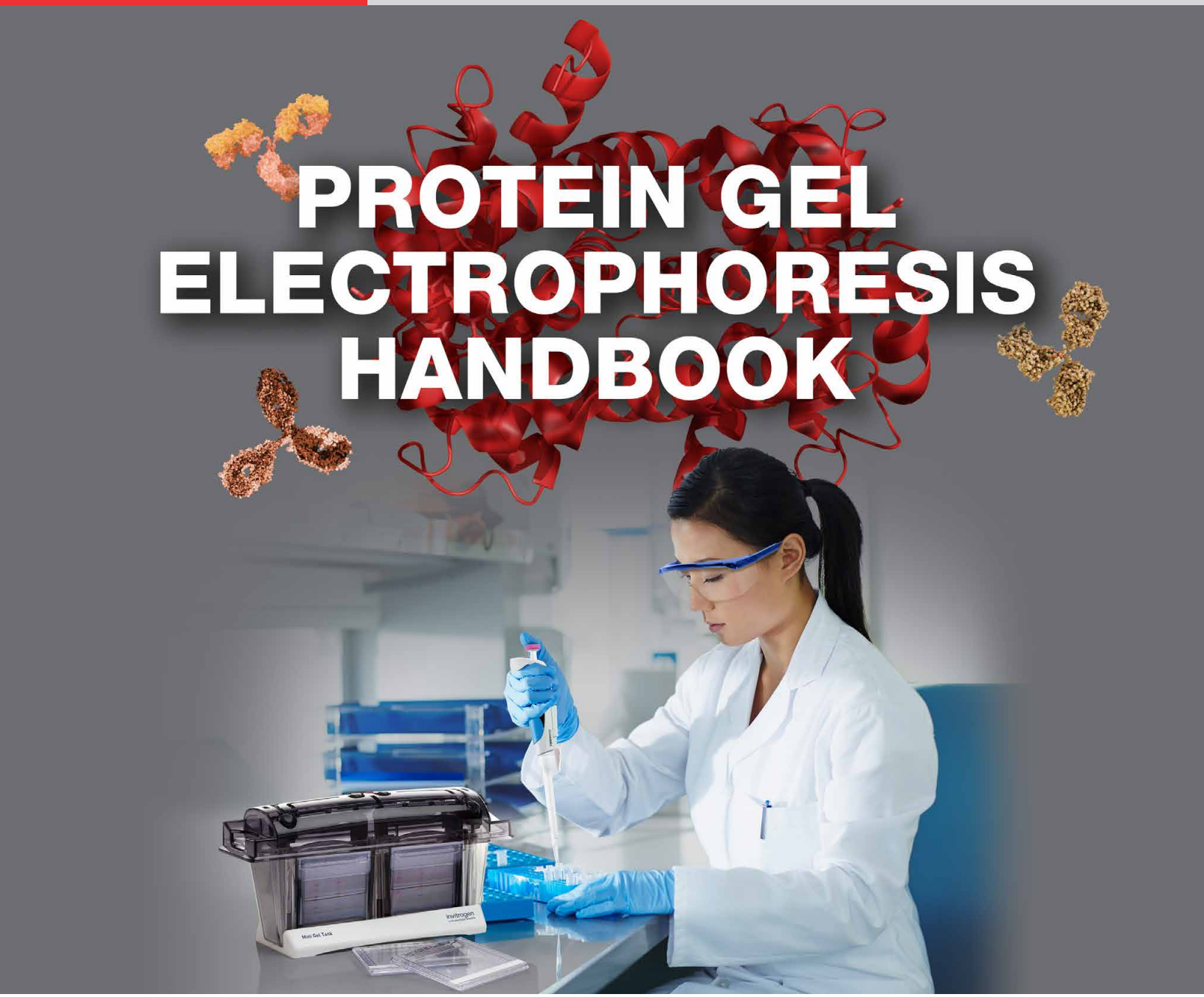
PROTEIN GEL ELECTROPHORESIS HANDBOOK



Techniques and tools for publication-quality results

ThermoFisher SCIENTIFIC

Strategies and solutions to drive your success

Protein gel electrophoresis is a simple way to separate proteins prior to downstream detection or analysis, and is a critical step in most workflows that isolate, identify, and characterize proteins. In this handbook, you will find information on selecting the appropriate separation scheme, choosing the right gel and equipment, and preparing samples for analysis. Also included are troubleshooting guides, how-to videos, selection guides, and buffer recipes to help you achieve optimal results. Our portfolio of high-quality protein electrophoresis products unites gels, stains, molecular weight markers, running buffers, and blotting products to provide you with a range of options designed for your experiments.

See a complete listing of all available products and more at **thermofisher.com/separate**

Contents

Electrophoresis overview

Electrophoresis is defined as the transport of charged molecules through a solvent by an electric field. Electrophoresis is a simple, rapid, and sensitive analytical tool for separating proteins and nucleic acids. Any charged ion or molecule will migrate when placed in an electric field. Most biological molecules carry a net charge at any pH other than at their isoelectric point and will migrate at a rate proportional to their charge density.

The mobility of a biological molecule through an electric field will depend on the following factors:

- Field strength
- Net charge on the molecule
- Size and shape of the molecule
- Ionic strength
- Properties of the matrix through which the molecules migrate (e.g., viscosity, pore size)

Support matrix

Two types of support matrices are commonly used in electrophoresis—polyacrylamide and agarose. The support matrices act as porous media and behave like a molecular sieve. Separation of molecules is dependent upon the gel pore size of the support matrix used. Agarose has a large pore size and is ideal for separating macromolecules such as nucleic acids and protein complexes. Polyacrylamide has a smaller pore size and is ideal for separating most proteins, peptides, and smaller nucleic acids.

Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gels are generated by the polymerization of acrylamide monomers. These monomers are crosslinked into long chains by the addition of bifunctional compounds such as N,N´-methylenebisacrylamide (bis), which react with the free functional groups of the chain termini. The concentration of acrylamide and bisacrylamide determines the pore size of the gel. The higher the acrylamide concentration, the smaller the pore size, resulting in resolution of lower molecular weight molecules and vice versa.

PAGE allows one to separate proteins for different applications based on:

- The acrylamide matrix
- Buffer systems
- Electrophoresis conditions

Did you know

Arne Tiselius won the Nobel Prize in Chemistry for electrophoretic analysis of serum proteins in 1948.

The acrylamide matrix

Linear vs. gradient gels

Gels that have a single acrylamide percentage are referred to as linear gels, and those with a range are referred to as gradient gels. The advantage of using a gradient gel is that it allows the separation of a broader range of proteins than does a linear gel.

Continuous vs. discontinuous gels

Researchers occasionally refer to gels as continuous or discontinuous. A continuous gel is a gel that has been formed from a single acrylamide solution in the entire gel cassette. A discontinuous gel is formed from two acrylamide solutions: a small, low-percentage stacking gel where the protein wells reside, and a larger portion of gel that separates the proteins. In the traditional Tris-glycine protein gel system, the proteins are stacked in the stacking gel between the highly mobile leading chloride ions (in the gel buffer) and the slower, trailing glycine ions (in the running buffer). The reason for using the stacking gel is to improve the resolution of the bands in the gel. These stacked protein bands undergo sieving once they reach the separating gel.

Mini vs. midi protein gels

Commercial gels are available in two size formats: mini gels and midi gels. Both gels have similar run lengths, but midi gels are wider than mini gels, allowing midi gels to have more wells or larger wells. The additional wells in the midi gels permit more samples or large sample volumes to be loaded onto one gel.

Buffer systems

Electrophoresis is performed using continuous or discontinuous buffer systems. A continuous buffer system utilizes only one buffer in the gel and running buffer. A discontinuous buffer system utilizes a different gel buffer and running buffer [1]. This system may also use two gel layers of different pore sizes and different buffer composition (the stacking and separating gel). Electrophoresis using a discontinuous buffer system results in concentration of the sample in the stacking gel and higher resolution as a result.

Electrophoresis conditions

The separation of proteins is dependent on the electrophoresis conditions used, some of which are described below.

Denaturing conditions (SDS-PAGE)

Electrophoresis is performed under denaturing conditions using an anionic detergent such as sodium dodecyl sulfate (SDS). SDS denatures and unfolds the protein by wrapping around the hydrophobic portions. SDS binds at a ratio of ~1.4 g SDS per gram of protein. The resultant SDS–protein complexes are highly negatively charged and are resolved in the gel based on their size.

Nondenaturing conditions (native PAGE)

Electrophoresis is performed under nondenaturing (native) conditions using buffer systems that maintain the native protein conformation, subunit interaction, and biological activity. During native electrophoresis, proteins are separated based on their charge-to-mass ratios.

Reducing conditions

Electrophoresis is performed under reducing conditions using reducing agents such as dithiothreitol (DTT), β-mercaptoethanol (β-ME), or tris(2-carboxyethyl) phosphine (TCEP). The reducing agents completely unfold the denatured proteins into their subunits by cleaving the disulfide bonds between cysteine residues.

1D vs. 2D PAGE

The most common form of protein gel electrophoresis is comparative analysis of multiple samples by one-dimensional (1D) electrophoresis, in which samples are loaded into wells, a current is applied to separate the proteins, and the resulting migration of the protein bands is visualized by staining or on a western blot.

Multiple components of a single sample can be resolved most completely by two-dimensional electrophoresis (2D-PAGE). The first dimension separates proteins according to their native isoelectric point (pI) using a form of electrophoresis called isoelectric focusing (IEF). The second dimension separates proteins by mass using ordinary SDS-PAGE. 2D PAGE provides the highest resolution for protein analysis and is an important technique in proteomic research, where resolution of thousands of proteins on a single gel is sometimes necessary. The main focus of this handbook is on 1D electrophoresis.

Protein gel chemistries

PAGE utilizes a discontinuous buffer system to concentrate or "stack" samples into a very sharp zone in the stacking gel at the beginning of the run. In a discontinuous buffer system, the primary anion in the gel is different (or discontinuous) from the primary anion in the running buffer. Invitrogen™ Bolt™ Bis-Tris Plus gels, Invitrogen™ NuPAGE™ Bis-Tris and Tris-Acetate gels, and the Laemmli system–based Invitrogen™ Novex™ Tris-Glycine gels are examples of discontinuous buffer systems and work in a similar fashion. However, Bis-Tris and Tris-acetate systems operate at a lower pH as a result of the ions that are in the system.

Tris-glycine chemistry

In a Tris-glycine system (Figure 1), three ions are primarily involved:

- Chloride (–), supplied by the gel buffer, serves as the leading ion because it has the highest attraction to the anode relative to other anions in the system.
- Glycine (–), the primary anion provided by the running buffer, serves as the trailing ion, because it is only partially negatively charged and remains behind the more highly charged chloride ions in a charged environment.
- Tris base (+), a common ion present in both the gel and the running buffers. During electrophoresis, the gel and buffer ions in the Tris-glycine system form an operating pH of 9.5 in the separating region of the gel.

Bis-Tris chemistry

In the case of the Bis-Tris system (Figure 2), three ions are primarily involved:

- Chloride (–) supplied by the gel buffer, serves as the fast-moving leading ion.
- MES or MOPS (–) (depending on the running buffer choice) serves as the trailing ion.
  - MES: 2-(N-morpholino) ethane sulfonic acid
  - MOPS: 3-(N-morpholino) propane sulfonic acid
- Bis-Tris (+) acts as the common ion present in the gel while Tris (+) is provided by the running buffer.

The combination of a lower-pH gel buffer (pH 6.4) and running buffer (pH 7.3–7.7) leads to a significantly lower operating pH (pH 7.0) during electrophoresis, resulting in better sample integrity and gel stability.

Tris-acetate chemistry

With the Tris-acetate system (Figure 3), three ions are primarily involved:

- Acetate (–), the leading ion from the gel buffer
- Tricine (–), the trailing ion from the running buffer
- Tris (+), the common ion (in both gel and running buffer)

This system also operates at a significantly lower pH than the Tris-glycine system, resulting in fewer gel-induced protein modifications.

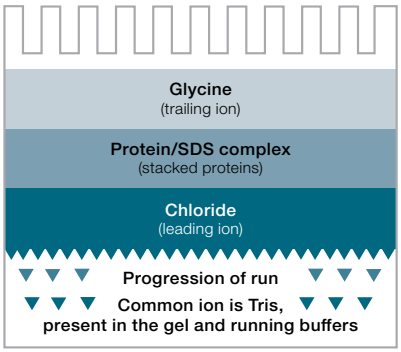


Figure 1. Tris-glycine gel system.

- Gel buffer ions are Tris and chloride (pH 8.7)
- Running buffer ions are Tris, glycine, and SDS (pH 8.3)
- Gel operating pH is 9.5

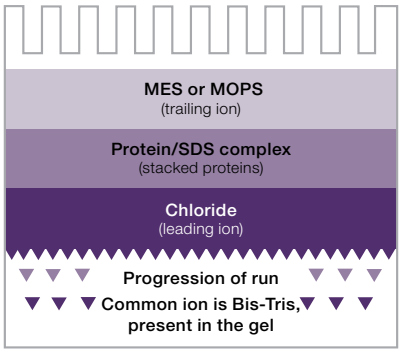


Figure 2. Bis-Tris gel system.

- Gel buffer ions are Bis-Tris and chloride (pH 6.4)
- Running buffer ions are Tris, MES or MOPS, and SDS (pH 7.3)
- Gel operating pH is 7.0

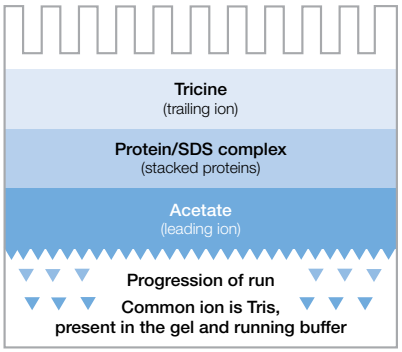


Figure 3. Tris-acetate gel system.

- Gel buffer ions are Tris and acetate (pH 7.0)
- Running buffer ions are Tris, tricine, and SDS (pH 8.3)
- Gel operating pH is 8.1

Tricine chemistry

The tricine system (not pictured) is a modification of the traditional Tris-glycine gel system that uses a discontinuous buffer system specifically designed for the resolution of low molecular weight proteins in the range of 2–20 kDa. As a result of reformulating the Laemmli running buffer and using tricine in place of glycine, SDS-polypeptides form behind the leading ion front rather than running with the SDS front, thus allowing for their separation into discrete bands.

Choosing the right protein gel

Bis-Tris chemistry vs. Tris-glycine chemistry

The most widely used gel system for separating a broad range of proteins by SDS-PAGE is Tris-glycine gels (Laemmli system), comprising a stacking gel component that helps focus the proteins into sharp bands at the beginning of the electrophoretic run and the resolving gel component that separates the proteins based on size. This classic system uses a discontinuous buffer system where the pH and ionic strength of the buffer used for running the gel (Tris, pH 8.3) is different from the buffers used in the stacking gel (Tris, pH 6.8) and resolving gel (Tris, pH 8.8). The highly alkaline operating pH of the Laemmli system may cause band distortion, loss of resolution, or artifact bands (Figure 4).

The major causes of poor band resolution with the Laemmli system are:

- Hydrolysis of polyacrylamide at the high pH of the resolving gel, resulting in a short shelf life of 8 weeks
- Chemical alterations such as deamination and alkylation of proteins due to the high pH of the resolving gel
- Reoxidation of reduced disulfides from cysteine-containing proteins, as the redox state of the gel is not constant
- Cleavage of Asp-Pro bonds of proteins when heated at 100°C in Laemmli sample buffer, pH 5.2

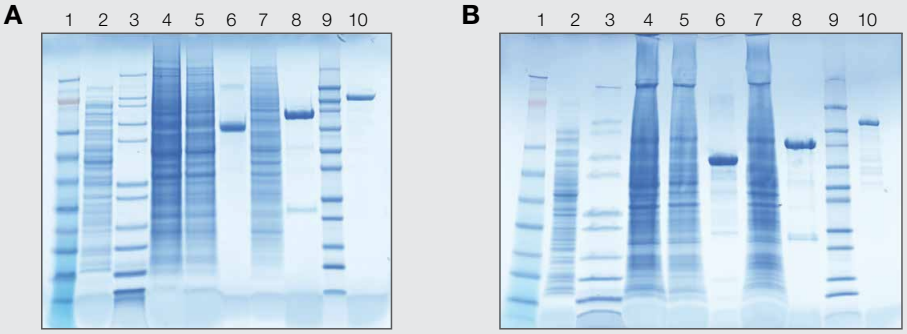


Figure 4. Protein separation using (A) an Invitrogen™ Bolt™ Bis-Tris Plus gel and (B) Bio-Rad's Tris-glycine gel.

Unlike traditional Tris-glycine gels, Invitrogen™ NuPAGE™ and Bolt™ gels are Bis-Tris HCl–buffered (pH 6.4) and have an operating pH of about 7.0. The neutral operating pH of the Bis-Tris system provides the following advantages over the Laemmli system:

- Longer shelf life of 8–12 months, due to improved gel stability
- Improved protein stability during electrophoresis at neutral pH, enabling sharper band resolution and accurate results [2]
- Complete reduction of disulfides under mild heating conditions (70°C for 10 minutes) and absence of cleavage of Asp-Pro bonds
- Reduced state of the proteins maintained during electrophoresis and blotting of the proteins when using Invitrogen™ NuPAGE™ Antioxidant or Invitrogen™ Bolt™ Antioxidant

Denaturing gel systems

Invitrogen NuPAGE Bis-Tris and Bolt Bis-Tris are well suited for separating a broad range of protein sizes. To separate high-abundance proteins, select our robust Invitrogen™ Novex™ Tris-Glycine gel chemistry. Tris-acetate gel chemistry, offered in Invitrogen™ NuPAGE™ Tris-Acetate gels, is recommended for the separation of high molecular weight proteins up to 500 kDa. Tricine gel chemistry is designed for the separation of low molecular weight proteins and peptides. Invitrogen™ Novex™ tricine gels provide increased resolution of proteins with molecular weights as low as 2.5 kDa.

