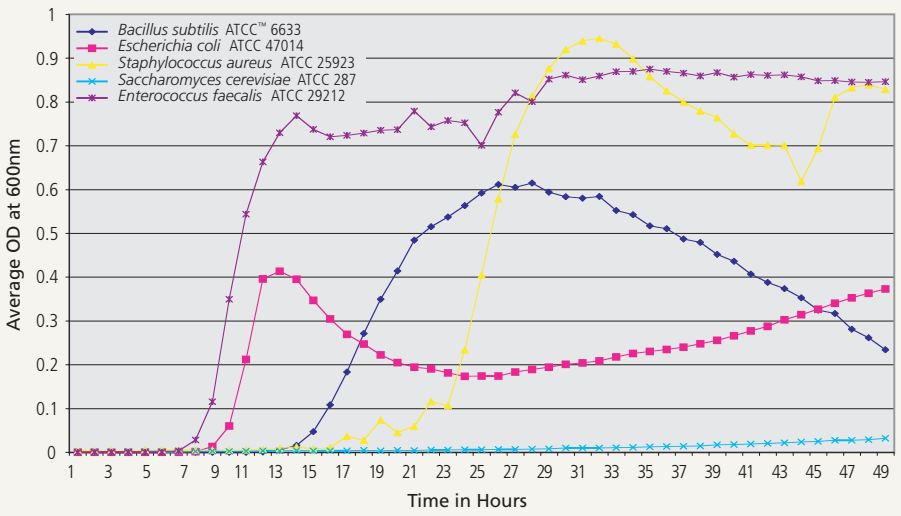
Molecular Weight

BiTek™ Proteose Peptone No. 3



Growth Curve

1% Bacto™ Proteose Peptone No. 2 in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



report that Proteose Peptone No. 3 in addition to calf serum was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Cell culture manufacturers have found significant yield improvements in using Proteose Peptone No. 3.

BiTek™ Proteose Peptone No. 3 was developed to provide an alternative product to Bacto Proteose Peptone No. 3 with growth characteristics similar to Bacto Proteose Peptone No. 3.

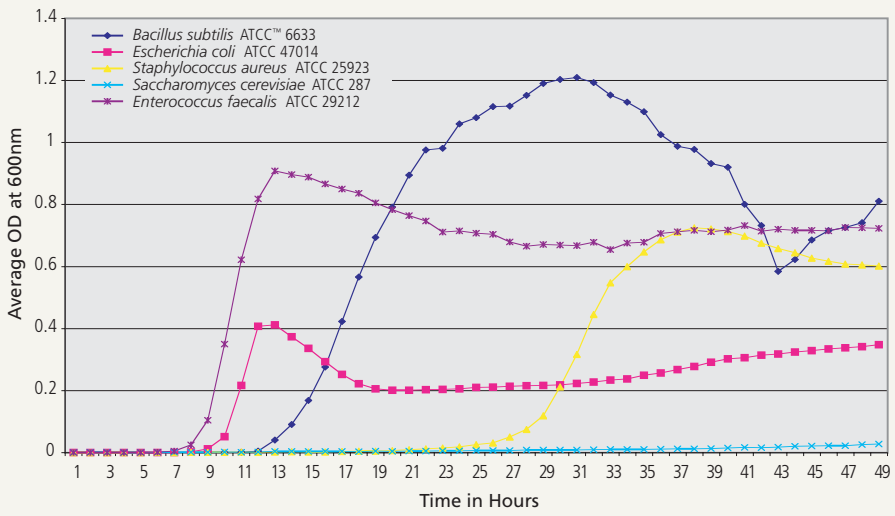
Bacto™ Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone. It offers the same beneficial nutrients as Proteose Peptone for growth promotion and toxin production with a wide range of fastidious microorganisms.

Availability

Product Description	Cat. No.	Qty.
Bacto™ Proteose Peptone	211684	500 g
Bacto™ Proteose Peptone	212010	10 kg
BiTek™ Proteose Peptone	253310	10 kg
Bacto™ Proteose Peptone No. 2	212120	500 g
Bacto™ Proteose Peptone No. 2	212110	10 kg
Bacto™ Proteose Peptone No. 3	211693	500 g
Bacto™ Proteose Peptone No. 3	212220	2 kg
Bacto™ Proteose Peptone No. 3	212230	10 kg
Bacto™ Proteose Peptone No. 3	211692	50 kg
BiTek™ Proteose Peptone No. 3	253720	25 kg
Bacto™ Proteose Peptone No. 4	211715	10 kg

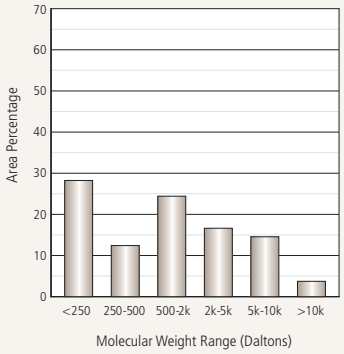
Growth Curve

1% Bacto™ Proteose Peptone No. 3 in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



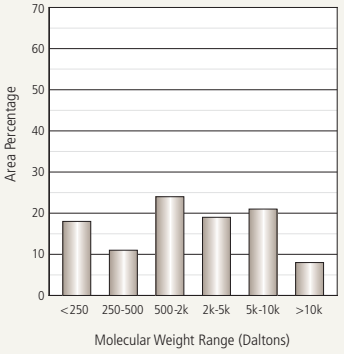
Molecular Weight

Bacto™ Proteose Peptone No. 3



Molecular Weight

Bacto™ Proteose Peptone No. 4



Physical Characteristics

Bacto™ Proteose Peptone appears as tan, free-flowing granules.

BiTek™ Proteose Peptone is a tan, free-flowing, homogeneous powder.

Bacto™ Proteose Peptone No. 2 appears as tan, free-flowing granules.

Bacto™ Proteose Peptone No. 3 appears as golden tan, free-flowing granules.

BiTek™ Proteose Peptone No. 3 is a light beige, free-flowing, homogeneous powder.

Bacto™ Proteose Peptone No. 4 is a light beige, free-flowing, homogeneous powder.

References

1. Kirkbride, Berthelsen and Clark. 1931. Comparative studies of infusion and infusion-free diphtheria toxin in antitoxin production and in standardization by the flocculation, subcutaneous, and intracutaneous tests. J. Immunol. *21*:1-20.
2. Hazen and Heller. 1931. Further studies upon the effect of various carbohydrates on production of diphtheria toxin with special reference to its flocculating titer and final pH. J. Bacteriol. *23*:195-209.
3. Nelson. 1927. The relationship between the intracellular globulin and the toxin of *C. botulinum*. J. Infect. Dis. *41*:9-12.
4. Mollby and Holme. 1976. Production of phospholipase C (alpha-toxin), haemolysins and lethal toxins by *Clostridium perfringens* types A to D. J. Gen. Microbiol. *96*:137-144.
5. Kirkbride and Wheeler. 1926. Studies of the toxins of the hemolytic streptococci associated with scarlet fever. J. Immunol. *11*:477-497.
6. Kneeland and Dawes. 1932. Studies on the common cold: V. The relationship of pathogenic bacteria to upper respiratory diseases in infants. J. Exp. Med. *55*:735-744.
7. Hanks and Rettger. 1931. Bacterial endotoxin; search for a specific intracellular toxin in *S. pullorum*. J. Immunol. *22*:283-314.
8. Berg, Nord and Wadstrom. 1978. Formation of glycosidases in batch and continuous culture of *Bacteroides fragilis*. Appl. Environ. Microbiol. *35*:269-273.
9. Mamo and Gessesse. 1999. Production of raw-starch digesting amyloglucosidase by *Aspergillus* sp. GP-21 in solid state fermentation. J. Ind. Microbiol. Biotechnol. *22*:622-626.
10. Hezayen, Rehm, Eberhardt and Steinbuchel. 2000. Polymer production by two newly isolated extremely halophilic archaea: application of a novel corrosion-resistant bioreactor. Appl. Microbiol. Biotechnol. *54*:319-325.
11. Jan, Jones, Emery and Al-Rubeai. 1994. Peptone, a low-cost growth-promoting nutrient for intensive animal cell culture. Cytotechnol. *16*:17-26.
12. Shukla, Kaul and Mehlotra. 1989. Development of improved media for axenic cultivation of *Acanthamoeba culbertsoni*, Singh and Das 1970. Indian J. Exp. Biol. *27*:785-791.
13. Bunney and Thomas. 1936. Diphtheria toxin-production on broths made from dried complete media. J. Immunol. *31*:95-102.
14. Ifediba and Vanderberg. 1980. Peptones and calf serum as a replacement for human serum in the cultivation of *Plasmodium falciparum*. J. Parasitol. *66*:236-239.

BBL™ Thiotone™ E Peptone

Product Description

BBL™ Thiotone™ E Peptone is an enzymatic digest of animal tissue. Thiotone E Peptone contains a wide range of peptide sizes, including the large molecular weight peptides which support fastidious organisms.

Potential Applications

Thiotone E Peptone is a source of nitrogen, amino acids and vitamins in microbiological culture media. It has been recommended for use in blood agar formulae for hemolysis studies with pneumococci and streptococci. Thiotone E Peptone is high in sulfur amino acids and can be used in media to detect hydrogen sulfide production. Tortora[1] utilized Thiotone E Peptone as the nitrogen source in a medium promoting sporulation of *Clostridium perfringens* strains. Thiotone E Peptone is recommended for use in media for testing water samples for coliforms.[2] Kwinn[3] utilized Thiotone E Peptone as a supplement to her medium for *Corynebacterium glutamicum* to make the cells electrocompetent for transformations. Thiotone E Peptone has also been cited as an ingredient in media for non-bacterial organisms; Thiotone E Peptone is used in Modified HL5 Medium, one of the main media used for culturing the cellular slime mold *Dictyostelium discoideum*.

Physical Characteristics

BBL™ Thiotone™ E Peptone is a tan, fine, free-flowing, homogeneous powder.

Availability

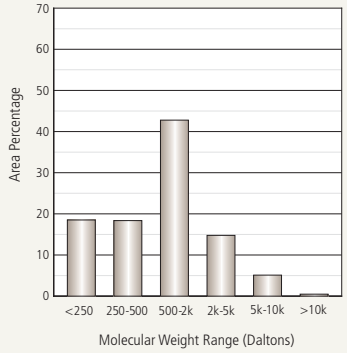
Product Description	Cat. No.	Qty.
BBL™ Thiotone™ E Peptone	212302	500 g

References

1. Tortora. 1984. Alternative medium for *Clostridium perfringens* sporulation. Appl. Environ. Microbiol. *47*:1172-1174.
2. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
3. Kwinn. 2001. Regulation of acetyl-CoA carboxylase in *Corynebacterium glutamicum*: isolation and cloning of the upstream region of the accBC gene. Bug Journal, Biology Department, Massachusetts Institute of Technology *4*:193-200.

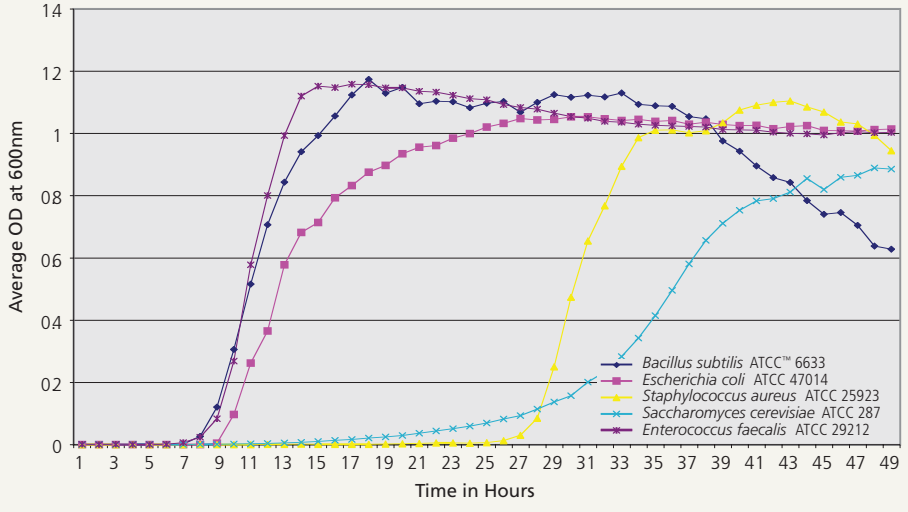
Molecular Weight

BBL™ Thiotone™ E Peptone



Growth Curve

1% BBL™ Thiotone™ E Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Bacto™ Tryptose

Product Description

Bacto™ Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of Tryptose results in assorted peptides of higher molecular weight suitable for long-chain amino acid requirements.