Other gel systems

Native gels

In native polyacrylamide gel electrophoresis, proteins are separated according to the net charge, size, and shape of their native structure. Electrophoretic migration occurs because most proteins carry a net negative charge in alkaline running buffers, with proteins of greater negative charge density migrating faster. At the same time, the sieving effect of the gel matrix regulates the migration of proteins according to their size and three-dimensional shape.

The Invitrogen™ NativePAGE™ Bis-Tris Gel System is based on the blue native polyacrylamide gel electrophoresis (BN PAGE) technique developed by Schägger and von Jagow, which overcomes the limitations of traditional native electrophoresis by providing a near-neutral operating pH and detergent compatibility. In this specific system, the Coomassie G-250 dye binds to proteins and confers a net negative charge while maintaining the proteins in their native state. NativePAGE gels are designed to separate proteins up to 10,000 kDa.

Because no denaturants are used in the NativePAGE system, protein subunits are generally retained and information can be gained about the quaternary structure. In addition, some proteins retain their enzymatic activity following separation using the NativePAGE system. Tris-glycine and Tris-acetate gel systems can also be used for native PAGE when used in the absence of SDS in sample and running buffers.

IEF gels

Isoelectric focusing (IEF) is a technique designed to separate proteins according to their isoelectric point (pI) rather than molecular weight. The pI is the pH at which a protein has no net charge and no longer moves in an electric field. These gels can be used to determine the pI or to detect minor changes in a protein due to deamination, phosphorylation, or glycosylation. They can also resolve different proteins of similar size that cannot be resolved on standard SDS-PAGE gels.

Zymogram gels

Invitrogen™ Zymogram gels are composed of gelatin and are used to characterize proteases that utilize gelatin as a substrate, such as matrix metalloproteases, lipases, and other proteases. Samples are run under denaturing conditions, but due to the absence of reducing agents, proteins can undergo renaturation under appropriate buffer conditions (e.g., Invitrogen™ Novex™ Zymogram Renaturing Buffer). Proteolytic proteins present in the sample consume the substrate in the presence of added divalent metal cations (e.g., Invitrogen™ Novex™ Zymogram Developing Buffer). The gels are then stained to generate clear bands where the substrate has been digested, against a background stained blue.

High-throughput gel electrophoresis

High-throughput gel electrophoresis expands the number of protein samples that can be analyzed in a given time and is especially useful for screening recombination products and protein profiling.

While midi gels enable higher-throughput electrophoresis and western blotting than mini gels, the Invitrogen™ E-PAGE™ High-Throughput (HTP) Precast Gel System is specially designed for fast, bufferless HTP protein analysis. Invitrogen™ E-PAGE™ gels are self-contained, precast gels that include a gel matrix and electrodes packaged inside a disposable cassette. E-PAGE gels are available in 48-well or 96-well formats. These gels use a compact and automated platform, the Invitrogen™ Mother E-Base™ Device.

Precast vs. handcast gels

Traditionally, researchers poured their own gels using standard recipes that are widely available in the protein methods literature. Today, more researchers rely on the convenience and consistency of commercially available, ready-to-use precast gels. We offer precast gels in a variety of percentages, including difficult-to-pour gradient gels that provide excellent resolution and that separate proteins over the widest possible range of molecular weights. Precast gels are also available with several different buffer formulations (e.g., Tris-glycine, Tris-acetate, Bis-Tris, and tricine), which are designed to optimize shelf life, run time, and protein resolution. More importantly, precast polyacrylamide gels eliminate the need to work with acrylamide, which is a known neurotoxin and suspected carcinogen.

However, some scientists need unique gel formulations not available in precast gel formats. In those cases, the gel-pouring process can be made easier by using newer, leak-free handcast systems, such as the Invitrogen™ SureCast™ system, or by using preassembled empty gel cassettes.

Did you know

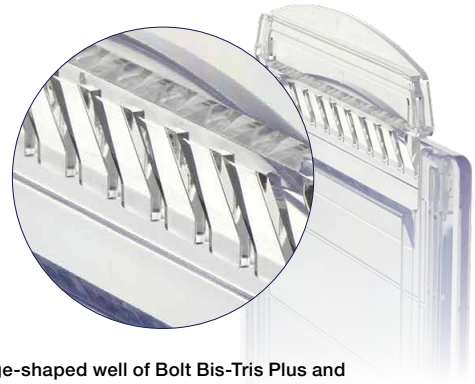
Over 45 years ago, Ulrich K. Laemmli first published on SDS-PAGE as a method for cleavage analysis of structural proteins in bacteriophage T4.

Choosing the right gel percentage

In general, the size of the molecule being separated should dictate the acrylamide or agarose percentage you choose. Use a lower percentage gel to resolve larger molecules and a higher percentage gel to resolve smaller ones. The exception to this rule is when performing isoelectric focusing. Refer to the gel migration charts throughout this chapter to find the gel best suited for your application. As a general rule, molecules should migrate through about 70% of the length of the gel for the best resolution. When protein molecular weights are wide ranging or unknown, gradient gels are usually the best choice.

Choosing a well format and gel thickness

Two thicknesses (1.0 mm and 1.5 mm) are available for popular gel types. If loading large sample volumes (>30 µL), a thicker gel (1.5 mm) with fewer wells (e.g., 5-well) may be more appropriate. Another consideration for large sample volumes is choosing a wedge-well format that is found in our Bolt Bis-Tris Plus or Novex Tris-Glycine gels. When blotting, remember that proteins will transfer more easily from a 1.0 mm thick gel than from a 1.5 mm thick gel. We offer most of our polyacrylamide gels in nine different well formats (17-well, 15-well, 12-well, 10-well, 9-well, 5-well, 1-well, 2D/preparative well, IPG well).



Wedge-shaped well of Bolt Bis-Tris Plus and Novex Tris-Glycine WedgeWell format gels.

Gel selection guide

Getting started

Use the tables below to guide your choice of gel based on sample type, separation type, and molecular weight. For help in choosing the best gel for your experiment, use our interactive product selector at **thermofisher.com/proteingelguide**. If you would like to replace your current precast gels from another supplier with Invitrogen™ gels, go to **thermofisher.com/proteingelconversion**.

Denaturing separation*			
Molecular weight range	**Sample type**		
	Low-abundance proteins and posttranslationally modified proteins		**High-abundance proteins**
Broad range molecular weight proteins (6–400 kDa)	**Bis-Tris gel chemistry**		**Tris-glycine gel chemistry**
	Bolt Bis-Tris Plus Mini gels (load up to 60 µL samples)	NuPAGE Bis-Tris gels	Novex Tris-Glycine gels
High molecular weight proteins (40–500 kDa)	**Tris-acetate gel chemistry**		
	NuPAGE Tris-Acetate gels		
Low molecular weight proteins (2.5–40 kDa)	**Tricine gel chemistry**		
	Novex Tricine Mini gels		

* Low-throughput applications. For medium or high throughput, see Invitrogen™ E-PAGE™ 48-well or 96-well gels at **thermofisher.com/specialtygels**

	Native separation				
	Molecular weight			**Isoelectric point**	
1st dimension	NativePAGE Bis-Tris gels	Novex Tris-Glycine gels	NuPAGE Tris-Acetate gels	Novex IEF gels	ZOOM IPG strips
2nd dimension	NuPAGE Bis-Tris gels, 2D well	Novex Tris-Glycine gels, 2D well	Novex Tris-Glycine gels, 2D well	Novex Tris-Glycine gels, 2D well or NuPAGE Bis-Tris gels, 2D well	Novex Tris-Glycine ZOOM gels, IPG well or NuPAGE Bis-Tris ZOOM gels, IPG well

Protease activity
Novex Zymogram gels (gelatin substrates)

Protein gel performance guarantee

We stand behind the quality of our high-performance protein gels. Purchase Invitrogen protein gels with confidence, knowing that our gels are backed by our protein gel performance guarantee. If an Invitrogen protein gel does not perform in your experiment as described on our website or Certificate of Analysis, we will replace the product at no cost to you, or we will provide you with a credit for future purchase.

Learn about our protein gel performance guarantee at **thermofisher.com/proteingelguarantee**

Protein gel welcome packs

Protein gel welcome packs contain the components for outstanding protein separation and are available for mini- and midi-format protein gels. The typical protein gel welcome pack provides all of the necessary components you need, including:

- Invitrogen™ Mini Gel Tank or Invitrogen™ SureLock™ Tandem Midi Gel Tank
- Invitrogen™ precast mini- or midi-format protein gels
- Running buffer
- SDS sample buffer
- Invitrogen™ Bolt™ or NuPAGE™ Sample Reducing Agent (10X)
- Prestained protein ladder

Educational and product support at your fingertips

Protein electrophoresis and western blotting education center

Gaining publication-quality results immediately is not exactly the norm when performing western blotting. Access resources to learn about protein gel electrophoresis and western blotting methods, from webinars to quick tips and tricks. Whether you are new to protein electrophoresis and western blotting or an experienced researcher wanting to confirm your knowledge, consider this center to help you get better results and succeed sooner.

Access resources at
thermofisher.com/westerneducation

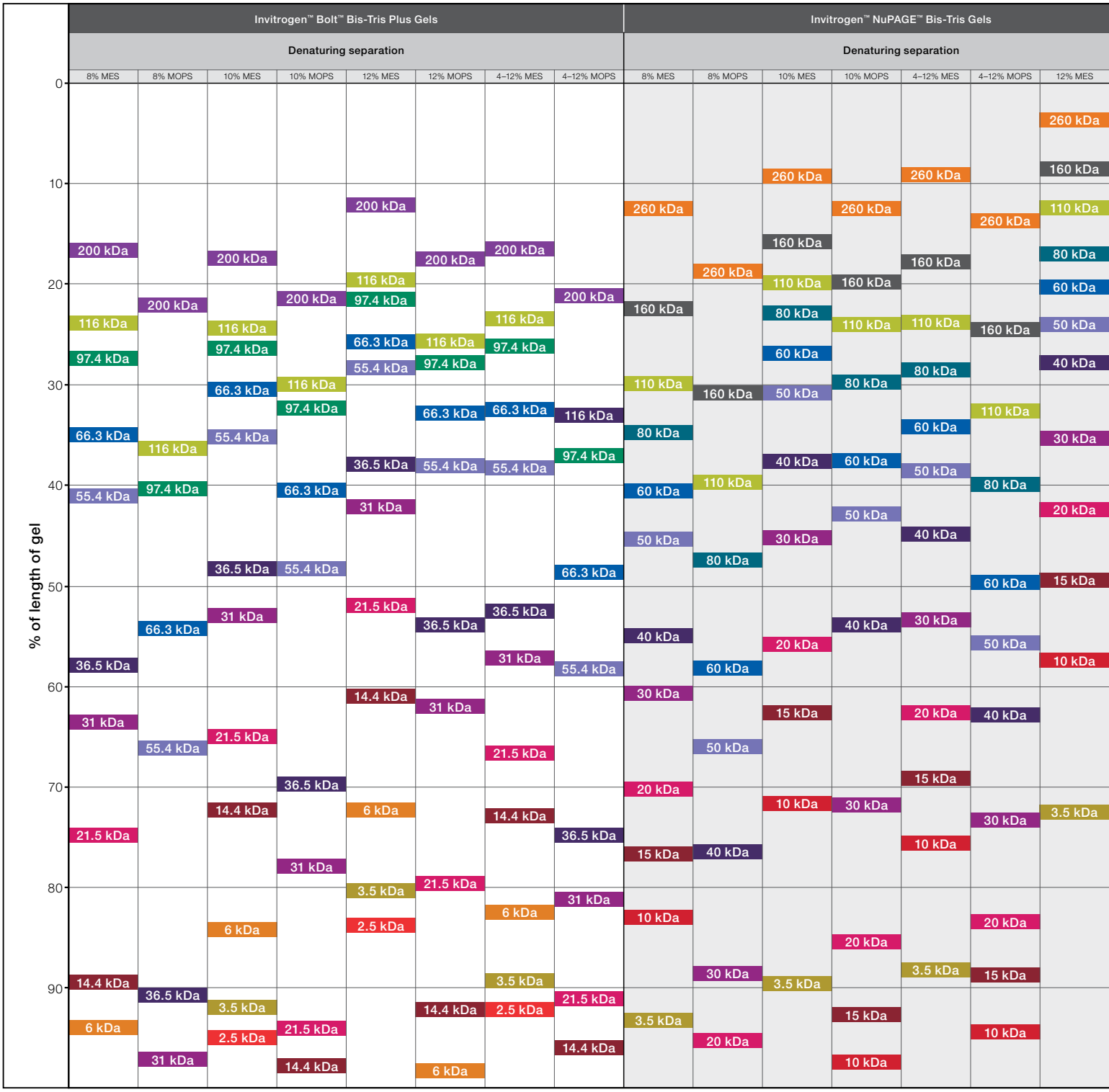
Invitrogen™ BlotBuilder™ Interactive Western Product Selection Tool

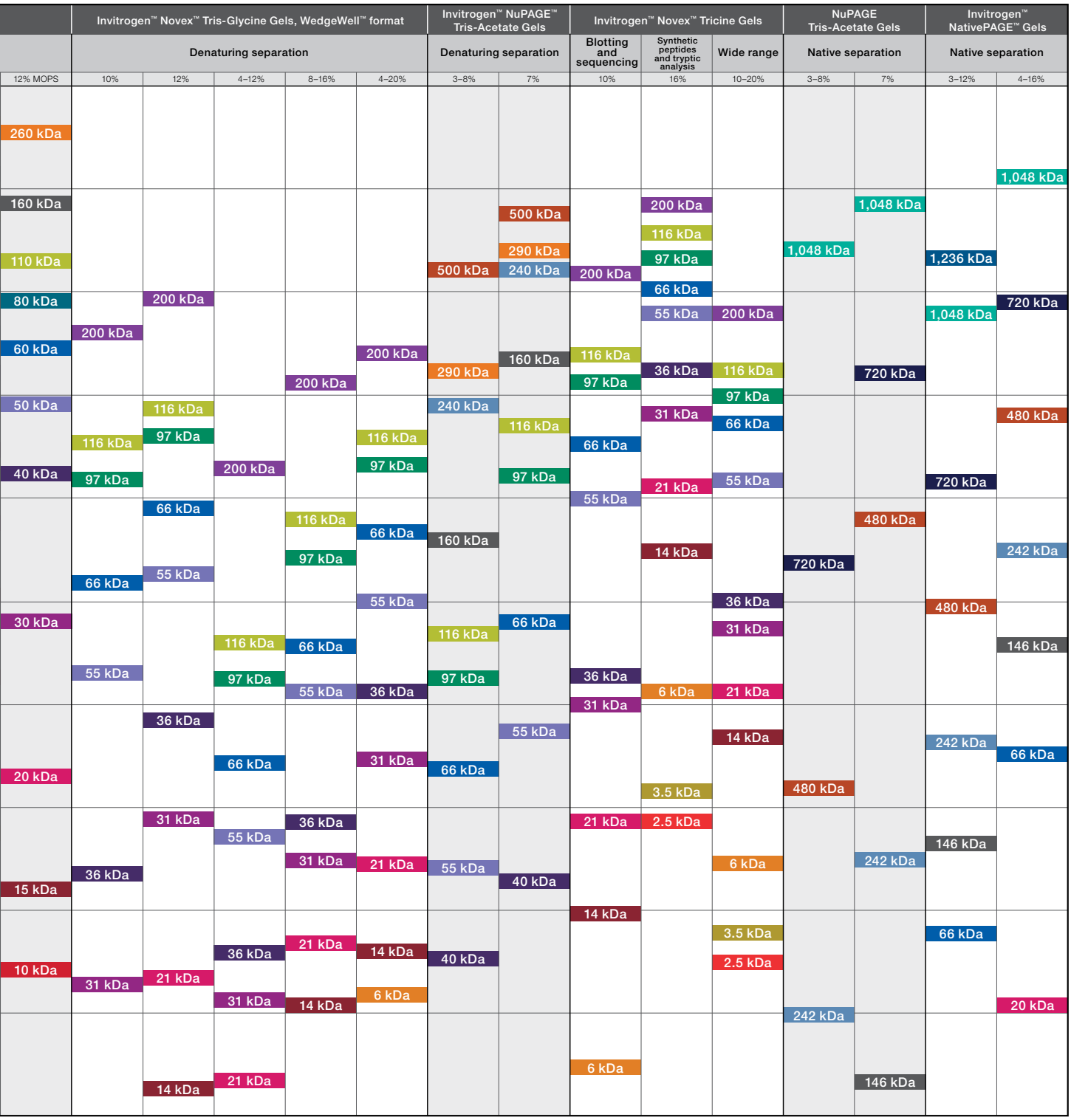
Let us help you select the right tools specially for your protein and experimental needs. Simply answer a few questions about your protein of interest and review a set of recommended products with a personalized western blot protocol.

Access the tool at **thermofisher.com/blotbuilder**

Protein mini gel migration chart

Use this chart to compare migration patterns of proteins of various molecular weights across the Invitrogen™ precast gel product line.




Invitrogen™ Novex™ Tris-Glycine Gels, WedgeWell™ format
Invitrogen™ NuPAGE™ Tris-Acetate Gels
Invitrogen™ Novex™ Tricine Gels
NuPAGE Tris-Acetate Gels
Invitrogen™ NativePAGE™ Gels
Denaturing separation
Denaturing separation
Blotting and sequencing
Synthetic peptides and tryptic analysis
Wide range
Native separation
Native separation
12% MOPS
10%
12%
4–12%
8–16%
4–20%
3–8%
7%
10%
16%
10–20%
3–8%
7%
3–12%
4–16%
260 kDa
160 kDa
110 kDa
80 kDa
60 kDa
50 kDa
40 kDa
30 kDa
20 kDa
15 kDa
10 kDa
1,048 kDa
1,236 kDa
720 kDa
480 kDa
242 kDa
146 kDa
66 kDa
20 kDa
500 kDa
290 kDa
240 kDa
200 kDa
116 kDa
97 kDa
66 kDa
55 kDa
36 kDa
31 kDa
21 kDa
14 kDa
6 kDa
3.5 kDa
2.5 kDa
1,048 kDa
720 kDa
480 kDa
242 kDa
146 kDa

Bolt Bis-Tris Plus and NuPAGE Bis-Tris gels

Neutral-pH gel systems for optimal separation

Bolt Bis-Tris Plus and NuPAGE Bis-Tris gels are precast polyacrylamide gels designed for optimal separation of a broad molecular weight range of proteins under denaturing conditions during gel electrophoresis (Figures 6–10). These gels help deliver consistent performance with a neutral-pH environment to minimize protein degradation, resulting in sharper bands without typical gel "smiling". Additionally, preserving protein integrity becomes particularly important when separating low-abundance proteins. The unique wedge-well design of Bolt Bis-Tris Plus gels (Figure 5) allows loading up to twice the sample volume of other precast gels. Bolt Bis-Tris Plus and NuPAGE Bis-Tris gels are ideal for western blot transfer and analysis along with any other technique where protein integrity is crucial. Bolt Bis-Tris Plus gels are available in the mini gel format and NuPAGE Bis-Tris gels are available in mini and midi gel formats, as well as multiple thicknesses.