Potential Applications

Bacto Tryptose was originally developed as a peptone particularly adapted to the growth requirements of *Brucella*. Tryptose is very useful for cultivation of streptococci, pneumococci, meningococci and other fastidious organisms, and was found to be superior to meat infusion peptone media previously used for these organisms.[1,2] Mobley et al.[3] reported that Tryptose Broth was the preferred medium for strains of *Bordetella bronchiseptica* in studies of phosphatase activity.

Tryptose has been reported as beneficial for cell culture applications. Litwin[4] found Tryptose suited to supplementing a serum-free medium for growing human diploid fibroblasts. Vaughn and Fan[5] established that Tryptose provided free amino acids necessary for growth of *Spodoptera frugiperda* and *Lymantria dispar* insect cell lines. Tryptose Peptone is often used as a biomass enhancer for recombinent *Escherichia coli* production.

Tryptose is the major ingredient and only peptone in the formulation, Tryptose Phosphate Broth, an often-used medium for various culture applications. Hata and Kojima[6] have shown Tryptose Phosphate Broth (TPB) to be a useful supplement in culturing the nematode, *Angiostrongylus cantonensis*, *in vitro*. TPB was also reported as a supplement to a medium for cultivating a protozoan parasite, which parasitizes vectors of Chagas' disease, on its insect cell host.[7] *Spodoptera frugiperda*, a cotton pest in Argentina[8] and several tick cell lines have also been grown using a TPB supplemented medium.[9] Tryptose Phosphate Broth has been reported as a suitable supplement for growth of baby hamster kidney cells[10] and porcine kidney cells.[11]

Physical Characteristics

Bacto™ Tryptose appears as tan, free-flowing granules.

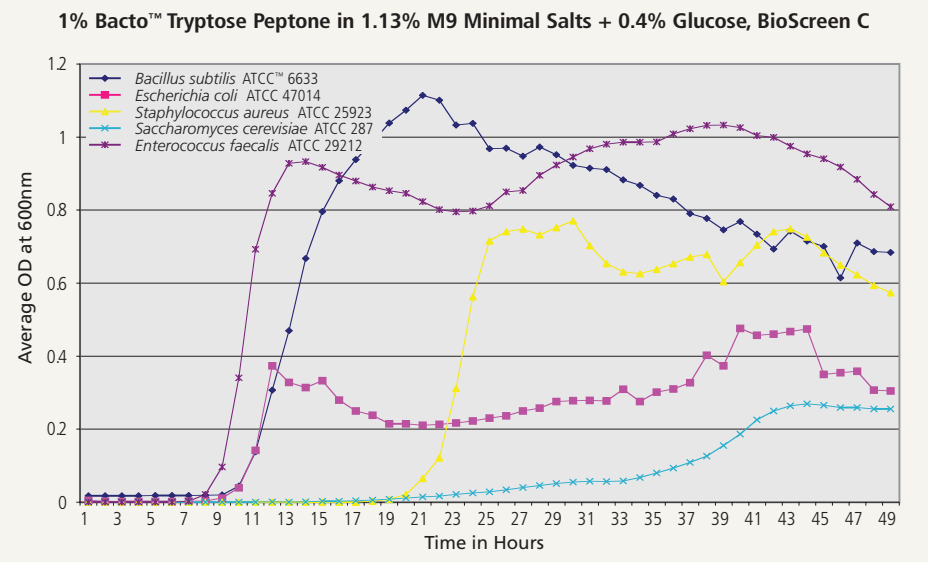
Availability

Product Description	Cat. No.	Qty.
Bacto™ Tryptose	211713	500 g
Bacto™ Tryptose	211709	10 kg

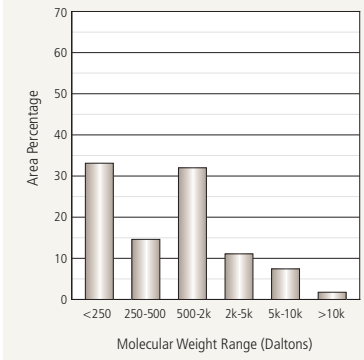
References

1. Casman. 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. J. Bacteriol. *43*:33.
2. Casman. 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. *17*:281-289.
3. Mobley, Chengappa, Kadel and Stuart. 1984. Effect of pH, temperature and media on acid and alkaline phosphatase activity in "clinical" and "nonclinical" isolates of *Bordetella bronchiseptica*. Can. J. Comp. Med. *48*:175-178.
4. Litwin. 1985. Further studies on a tryptose based serum-free medium for human diploid fibroblasts. Dev. Biol. Stand. *60*:25-33.
5. Vaughn and Fan. 1997. Differential requirements of two insect cell lines for growth in serum-free medium. *In Vitro* Cell. Dev. Biol. Anim. *33*:479-482.
6. Hata and Kojima. 1990. *Angiostrongylus cantonensis*: *in vitro* cultivation from the first-stage to infective third-stage larvae. Exp. Parasitol. *70*:467-482.
7. Reduth, Schaub and Pudney. 1989. Cultivation of *Blastocrithidia triatomae* (*Trypanosomatidae*) on a cell line of its host *Triatoma infestans* (*Reduviidae*). Parasitology *98*:387-393.
8. Deutschmann and Jager. 1994. Optimization of the growth conditions of Sf21 insect cells for high-density perfusion culture in stirred-tank bioreactors. Enzyme Microb. Technol. *16*:506-512.
9. Munderloh and Kurtti. 1989. Formulation of medium for tick cell culture. Exp. Appl. Acarol. *7*:219-229.
10. Prodafikas and Plavsic. 2000. Effects of medium supplements on BHK-21 cell growth and bluetongue virus production. Focus *22*:35.
11. Sakoda and Fukusho. 1998. Establishment and characterization of a porcine kidney cell line, FS-L3, which forms unique multicellular domes in serum-free culture. *In Vitro* Cell Dev. Biol. Anim. *34*:53-57.

Growth Curve



Molecular Weight



Meat Peptones Typical Analyses Table

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Total Carbohydrate (mg/g)	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (1% Solution)	Calcium (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)
Beef Extract Powder, BBL™	12.4	2.3	0.19	56.10	9.3	3.5	0.3	6.9	264	27.4	285	28793	18510	0.00	0.53	3.22	1.8	4.0	2.8	2.8	0.6	0.6
Beef Extract, Desiccated, Bacto™	13.9	2.0	0.14	9.80	7.7	1.8	1.7	6.9	53	19.2	92	31423	21645	1.62	0.70	0.43	1.1	7.1	1.3	4.2	0.1	0.3
Gelysate™ Peptone, BBL	17.0	2.9	0.17	11.58	3.8	4.9	0.2	6.9	381	11.8	150	656	11090	0.00	1.66	0.18	0.8	8.8	3.1	6.3	0.1	0.1
Neopeptone, Bacto	13.6	3.2	0.20	13.13	6.9	4.0	1.4	7.4	77	5.3	28	8945	36313	0.48	0.45	2.59	0.5	4.3	0.5	2.6	0.2	0.3
Peptone, Bacto	15.4	3.5	0.20	6.29	3.8	2.7	1.7	7.1	30	7.8	17	2487	18127	0.90	0.32	0.40	1.2	9.2	2.8	5.8	0.3	0.3
Polypeptone™ Peptone, BBL	13.1	5.2	0.40	8.06	9.7	4.9	2.7	7.3	271	16.7	342	7340	44257	1.00	0.40	3.40	1.2	4.1	2.4	3.3	0.4	0.4
Proteose Peptone, Bacto	14.3	2.8	0.20	12.02	7.8	3.0	4.9	6.7	120	13.5	261	9123	29730	2.65	0.19	0.64	0.5	6.0	0.4	4.7	0.1	0.4
Proteose Peptone, BiTek™	13.1	3.1	0.24	10.30	13.1	4.8	10.3	6.8	219	12.0	680	7390	44750	4.93	1.01	0.94	0.8	7.0	0.4	4.4	0.1	0.6
Proteose Peptone No. 2, Bacto	12.9	5.0	0.39	18.07	12.1	3.5	7.1	7.3	151	10.2	212	13313	47610	3.86	0.38	1.88	1.6	5.2	1.4	4.1	0.5	1.1
Proteose Peptone No. 3, Bacto	13.4	3.7	0.28	17.94	10.5	2.3	6.6	7.4	132	23.7	103	13160	38113	2.54	0.37	1.51	0.9	5.2	0.8	4.3	0.3	0.6
Proteose Peptone No. 3, BiTek	12.8	3.1	0.24	12.35	13.1	1.3	12.5	6.7	129	10.6	214	8682	50153	9.40	0.17	1.22	0.8	6.4	0.8	5.1	0.1	0.7
Proteose Peptone No. 4, Bacto	14.3	2.7	0.19	12.17	7.8	3.3	3.9	7.0	169	12.5	280	9109	35280	2.63	0.34	0.72	0.5	6.5	0.4	4.6	0.1	0.3
Thiotone™ E Peptone, BBL	13.4	3.4	0.25	30.71	11.4	4.8	8.2	6.7	196	20.2	270	9629	46683	4.17	0.81	0.65	1.0	6.7	0.9	4.3	0.1	0.9
Tryptose, Bacto	13.3	4.5	0.34	10.56	8.8	3.2	3.2	7.3	191	34.2	110	9292	37740	1.61	0.23	2.05	1.2	4.3	1.9	3.5	0.4	0.5

LEGEND

* = Partially destroyed during hydrolysis
0.0 = Below limit of detection
■ = Free Amino Acids
■ = Total Amino Acids

For analytical methods, see Methods of Detection

Aspartic Acid (% Total)	Cystine (% Free)	Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)	Methionine (% Total) *	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)*	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
5.5	0.2	2.5	14.6	0.1	0.5	2.3	0.4	2.1	1.3	5.1	3.8	7.2	4.0	5.7	0.8	1.6	2.5	5.0	0.3	5.7	0.8	2.1	0.6	1.8	0.7	0.6	1.5	1.4	5.4
2.4	0.0	0.6	6.4	0.0	1.0	8.2	0.1	1.4	0.2	1.3	0.4	2.8	0.6	2.5	0.3	0.7	0.2	1.5	0.4	7.2	0.3	0.3	0.2	0.4	0.2	0.3	0.8	0.2	2.0
4.7	0.3	0.2	7.9	0.1	0.5	16.8	0.3	1.0	0.5	1.6	0.9	3.2	2.0	3.3	0.3	0.8	1.1	2.4	0.1	9.7	0.2	1.8	0.1	0.9	0.0	0.5	0.6	0.3	2.3
4.2	0.4	0.6	7.4	0.0	0.2	3.4	0.1	1.2	0.3	2.3	1.6	4.6	0.8	4.0	0.5	1.0	1.3	2.7	0.1	4.7	0.3	0.8	0.2	0.9	0.3	0.8	2.2	0.3	2.9
5.0	0.0	0.7	8.1	0.0	0.7	15.9	0.2	0.8	0.6	2.1	1.6	3.8	2.2	3.4	0.3	0.7	1.4	2.8	0.3	8.8	0.4	1.5	0.3	1.1	0.3	0.5	0.6	0.7	2.8
6.1	0.3	0.9	12.6	0.1	0.5	3.0	0.4	2.1	1.1	3.8	3.9	6.2	3.6	6.2	1.0	1.9	2.4	3.6	0.3	5.4	0.7	2.1	0.7	1.9	0.6	0.7	1.6	1.3	4.7
5.3	0.4	0.7	8.4	0.0	0.2	8.2	0.1	1.3	0.3	3.3	1.4	5.7	0.8	4.2	0.3	1.4	1.0	3.6	0.1	4.6	0.2	1.7	0.2	1.5	0.1	0.6	1.8	0.2	3.7
3.9	0.4	0.4	6.3	0.1	0.4	7.3	0.1	0.8	0.4	2.0	1.4	4.2	0.9	3.4	0.6	1.0	1.1	2.3	0.1	6.3	0.2	0.3	0.1	0.7	0.1	0.5	1.2	0.4	2.8
5.5	1.0	1.8	7.5	0.1	0.9	6.2	0.3	1.3	1.1	3.7	3.3	6.2	2.5	4.2	0.8	1.2	2.2	3.9	0.5	3.8	0.8	1.9	0.6	1.7	0.5	0.7	1.3	1.0	4.0
5.1	0.6	1.2	8.0	0.0	0.4	6.5	0.1	1.3	0.6	3.2	2.3	5.6	1.5	4.2	0.6	1.3	1.5	3.5	0.3	3.8	0.5	1.6	0.4	1.5	0.3	0.8	1.6	0.5	3.5
5.7	1.2	0.4	11.3	0.1	0.3	1.1	0.1	1.1	0.3	2.5	1.5	4.7	0.3	4.2	0.7	1.2	0.9	2.6	0.7	6.5	0.3	1.6	0.4	0.5	0.0	1.0	1.9	0.7	3.6
4.4	0.3	0.6	6.5	0.0	0.2	5.9	0.1	1.1	0.3	2.2	1.2	4.3	0.7	4.0	0.5	1.1	0.9	2.3	0.1	5.0	0.2	0.4	0.2	0.8	0.2	0.5	1.6	0.2	2.9
4.4	0.6	0.6	7.4	0.0	0.4	10.7	0.1	0.8	0.5	2.8	1.8	5.5	1.4	2.8	0.5	1.1	1.4	3.6	0.1	6.2	0.3	1.6	0.2	1.2	0.1	0.6	1.2	0.6	3.5
5.1	0.4	1.3	10.6	0.0	0.4	4.4	0.3	1.5	1.0	4.0	3.5	6.4	3.5	4.9	0.9	1.6	2.2	4.0	0.4	4.8	0.7	1.8	0.6	1.6	0.5	0.6	1.4	1.3	4.4

Casein and Whey Peptones

Casein and whey peptones are hydrolysates of bovine milk proteins. Milk is a complex material, consisting of water, lactose, lipids, salts and proteins. Casein (80%) and whey (20%) are the fundamental protein components in milk. After the cream, or fat, has been removed from bovine milk, hydrochloric or sulfuric acid is added in order to precipitate out casein, the insoluble portion.[1,2] The casein recovered is known as acid casein and is insoluble in water. Generally, the acid casein is dissolved in a suitable hydroxide such as NaOH, to make it soluble in water. The resulting sodium caseinate is then used as the basis for hydrolyzed caseins. Sodium caseinate typically consists of 87% to 90% protein.[3] Casein, which can make up about 3% of the total components in bovine milk, is one of the most nutritive of the milk proteins, as it contains all of the common amino acids and is rich in the essential ones. Casein peptones are manufactured by either acid or enzymatic hydrolysis (described in the Hydrolysis to Hydrolysate section).

The soluble supernatant material separated from milk after casein precipitates is whey, also called milk plasma. Whey contains the lactalbumin and lactoglobulin proteins and is a by-product of casein (and cheese) production. Whey protein concentrates and isolates are recovered using various separation technologies such as ion exchange and filtration; lactalbumin is recovered by heat denaturing and then separation.[1] Whey peptones are manufactured using the process of enzymatic hdrolysis on the proteins isolated from whey. The whey peptones contain free amino acids and peptides, as well as carbohydrates, vitamins, and minerals.

References

1. Huffman and Harper, W.J. 1999. Maximizing the value of milk through separation technologies. J. Dairy Sci. *82*:2238-2244.
2. Haurowitz, F. 1963, The chemistry and function of proteins, 2nd edition. Academic Press, New York.
3. Dziuba, Babuchowski, Smoczynski and Smietana. 1999. Fractal analysis of caseinate structure. Int. Dairy J. *9*:287-292.

BBL™ Acidicase™ Peptone
Bacto™ Casamino Acids
Bacto™ Casamino Acids, Technical

Acid Hydrolysates of Casein Product Description

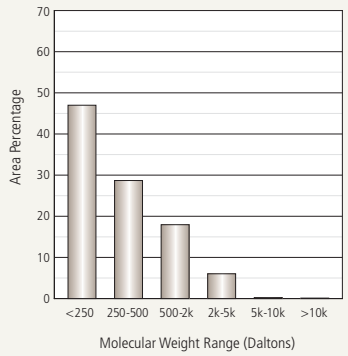
BBL™ Acidicase™ Peptone is a hydrochloric acid hydrolysate of casein. The manufacturing process produces a casein hydrolysate that has a high salt content of approximately 37% and nitrogen content of approximately 8%. The hydrolysis of the casein, a milk protein rich in amino acid nitrogen, is carried out until all the nitrogen is converted to amino acids or other compounds of relative simplicity. It is deficient in cystine, because casein contains little cystine, and in tryptophan, which is destroyed by the acid treatment.

Bacto™ Casamino Acids is an acid hydrolysate of casein, prepared according to the method described by Mueller and Miller.[1] The method described, reduces the sodium chloride and iron content of the hydrolyzed casein. This hydrolyzed casein, supplemented with inorganic salts, growth factors, cystine, maltose and an optimum amount of iron was used by Mueller and Miller to prepare diptheria toxin. Bacto Casamino Acids duplicate this specially treated hydrolyzed casein.

Bacto Casamino Acids, Technical is prepared similarly to Bacto Casamino Acids but is a less refined product, leaving a higher sodium chloride and iron content than in Bacto Casamino Acids.

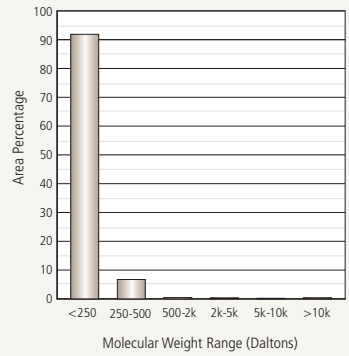
Molecular Weight

BBL™ Acidicase™ Peptone



Molecular Weight

Bacto™ Casamino Acids



Potential Applications

BBL Acidicase Peptone is intended for use as a nutritional supplement in vitamin assay, susceptibility testing and other laboratory media and microbial fermentation where the high salt content will not interfere.

Bacto Casamino Acids, due to the nearly complete hydrolysis of casein and the low sodium chloride and iron content, make an excellent supplement for many media formulations where nitrogen requirements are minimal. It has been recommended as a compromise for the replacement of pure amino acids in a defined medium for the growth of *Lactobacillus*, thus eliminating the complexity of preparation.[2] Additionally, it has been successfully used, along with Tryptone Peptone in nutritional studies to determine a bacterium's growth requirement for peptides or amino acids.[3,4] It also works well as a component in laboratory media. It has been utilized in such diverse applications as TYI-S-33 media for the parasite *Entamoeba histolytica* and LCM medium for the growth of a nematode-bacterium complex.[5]

Bacto Casamino Acids, Technical provides similar benefits to Bacto Casamino Acids, for applications requiring a less refined hydrolysate.

Physical Characteristics

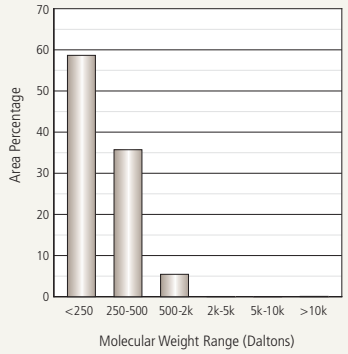
BBL™ Acidicase™ Peptone is a light beige, fine, homogeneous, free-flowing powder.

Bacto™ Casamino Acids is a very light beige, fine, homogeneous, free-flowing powder.

Bacto™ Casamino Acids, Technical, is a very light beige, fine, homogeneous, free-flowing powder.

Molecular Weight

Bacto™ Casamino Acids, Technical



Availability

Product Description	Cat. No.	Qty.
BBL™ Acidicase™ Peptone	211843	500 g
Bacto™ Casamino Acids	223050	500 g
Bacto™ Casamino Acids	223020	2 kg
Bacto™ Casamino Acids	223030	10 kg
Bacto™ Casamino Acids, Technical	223120	500 g
Bacto™ Casamino Acids, Technical	223110	10 kg

References

1. Mueller and Miller. 1941. Production of diphtheria toxin of high potency (100 lf) on a reproducible medium. J. Immunol. *40*:21-32.
2. Van Niel and Hahn-Hägerdal. 1999. Nutrient requirements of lactococci in defined growth media. Appl. Microbiol. Biotechnol. *52*:617-627.
3. Takahashi, Sato and Yamada. 2000. Metabolic pathways for cytotoxic end product formation from glutamate- and aspartate-containing peptides by *Porphyromonas gingivalis*. J. Bacteriol. *182*:4704-4710.
4. Attwood, Klieve, Ouwerkerk and Patel. 1998. Ammonia-hyperproducing bacteria from New Zealand ruminants. Appl. Environ. Microbiol. *64*:1796-1804.
5. Strauch and Ehlers. 2000. Influence of the aeration rate on the yields of the biocontrol nematode *Heterorhabditis megidis* in monoxenic liquid cultures. Appl. Microbiol. Biotechnol. *54*:9-13.

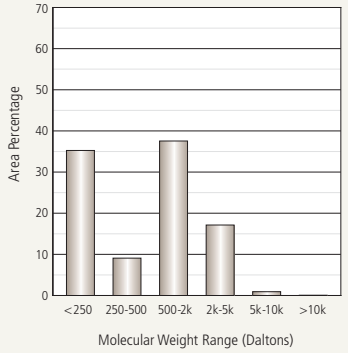
BBL™ Biosate™ Peptone

Product Description

BBL™ Biosate™ Peptone is a mixed hydrolysate comprised of 65% pancreatic digest of casein and 35% yeast extract.

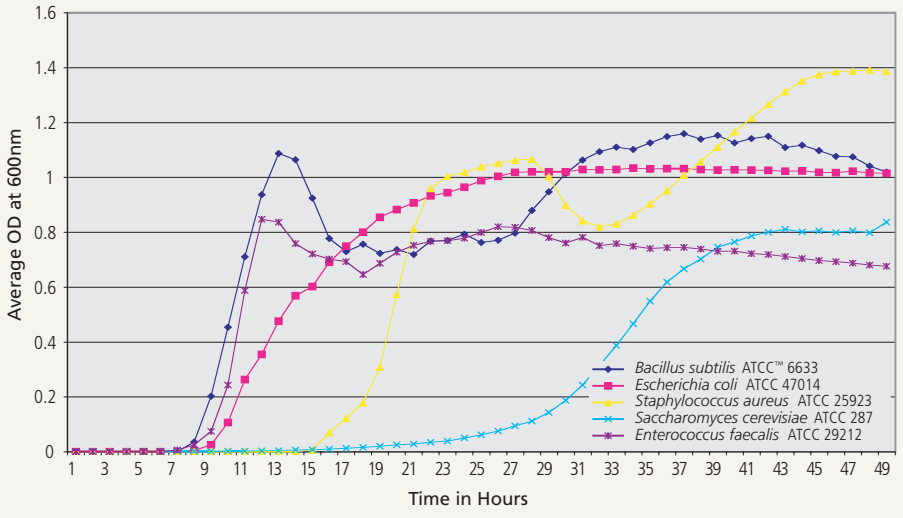
Molecular Weight

BBL™ Biosate™ Peptone



Growth Curve

1% BBL™ Biosate™ Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



Potential Applications

Biosate™ Peptone can be used as a component in microbiological media or in fermentation applications. The synergistic effect of two or more types of hydrolysates is well documented and has been utilized for decades in culture media formulation. The combination of pancreatic digest of casein and yeast extract provides nutritional benefits that are not provided by the components alone. It has been reported that the combined use of these two peptones has shown improved toxin production in clostridia.[1,2] Additionally, the combination of pancreatic digest of casein and yeast extract has been used successfully as components in media which supported the first-time culturing of a nematode without the need of its symbiotic bacteria.[3]

Physical Characteristics

BBL™ Biosate™ Peptone is a yellow-tan, fine, homogeneous, free-flowing powder.