Bolt Bis-Tris Plus and NuPAGE Bis-Tris gels offer:

- **Preserved protein integrity**—neutral-pH formulation minimizes protein modifications (Figure 8)
- **Superior band quality and band volume**—Bis-Tris gel chemistry is designed to deliver sharp, straight bands with higher band volume
- **High sample volume capacity**—wedge-well design of Bolt Bis-Tris mini gels allows detection of proteins in very dilute samples or measurement of low-abundance proteins
- **More efficient western blot transfer**—neutral pH prevents reoxidation of reduced samples during protein transfer
- **High lot-to-lot consistency**—coefficient of variation (CV) of only 2% for Rf values (migration)

Figure 5. Unique wedge-shaped well design of Bolt Bis-Tris Plus gels. The WedgeWell feature enables larger sample loads and easier loading.

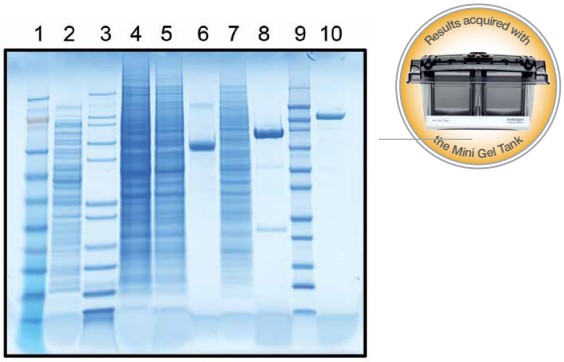


Figure 6. Bolt Bis-Tris Plus gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes in a Bolt 4–12% Bis-Tris Plus gel. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands with consistent migration patterns were observed after staining with Invitrogen™ SimplyBlue™ SafeStain. Images were acquired using a flatbed scanner. **Lane 1:** Invitrogen™ SeeBlue™ Plus2 Prestained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** Invitrogen™ Mark12™ Unstained Standard (blend of 12 purified proteins); **lane 4:** 40 µg HeLa cell lysate; **lane 5:** 20 µg HeLa cell lysate; **lane 6:** 5 µg BSA; **lane 7:** 40 µg Jurkat cell lysate; **lane 8:** 5 µg GST fusion protein; **lane 9:** Invitrogen™ Novex™ Sharp Unstained Protein Standard; **lane 10:** 5 µg β-galactosidase.

View the video to see how easy it is to run an Invitrogen gel **here**.
Review the quick reference protocol for Bolt gels **here**.
Review the quick reference protocol for NuPAGE Bis-Tris mini gels **here**.

Learn more at **thermofisher.com/nupage**

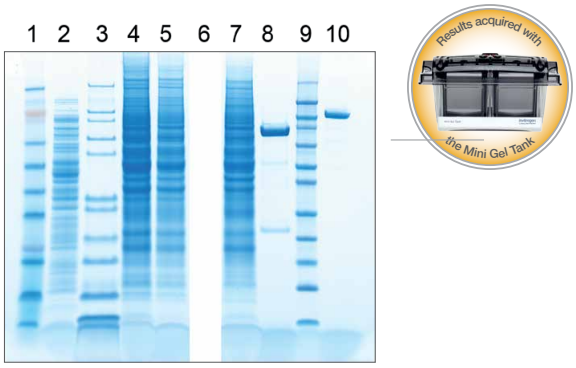
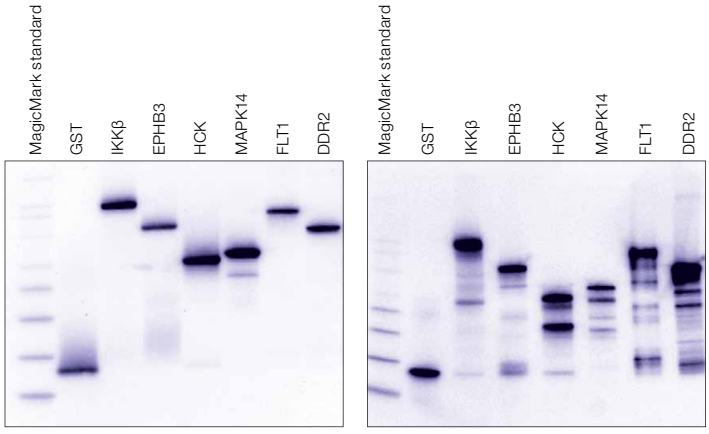


Figure 7. NuPAGE Bis-Tris gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes in an Invitrogen™ NuPAGE™ 4–12% Bis-Tris gel. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands were observed after staining with SimplyBlue SafeStain. Images were acquired using a flatbed scanner. **Lane 1:** SeeBlue Plus2 Prestained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** Mark12 Unstained Standard (blend of 12 purified proteins); **lane 4:** 40 µg HeLa cell lysate; **lane 5:** 20 µg HeLa cell lysate; **lane 6:** not used; **lane 7:** 40 µg Jurkat cell lysate; **lane 8:** 5 µg GST fusion protein; **lane 9:** Novex Sharp Unstained Protein Standard; **lane 10:** 5 µg β-galactosidase.

NuPAGE Bis-Tris gels have been referenced in >20,000 publications.



Bolt Bis-Tris Plus gel **Bio-Rad TGX gel**

Figure 8. Bolt Bis-Tris Plus mini gels help provide better western blotting results. A western blot of a Bolt gel shows clean, sharp protein signals corresponding to only full-length proteins, whereas a western blot of a Bio-Rad™ TGX™ gel shows multiple low molecular weight degradation products. Protein kinases implicated in cancer (IKKβ, EPHB3, HCK, MAPK14, FLT1, and DDR2) were analyzed on a Bolt Bis-Tris Plus gel and a Bio-Rad TGX Tris-glycine gel. The purified kinases (50 ng each), along with Invitrogen™ MagicMark™ XP Western Protein Standard and purified recombinant GST protein, were loaded on a 10-well, 4–12% Bolt gel and a 10-well, 4–20% Bio-Rad TGX gel. The samples were separated and transferred to 0.45 µm PVDF membranes using the respective manufacturers' protocols. Immunodetection was performed using an anti-GST antibody and Invitrogen™ WesternBreeze™ chemiluminescent detection. The blots were imaged using an LAS-1000 system (FujiFilm).

Bolt Welcome Pack

Bolt Bis-Tris Plus mini gel specifications

- Shelf life: 16 months
- Average run time: 35 minutes
- Separation range: 0.3–260 kDa
- Polyacrylamide concentrations: fixed 8%, 10%, and 12%; gradient 4–12%
- Gel dimensions: 8 x 8 cm (1 mm thick)
- Maximum sample volume per 10-well gel: ~60 µL, or two-thirds of the sample well volume

> The new Bolt™ system is wonderful. I am still amazed that I can run a PAGE gel in 23 minutes. The entire system is incredibly user-friendly, from the Bolt™ precast gels with wedged wells for ease of loading to the Mini Gel Tank system. The bands produced from the westerns were sharp and straight. I would and have highly recommended this system to anyone doing protein work.
>
> —Crystal M., large Canadian university

> For one of our projects in the lab, we resolve proteins by electrophoresis to determine the accumulation of ubiquitinated proteins following treatment with a proteasome inhibitor. When we resolved the ubiquitinated proteins using the Tris-glycine gels, we observed a smear. However, when we switched to resolving the ubiquitinated proteins using the Bolt Bis-Tris gels, we were delightfully surprised to observe individual protein bands in place of the smear.
>
> —Susan S., large U.S. university

Bolt Bis-Tris Plus gels

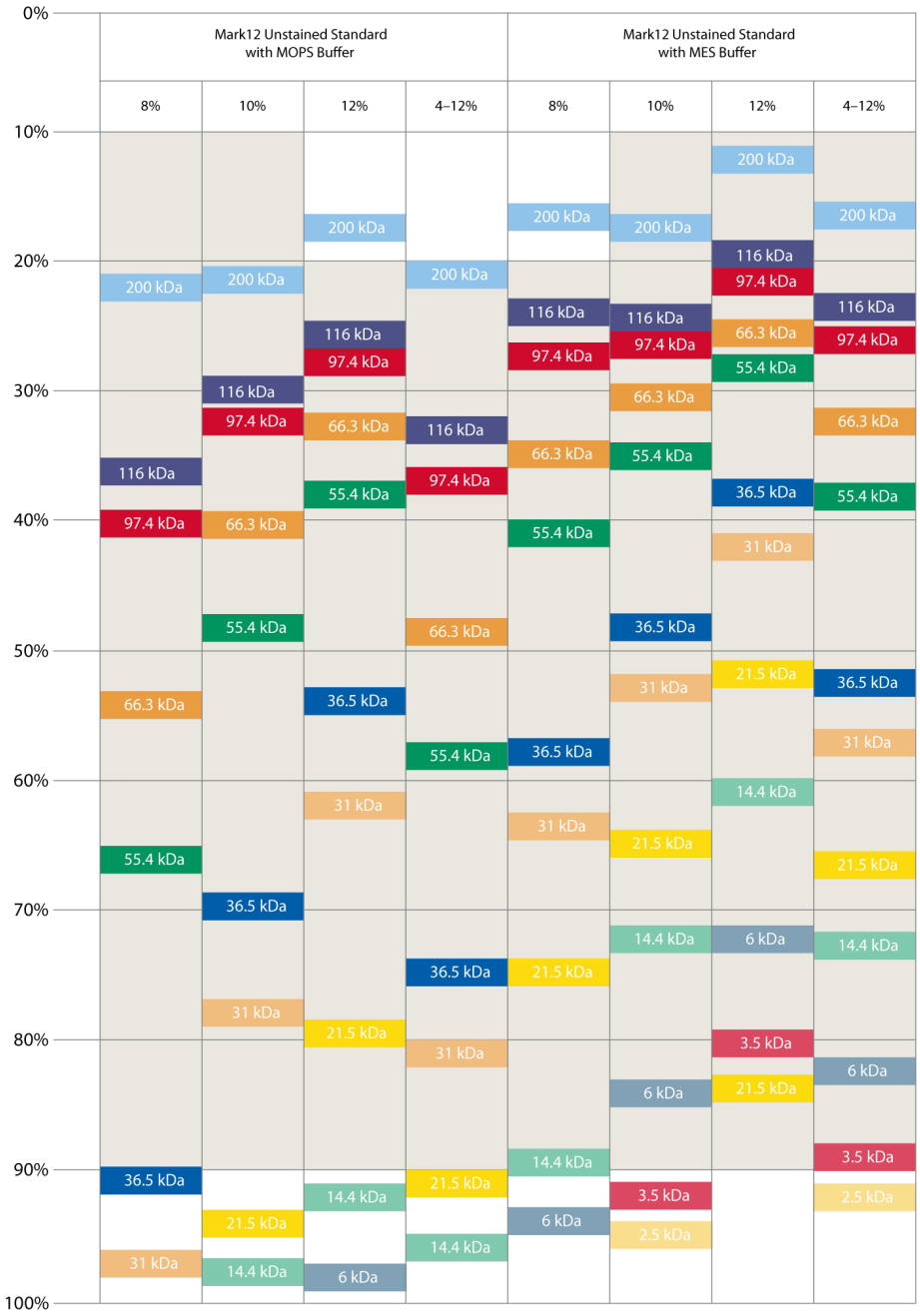


Figure 9. Bolt Bis-Tris Plus gel migration chart. Optimal separation range is shown within the gray areas.

NuPAGE Bis-Tris gel specifications

- Shelf life: 12 months
- Average run time: 35 minutes
- Separation range: 1.5–300 kDa
- Polyacrylamide concentrations: fixed 8%, 10%, and 12%; gradient 4–12%
- Gel dimensions:
  - Mini: 8 x 8 cm (1 or 1.5 mm thick)
  - Midi: 8 x 13 cm (1 mm thick)
- Maximum sample volume per 10-well mini gel: 25 µL (1 mm thick); 37 µL (1.5 mm thick)

Recommended products

Thermo Scientific™ PageRuler™, PageRuler™ Plus, and **Spectra™ prestained protein ladders** are recommended for use with **NuPAGE Bis-Tris gels** for easy molecular weight determination.

Visualize with **Coomassie stain, silver stain, or fluorescent protein stains** after electrophoresis (see "Staining gels" on **page 63**).

Did you know

Timothy Updyke and Sheldon Engelhorn filed a patent for the neutral-pH Bis-Tris gel system in 1996.

NuPAGE Bis-Tris gels (denaturing separation)

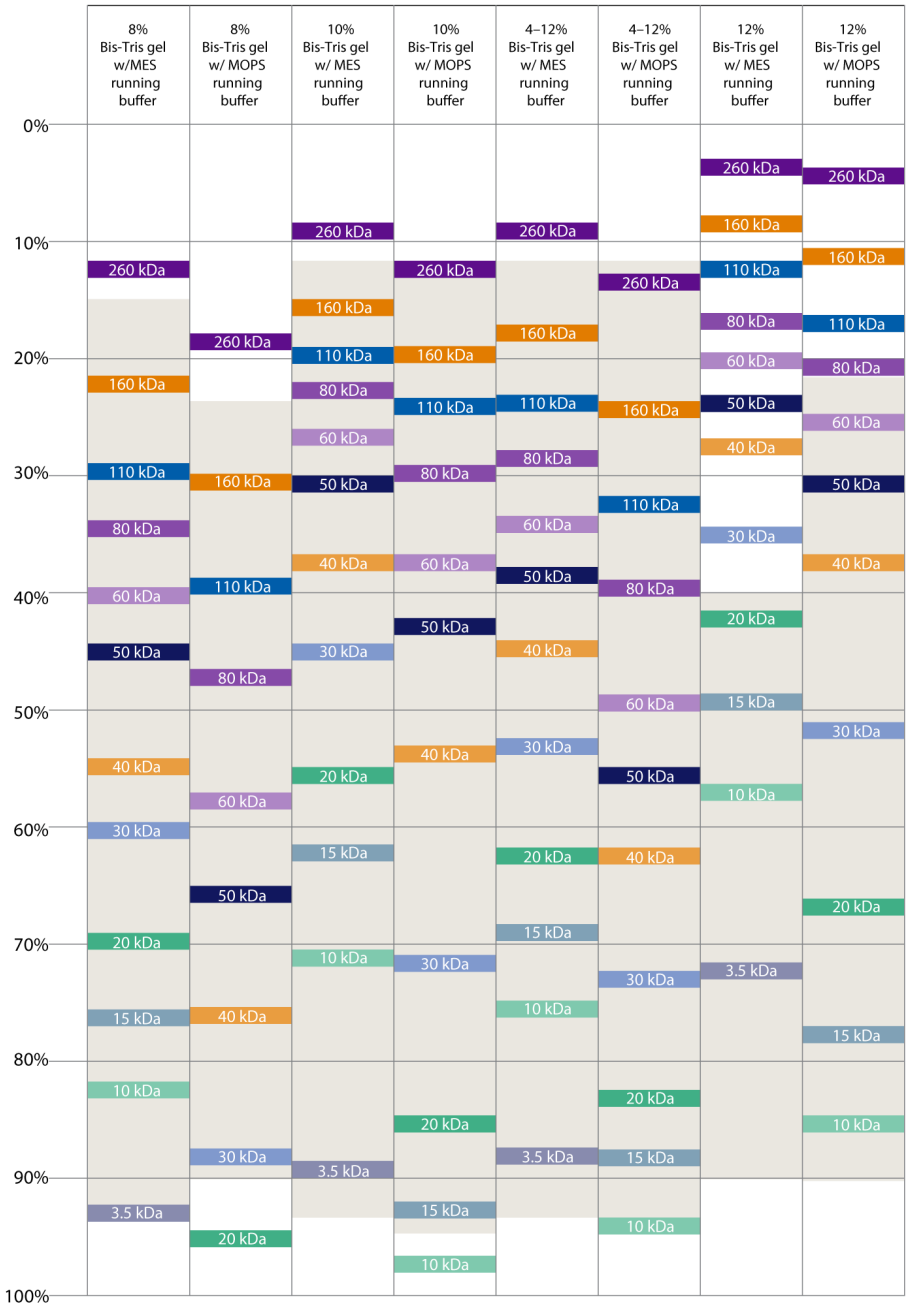


Figure 10. Migration patterns in NuPAGE Bis-Tris gels using Invitrogen™ Novex™ Sharp Prestained Protein Standard or Novex™ Sharp™ Unstained Protein Standard. The optimal separation range is shown within the gray areas.

Review the quick reference guide for NuPAGE Bis-Tris Mini Gels **here**.