Availability

Product Description	Cat. No.	Qty.
BBL™ Biosate™ Peptone	211862	454 g
BBL™ Biosate™ Peptone	294312	25 lb (11.3 kg)

References

1. Artemenko, Ivanova, Nenashev, Kuznetsova and Ochkina. 1985. Use of experimental analytical method for equilibrating nutrient broths for *Clostridium perfringens* type A growth and toxin production. Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii. *11*:37-41.
2. Siegel and Metzger. 1980 Effect of fermentation conditions on toxin production by *Clostridium botulinum* type B. Appl. Environ. Microbiol. *40*:1023-1026.
3. Dorsman and Bijl. 1985. Cultivation of free-living stages of *Trichostrongylus colubriformis* in media without bacteria, animal tissue extract, or serum. J. Parasitol. *71*:200-203.

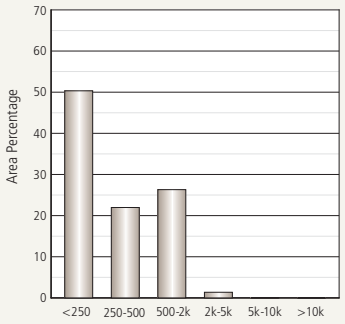
Difco™ Casein Digest

Product Description

Difco™ Casein Digest is an enzymatic digest of casein similar to NZ Amine A. Casein Digest is hydrolyzed under conditions different from other enzymatic digests of casein such as Tryptone and Casitone.

Molecular Weight

Difco™ Casein Digest



Potential Applications

Difco™ Casein Digest is applicable as a component in microbiological culture media. It was developed for use in molecular genetics media and is a component in NZCYM Broth, NZYM Broth and NZM Broth, which are used for cultivating recombinant strains of *Escherichia coli*. *E. coli* grows rapidly in these rich media because they provide amino acids, nucleotide precursors, vitamins and other metabolites that the cells would otherwise have to synthesize.[1]

Physical Characteristics

Difco™ Casein Digest is a light beige homogeneous, free-flowing powder.

Availability

Product Description	Cat. No.	Qty.
Difco™ Casein Digest	211610	500 g

Reference

1. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol.1. Current Protocols, New York, N.Y.

Bacto™ Casitone
BBL™ Trypticase™ Peptone
Bacto™ Tryptone
BiTek™ Tryptone

Enzymatic Digests of Casein Product Description

Bacto™ Casitone is a pancreatic digest of casein. The manufacturing process for an enzymatic digest of casein is not as destructive as an acid hydrolysis. Thus, the casein is not broken down as completely into its constituent components. In many cases this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

BBL™ Trypticase™ Peptone is a pancreatic digest of casein and is the primary nitrogen source in Trypticase Soy Broth and Agar.

Bacto™ Tryptone is a pancreatic digest of casein. It was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria. It is also notable for the absence of detectable levels of carbohydrates.

BiTek™ Tryptone is prepared similarly to Bacto Tryptone but the final product goes through fewer refinement steps during processing.

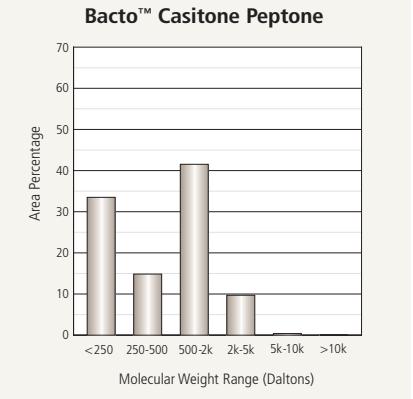
Potential Applications

Bacto™ Casitone can be used as a component in microbiological media or in fermentation applications. A recent publication has also reported that the stability of lyophilized influenza virus vaccine was augmented by the addition of 2% Casitone.[1]

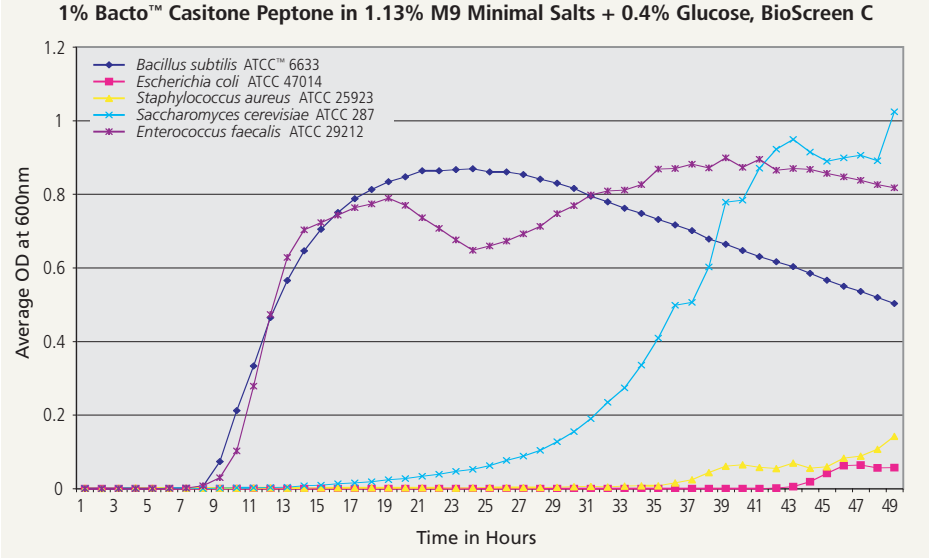
BBL™ Trypticase™ Peptone is recommended for use in media formulations, where good growth of fungi and bacteria is required. It is referenced in *Official Methods of Analysis of AOAC International* and meets the USP specifications for pancreatic digest of casein.[2,3]

Bacto™ Tryptone has been used in conjunction with Casamino Acids in nutritional studies to determine amino acids vs. peptide utilization.[4,5] It is included in standard methods manuals

Molecular Weight



Growth Curve



applications and is listed in the "Reagent" section of *The United States Pharmacopeia*, as meeting the specifications for pancreatic digest of casein, a component in many of the media listed.[2,3,6-9] *The European Pharmacopoeia* also lists pancreatic digest of casein as a component in many of the recommended media.[10] Bacto™ Tryptone also works well in fermentation applications. It has been used successfully with commonly used organisms such as *Escherichia coli*,[11] as well as uncommon organisms such as the diatom *Nitzschia laevis*.[12]

BiTek™ Tryptone provides some of the same benefits as Bacto Tryptone in instances where a less refined hydrolysate can be utilized.

Physical Characteristics

Bacto™ Casitone appears as tan, free-flowing granules.

BBL™ Trypticase™ Peptone is a very light beige, fine, homogeneous, free-flowing powder.

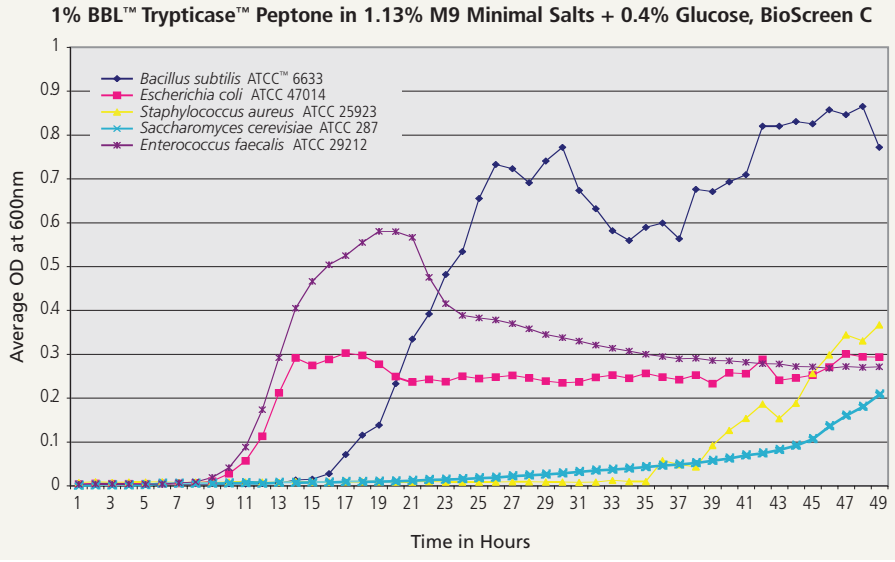
Bacto™ Tryptone is a light beige, homogeneous, free-flowing powder.

BiTek™ Tryptone is a light beige, homogeneous, free-flowing powder.

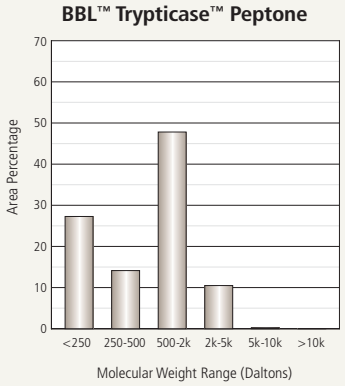
Availability

Product Description	Cat. No.	Qty.
Bacto™ Casitone	225930	500 g
Bacto™ Casitone	225910	10 kg
BBL™ Trypticase™ Peptone	211921	454 g
BBL™ Trypticase™ Peptone	211922	5 lb (2.3 kg)
BBL™ Trypticase™ Peptone	211923	25 lb (11.3 kg)
Bacto™ Tryptone	211705	500 g
Bacto™ Tryptone	211699	2 kg
Bacto™ Tryptone	211701	10 kg
BiTek™ Tryptone	251420	10 kg

Growth Curve

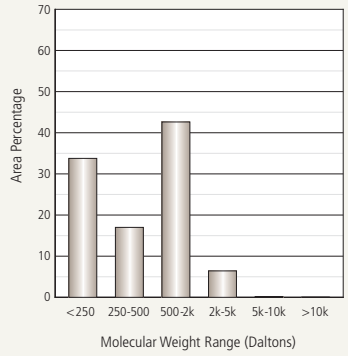


Molecular Weight



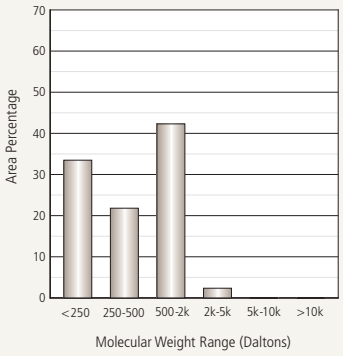
Molecular Weight

Bacto™ Tryptone Peptone



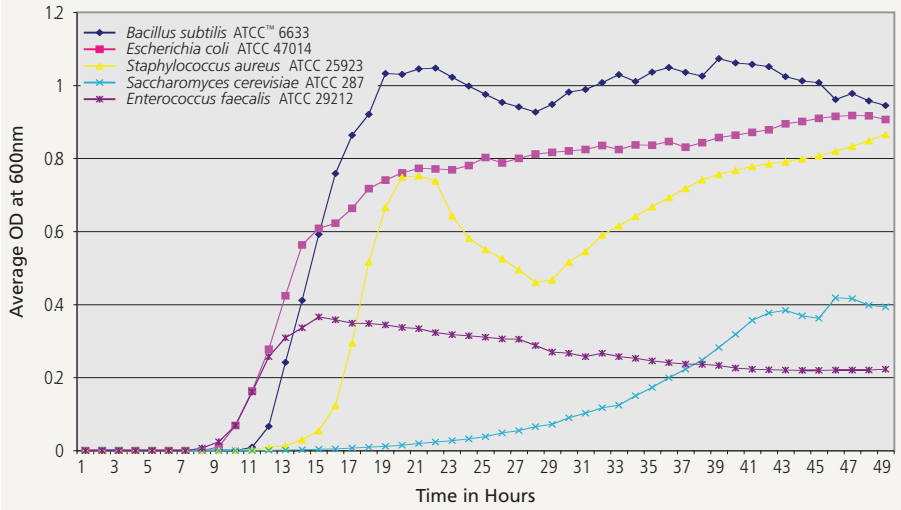
Molecular Weight

BiTek™ Tryptone Peptone



Growth Curve

1% Bacto™ Tryptone Peptone in 1.13% M9 Minimal Salts + 0.4% Glucose, BioScreen C



References

1. Yannarell, Goldberg and Hjorth. 2002 Stabilizing cold-adapted influenza virus vaccine under various storage conditions. J. Virol. Methods. Apr;*102*(1-2):15-25.
2. Horowitz. (ed.). 2005. Official methods of analysis of AOAC international. 18th ed. AOAC International, Gaithersburg, Md.
3. United States Pharmacopeial Convention, Inc. 2006. The United States Pharmacopeia 29/The national formulary 24—2006. United States Pharmacopeial Convention, Inc., Rockville, Md.
4. Takahashi and Yamada. 2000. Metabolic pathways for cytotoxic end product formation from glutamate- and aspartate-containing peptides by *Porphyromonas gingivalis* J. Bacteriol. *182*:4704-4710.
5. Nagel, Oostra, Tramper and Rinzema. 1999. Improved model system for solid-substrate fermentation: effects of pH, nutrients and buffer on fungal growth rate. Process Biochem. *35*:69-75.
6. Downes and Ito. (ed.).2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association. Washington D.C.
7. U.S. Food and Drug Administration. 1998. Bacteriological analytical manual, 8th ed., rev. A. AOAC International, Gaithersburg, Md.
8. Clesceri, Greenberg and Eaton. (ed.). 1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, Washington D.C.
9. Wehr (ed.). 2004. Standard methods for the examination of dairy products. 17th ed. American Public Health Association. Washington D.C.
10. European Directorate for the Quality of Medicines. 2006. Supplement 5.5, European Pharmacopoeia 5th ed. Council Of Europe, Strasbourg.
11. Sivakesavs, Chen, Hackett, Huang, Lam, Lam, Siu, Wong and Wong. 1999. Production of excreted human epidermal growth factor (hEGF) by an efficient recombinant *Escherichia coli* system. Process Biochem. *34*:893-900.
12. Wen and Chen. 2001. Optimization of nitrogen sources for heterotrophi production of eicosapentaenoic acid by the diatom *Nitzschia laevis*. Enzyme Microbia Technol. *29*:341-347.

Bacto™ TC Lactalbumin Hydrolysate

Product Description

Bacto™ TC Lactalbumin Hydrolysate is the enzymatically hydrolyzed protein portion of milk whey, which is recognized as a complete protein source. This product is a mixture of peptides, amino acids and carbohydrates, both simple and complex.

Potential Applications

Bacto TC Lactalbumin Hydrolysate is intended as a nutritional supplement for bacterial, insect and mammalian cell culture. For years, TC Lactalbumin Hydrolysate has been used as a nutritional source for lactobacilli. It is also useful for indole testing because of its high tryptophan content. TC Lactalbumin is frequently used in mammalian cell culture media as an amino acid supplement.[1]

Physical Characteristics

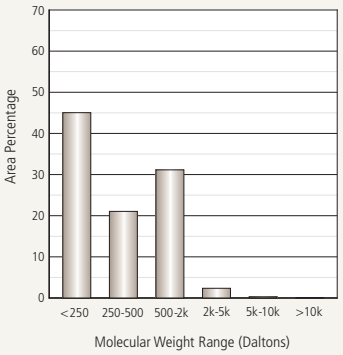
Bacto™ TC Lactalbumin Hydrolysate is a buff to tan homogeneous, free-flowing powder.

Availability

Product Description	Cat. No.	Qty.
Bacto™ TC Lactalbumin Hydrolysate	259962	500 g
Bacto™ TC Lactalbumin Hydrolysate	259961	10 kg

Molecular Weight

Bacto™ TC Lactalbumin Hydrolysate



Reference

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. *In* Norris and Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, New York.

Casein and Whey Peptones

Product Name	Total Nitrogen (%)	Amino Nitrogen (%)	AN/TN	Total Carbohydrate (mg/g)	Ash (%)	Loss on Drying (%)	NaCl (%)	pH (1% Solution)	Calcium (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% Free)	Alanine (% Total)	Arginine (% Free)	Arginine (% Total)	Asparagine (% Free)	Aspartic Acid (% Free)
Acidicase™ Peptone, BBL™	8.5	6.2	0.73	0.29	36.8	5.3	32.3	6.8	229	4.9	36	383	140900	16.99	0.25	1.42	1.6	2.1	1.3	1.9	0.0	3.4
Biosate™ Peptone, BBL	13.4	6.0	0.45	32.98	7.7	6.6	0.3	7.1	258	56.2	398	21320	17100	0.07	0.43	3.19	2.4	4.2	2.1	2.9	0.9	0.9
Casamino Acids, Bacto™	10.8	9.4	0.87	0.00	18.3	4.8	12.1	6.4	59	1.3	143	4098	88090	6.74	0.55	2.56	3.0	3.0	2.4	2.5	0.0	0.7
Casamino Acids, Technical, Bacto	8.3	5.9	0.71	0.15	36.0	1.2	30.1	6.9	110	6.2	48	1361	145667	18.25	0.26	1.53	2.1	4.4	1.1	1.7	0.0	3.1
Casein Digest, Difco	13.2	7.0	0.53	1.44	10.0	5.0	0.0	3.7	163	2.8	49	1091	23923	0.26	0.10	0.57	1.6	2.7	2.9	5.1	1.6	1.1
Casitone, Bacto	13.5	5.0	0.37	3.54	6.4	2.0	0.0	7.0	111	23.5	213	3480	34090	0.10	0.40	2.48	0.9	3.4	2.6	2.8	0.5	0.2
TC Lactalbumin Hydrolysate, Bacto	13.0	6.3	0.48	21.01	7.2	4.6	0.3	7.0	1620	50.3	340	17200	14800	0.80	1.20	4.10	2.3	4.7	2.2	2.5	0.9	0.9
Trypticase™ Peptone, BBL	14.2	5.2	0.37	3.99	5.7	4.0	0.1	7.2	295	33.5	110	588	26600	0.09	0.18	2.54	0.9	5.7	2.3	4.8	0.5	0.2
Tryptone, Bacto	13.3	5.3	0.40	4.30	6.6	2.3	0.0	7.3	256	23.0	195	3257	33910	0.06	0.33	2.58	1.0	3.2	2.2	5.0	0.6	0.4
Tryptone, BiTek™	13.1	5.6	0.43	8.42	5.8	5.0	0.0	7.1	387	7.3	100	620	26970	0.35	0.22	2.25	0.6	5.0	3.8	2.6	0.5	0.1

LEGEND

* = Partially destroyed during hydrolysis
0.0 = Below limit of detection
■ = Free Amino Acids
■ = Total Amino Acids

For analytical methods, see Methods of Detection

Typical Analyses Table

Aspartic Acid (% Total)	Cystine (% Free)	Glutamic Acid (% Free)	Glutamic Acid (% Total)	Glutamine (% Free)	Glycine (% Free)	Glycine (% Total)	Histidine (% Free)	Histidine (% Total)	Isoleucine (% Free)	Isoleucine (% Total)	Leucine (% Free)	Leucine (% Total)	Lysine (% Free)	Lysine (% Total)	Methionine (% Free)	Methionine (% Total) *	Phenylalanine (% Free)	Phenylalanine (% Total)	Proline (% Free)	Proline (% Total)	Serine (% Free)	Serine (% Total)*	Threonine (% Free)	Threonine (% Total)	Tryptophan (% Free)	Tyrosine (% Free)	Tyrosine (% Total)	Valine (% Free)	Valine (% Total)
3.9	0.8	8.3	11.6	0.0	0.8	1.0	0.8	1.6	1.6	4.0	3.9	6.3	4.4	4.6	0.9	1.4	2.5	3.5	3.3	5.3	2.1	2.5	0.9	1.4	0.0	1.0	1.4	1.8	4.4
5.9	0.3	3.5	16.1	0.3	0.6	2.2	0.6	2.0	1.6	5.8	4.7	7.7	3.5	5.9	1.0	1.9	2.9	5.5	0.5	6.2	1.0	2.2	0.8	1.9	0.7	0.5	1.4	1.9	6.1
2.4	0.1	15.1	15.9	0.0	1.4	1.4	0.2	0.8	3.1	4.0	4.6	5.0	2.1	5.2	1.4	1.4	3.4	3.6	7.5	8.0	0.4	2.1	0.5	1.5	0.0	0.4	0.4	4.7	5.6
3.4	0.4	5.1	8.4	0.0	0.8	1.1	0.5	1.1	1.2	2.7	2.7	4.6	4.0	4.6	0.9	1.2	1.4	1.9	2.9	5.7	2.1	1.6	0.9	0.5	0.0	1.5	1.6	1.6	3.4
6.0	2.4	3.6	16.8	0.1	0.5	1.7	1.3	2.2	2.5	3.9	6.8	7.8	5.4	6.7	2.3	2.7	3.5	4.0	1.1	7.4	1.7	4.2	2.0	2.2	7.2	3.4	3.6	3.1	5.5
5.5	0.0	0.9	16.0	0.0	0.2	1.7	0.4	1.9	1.1	5.9	4.7	7.9	4.5	5.9	1.1	2.2	2.7	5.5	0.3	7.1	0.8	2.1	0.5	1.9	0.8	0.5	1.6	1.3	6.3
6.5	0.2	6.6	8.7	0.3	1.3	2.7	0.5	1.1	2.1	3.6	3.5	4.9	2.5	8.4	1.6	2.5	0.8	2.3	0.5	1.1	1.5	4.2	1.3	1.4	0.6	0.8	0.9	2.4	3.7
7.7	0.3	1.1	13.2	0.1	0.1	6.3	0.5	4.8	1.1	8.3	5.3	10.4	3.3	10.6	1.1	2.5	2.7	7.1	0.2	10.9	0.4	2.5	0.6	2.4	0.8	0.4	1.6	1.5	9.1
5.2	0.3	1.4	15.1	0.1	0.2	1.7	0.5	1.9	1.3	5.5	4.8	7.5	5.5	6.2	1.0	2.1	3.0	5.2	0.2	6.6	0.7	2.2	0.7	1.8	0.8	0.5	1.3	1.7	5.9
3.9	0.4	0.7	9.8	0.1	0.1	1.4	0.6	1.6	1.1	3.8	4.2	6.0	5.4	5.9	0.7	1.4	2.8	3.4	0.1	7.3	0.7	0.3	0.7	0.8	0.8	0.4	1.2	1.5	4.6

BD Bionutrients™ Media

BD offers a wide variety of media products for cell culture and microbial fermentation applications supplying the biotechnology, pharmaceutical, animal and human vaccine, and bioremediation markets worldwide. This section of the BD Bionutrients™ Technical Manual highlights BD Cell™ MAb liquid media, Select APS™ powdered media, and chemically defined powdered media.

BD Bionutrients™ Media Products:

- BD Cell™ MAb Medium, Animal Component Free
- BD Cell™ MAb Medium, Quantum Yield
- BD Cell™ MAb Medium, Serum Free
- BBL™ Select APS™ LB Broth Base
- Difco™ Select APS™ Super Broth
- Difco™ M9 Minimal Salts, 5×
- Difco™ Yeast Nitrogen Base
- Difco™ Yeast Nitrogen Base w/o Amino Acids
- Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Chemically Defined Media Products:

- BD Cell™ MAb Medium, Quantum Yield
- Difco™ M9 Minimal Salts, 5×
- Difco™ Yeast Nitrogen Base
- Difco™ Yeast Nitrogen Base w/o Amino Acids
- Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

BD Cell™ MAb Medium, Animal Component Free
BD Cell™ MAb Medium, Quantum Yield
BD Cell™ MAb Medium, Serum Free

Product Description

BD Cell MAb media are formulated to produce high-yield monoclonal antibody secretion. BD Cell media show an increase in yield of 5-25 times more antibody than with conventional media. The amount of increased expression will be cell line dependent. BD Cell media not only increase yield, but also dramatically reduce media consumption and labor cost. Cells will retain viability for longer periods of time and require much less handling than is needed with traditional media.

BD Cell MAb media will support the growth of a wide variety of myeloma fusion partners and hybridomas including: Sp2/0, NS-1, P3X63Ag9, and FOX-NY as well as other secreting cell lines such as CHO. BD Cell media are HEPES based and can be used in either CO_2 incubators or non-CO_2 incubators in a closed system. Always pre-warm medium to 37°C before use.