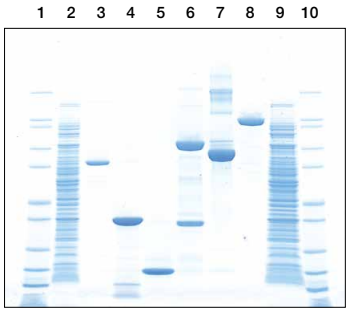
Novex Tris-Glycine gels

Load up to 60 µL of sample

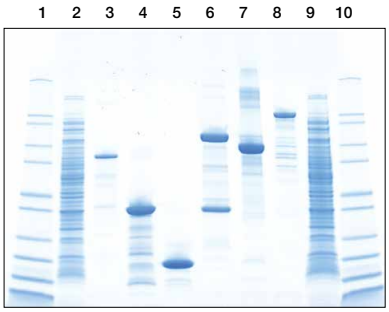
The Invitrogen™ Novex™ Tris-Glycine mini gels, WedgeWell™ format, and Invitrogen™ Novex™ Tris-Glycine Plus midi gels are polyacrylamide gels based on traditional Laemmli chemistry that enable the use of Laemmli sample and running buffers. Novex Tris-Glycine gels provide high-quality performance and separation of a wide range of proteins into well-resolved bands (Figures 11 and 12).

Highlights:

- **Wedge-shaped wells**—easily load up to 60 µL of sample without sacrificing gel width or length (mini gel only)
- **High performance**—excellent protein band resolution and sharpness
- **Improved shelf life**—store gels for up to 12 months at 4°C
- **Fast run conditions**—quickly separate your proteins using constant voltage in less than 60 minutes
- **Flexible**—compatible with native and denatured protein samples



Novex 4–20% Tris-Glycine Mini Gel, WedgeWell format



Bio-Rad TGX 4–20% Gel

Figure 11. Better protein resolution and band sharpness with Novex Tris-Glycine Mini Gels, WedgeWell format. Protein ladder, purified proteins, and *E. coli* lysate were loaded on a gradient Novex 4–20% Tris-Glycine Mini Gel, WedgeWell format, and a Bio-Rad TGX 4–20% gradient gel. The Bio-Rad TGX gel displays numerous low molecular weight protein degradation products below major bands in lanes 3, 4, 7, 8. These are not seen in the Novex Tris-Glycine gel. The Novex gel also displays better protein band sharpness and resolution of lysate than the Bio-Rad gel. **Lanes 1, 10:** 5 µL Mark12 Unstained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** 6 µg catalase; **lane 4:** 6 µg carbonic anhydrase; **lane 5:** 6 µg lysozyme; **lane 6:** 6 µg hIgM; **lane 7:** 6 µg BSA; **lane 8:** 6 µg β-galactosidase; **lane 9:** 20 µg *E. coli* lysate. The sample volume for lanes 2–9 was 10 µL.

Review the quick reference protocol for the Novex Tris-Glycine mini gels, WedgeWell format, **here**.

Learn more at **thermofisher.com/novexwedge**

Novex Tris-Glycine gel specifications

- Shelf life: up to 12 months at 4°C
- Average run time: 60 minutes
- Separation range: 8 kDa to 260 kDa
- Polyacrylamide concentrations: fixed 6%, 8%, 10%, 12%, 14%, 16%; gradient 4–12%, 4–20%, 8–16%, 10–20%
- Gel dimensions:
  - Mini: 8 x 8 cm (1 mm thick)
  - Midi: 8 x 13 cm (1 mm thick)
- Maximum sample volume:
  - In 10-well mini gel, WedgeWell format: 60 µL
  - In 20-well midi gel with standard well format: 25 µL

Novex Tris-Glycine gels

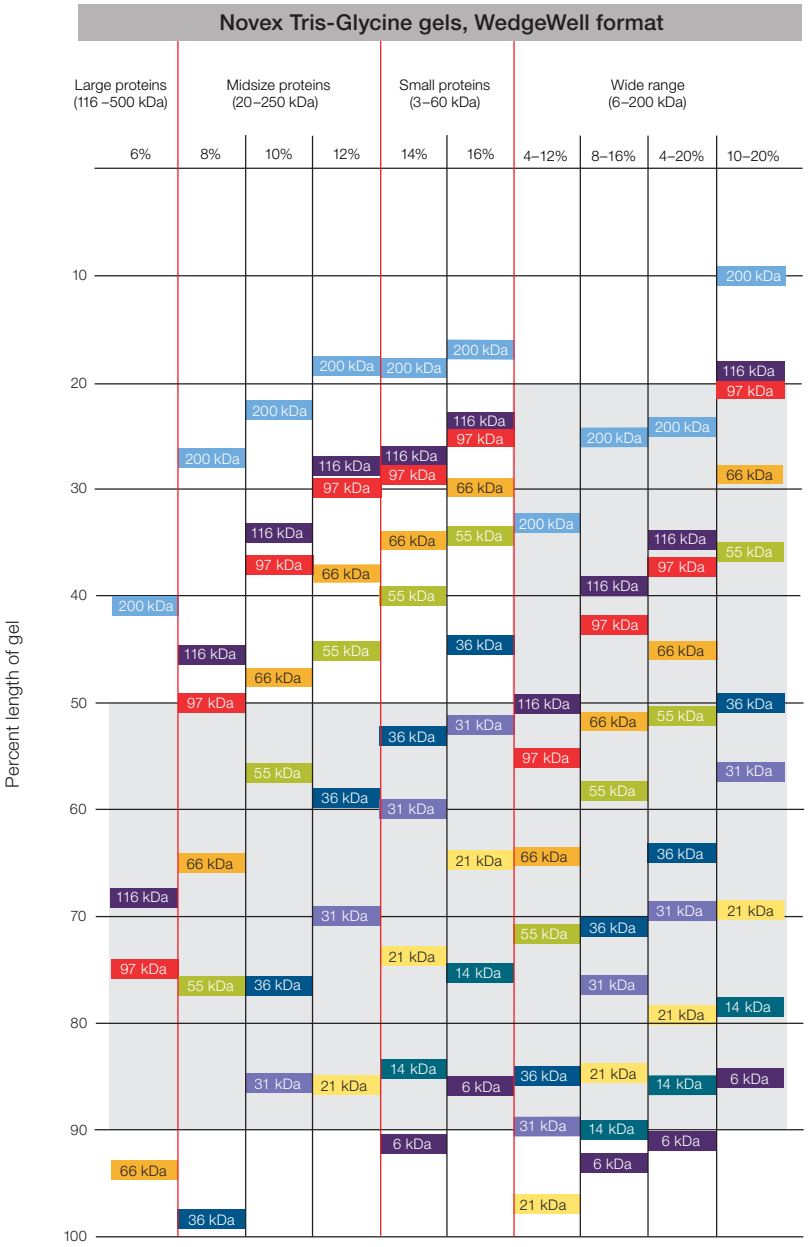


Figure 12. Migration patterns of a protein standard on Novex Tris-Glycine gels, WedgeWell format. Use this chart to select the proper gel for separating proteins based on size. Optimal resolution is achieved when protein bands migrate within the shaded regions. The standard represented here is the Mark12 Unstained Standard under denaturing conditions.

Recommended products

For sample clean-up prior to electrophoresis, we recommend using the **Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit**.

Buffers for denatured proteins: **Invitrogen™ Novex™ Tris-Glycine SDS Sample Buffer and Tris-Glycine SDS Running Buffer**.

Buffers for native proteins: **Invitrogen™ Novex™ Tris-Glycine Native Sample Buffer and Tris-Glycine Native Running Buffer**.

PageRuler, PageRuler Plus, and Spectra protein ladders are recommended for molecular weight determination with **Novex Tris-Glycine gels**.

NuPAGE Tris-Acetate gels

High molecular weight protein separation

Tris-acetate gel chemistry enables the optimal separation of high molecular weight proteins. NuPAGE Tris-Acetate gels offer a pH 8.1 environment that minimizes protein modifications and results in sharper bands. NuPAGE Tris-Acetate gels can also be run with Novex Tris-Glycine Native Running Buffer to resolve native proteins more effectively than a Tris-glycine gel system.

NuPAGE Tris-Acetate gels and buffers are designed to allow:

- Optimal separation of high molecular weight proteins (Figures 13 and 15)
- Preservation of protein sample integrity using optimized sample preparation processes
- More efficient western blot transfer—neutral pH prevents reoxidation of reduced samples during protein transfer (Figure 14)

Specifications

- Shelf life: 8 months
- Average run time: 35 minutes
- Separation range: 30–400 kDa
- Polyacrylamide concentrations: fixed 7%; gradient 3–8%
- Gel dimensions:
  - Mini: 8 x 8 cm (1 or 1.5 mm thick)
  - Midi: 8 x 13 cm (1 mm thick)
- Maximum sample volume per 10-well mini gel: 25 µL (1 mm thick); 37 µL (1.5 mm thick)

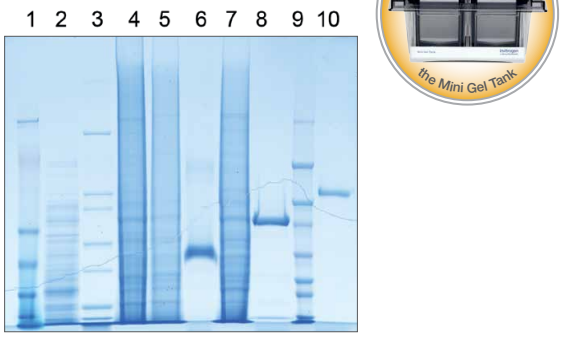
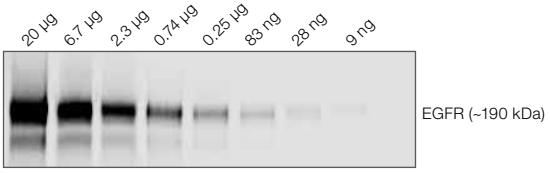
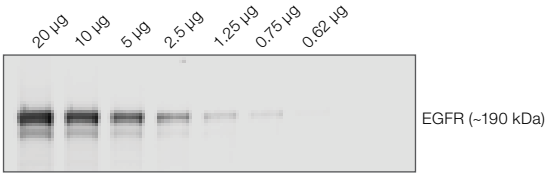


Figure 13. NuPAGE Tris-Acetate gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes in an Invitrogen™ NuPAGE™ 3–8% Tris-Acetate gel. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands were observed after staining with SimplyBlue SafeStain. Images were acquired using a flatbed scanner. **Lane 1:** SeeBlue Plus2 Prestained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** Mark12 Unstained Standard (blend of 12 purified proteins); **lane 4:** 40 µg HeLa cell lysate; **lane 5:** 20 µg HeLa cell lysate; **lane 6:** 5 µg BSA; **lane 7:** 40 µg Jurkat cell lysate; **lane 8:** 5 µg GST fusion protein; **lane 9:** Novex Sharp Unstained Protein Standard; **lane 10:** 5 µg β-galactosidase.



NuPAGE 3–8% Tris-Acetate mini gel



Novex 4–20% Tris-Glycine mini gel, WedgeWell format

Figure 14. Improved transfers of high molecular weight proteins enhance western detection sensitivity. Western blotting analysis of EGFR from A431 lysates transferred from a Novex 4–20% Tris-Glycine mini gel, WedgeWell format, and a NuPAGE 3–8% Tris-Acetate mini gel using the iBlot 2 Gel Transfer Device.

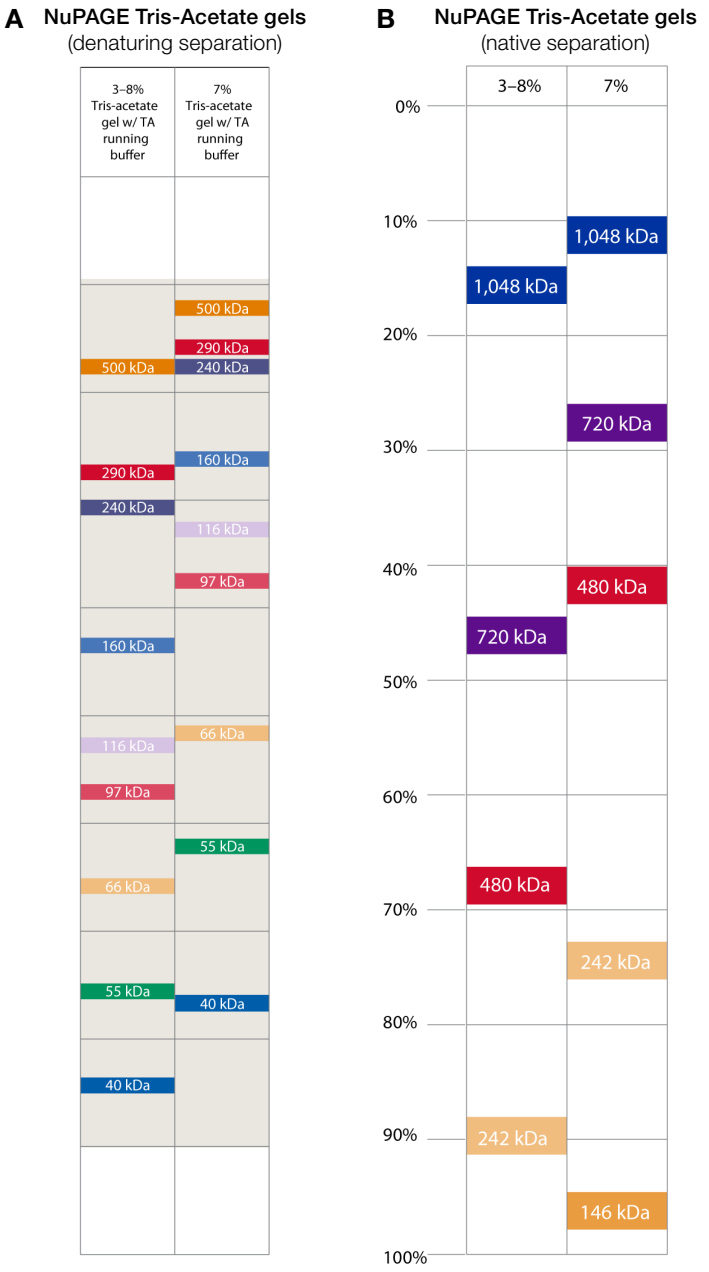


Figure 15. Migration patterns in NuPAGE Tris-Acetate gels. For optimal results, protein bands should migrate within the gray shaded areas. **(A)** Migration pattern of Invitrogen™ HiMark™ Unstained Protein Standard on a NuPAGE Tris-Acetate gel under denaturing conditions. **(B)** Migration pattern of Invitrogen™ NativeMark™ Unstained Protein Standard on a NuPAGE Tris-Acetate gel under native conditions.

Recommended products

Invitrogen™ HiMark™ Unstained and Prestained Protein Standards are specifically designed for large protein analysis on NuPAGE Tris-Acetate gels under denaturing conditions. Both standards offer a ready-to-load format and consist of 9 proteins with a size range of 40–500 kDa.

Novex Tricine gels

High-resolution gels for peptide analysis and low molecular weight proteins

The Invitrogen™ Novex™ tricine gel system is a modification of the Tris-glycine system in which tricine replaces glycine in the running buffer. This system uses a discontinuous buffer system specifically designed for the resolution of low molecular weight proteins (Figure 16).

Advantages of Novex Tricine gels over Tris-glycine gels include:

- Increased resolution of proteins with molecular weights as low as 2 kDa (Figure 17)
- Improved compatibility with direct protein sequencing applications after transferring to PVDF membranes
- Minimized protein modifications, due to the lower pH of the tricine buffering system

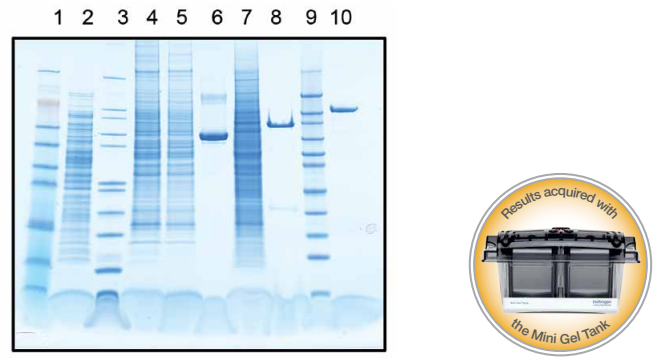


Figure 16. Novex Tricine gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes on Invitrogen™ Novex™ 10–20% Tricine Protein Gels. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands were observed after staining with SimplyBlue SafeStain. Image was acquired using a flatbed scanner. **Lane 1:** SeeBlue Plus2 Prestained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** Mark12 Unstained Standard (blend of 12 purified proteins); **lane 4:** 40 µg HeLa cell lysate; **lane 5:** 20 µg HeLa cell lysate; **lane 6:** 5 µg BSA; **lane 7:** 40 µg Jurkat cell lysate; **lane 8:** 5 µg GST fusion protein; **lane 9:** Novex Sharp Unstained Protein Standard; **lane 10:** 5 µg β-galactosidase.

Good to know

How Novex Tricine gels work

In the traditional Tris-glycine protein gel system, the resolution of smaller proteins (<10 kDa) is hindered by the continuous accumulation of free dodecyl sulfate (DS) ions from the SDS sample and running buffers in the stacking gel, which causes mixing of the DS ions with smaller proteins and results in fuzzy bands and decreased resolution. The mixing also interferes with the fixing and staining of smaller proteins. The Novex Tricine gel system uses a low pH in the gel buffer and substitutes tricine for glycine in the running buffer. The smaller proteins and peptides that migrate with the stacked DS ions in the Tris-glycine gel system are well separated from DS ions in the Novex Tricine gel system, offering sharper bands and higher resolution.

Review the protocol for Novex Tricine gels **here**.

Learn more at **thermofisher.com/tricine**

Novex Tricine gels

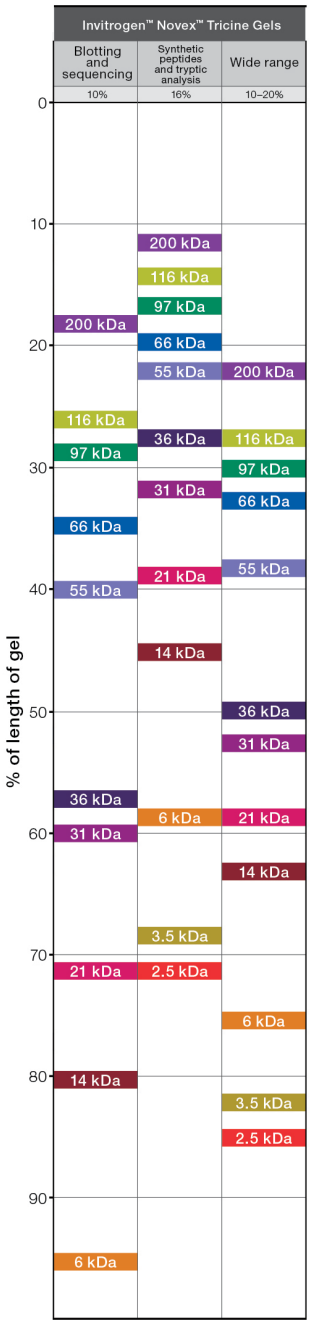
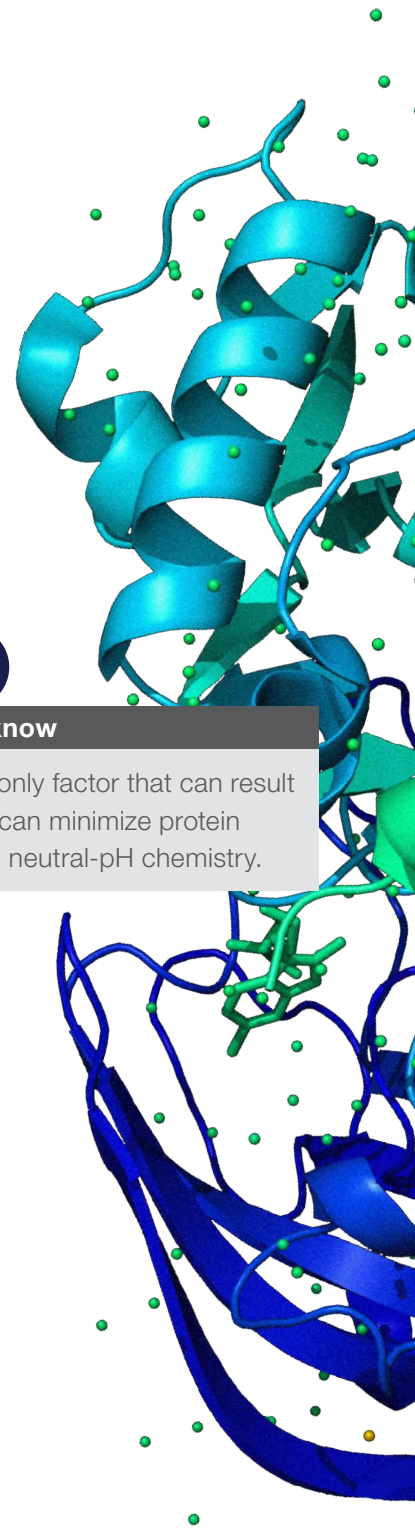


Figure 17. Novex Tricine gel migration chart. For optimal resolution, protein bands should migrate within the shaded areas.



Did you know

Sample preparation is not the only factor that can result in poorly resolved bands. You can minimize protein degradation by using gels with neutral-pH chemistry.

Recommended products

Use Novex Tricine gels with the **Thermo Scientific™ In-Gel Tryptic Digestion Kit** for separation and digestion of peptides for mass spectrometry.

NativePAGE Bis-Tris gels

Superior resolution of native proteins and protein complexes

The Invitrogen™ NativePAGE™ Bis-Tris gel system is based on the blue native polyacrylamide gel electrophoresis (BN PAGE) technique that uses Coomassie G-250 dye as a charge shift molecule that binds to proteins and confers a negative charge without denaturing the proteins (Figure 18). This technique overcomes the limitations of traditional native electrophoresis by providing a near-neutral operating pH and detergent compatibility. The near-neutral (pH 7.5) environment of the NativePAGE gel system during electrophoresis results in maximum protein and gel matrix stability, enabling better band resolution than other native gel systems. A gel migration chart is shown in Figure 19.

The NativePAGE gel system is designed for:

- **A wide resolving range**—from 15 kDa to >10 MDa, regardless of isoelectric point
- **Neutral-pH separation**—the native state of protein complexes is better preserved
- **Superior performance**—higher resolution than Tris-glycine native electrophoresis

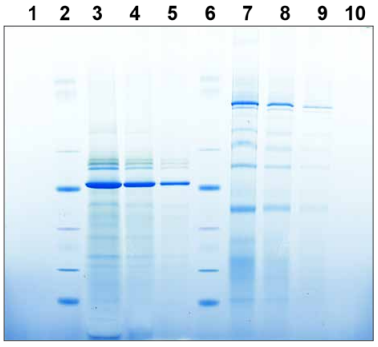
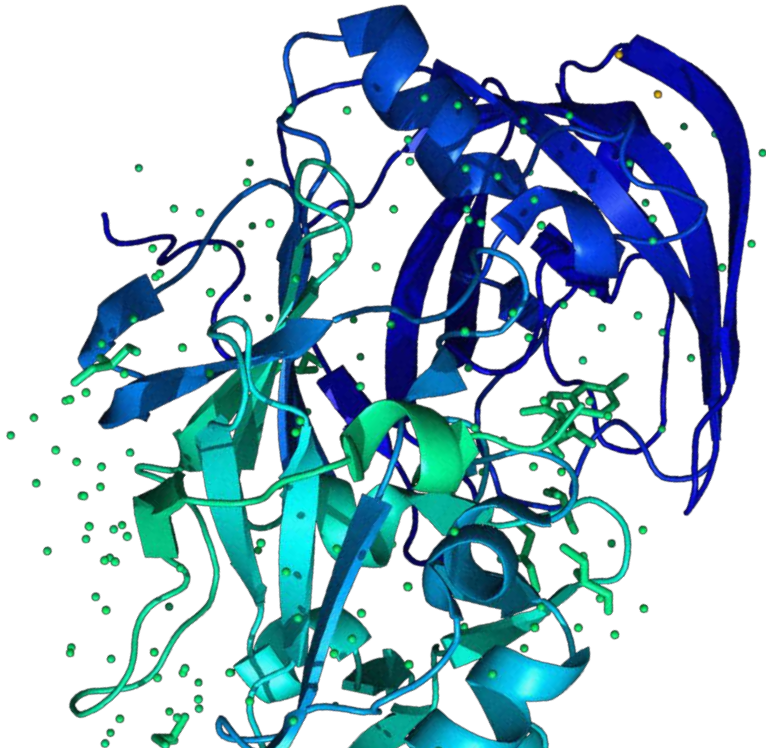


Figure 18. NativePAGE gel electrophoresis. Two-fold dilution series of protein extracts were run on an Invitrogen™ NativePAGE™ 3–12% Bis-Tris Protein Gel using a Mini Gel Tank. Following electrophoresis, the gel was stained with Coomassie dye and imaged using a flatbed scanner. **Lanes 1, 10:** blank; **lanes 2, 6:** 5 µL NativeMark Unstained Protein Standard; **lanes 3–5:** 10, 5, and 2.5 µg spinach chloroplast extract; **lanes 7–9:** 10, 5, and 2.5 µg bovine mitochondrial extract.



NativePAGE gels

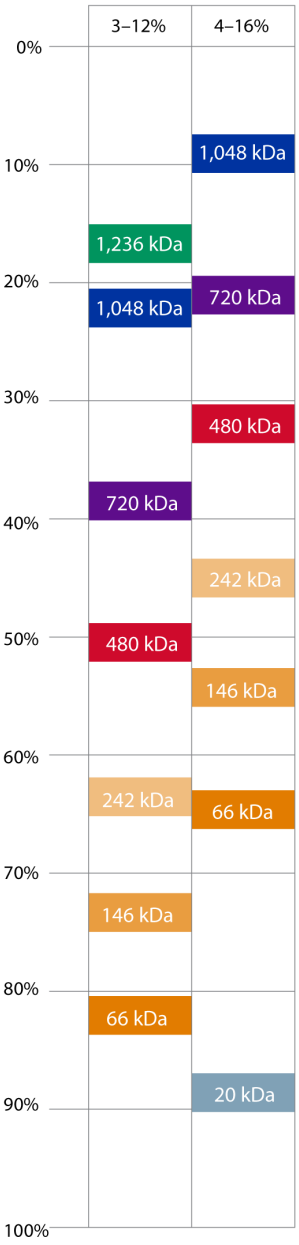
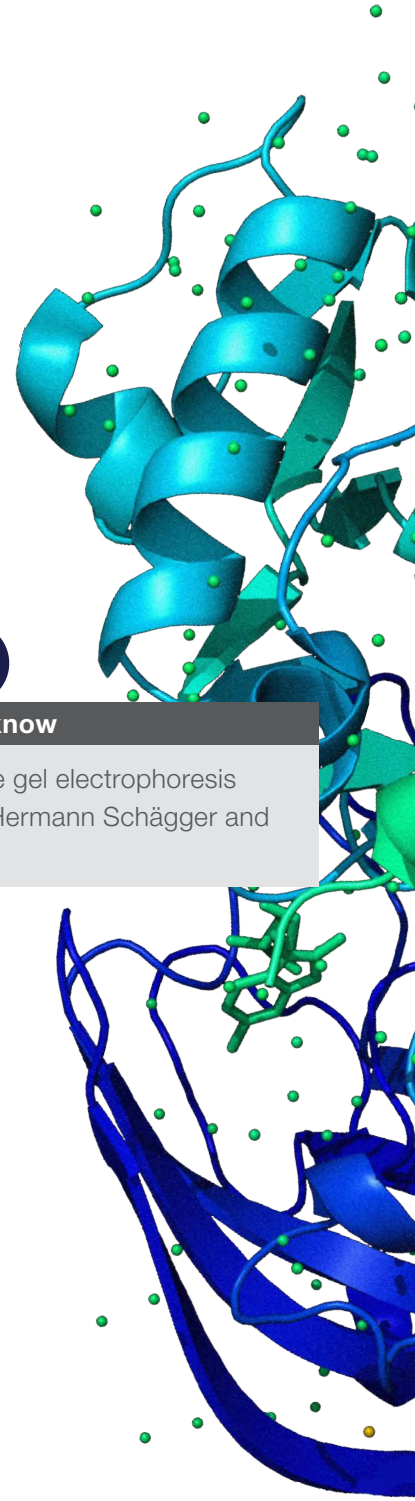


Figure 19. NativePAGE gel migration chart. Migration patterns of the NativeMark Unstained Protein Standard on NativePAGE gels are shown.



Did you know

The blue native polyacrylamide gel electrophoresis technique was developed by Hermann Schägger and Gebhard von Jagow in 1991.

Recommended products

The **NativeMark Unstained Protein Standard** is recommended for use with native gel chemistries, including our **Tris-glycine, Tris-acetate, and NativePAGE gel systems**. This standard offers a wide molecular weight range of 20–1,200 kDa, and the 242 kDa β-phycoerythrin is visible as a red band after electrophoresis for reference (prior to staining).

Novex IEF gels

Precast gels for isoelectric point determination

Isoelectric focusing (IEF) is an electrophoresis technique that separates proteins based on their isoelectric point (pI). The pI is the pH at which a protein has no net charge and does not move in an electric field. Invitrogen™ Novex™ IEF gels effectively create a pH gradient so proteins separate according to their unique pI (Figures 20 and 21). These gels can be used for pI determination or for detection of minor changes in a protein due to deamination, phosphorylation, or glycosylation, and can resolve different proteins of similar size that cannot be resolved on standard SDS-PAGE gels.

When used with our convenient, preoptimized buffers, solubilizers, and molecular weight markers, Novex IEF gels can provide:

- **Accurate pI determination**
- **Clear, sharp bands** for easy identification of protein modifications
- **Higher resolution** of slight differences in size when used in combination with SDS-PAGE for 2D electrophoresis

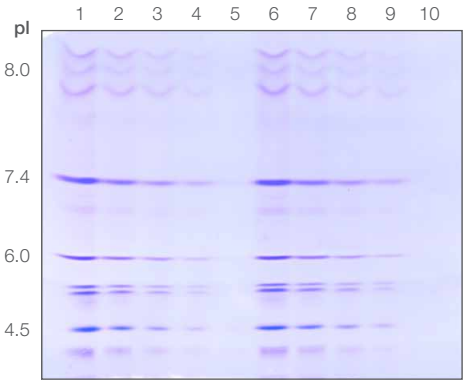
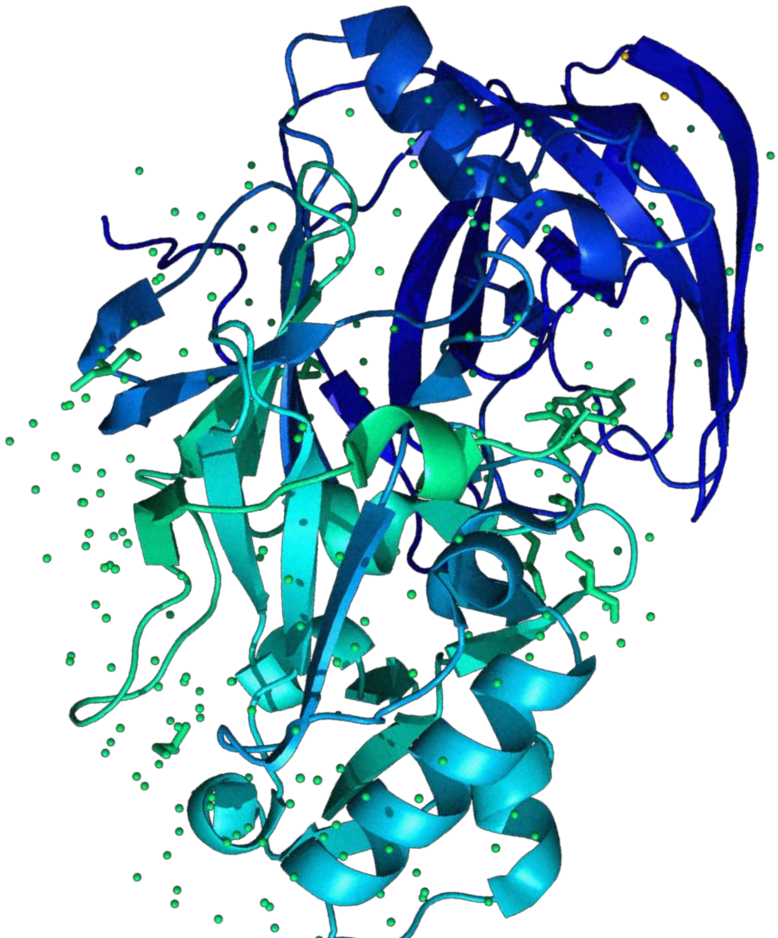


Figure 20. Novex IEF gel electrophoresis. A 2-fold dilution series of Invitrogen™ IEF Marker 3–10 was run in duplicate on an Invitrogen™ Novex™ pH 3–10 IEF Protein Gel using a Mini Gel Tank. The IEF Marker 3–10 consists of proteins with a variety of isoelectric points; these proteins include lectin (pI = 7.8, 8.0, and 8.3), myoglobin from horse muscle (pI = 6.9 and 7.4), carbonic anhydrase from bovine erythrocytes (pI = 6.0), β-lactoglobulin from bovine milk (pI = 5.2 and 5.3), soybean trypsin inhibitor (pI = 4.5), and glucose oxidase (pI = 4.2). After electrophoresis, the gel was fixed and stained using Coomassie R-250 dye. Gel imaging was performed with a flatbed scanner. Volumes of IEF Marker 3–10 loaded: **Lanes 1, 6:** 20 µL; **lanes 2, 7:** 10 µL; **lanes 3, 8:** 5 µL; **lanes 4, 9:** 2.5 µL; **lanes 5, 10:** blank.

Specifications

- Shelf life: 2 months
- Average run time: 2.5 hours
- Separation range:
  - pH 3–10 gels: pI performance range is 3.5–8.0
  - pH 3–7 gels: pI performance range is 3.0–7.0
- Polyacrylamide concentration: fixed 5%
- Gel dimensions: 8 x 8 cm (1 mm thick)
- Maximum sample volume per 10-well gel: 20 µL



Novex IEF gels

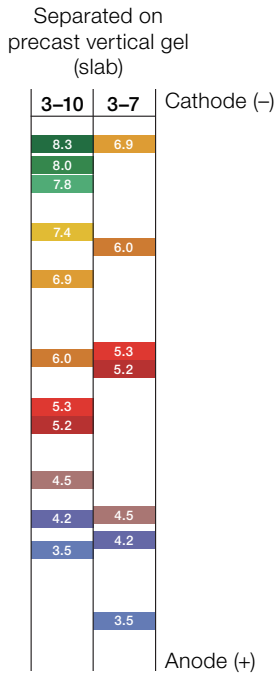
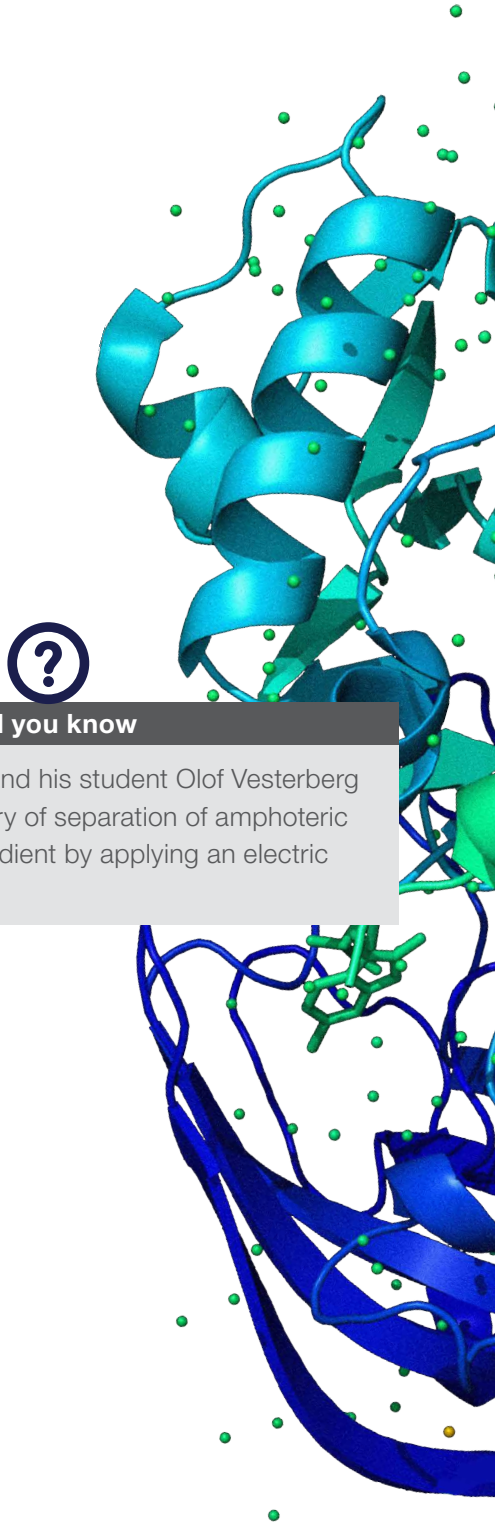


Figure 21. Novex IEF gel migration chart using the IEF marker. Proteins shown are amyloglucosidase (*Aspergillus niger*), pI = 3.5; glucose oxidase (*Aspergillus niger*), pI = 4.2; trypsin inhibitor (soybean), pI = 4.5; β-lactoglobulin (bovine, milk), pI = 5.2 and 5.3; carbonic anhydrase (bovine, erythrocytes), pI = 6.0; myoglobin (horse, muscle), pI = 6.9 and 7.4; lectin (*Lens culinaris*), pI = 7.8, 8.0, and 8.3; ribonuclease A (bovine, pancreas), pI = 9.5; and cytochrome c (horse, heart), pI = 10.7.