If you supplement your current medium with special growth factors, cholesterol, or lipids, etc., your cells may still need these supplements. A trial period with and without the supplements is recommended.

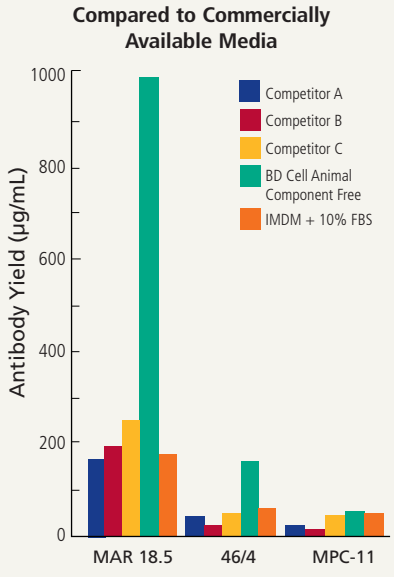
BD Cell™ MAb Medium, Animal Component Free, is a complete medium designed to enhance monoclonal antibody production *in vitro*. BD Cell™ Animal Component Free contains L-glutamine and is supplemented with 0.3% Select Soytone, an enzymatic digest of soybean. It does not contain phenol red or pluronic acid or other surfactants. It does not contain attachment factors, which will need to be added if this medium is used with attached dependent cell lines.

- BD Cell™ Animal Component Free is a 1× sterile medium with a shelf life of six months if stored at 4°C in the dark.
- pH at 25°C: 7.0 to 7.4
- Osmolality: 315 to 365 mOsm
- Endotoxin High limit: 10.0 EU/mL

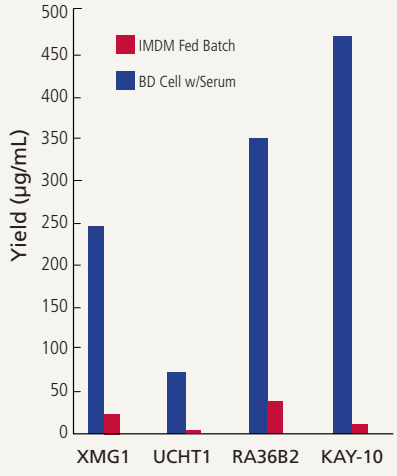
BD Cell™ MAb Medium, Quantum Yield, is a chemically defined basal cell culture medium designed to enhance monoclonal antibody production *in vitro*. BD Cell™ Quantum Yield contains L-glutamine and phenol red. It does not contain pluronic acid or other surfactants. BD Cell™ Quantum Yield is a basal medium and must be supplemented in the same way that you supplement your traditional basal medium (i.e., serum or serum-free supplements, antibiotics, etc.).

- BD Cell™ Quantum Yield is a 1× sterile medium with a shelf life of one year if stored at 4°C in the dark.
- pH at 25°C: 7.0 to 7.4
- Osmolality: 315 to 365 mOsm
- Endotoxin High limit: 1.0 EU/mL

BD Cell™ Animal Component Free

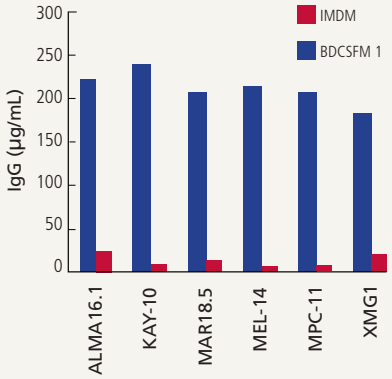


BD Cell™ Quantum Yield



BD Cell™ Serum Free

BDCSFM 1 compared to IMDM



BD Cell™ MAb Medium, Serum Free, is a complete medium designed to enhance monoclonal antibody production *in vitro*. BD Cell™ Serum Free contains L-glutamine and phenol red and has a total protein content of 1.1 mg/mL. The majority of the protein is comprised of bovine-serum albumin. It does not contain pluronic acid or other surfactants.

- BD Cell™ Serum Free is a 1× sterile medium with a shelf life of six months if stored at 4°C in the dark.
- pH at 25°C: 7.0 to 7.4
- Osmolality: 315 to 365 mOsm
- Endotoxin High limit: 1.0 EU/mL

NOTE: Do not freeze BD Cell media. Please note that BD Cell Quantum Yield and Serum Free media should be more orange-yellow in color than other basal and serum free media such as DMEM, IMDM or RPMI 1640.

Application

Production

Some cell lines may adapt readily to BD Cell™ Animal Component Free medium or Serum Free medium while other cell lines may require adaptation first to BD Cell Quantum Yield medium followed by adaptation to the animal free or serum free medium (see Adaptation of Cells into BD Cell MAb Medium). Protocols for various culture systems such as roller bottle, hollow fiber and stirred tank are available upon request or can be accessed at **www.bd.com/industrial**.

NOTE: Adaptation cultures will not be a true indicator of antibody production potential.

Fusions

BD Cell media have been found to work well in cell fusions thus eliminating the adaptation process for new clones. Normal fusion procedures can be followed with the substitution of BD Cell media for the traditional media.

Downstream Processing

High speed centrifugation or filtration procedures such as tangential flow filtration can be used for cellular material removal prior to purification.

Purification of products produced using BD Cell™ Animal Component Free medium may be greatly simplified due to the low molecular weight of the Select Soytone supplementation. Ammonium sulfate precipitation or diafiltration can be utilized to extract the protein of interest rather than usage of Protein A or Protein G affinity purification.

Normal purification procedures such as Protein A or Protein G work well for processing supernatants produced using BD Cell™ Quantum Yield.

Normal purification procedures such as Protein A or Protein G also work well for processing supernatants produced using BD Cell™ Serum Free medium. The purity of the product should be higher with BD Cell™ Serum Free medium as compared to product produced with serum supplemented media. The use of bovine serum albumin in BD Cell™ Serum Free medium eliminates the co-purification of bovine IgGs that occurs with serum supplemented media.

Availability

Product Description	Cat. No.	Qty.
BD Cell™ MAb Medium, Animal Component Free	220513	1000 mL
BD Cell™ MAb Medium, Quantum Yield	220511	1000 mL
BD Cell™ MAb Medium, Serum Free	220509	1000 mL

Custom packaging is available upon request.

Adaptation of Cells into BD Cell™ MAb Medium

Some cell lines may adapt readily to BD Cell™ MAb media, but most will require a brief period of adaptation. Cell lines may necessitate adaptation first to BD Cell™ Quantum Yield basal medium followed by adaptation to BD Cell™ Animal Component Free medium or Serum Free medium. It is recommended that adaptation to BD Cell™ media be performed before changing to a new growth system.

Cells perform best in BD Cell™ medium when grown in an aerated system such as a roller bottle, spinner, CELLine™ flask, etc., but adaptation can be done in a stationary system if desired. A T-flask or small roller bottle is easy to handle and uses minimal medium. Use of CO_2 is optional if using a closed (non-vented) system. When seeding the cells, it is best to take an aliquot from an existing culture and seed into the new media combination. This enables cell-produced growth factors to assist the cells in adaptation to the new medium. Likewise, small splits at 1:2 or 1:3 are better than 1:5 or 1:10 as this will also retain the cell-generated growth factors and help keep cells in log phase growth. Centrifuging off the current medium is not recommended.

Procedure

From cells that are in growth phase with at least 80% viability, seed the adaptation culture vessel at 2×10^5/mL in 50% BD Cell™ medium and 50% current culture medium. Pass the cells for at least two days in this media combination. If cells retain normal doubling time and good viability they are ready for the next medium combination. It is best to split the cells down no lower that $2\text{-}3 \times 10^5$/mL and work with cells daily. For weekend splits, the cells may be reduced to 1×10^5/mL.

Next, seed cells at 2×10^5/mL in 75% BD Cell™ medium and 25% current culture medium. Pass the cells again for at least two days keeping the densities as suggested above. The cells may begin to slow down in their doubling time. If this happens, give the cells more time to adapt at this medium combination.

When cells are growing well in the 75/25% combination, they are ready to be seeded into 100% BD Cell™ medium. Again, keep the cell density high to help with the adaptation. Allow at least 2 to 3 cell passages at 100% BD Cell™ medium before moving cells to the production system. It is best not to change to 100% BD Cell™ medium over a weekend but early in the week so cells can be passed every day at higher cell densities.

While the cells are being adapted, production can be monitored but until the cells are placed in an aerated production system, the results will not be indicative of the true production performance of BD Cell™ medium.

BBL™ Select APS™ LB Broth Base
Difco™ Select APS™ Super Broth

Product Description

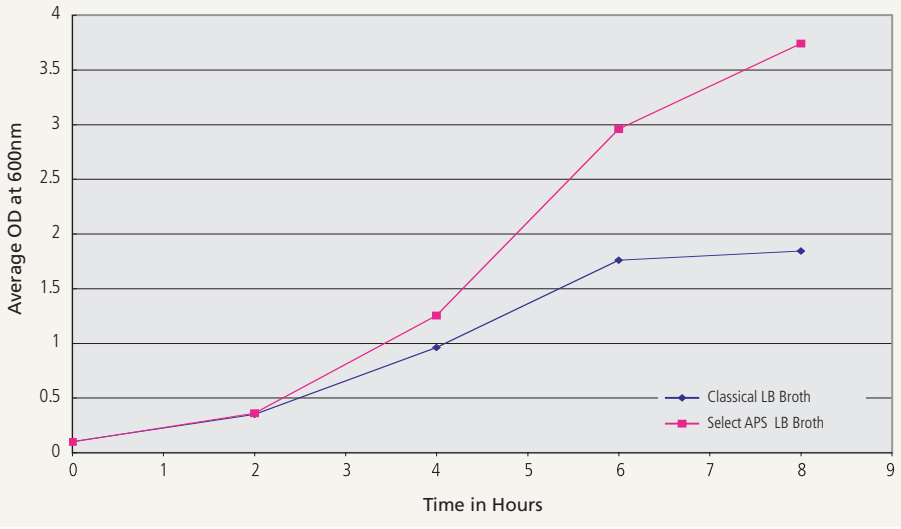
The Select Alternative Protein Source (APS) media were designed as alternatives to classical animal-based media for the maintenance and propagation of *Escherichia coli* strains in molecular genetics procedures. Select APS™ media are manufactured from animal-free ingredients in order to minimize the risk of bovine spongiform encephalopathy in culture media containing animal, and especially bovine, materials.

Select APS LB Broth Base is based on the LB Broth Lennox formulation (1% tryptone, 0.5% yeast extract and 0.5% sodium chloride) with 5.0 g/L sodium chloride, which was developed by Lennox for the growth and maintenance of recombinant strains of *E. coli*.[1] The tryptone in the classical LB Lennox formulation is replaced by a combination of soy hydrolysate and yeast extract in the Select APS LB Broth Base. Soy peptone provides nitrogen and carbon essential for bacterial metabolism. Yeast extract supplies vitamins, amino acids and trace elements which enhance bacterial growth and plasmid yield. Sodium chloride provides sodium ions for transport and osmotic balance.

Select APS Super Broth, like BBL Super Broth II, is based on the Terrific Broth formulation designed by Tartof and Hobbs[2] to improve yield of plasmid-bearing *E. coli* strains over that of LB Broth. The 1.2% tryptone in Terrific Broth was replaced with soy hydrolysate in the same concentration in Select APS Super Broth. The 2.4% yeast extract and recommended addition of 5 mL/L glycerol is the same for Select APS Super Broth as is used in classical Terrific Broth. The buffering system, 1.14% dipotassium phosphate and 0.17% monopotassium phosphate, is altered from that of classical Terrific Broth. As with Select APS LB Broth, soy hydrolysate provides nitrogen and carbon compounds for bacterial metabolism. Yeast extract supplies vitamins, amino acids and trace elements which enhance bacterial growth and plasmid yield. The phosphate buffering system prevents cell death caused by pH drop. Glycerol is added as a carbon and energy source which, unlike glucose, is not fermented to acetic acid.

Growth Curve

Growth Performance of Classical LB Broth Base vs. Select APS™ LB Broth *E. coli* DH5α



Applications

Select APS™ media are nutrient-rich formulations designed to out-perform classical animal-based molecular genetics media formulations.