Did you know

Harry Svensson-Rilbe and his student Olof Vesterberg first described the theory of separation of amphoteric proteins along a pH gradient by applying an electric field in the 1960s.

Recommended products

Invitrogen™ Novex™ IEF buffer kits include optimized cathode, anode, and sample buffers to help reduce variability and enable consistent results.

IEF Marker 3–10 are ready to use and give accurate results.

Zymogram gels

Easy in-gel protease analysis

Invitrogen™ Novex™ 10% Zymogram Plus (gelatin) gels are useful for the detection and characterization of proteases that use gelatin as a substrate. The proteases are run under denaturing conditions and visualized as clear bands against a dark background using a simple renaturing, developing, and staining protocol. Zymogram gels are commonly used to detect matrix metalloproteases. Novex 10% Zymogram Plus gels are highly sensitive, detecting as little as 5 x 10^{-6} units of collagenase.

Novex Zymogram Plus (gelatin) gel characteristics.

	Zymogram Plus gelatin gel
Gel composition	10% Tris-glycine gel
Substrate	0.1% gelatin
Sensitivity	5 x 10^{-6} units of collagenase
Post-staining required?	Yes
Separation range	20–220 kDa

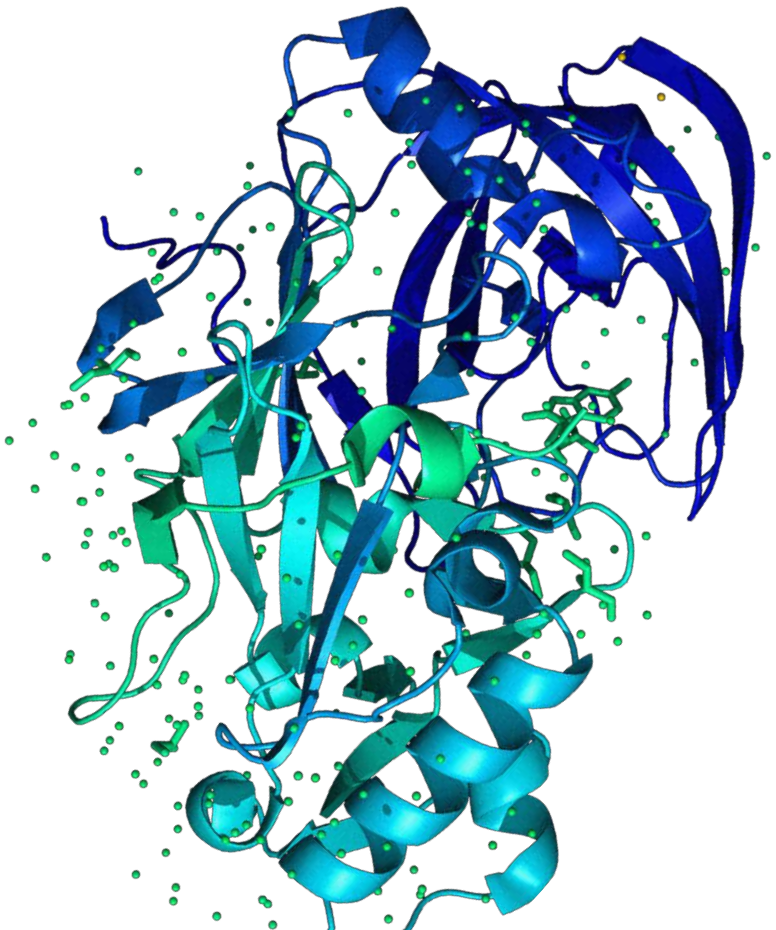
Specifications

- Shelf life: 2 months
- Average run time: 90 minutes
- Separation range: 20–220 kDa (Figure 22)
- Polyacrylamide concentrations: fixed 10% (with gelatin)
- Gel dimensions: 8 x 8 cm (1 mm thick)
- Maximum sample volume per well: 20 µL

Good to know

How do Novex zymogram gels work?

Protease samples are denatured in SDS buffer under nonreducing conditions and without heating, and run on a Novex Zymogram Plus gel in Tris-Glycine SDS Running Buffer. After electrophoresis, the proteases are renatured by incubating the gel in Invitrogen™ Novex™ Zymogram Renaturing Buffer, which contains a nonionic detergent. The gels are then equilibrated in Invitrogen™ Novex™ Zymogram Developing Buffer to add divalent metal cations required for enzymatic activity, and then stained and destained. Regions of protease activity appear as clear bands against a dark blue background where the protease has digested the substrate.



Novex Zymogram gel

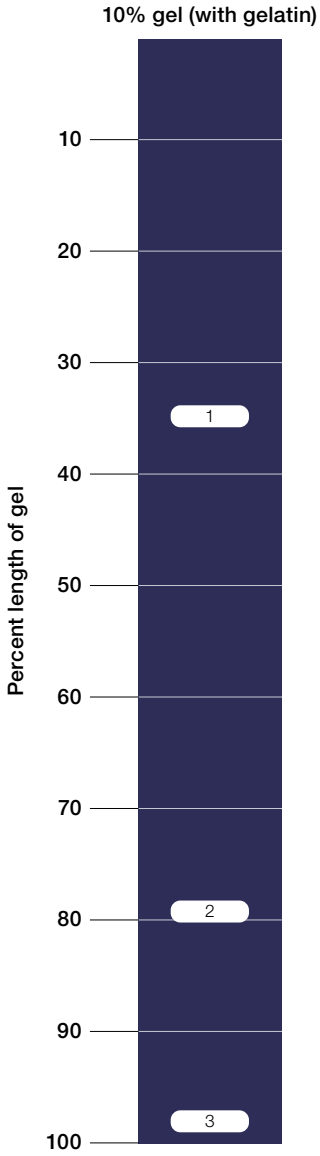
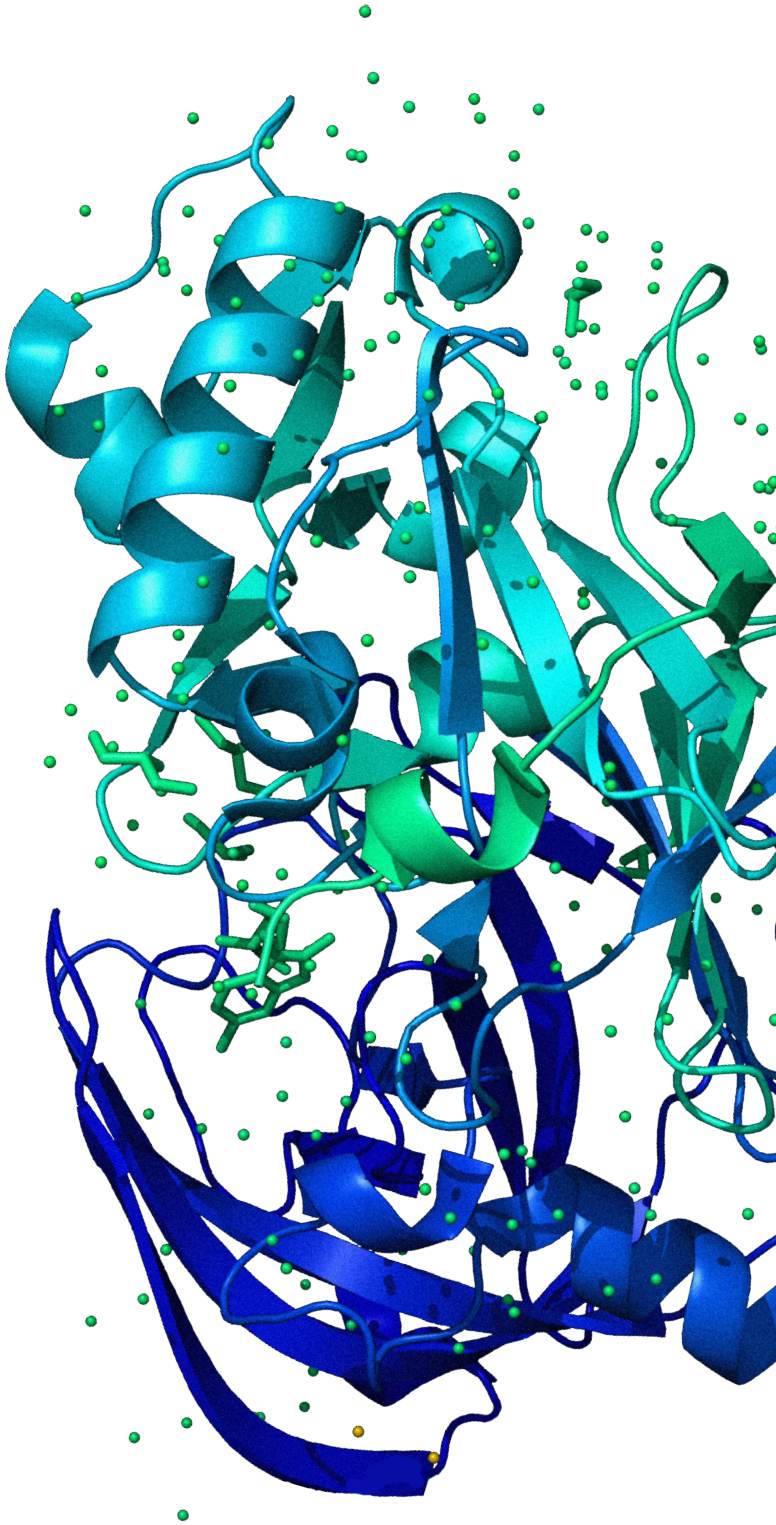


Figure 22. Novex Zymogram Plus (gelatin) gel migration chart.
The numbered bands refer to the following proteases:
Band 1: Collagenase type I (140 kDa)
Band 2: Thermolysin (37 kDa)
Band 3: Trypsin (19 kDa)



Recommended products

After electrophoresis, incubate the gel in **Novex Zymogram Renaturing Buffer (10X)** to renature the enzyme. The gel is then equilibrated in **Novex Zymogram Developing Buffer (10X)** to add divalent metal cations required for enzymatic activity.

E-PAGE High-Throughput Precast Gel System

Protein separation and analysis for increased sample throughput

The Invitrogen™ E-PAGE™ High-Throughput Precast Gel System is designed for fast, bufferless medium- and high-throughput protein analysis. Invitrogen™ E-PAGE™ 48-well and 96-well precast gels consist of a buffered gel matrix and electrodes packaged inside a disposable, UV-transparent cassette. Each cassette is labeled with a unique barcode to facilitate identification of the gel using commercial barcode readers. These gels can be loaded with a multichannel pipettor or automated loading system. The E-PAGE system also includes Invitrogen™ E-Base™ integrated devices to run the gels, an E-Holder™ platform for optional robotic loading, and free E-Editor™ 2.0 Software to align images for easy comparison.

Advantages of using the E-PAGE High-Throughput Precast Gel System include:

- **Ease of use**—quick setup and fast protein separation in about 23 minutes
- **Fast loading**—compatible with multichannel pipettors and robotic loading
- **Efficient western blotting and staining**—optimized protocols and reagents

Specifications

- Shelf life: 6 months
- Average run time: 23 minutes
- Separation range: 10–200 kDa (Figure 24)
- Polyacrylamide concentrations:
  - E-PAGE 48 gel: fixed 8%
  - E-PAGE 96 gel: fixed 6%
- Gel dimensions: 13.5 x 10.8 cm (3.7 mm thick)
- Maximum sample volume per well:
  - E-PAGE 48 gel: 20 µL
  - E-PAGE 96 gel: 15 µL

Good to know

How do E-PAGE gels work?

E-PAGE™ gels run in the Invitrogen™ E-Base™ electrophoresis device, which has an integrated power supply for direct connection to an electrical outlet. Use the Invitrogen™ Mother E-Base™ Device for a single E-PAGE gel, or use the Mother E-Base Device in conjunction with two or more Invitrogen™ Daughter E-Base™ Devices for running multiple gels simultaneously (Figure 23).

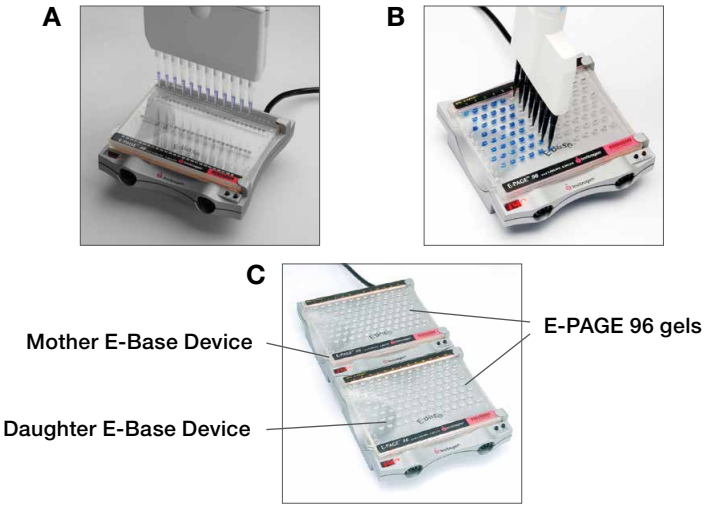


Figure 23. Loading and running E-PAGE gels. (A) Loading an E-PAGE 48 gel using a multichannel pipettor. **(B)** Loading an E-PAGE 96 gel using a multichannel pipettor. **(C)** The Mother/Daughter E-Base Device combination.

E-PAGE gels

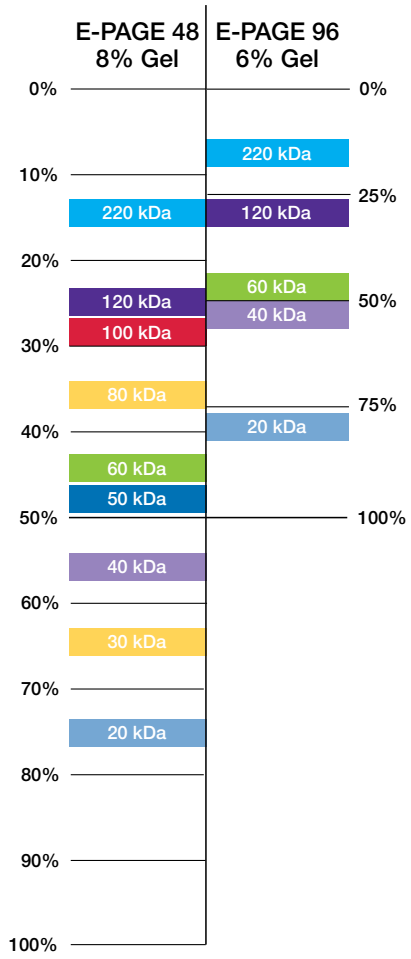


Figure 24. E-PAGE gel migration chart. Migration patterns of the Invitrogen™ E-PAGE™ MagicMark™ Unstained Protein Standard are shown.

Recommended products

The **Invitrogen™ E-PAGE™ SeeBlue™ Prestained Protein Standard** and **E-PAGE MagicMark Unstained Protein Standard** are specifically designed for use with E-PAGE gels.

Did you know

Our E-Base devices are compatible with the Society for Biomolecules Screening (SBS) standard plate size and can be conveniently mounted on liquid handling robot decks.