Select APS™ LB Broth Base is an excellent all-purpose growth medium for the propagation and maintenance of *E. coli* in molecular biology procedures. Figure 1 shows *E. coli* DH5α growth curves comparing the classical LB Broth Base formulation to Select APS LB Broth Base in shaker flask culture. The Select APS LB Broth allowed for faster growth of the plasmid carrying *E. coli* strain and showed twice the optical density (OD) after nine hours as did the classical LB Broth formulation containing tryptone.

Select APS™ Super Broth is a molecular genetics medium designed to grow *Escherichia coli* to a high cell density. There is no glucose in the formulation thus preventing acetate build-up in the fermentation of the organism.[3] Figure 2 shows *E. coli* DH5α growth curves comparing the classical Super Broth formulation to Select APS Super Broth in shaker flask culture. The Select APS formulation allowed comparable log growth to the BBL Super Broth II and held lag OD through at least 15 hours while the Super Broth II culture OD declined.

Formulae

BBL™ Select APS™ LB Broth Base

Approximate Formula* Per Liter

Soy Hydrolysate	2.5 g
Yeast Extract	12.5 g
Sodium Chloride	5.0 g

Dissolve 20.0 g of the Select APS LB Broth Base powder in 1 L of purified water. Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures. Final pH 6.6 – 7.1

*Adjusted and/or supplemented as required to meet performance criteria.

Difco™ Select APS™ Super Broth

Approximate Formula* Per Liter

Soy Hydrolysate	12.0 g
Yeast Extract	24.0 g
Dipotassium Phosphate	11.4 g
Monopotassium Phosphate	1.7 g

Suspend 49.1 g of the Select APS Super Broth powder and 5 mL of glycerol in 1 L of purified water. Mix thoroughly. Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures. Final pH 6.8 – 7.5

*Adjusted and/or supplemented as required to meet performance criteria.

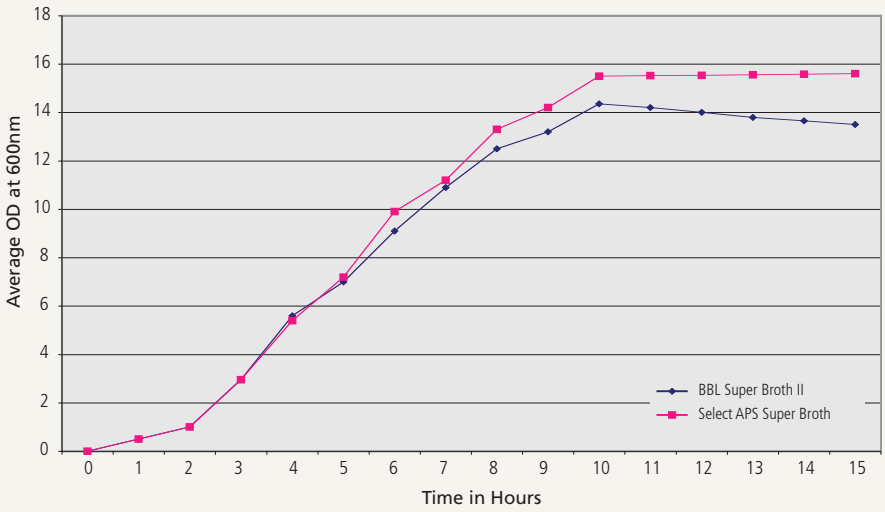
Physical Characteristics

Select APS Super Broth is a tan, free-flowing powder.

Select APS LB Broth is a tan, free-flowing powder.

Growth Curve

Growth Performance of BBL™ Super Broth II vs. Select APS™ Super Broth *E. coli* DH5α



Availability

Product Description	Cat. No.	Qty.
BBL™ Select APS™ Super Broth	212485	500 g
BBL™ Select APS™ Super Broth	212486	10 kg
Difco™ Select APS™ LB Broth	292438	500 g
Difco™ Select APS™ LB Broth	212484	10 kg

Reference

1. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology *1*:190-206.
2. Tartof and Hobbs. 1987. Improved media for growing plasmid and cosmid clones. Bethesda Research Laboratories Focus *9*:12.
3. Swartz. 2001. Advances in *Escherichia coli* production of therapeutic proteins. Curr. Opinion Biotechnology *12*:195-201.

Chemically Defined Media

Chemically defined media contain known quantities of only chemically defined ingredients added to purified water for the cultivation of microorganisms, mammalian or insect cells. Defined media include no complex ingredients such as proteins, hydrolysates, animal-derived ingredients, or constituents of unknown composition. There are benefits to using chemically defined media that vary depending on the type of cells cultured and the purpose and scope of application, which range from laboratory scale metabolic studies to production scale fermentation or cell culture.

The absence of animal-derived components, desirable from a regulatory standpoint due to concerns over BSE/TSE, is a major advantage to chemically defined media. Reproducibility is another advantage to using chemically defined media; all the components of a defined medium have known chemical structures, which allows for consistent performance of cells in the medium. Use of a chemically defined media formulation also offers greater simplicity of both downstream processing and the analysis of products.

Despite the advantages, chemically defined media are rarely used in industrial fermentations because complex media usually allow higher yields at lower cost.[1] Complex ingredients that are inexpensive by-products of food and agriculture industries will provide a majority of nutrients needed for bacterial and yeast fermentation. Each microorganism has a specific set of nutritional requirements that may add a long list of expensive growth factors such as L-amino acids and vitamins to a defined formulation.[2] Chemically defined media must be optimized specifically for each individual organism, and the design time may be quite lengthy and expensive. Even then, after an extended media development period, the optimized defined media may produce lower yields than will a complex medium.

Semi-defined media can provide a balance between maximum performance and minimum downstream processing issues. Semi-defined media are prepared by adding a small amount (from 0.05 to 0.5%) of a complex ingredient, such as a protein hydrolysate or yeast extract, to a chemically defined medium. The small amount of complex material in a semi-defined medium may provide enough nutrients to enhance growth of microorganisms without interfering with recovery or analysis of products.[1,3] Utilizing ultrafiltered peptones or extracts may reduce difficulties with downstream processing while providing cells with necessary nutrients. Besides reducing endotoxin levels, ultrafiltration contributes to solubility and ease of filtration of a protein product.

Chemically defined media are very well suited to research purposes at laboratory scale. Reproducibility from working with known constituents makes chemically defined media useful for studying cells' metabolic pathways and nutritional requirements for growth and product formation. Chemically defined media can be optimized for yield or performance by individually controlling the ingredients, especially any possible limiting nutrients, in the formulation. Chemically defined media are also valuable as basal media for screening various complex ingredients such as hydrolysates and extracts. Knowledge gained from biochemical studies and peptone analyses can drive further media improvement and scale-up to production.

BD offers several chemically defined media formulated from components chosen based on purity and quality standards. M9 Minimal Salts, 5×, is a minimal chemically defined dehydrated culture medium that—with the addition of dextrose—is optimized for *Escherichia coli*. The Yeast Nitrogen Base products are minimal chemically defined dehydrated culture media used for yeast molecular genetics studies. M9 Minimal Salts and the Yeast Nitrogen Base products are tested to ensure product quality and lot-to-lot consistency using various physical, chemical and growth support tests.

References

1. Dahod. 1999. Raw materials selection and medium development for industrial fermentation processes. *In* Demain, Davies (ed.), Manual of industrial microbiology and biotechnology, 2nd ed. American Society for Microbiology, Washington, D.C.
2. Cote. 1999. Media composition, microbial laboratory scale. *In* Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York.
3. Michels and Rosazza. 1999. Methods for biocatalysis and biotransformations. *In* Demain, Davies (ed.), Manual of industrial microbiology and biotechnology, 2nd ed. American Society for Microbiology, Washington, D.C.

Difco™ M9 Minimal Salts, 5×

Product Description

Difco™ M9 Minimal Salts, 5× is used in preparing M9 Minimal Medium which is used for cultivating recombinant strains of *Escherichia coli*. M9 Minimal Salts, 5× is a minimal chemically defined dehydrated culture media comprised only of ingredients with known chemical structures.

Sodium phosphate and potassium phosphate are present as buffering agents. Ammonium chloride is a source of nitrogen for cellular systems. Sodium chloride provides essential ions. Glucose may be added as a source of carbohydrate. Supplementing the medium with magnesium and calcium increases the growth of recombinants.

Applications

M9 Minimal Salts, 5× is a 5× concentrate that is diluted to a 1× concentration and supplemented with an appropriate carbon and energy source, such as dextrose, to provide a minimal, chemically defined medium that contains only those ingredients essential for the growth of *E. coli*. The medium will support the growth of "wild-type" strains of *E. coli* and various other bacteria. M9 Minimal Medium may be supplemented with magnesium sulfate, calcium chloride, and other nutrients for increased growth of microorganisms.

M9 Minimal Medium is useful for maintaining positive selection pressure on plasmids coding for the ability to produce essential substances such as amino acids or vitamins. M9 Minimal Medium is also used to maintain stocks of F′-containing bacteria for use with M13. The medium can be supplemented with specific amino acids or other metabolites, allowing for selection of specific auxotrophs. Consult appropriate references for recommended test procedures.[1-3]

M9 Minimal Medium may be used as a chemically defined basal medium for screening peptones as nitrogen sources for the cultivation of various microorganisms.

Formulae

M9 Minimal Salts, 5×

Approximate Formula* Per Liter

Disodium Phosphate (anhydrous)	33.9 g
Monopotassium Phosphate	15.0 g
Sodium Chloride	2.5 g
Ammonium Chloride	5.0 g

Final pH 6.6 to 7.0 at 25°C

*Adjusted and/or supplemented as required to meet performance criteria.

Physical Characteristics

Difco™ M9 Minimal Salts, 5× is a white, free-flowing, homogeneous powder.

Availability

Product Description	Cat. No.	Qty.
Difco™ M9 Minimal Salts, 5×	248510	500 g

References

1. Davis, Dibner and Battey. 1986. Basic methods in molecular biology. Elsevier, New York, NY.
2. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
3. Treco and Lundblad. 1997. Preparation of yeast media. *In* Ausubel (ed.), Short protocols in molecular biology, Wiley, New York, NY.

Difco™ Yeast Nitrogen Base
Difco™ Yeast Nitrogen Base w/o Amino Acids
Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Product Description

The yeast nitrogen bases are minimal chemically defined dehydrated culture media.

Yeast Nitrogen Base (YNB) contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of carbon. YNB is a defined base composed of salts, vitamins, amino acids and trace elements with ammonium sulfate as the sole nitrogen source in this basal medium. Addition of a carbon source is required.

Yeast Nitrogen Base w/o Amino Acids contains all essential vitamins and inorganic salts necessary for the cultivation of yeasts except the amino acids histidine, methionine, tryptophan and a source of carbon. As in YNB, ammonium sulfate is the sole source of nitrogen in this basal medium. Addition of a carbon source is required.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate has the same formulation as the YNB w/o Amino Acids except that ammonium sulfate as a source of nitrogen has been omitted. Addition of a carbon source is required.

Applications

Chemically defined growth media are useful tools for screening yeast strains and selecting for growth requirements.

Yeast Nitrogen Base is used for classifying yeasts based on carbon assimilation.

Yeast Nitrogen Base w/o Amino Acids, which lacks the amino acids histidine, methionine and tryptophan, is used for classifying yeasts based on amino acid and carbohydrate requirements.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate, which lacks amino acids and ammonium sulfate, is used for classifying yeasts based on carbon and nitrogen requirements.

Yeast Nitrogen Base w/o Amino Acids and Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate are prepared according to modifications of Wickerham's Yeast Nitrogen Base formulation.[1-3] These media are utilized in many applications for the study of yeasts in molecular genetics. Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate is recommended as a base in preparing several synthetic minimal media and synthetic complete and dropout media for yeast studies.[4-6]

Physical Characteristics

Yeast Nitrogen Base is off-white, free-flowing, homogeneous.

Yeast Nitrogen Base w/o Amino Acids is off-white, free-flowing, homogeneous.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate is light yellowish beige, free-flowing, homogeneous.

References

1. Wickerham. 1951. Taxonomy of yeasts. Technical bulletin No. 1029, U.S. Dept Agriculture, Washingon, D.C.
2. Wickerham. 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. *52*:293.
3. Wickerham and Burton. 1948. Carbon assimilation tests for the classification of yeasts. J. Bacteriol. *56*:363.
4. Treco and Lundblad. 1997. Preparation of yeast media. In Ausubel (ed.), Short protocols in molecular biology, Wiley, New York, NY.
5. Sherman. 1991. Getting started with yeast. *In* Guthrie and Fink (ed.), Methods in enzymology, vol. 194, guide to yeast genetics and molecular biology, Academic Press, Inc., New York, NY.
6. Warren. 2003. *Candida*, *Cryptococcus* and other yeasts of medical importance. *In* Murray (ed.), Manual of clinical microbiology, 8th ed., American Society for Microbiology, Washington, D.C.

Approximate Formula* Per Liter

Nitrogen Sources	Nitrogen Base	Yeast Nitrogen Base w/o Amino Acids	Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate
Ammonium Sulfate	5.0 g	5.0 g	--
Amino Acids			
L-Histidine Monohydrochloride	10 mg	--	--
LD-Methionine	20 mg	--	--
LD-Tryptophan	20 mg	--	--
Vitamins			
Biotin	2 µg	2 µg	2 µg
Calcium Pantothenate	400 µg	400 µg	400 µg
Folic Acid	2 µg	2 µg	2 µg
Inositol	2000 µg	2000 µg	2000 µg
Niacin	400 µg	400 µg	400 µg
p-Aminobenzoic Acid	200 µg	200 µg	200 µg
Pyridoxine Hydrochloride	400 µg	400 µg	400 µg
Riboflavin	200 µg	200 µg	200 µg
Thiamin Hydrochloride	400 µg	400 µg	400 µg
Compounds Supplying Trace Elements			
Boric Acid	500 µg	500 µg	500 µg
Copper Sulfate	40 µg	40 µg	40 µg
Potassium Iodide	100 µg	100 µg	100 µg
Ferric Chloride	200 µg	200 µg	200 µg
Manganese Sulfate	400 µg	400 µg	400 µg
Sodium Molybdate	200 µg	200 µg	200 µg
Zinc Sulfate	400 µg	400 µg	400 µg
Salts			
Monopotassium Phosphate	1.0 g	1.0 g	1.0 g
Magnesium Sulfate	0.5 g	0.5 g	0.5 g
Sodium Chloride	0.1 g	0.1 g	0.1 g
Calcium Chloride	0.1 g	0.1 g	0.1 g
Final pH	**5.2 to 5.6 at 25°C**	**5.2 to 5.6 at 25°C**	**4.3 to 4.7 at 25°C**

*Adjusted and/or supplemented as required to meet performance criteria.

Availability

Product Description	Cat. No.	Qty.
Difco™ Yeast Nitrogen Base	239210	100 g
Difco™ Yeast Nitrogen Base w/o Amino Acids	291940	100 g
Difco™ Yeast Nitrogen Base w/o Amino Acids	291920	2 kg
Difco™ Yeast Nitrogen Base w/o Amino Acids	291930	10 kg
Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate	233520	100 g
Difco™ Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate	233510	10k g

Definition of Methods

Analytical tests used in data gathering for this manual are described below.

The **Amino Nitrogen (AN)** test procedure is based on the AOAC Sorensen method.

The **AN/TN** ratio gives an estimate of the degree of protein hydrolysis.

Ash values were measured after heating at 650°C overnight. Ash values refer to the non-combustible portion of the sample and roughly correspond to the mineral content of the sample.

Total Carbohydrate percentage was calculated by colorimetric assay.

Chloride, Sulfate and Phosphate percentages were determined by ion chromatography.

Elemental analysis was determined by ICP (Inductively Coupled Plasma) using a Thermo Jarrell Ash instrument or equivalent.

Endotoxin values were determined by a quantitative kinetic chromogenic method.

Free Amino Acids are defined as amino acids that are not part of a protein or peptide chain. The amino acids were measured using the Waters AccQ•Tag™ Method. The AccQ•Tag Method is based on the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimide-activated heterocyclic carbamate.

Labsystems BioScreen C is a 200-well incubating kinetic optical density reader. Media were inoculated with approximately 100 CFU per 200 µL fill in each well. OD readings were averaged from 4 duplicate wells.

Loss on Drying is a measurement of moisture in the dehydrated sample. The test procedure is based on the method described in *The United States Pharmacopeia* with modifications.[1]

Molecular weight distribution, which is an indication of degree of protein digestion, was determined by size-exclusion chromatography using an agarose/dextran matrix based column and a TFA/acetonitrile based mobile phase.

Nucleoside quantitation (hypoxanthine & thymidine) was determined by reverse-phase HPLC using a silica-based column and a phosphate/methanol gradient.

pH was measured potentiometrically at room temperature in a 1% solution after autoclaving.

Sodium Chloride was determined by silver nitrate/potassium thiocyanate titration method.

Total Amino Acids were measured by the same method as the Free Amino Acids after an acid hydrolysis at 110°C for 20 hours 45 minutes using a CEM microwave. Asparagine, cystine, glutamine and tryptophan are destroyed during the hydrolysis. The asparagine, cystine, glutamine and tryptophan values are not reported for Total Amino Acids. Methionine and serine are partially destroyed during the hydrolysis.

Total Nitrogen (TN) content is determined by the Kjeldahl Method.

References:

1. United States Pharmacopeial Convention, Inc. 2006. The United States pharmacopeia 29/The national formulary 24—2006. United States Pharmacopeial Convention, Inc., Rockville, Md.

Regulatory Documentation

BD prides itself on the investment it has made in regulatory compliance, based on government agency guidance and customer feedback. Our strong commitment to quality products, reliably delivered with the appropriate documentation, has resulted in the provision of the following services.

Certificates of Analysis

Certificates of Analysis (COA) are available on all production products and include animal source information when applicable (see Exhibit 1). As a leader in manufacturing and sourcing meat-based products, BD has invested in a very intensive documentation program.

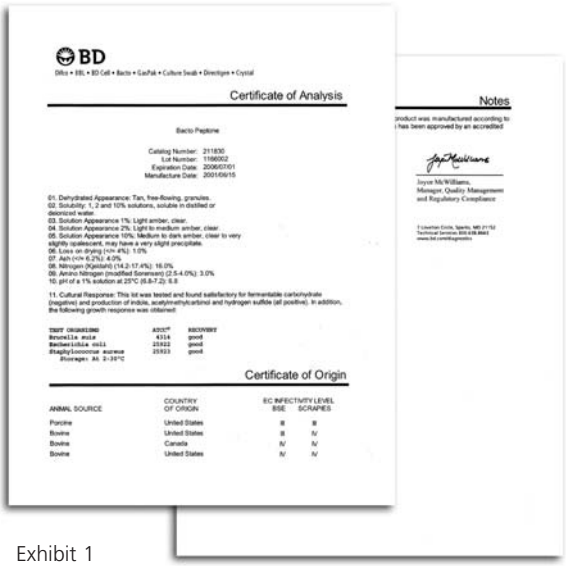


Exhibit 1

In order to streamline the communication and transmission of COAs and the animal origin information they contain, BD provides COAs and other regulatory documentation on the Internet, 24 hours a day, 7 days a week. With specific catalog number and lot number information, you can access certificates on the BD web site at **www.bdregdocs.com**.

Drug Master Files (DMF)

BD maintains Drug Master Files (DMF) on certain key proprietary products used in the manufacture of bio-therapeutics.

A DMF is a submission to the Food and Drug Administration (FDA) that contains confidential information on the manufacturing, process and packaging of a raw material used in the production of a drug. The information contained in the DMF may be used to support an Investigational New Drug Application (IND). The FDA reviews DMF information upon written request by the DMF holder in support of another regulatory application.

For more information on DMF availability and permission to reference, please contact your local BD representative.

Change Notification Program

BD offers an Automated Change Notification Program to customers who require notification of agreed-upon manufacturing and process changes. The program provides greater assurances that these changes are occurring under our ISO-certified Quality Systems.

To request a Change Notification Program packet, please e-mail **ProductInfo@bd.com**.

Certificates of Suitability

BD participates in the European Pharmacopeia program for Certificates of Suitability (COS), for animal derived products. Under the procedure, based on Resolution of the Public Health Committee (Partial Agreement, Resolution AP-CSP (99) 4), BD has applied for certificates concerning: evaluation of the suitability of the control of the chemical purity and micro-biological quality of the substance according to the corresponding specific monograph; or the evaluation of reduction of Transmissible Spongiform Encephalopathy (TSE) risk.

Although each of our products with a COS contain this cross reference in the body of the respective COA, if you would like a complete list of products certified or under application, please contact your local BD representative.

Product Listing

Product Name	100 g	454 g	500 g	2 kg	5 lb (2.3 kg)	10 kg	25 lb (11.3 kg)	25 kg	50 kg
Acidicase™ Peptone, BBL™			211843						
Beef Extract Powder, BBL™			212303						
Beef Extract, Desiccated, Bacto™			211520						
Biosate™ Peptone, BBL™		211862					294312		
Casamino Acids, Bacto™			223050	223020		223030			
Casamino Acids, Technical, Bacto™			223120			223110			
Casein Digest, Difco™			211610						
Casitone, Bacto™			225930			225910			
Gelysate™ Peptone, BBL™		211870							
Malt Extract, Bacto™			218630			218610			
Neopeptone, Bacto™			211681			211680			
Peptone, Bacto™			211677	211820		211830			
Phytone™ Peptone, BBL™		211906			298147	292450			
Polypeptone™ Peptone, BBL™		211910				297108			
Proteose Peptone No. 2, Bacto™			212120			212110			
Proteose Peptone No. 3, Bacto™			211693	212220		212230			211692
Proteose Peptone No. 3, BiTek™								253720	
Proteose Peptone No. 4, Bacto™						211715			
Proteose Peptone, Bacto™			211684			212010			
Proteose Peptone, BiTek™						253310			
Select Phytone™ UF, Difco™			210931			210936			
Select Soytone, Difco™			212488			212489			
Soytone, Bacto™			243620			243610			
TC Lactalbumin Hydrolysate, Bacto™			259962			259961			
TC Yeastolate, Bacto™	255772					255771		292731	
TC Yeastolate, UF, Difco™			292804			292805			
Thiotone™ E Peptone, BBL™			212302						
Trypticase™ Peptone, BBL™		211921			211922		211923		
Tryptone, Bacto™			211705	211699		211701			
Tryptone, BiTek™						251420			
Tryptose, Bacto™			211713			211709			
Yeast Extract, Bacto™			212750	212720		212730			212710
Yeast Extract, BBL™		211929			211930		211931		
Yeast Extract, Technical, Bacto™			288620			288610			
Yeast Extract, UF, Difco™			210929			210934			

	Powdered Media				Liquid
Bionutrients Media Product	100 g	500 g	2 kg	10 kg	1000 mL
BD Cell™ MAb Medium, Animal Component Free					220513
BD Cell™ MAb Medium, Quantum Yield					220511
BD Cell™ MAb Medium, Serum Free					220509
M9 Minimal Salts 5×, Difco™		248510			
Select APS™ LB Broth Base, BBL™		292438		212484	
Select APS™ Super Broth, Difco™		212485		212486	
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate, Difco™	233520			233510	
Yeast Nitrogen Base w/o Amino Acids, Difco™	291940		291920	291930	
Yeast Nitrogen Base, Difco™	239210				

BD Biosciences
7 Loveton Circle
Sparks, MD 21152-0999
800.638.8663
www.bd.com/ds

2771 Bristol Circle
Oakville, Ontario
Canada L6H 6R5
Tel: 800.268.5430

Monte Pelvoux 111, 9th Floor
Col. Lomas de Chapultepec
11000 México D.F.
Tel: 52.55.59.99.82.00

11 rue Aristide Bergès
38800 Le Pont de Claix, France
Tel: 33.4.7668.3636

Akasaka DS Building
5-26 Akasaka 8-chome
Minato-ku
Tokyo, 107 Japan
Tel: 81.24.593.5405

30 Tuas Avenue 2
Singapore 639461
Tel: 65.6861.0633

Rua Alexandre Dumas 1976
04717-004 São Paulo, S.P. Brazil
Tel: 55.11.5185.9833

3-2547 October 2006 Printed in the USA