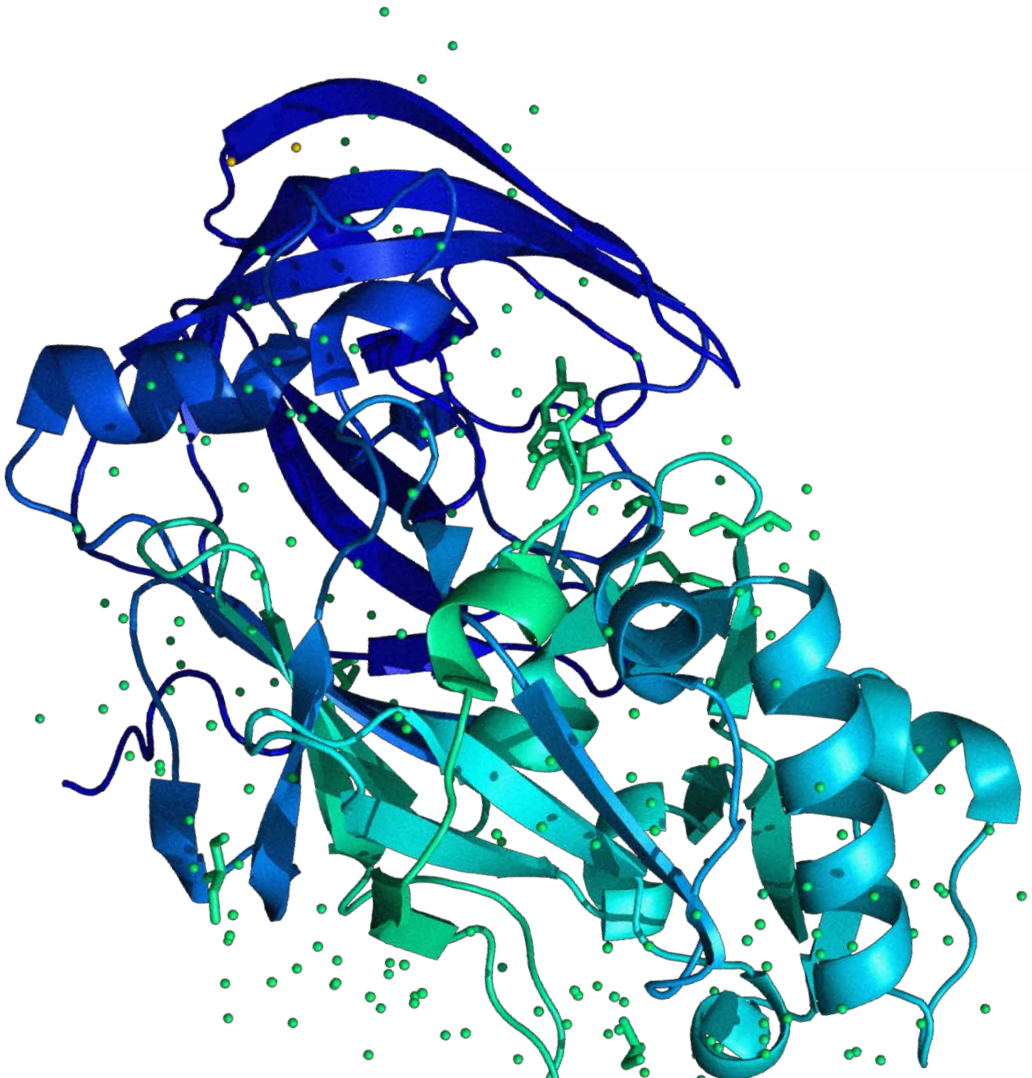
SureCast Gel Handcast System

100% leak-free*—avoid pouring another gel because of leaks

The Invitrogen™ SureCast™ Gel Handcast System is designed for leak-free, confident gel casting. The SureCast system is fully compatible with the Mini Gel Tank.

Benefits offered by the SureCast Gel Handcast System include:

- **Leak-free design**—less time wasted recasting after gels have leaked
- **Superior glass plate durability**—up to 20 times more durable than other suppliers' plates**
- **Unique tilt feature**—helps minimize spillage when pouring acrylamide solutions
- **Simple assembly of casting components**—a single-motion, load-and-lock mechanism
- **Optional multi-use tools**—all-in-one tools to guide gel loading, help open gel cassettes, and trim gels



* For details, go to thermofisher.com/surecastterms

** Based on internal testing.

SureCast gel handcast reagents

SureCast Stacking Buffer and Resolving Buffer

Invitrogen™ SureCast™ Stacking Buffer and Resolving Buffer are pouches of dry-blend powder, each sufficient to make 500 mL of stacking gel buffer (0.5 M Tris-HCl buffer, pH 6.8) or 500 mL of resolving gel buffer (1.5 M Tris buffer, pH 8.8) for handcasting polyacrylamide gels.

Benefits include:

- **Convenient pouches of dry-blend powder**—dissolve contents of a single packet in water and the buffer is ready to use
- **Time- and space-saving**—no weighing, no calculations, no pH adjustment, and no need to stock individual components
- **Long shelf life**—easy stocking and storage, as dry powder minimizes concerns about long-term stability of stock solutions

See the SureCast Gel Handcast System in action at **thermofisher.com/surecast**

SureCast Acrylamide Solution, 40%

Invitrogen™ SureCast™ Acrylamide Solution (40%) can be used to prepare single-percentage and gradient gels using the SureCast Gel Handcast System or other handcast systems.

Features include:

- Room-temperature storage
- Long shelf life
- High purity
- Safer alternative to powdered acrylamide
- Concentrated to enable a broader range of gel percentages to cast

Protein extraction and sample preparation for electrophoresis

Good sample preparation is critical for successful separation of protein bands in electrophoresis. No single sample preparation method or buffer will work for all sample types due to the diversity of protein samples. However, the following general guidelines below should help when preparing samples for protein electrophoresis.

- To minimize sample variability, keep sample preparation workflows simple, and use reagents optimized for the specific sample type and target proteins.
- Cell lysis disrupts cell membranes and organelles, resulting in unregulated enzymatic activity that can reduce protein yield and lead to degraded proteins. To prevent these negative effects, protease and phosphatase inhibitors should be added to the lysis reagents.
- Some buffer components may interfere with the chosen gel electrophoresis chemistry system (e.g., Tris-glycine, Bis-Tris) and cause a variety of artifacts when running the gel. Selecting a gel electrophoresis chemistry that is compatible with the buffer one's sample is prepared in is the simplest route. However, if one cannot change the gel electrophoresis chemistry system, one may need to perform sample clean-up to render the sample compatible with the given system (see "Protein clean-up methods" section). Certain clean-up methods are more favorable than others for reducing or removing specific interfering substances.
- To avoid under- or overloading samples, determine the protein concentration of each sample prior to electrophoresis with a compatible protein assay (see "Protein quantitation" section). Note that certain buffer components can interfere with the chemistry of a given protein assay, and these components may need to be reduced or removed from the sample prior to determining sample concentration. These interfering substances can cause artificially high or low concentration readings, depending on the substance and the given protein assay.

Protein sample preparation for electrophoresis

Step 1

Cellular disruption and protein extraction
Different samples (e.g., plant vs. mammalian cells) will require different disruption and extraction strategies. Use reagents that have been optimized for specific sample types. In addition, use protease and phosphatase inhibitors to minimize activity of proteases that can modify and reduce protein yields. For long-term storage of samples, we recommend freezing them to further inhibit protein degradation.

Step 2

Contaminant removal, desalting, concentration
Remove or reduce interfering substances that can negatively impact electrophoresis.

Step 3

Quantitation
Quantitate protein samples using a compatible protein assay. Concentrate or dilute samples if necessary.

Protein extraction and clean-up methods

Gentle formulations designed to maximize protein yield and activity

Obtain high protein yields from tissues, cells, or subcellular fractions using reagents and kits that are optimized for mammalian, bacterial, yeast, insect (baculovirus), and plant samples. These gentle formulations have been verified in multiple tissue types and cell lines, and generally eliminate the need for mechanical cell disruption.

Overview of sample types and recommended protein extraction reagents and kits.

Sample type	Goal	Recommended Thermo Scientific™ reagents or kits
Primary cultured or mammalian cells or tissues	Total protein extraction	M-PER™ Mammalian Protein Extraction Reagent T-PER™ Tissue Protein Extraction Reagent N-PER™ Neuronal Protein Extraction Reagent RIPA Lysis and Extraction Buffer Pierce™ IP Lysis Buffer
Cultured mammalian cells or tissues	Subcellular fractionation or organelle isolation	NE-PER™ Nuclear and Cytoplasmic Extraction Reagents Subcellular fractionation kits Mitochondria isolation kits Pierce™ Cell Surface Protein Isolation Kit Syn-PER™ Synaptic Protein Extraction Reagent Lysosome Enrichment Kit for Tissues and Cultured Cells
Bacterial cells	Total protein extraction	B-PER™ Complete Bacterial Protein Extraction Reagent
Yeast cells	Total protein extraction	Y-PER™ Yeast Protein Extraction Reagent Y-PER™ Plus Dialyzable Yeast Protein Extraction Reagent
Insect cells (baculovirus)	Total protein extraction	I-PER™ Insect Cell Protein Extraction Reagent
Plant tissue (leaf, stem, root, flower)	Total protein extraction	Pierce™ Plant Total Protein Extraction Kit

Did you know

Although RIPA buffer is a common lysis buffer used for preparing samples for SDS-PAGE and western blot analysis, RIPA buffer is a strong lysis buffer than contains components that may interfere with certain protein assays and gel electrophoresis chemistries. Cross reference your RIPA buffer recipe to the interfering substances lists for both gel electrophoresis and protein assays, as multiple variants on the traditional RIPA buffer recipe exist.

Protein electrophoresis interfering substances

Interfering substance	Potential issues	Solution
Excess salt	High salt results in increased conductivity that causes uneven sample lanes and lane widening	• Perform a sample clean-up method to lower salt concentration (see "Protein clean-up methods") • Make sure that the salt concentration does not exceed 50—100 mM
DNA contamination	Excess DNA causes the sample to become viscous resulting in protein clumping, which can result in narrow lanes that cannot be interpreted	Shear genomic DNA to reduce viscosity before loading the sample
High detergent concentration	Detergents can form mixed micelles with SDS and migrate through the gel. This can lead to lane widening, uneven sample lanes, and streaks in lower regions of lanes, limiting the ability to analyze proteins less than 40 kDa	• Dilute the sample to reduce the final concentration of detergents in the samples loaded • Remove excess detergent with detergent removal columns or SDS-PAGE sample prep kits
Sample type and protein load	High protein loads can cause several issues, such as loss of protein band resolution, lane streaking, and non-straight lanes	The maximum recommended sample load for optimal resolution in mini gels with 10, 12, 15, or 17 wells is 0.5 µg per band or about 10–15 µg of cell lysate per lane
Excess/incorrect reducing agent	Excess reducing reagent can cause shadows at lane edges	The final concentration of reducing agents for SDS-PAGE should be less than 50 mM for dithiothreitol (DTT) and Tris(2-carboxyethyl)phosphine (TCEP), and less than 2.5% for β-mercaptoethanol (β-ME)
Excess guanidine-HCl	Guanidine-HCl has high ionic strength and results in increased conductivity that causes uneven sample lanes and lane widening	Perform sample clean-up method

Protein clean-up methods

Many detergents and salts used in protein extraction formulations may have adverse effects on downstream analysis by protein electrophoresis. Therefore, it may be necessary to remove or reduce these contaminants following cell lysis or subsequent sample processing such as protein purification. Listed below are the different techniques that can be used to limit these interfering substances.

Dialysis

Dialysis is a classic separation technique that facilitates the removal of small, unwanted compounds from proteins in solution by selective diffusion through a semipermeable membrane. Proteins that are larger than the membrane pores are retained on the sample side of the membrane, but low molecular weight contaminants diffuse freely through the membrane and can be removed over multiple buffer exchanges. Thermo Scientific™ Slide-A-Lyzer™ dialysis cassettes and devices are ready to use and designed to eliminate potential sample leakage and maximize ease of use for specific applications.

Desalting

Size exclusion chromatography (also known as gel filtration) can be effectively utilized for protein desalting. A resin is selected with pores that are large enough for small contaminants (e.g., salts) to penetrate, but too small for the protein of interest to enter. This causes the migration of small contaminants to slow as they get trapped in the resin, while the larger, faster proteins emerge from the column first, allowing the protein of interest to be recovered separately from the small molecules retained on the column. Thermo Scientific™ Zeba™ desalting products contain a unique resin and are specifically designed to provide consistent performance over a wide range of protein concentrations and sample sizes. High recovery of protein can be achieved even for dilute protein samples.

Concentration

Protein concentration and diafiltration, similar to dialysis, uses a semipermeable membrane to separate macromolecules from low molecular weight compounds. Unlike dialysis, which relies on passive diffusion, concentration is achieved by forcing both liquid (buffers) and low molecular weight solutes through the membrane by centrifugation, where they are collected on the other side (filtrate). Macromolecules remain on the sample side of the membrane, where they become concentrated to a smaller volume (retentate). For buffer exchange, the retentate is diluted to the original volume with exchange buffer and centrifuged multiple times until the desired level of exchange has been achieved. Our high-performance Thermo Scientific™ Pierce™ Protein Concentrators enable rapid sample processing with high protein recovery.

Precipitation

Protein precipitation removes interfering substances by selectively precipitating proteins using trichloroacetic acid (TCA) or acetone. The solution containing the interfering substance is removed, and the protein is then resolubilized in an assay-compatible buffer. Commercially available kits simplify sample pretreatment for protein assays. Small molecules may be separated from large proteins in a sample via precipitation with acetone. When used in combination with 96-well centrifuge filter plates, this method is ideal for processing many samples at once.

Comparison of protein sample clean-up methods

Technique	Dialysis	Desalting	Concentration	Precipitation
Best for	Buffer exchange, desalting	Desalting, buffer exchange	Sample concentration, desalting, buffer exchange	Complete buffer replacement
Advantages	Maximum flexibility in processing volume, wide range of molecular weight cutoffs	Speed	Best for low- to medium-volume samples; fast compared to traditional dialysis	Low cost, good for small volumes
Disadvantages	Slow and multiple rounds of dialysis may be required	Limited sample volume can be processed at a time	Potential protein loss	Higher protein loss
Sample processing time	~2–24 hr	~5–10 min	~5–30 min	~5 min
Sample volume processing ranges	10 µL–250 mL	2 µL–4 mL	100 µL–100 mL	Any
Recommended sample type	Purified protein	Lysate or purified protein	Lysate or purified protein	Lysate or purified protein
Recommended product	**Thermo Scientific™ Slide-A-Lyzer™ Cassettes**	**Thermo Scientific Zeba™ Spin Desalting Columns**	**Thermo Scientific™ Pierce™ Protein Concentrators**	**Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit**

Download the **protein clean-up handbook** to learn more

Protein quantitation

Determining your sample's protein concentration

Protein quantitation is a necessary step before conducting analysis that compares one sample to another and to prevent under- or overloading of the gel. The choice of which method to use for protein quantitation will be based on the compatibility with common substances in the sample (detergents, reducing agents, inhibitors, salts, chaotropic agents) and the sensitivity needed. See the table below for the recommend assay based on common lysis buffers.

Recommended assays based on common lysis buffers

Lysis/extraction buffer	Recommended protein assay	If adding reducing agents or metal chelators (e.g., DTT above 1 mM, EDTA above 10 mM)
RIPA Lysis and Extraction Buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS)	BCA protein assay	Pierce BCA Protein Assay Kit – Reducing Agent Compatible
Invitrogen™ Lysis Buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate)	Pierce BCA Protein Assay Kit – Reducing Agent Compatible	Pierce BCA Protein Assay – Reducing Agent Compatible
M-PER Mammalian Protein Extraction Reagent	BCA protein assay	Pierce Coomassie Plus (Bradford) Assay Kit
B-PER Bacterial Protein Extraction Reagents	BCA protein assay	Pierce BCA Protein Assay Kit – Reducing Agent Compatible
NE-PER Nuclear and Cytoplasmic Extraction Reagents	BCA protein assay	Pierce Coomassie Plus (Bradford) Assay Kit

Good to know

BCA protein assays have a unique advantage over Coomassie dye–based assays (Bradford assays), as they are compatible with samples that contain up to 5% surfactants (detergents), and are affected much less by protein compositional differences, providing greater protein-to-protein uniformity and accuracy.

Tip

For quantitation of low-volume or very dilute samples, fluorescent assays can provide working ranges down to 10 ng/mL compared to 500 ng/mL for enhanced colorimetric assays and 2,000 ng/mL for standard colorimetric protocols.

Protein assay guide

	Thermo Scientific™ Pierce™ Rapid Gold BCA Protein Assay Kit	Thermo Scientific™ Pierce™ BCA Protein Assay Kit	Thermo Scientific™ Pierce™ BCA Protein Assay – Reducing Agent Compatible Kit	Thermo Scientific™ Pierce™ Coomassie Plus (Bradford) Assay Kit
Overview	5 min room-temperature BCA assay	Standard BCA assay with large linear response	Reducing agent–compatible BCA assay	Improved Bradford assay with linear response
Minimum sample volume	10 µL	25 µL	25 µL	10 µL
Working range	125–2,000 µg/mL	20–2,000 µg/mL	125–2,000 µg/mL	100–1,500 µg/mL
Compatibility	Detergents	Detergents	Detergents and reducing agents	Reducing agents
Incubation temperature	RT	37°C	37°C	RT
Assay time	5 min	30 min	50 min	10 min
Assay wavelength	480 nm	562 nm	562 nm	595 nm

Download the **technical reference guide** for all available protein assays

Preparing PAGE samples for gel loading

Before a sample can be loaded onto a gel for analysis, it must be properly prepared. Depending on the gel type, this may involve denaturing the proteins, reducing any disulfide bonds, and adding a sample buffer. Sample buffers contain glycerol so that they are heavier than water and sink neatly to the bottom of the buffer-submerged well when loaded onto a gel. If suitable, negatively charged, low molecular weight dye is also included in the sample buffer; it will migrate at the buffer front, enabling one to monitor the progress of electrophoresis. The most common tracking dye for sample buffers is bromophenol blue. General guidelines for preparing samples are provided below.

General guidelines for preparing samples for separation:
Prepare your sample in the appropriate sample buffer, sometimes referred to as loading buffer, such that the final concentration of the sample buffer is 1X. Recommended sample buffers are listed on **page 42**.

Running reduced and nonreduced samples: For optimal results, we do not recommend running reduced and nonreduced samples on the same gel. If you do choose to run reduced and nonreduced samples on the same gel, do not run reduced and nonreduced samples in adjacent lanes. The reducing agent may have a carry-over effect on the nonreduced samples if they are in close proximity.

Heating samples: Heating the sample at 100°C in SDS-containing buffer results in proteolysis [3]. We recommend heating samples for denaturing electrophoresis (reduced or nonreduced) at 70°C for 2–10 minutes for optimal results. Do not heat the samples for nondenaturing (native) electrophoresis or Invitrogen™ Novex™ Zymogram Plus gels.

Electrophoresis buffers

We offer reliable premixed SDS-PAGE buffers and reagents, including sample buffers, running buffers, reducing agents, and antioxidants.

Buffer and reagent selection guide

Gel type	Sample buffers optimized for use with the gel	Running buffers optimized for use with the gel
Bolt Bis-Tris Plus gel	• Bolt Sample Reducing Agent (10X) • 4X Bolt LDS Sample Buffer (nonreducing) • Bolt Antioxidant	• 20X Bolt MES SDS Running Buffer (3.5–160 kDa separations) • 20X Bolt MOPS SDS Running Buffer (15–260 kDa separations)
NuPAGE Bis-Tris gel	• NuPAGE Sample Reducing Agent (10X) • 4X NuPAGE LDS Sample Buffer (nonreducing) • NuPAGE Antioxidant	• NuPAGE MES SDS Running Buffer (20X) (3.5–160 kDa separations) • NuPAGE MOPS SDS Running Buffer (20X) (15–260 kDa separations)
NuPAGE Tris-Acetate gel	• 4X NuPAGE LDS Sample Buffer (nonreducing) • NuPAGE Sample Reducing Agent (10X) • Novex Tris-Glycine Native Sample Buffer (2X)*	• NuPAGE Tris-Acetate SDS Running Buffer (20X) • Novex Tris-Glycine Native Running Buffer (10X)*
Novex Tris-Glycine gel	• Novex Tris-Glycine SDS Sample Buffer (2X) • NuPAGE Sample Reducing Agent (10X) • Novex Tris-Glycine Native Sample Buffer (2X)*	• Novex Tris-Glycine SDS Running Buffer (10X) • Novex Tris-Glycine Native Running Buffer (10X)* • Pierce Tris-Glycine SDS Buffer (10X) • BupH Tris-Glycine Buffer Packs
Novex Tricine gel	• Novex Tricine SDS Sample Buffer (2X)	• Novex Tricine SDS Running Buffer (10X)
NativePAGE gel	• NativePAGE Sample Buffer (4X) • NativePAGE 5% G-250 Sample Additive	• NativePAGE Running Buffer (20X) • NativePAGE Cathode Buffer Additive (20X)
Novex IEF gel	• Novex IEF Sample Buffer, pH 3–10 (2X) • IEF Sample Buffer, pH 3–7 (2X)	• Novex IEF Anode Buffer (50X) • Novex IEF Cathode Buffer, pH 3–10 (10X) • Novex IEF Cathode Buffer, pH 3–7 (10X)
Novex Zymogram Plus gel**	• Novex Tris-Glycine SDS Sample Buffer (2X)	• Novex Tris-Glycine SDS Running Buffer (10X)

* Use this buffer when performing a native separation with this gel.

** Novex Zymogram Developing Buffer (10X) and Novex Zymogram Renaturing Buffer (10X) are available for visualizing the Zymogram Plus gels.

MES vs. MOPS running buffer

- Use MES SDS running buffers to resolve small molecular weight proteins.
- Use MOPS running buffers to resolve medium-sized proteins.
- MES has a lower pK_a than MOPS, enabling gels with MES running buffer to run faster than gels with MOPS SDS running buffer. The difference in ion migration affects stacking and results in a difference in protein separation range between these buffers.

Reducing agent

When preparing samples for reducing gel electrophoresis, any of the following reducing agents may be used:

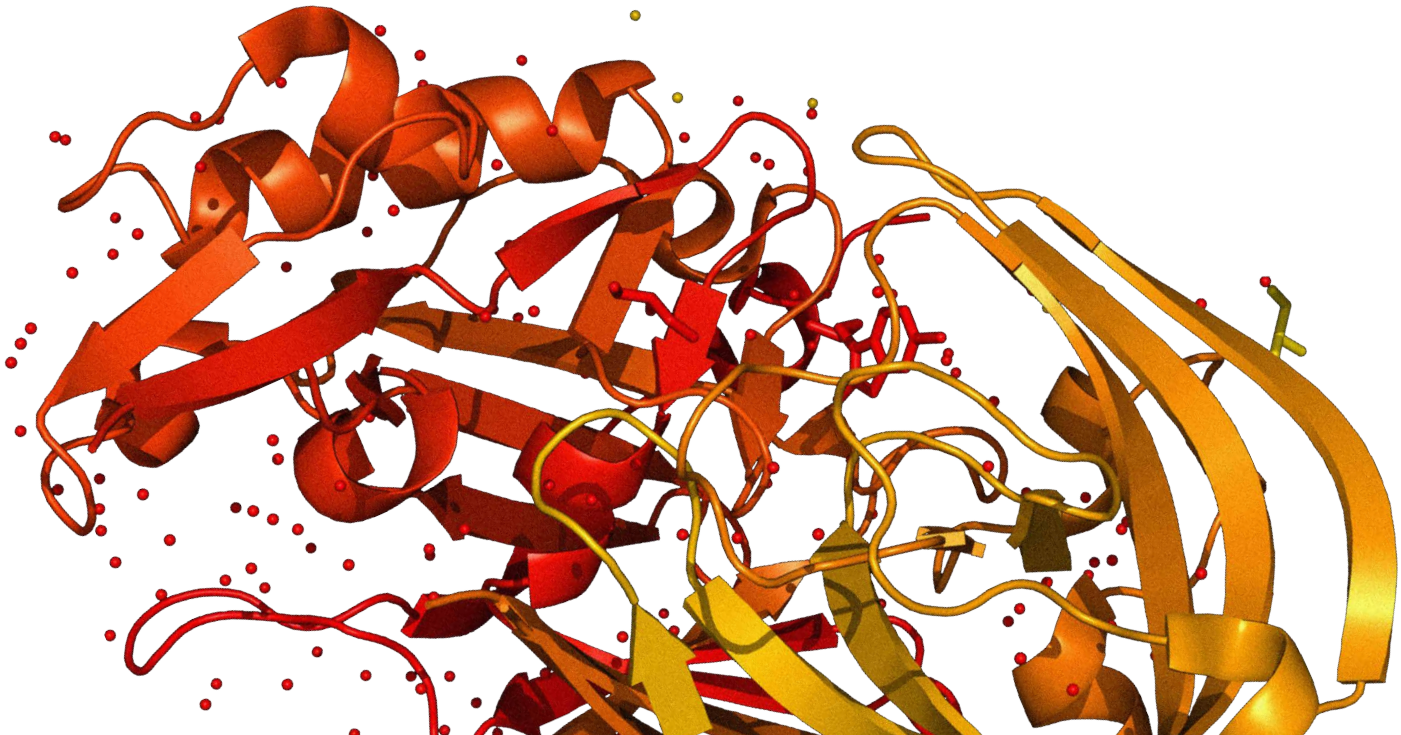
- Bolt Sample Reducing Agent
- NuPAGE Sample Reducing Agent
- Dithiothreitol (DTT), 50 mM final concentration
- β-mercaptoethanol (β-ME), 2.5% final concentration
- Tris-(2-carboxyethyl)phosphine (TCEP), 50 mM final concentration

Add the reducing agent to the sample up to an hour before loading the gel. Avoid storing reduced samples for long periods, even if they are frozen. Reoxidation of samples can occur during storage and produce inconsistent results.

Buffer recipes

Bolt Bis-Tris buffer recipes

Buffer	Storage	Component	Concentration (1X)
Bolt LDS Sample Buffer	4–25°C	Glycerol Tris base Tris-HCl LDS EDTA SERVA™ Blue G-250 Phenol red	10% 141 mM 106 mM 2% 0.51 mM 0.22 mM 0.175 mM (pH 8.5)
Bolt MOPS SDS Running Buffer	4–25°C	MOPS Tris base SDS EDTA	50 mM 50 mM 0.1% 1 mM (pH 7.7)
Bolt MES SDS Running Buffer	4–25°C	MES Tris base SDS EDTA	50 mM 50 mM 0.1% 1 mM (pH 7.3)
Bolt Transfer Buffer	4–25°C	Bicine Bis-Tris (free base) EDTA Chlorobutanol	25 mM 25 mM 1.0 mM 0.05 mM (pH 7.2)



NuPAGE Bis-Tris and Tris-Acetate buffer recipes

Buffer	Storage	Component	Concentration (1X)
NuPAGE LDS Sample Buffer	4–25°C	Glycerol Tris base Tris-HCl LDS EDTA SERVA Blue G-250 Phenol red	10% 141 mM 106 mM 2% 0.51 mM 0.22 mM 0.175 mM (pH 8.5)
NuPAGE MOPS SDS Running Buffer*	4–25°C	MOPS Tris base SDS EDTA	50 mM 50 mM 0.1% 1 mM (pH 7.7)
NuPAGE MES SDS Running Buffer*	4–25°C	MES Tris base SDS EDTA	50 mM 50 mM 0.1% 1 mM (pH 7.3)
NuPAGE Tris-Acetate SDS Running Buffer	4–25°C	Tris base Tricine SDS	50 mM 50 mM 0.1% (pH 8.24)
NuPAGE Transfer Buffer	4–25°C	Bicine Bis-Tris (free base) EDTA Chlorobutanol	25 mM 25 mM 1.0 mM 0.05 mM (pH 7.2)

* The premixed buffers (Cat. Nos. NP0001 and NP0002) also contain trace amounts of the proprietary NuPAGE Antioxidant (Cat. No. NP0005) for stability. Additional antioxidant may be required with specific protocols.

Novex Tris-Glycine buffer recipes

Buffer	Storage	Component	Concentration (1X)
Novex Tris-Glycine SDS Sample Buffer	4°C	Tris-HCl* Glycerol SDS Bromophenol blue Deionized water	63 mM 10% 2% 0.0025% — (pH 6.8)
Novex Tris-Glycine Native Sample Buffer	4°C	Tris-HCl* Glycerol Bromophenol blue Deionized water	100 mM 10% 0.0025% — (pH 8.6)
Novex Tris-Glycine SDS Running Buffer	Room temperature	Tris base Glycine SDS Deionized water	25 mM 192 mM 0.1% — (pH 8.3)
Novex Tris-Glycine Native Running Buffer	Room temperature	Tris base Glycine Deionized water	25 mM 192 mM — (pH 8.3)
Novex Tris-Glycine Transfer Buffer	Room temperature	Tris base Glycine Deionized water	12 mM 96 mM — (pH 8.3)

* Tris-HCl solutions are prepared from Tris base and pH-adjusted with 6 N HCl.

Novex Tricine buffer recipes

Buffer	Storage	Component	Concentration (1X)
Novex Tricine SDS Sample Buffer	+4˚C	Tris-HCl* Glycerol SDS Coomassie Blue G Phenol red Deionized water	450 mM 12% 4% 0.0075% 0.0025% – (pH 8.45)
Novex Tricine SDS Running Buffer	Room temperature	Tris base Tricine SDS Deionized water	100 mM 100 mM 0.1% – (pH 8.3)

* Tris-HCl solutions are prepared from Tris base and pH-adjusted with 6 N HCl.

Zymogram buffer recipes

Buffer	Storage	Component	Concentration (1X)
Novex Zymogram Plus Renaturing Buffer	Room temperature	Triton X-100 solution Deionized water	2.7% (w/v) in H_2O
Novex Zymogram Plus Developing Buffer	Room temperature	Tris-HCl* NaCl $CaCl_2 \cdot 2H_2O$ Brij 35 Deionized water	50 mM 200 mM 5 mM 0.006% (w/v) – (pH 7.6)

* Tris-HCl solutions are prepared from Tris base and pH-adjusted with 6 N HCl.

Isoelectric focusing buffer recipes

Buffer	Storage	Component	Concentration (1X)
Novex IEF Sample Buffer pH 3–7	4°C	Lysine (free base) Glycerol Deionized water	40 mM 15% —
Novex IEF Sample Buffer pH 3–10	4°C	Arginine (free base) Lysine (free base) Glycerol Deionized water	20 mM 20 mM 15% —
Novex IEF Cathode Buffer pH 3–7 (upper buffer chamber)	4°C	Lysine (free base) Deionized water	40 mM —
Novex IEF Cathode Buffer pH 3–10 (upper buffer chamber)	4°C	Arginine (free base) Lysine (free base) Deionized water	20 mM 20 mM — (pH 10.1)
Novex IEF Anode Buffer (for both pH ranges) (lower buffer chamber)	Room temperature	Phosphoric acid 85% Deionized water	7 mM — (pH 2.4)
Urea-thiourea-CHAPS (rehydration buffer for IPG strips)	–20°C	Deionized urea Deionized thiourea CHAPS Ampholytes Bromophenol blue Ultrapure water DTT	7 M 2 M 2–4% 0.2–2.0% 0.002% — 20 mM

Estimating protein sizes

Protein ladders and standards

Protein ladders, also known as protein markers or protein standards, are used to help estimate the size of proteins separated during electrophoresis. They serve as points of reference because they contain mixtures of highly purified proteins with known molecular weights and characteristics.

Protein ladders are loaded onto gels alongside samples and migrate during electrophoresis at a rate that is inversely proportional to their molecular sizes. When the run is complete, the proteins will appear as separate bands in the gel. A standard curve can be constructed from the distance each marker protein migrates through the gel versus the log of its molecular weight. After measuring the migration distance that an unknown protein travels through the same gel, its molecular weight can be determined graphically from the standard curve. Modern gel documentation instruments may have software algorithms that can make these mathematical calculations significantly more convenient. More often, however, protein ladders are used as a reference to help confirm the identity of proteins of interest whose sizes are already known.

Protein ladders are available with various protein molecular weight ranges and can be prestained, unstained, or labeled for different modes of detection and downstream applications. Read on to learn which protein ladder is best for your applications.

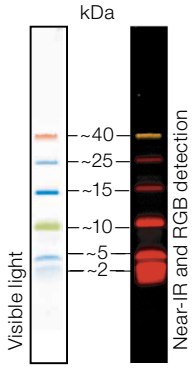
Prestained protein ladders

Prestained protein ladders contain proteins prelabeled with various dyes that allow them to be visible without additional staining. Prestained ladders are visible during electrophoresis, enabling the ability to monitor the progress of separation for the range of protein sizes that are most relevant to the particular experiment. Most prestained markers and ladders also remain visible after gel staining or transfer to membranes for detection by western blotting.

Prestained protein ladders can be used to:

- Monitor protein separation during polyacrylamide gel electrophoresis
- Quickly check visually whether protein transfer has occurred from gel to membrane (prestained protein ladder should transfer to membrane but should not be used to determine transfer efficiency; see "Good to know" notes)
- Estimate the approximate size of your target protein

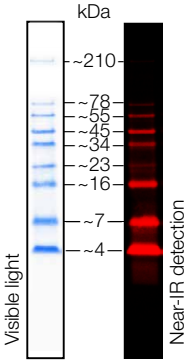
Low range



Novex
16% Tricine Gel

Thermo Scientific™ Spectra™ Multicolor Low Range Protein Ladder
Cat. No. 26628

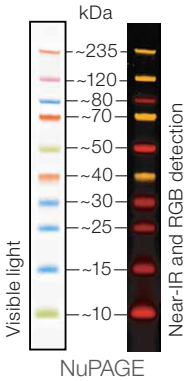
Low range



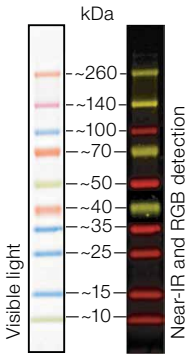
Novex
16% Tricine Gel

Invitrogen™ SeeBlue™ Prestained Protein Standard
Cat. No. LC5625

Broad range



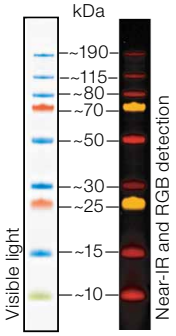
NuPAGE
4–12% Bis-Tris
Gel (MES buffer)



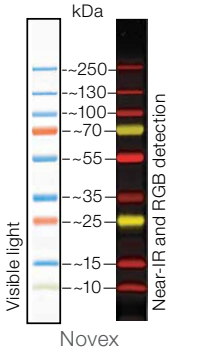
Novex
4–20% Tris-Glycine Gel

Thermo Scientific™ Spectra™ Multicolor Broad Range Protein Ladder
Cat. No. 26634

Broad range

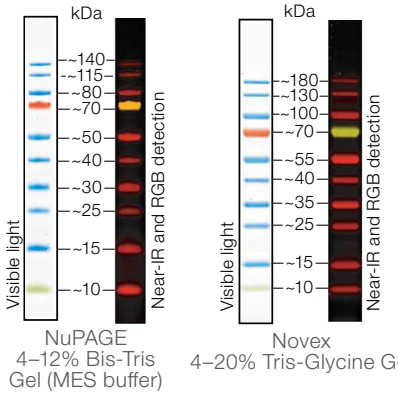
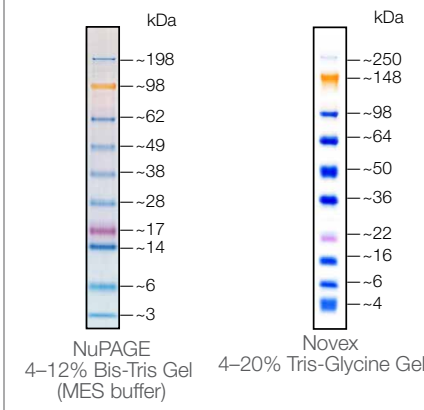


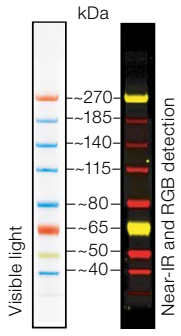
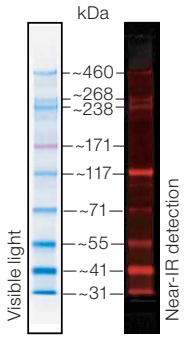
NuPAGE
4–12% Bis-Tris
Gel (MES buffer)



Novex
4–20% Tris-Glycine Gel

Thermo Scientific™ PageRuler™ Plus Prestained Protein Ladder
Cat. No. 26619

Broad range	Broad range
 NuPAGE 4–12% Bis-Tris Gel (MES buffer) Novex 4–20% Tris-Glycine Gel	 NuPAGE 4–12% Bis-Tris Gel (MES buffer) Novex 4–20% Tris-Glycine Gel
Thermo Scientific™ PageRuler™ Prestained Protein Ladder	SeeBlue Plus2 Prestained Protein Standard
Cat. No. 26616	Cat. No. LC5925

High range	High range
 NuPAGE 3–8% Tris-Acetate Gel	 NuPAGE 3–8% Tris-Acetate Gel
Thermo Scientific™ Spectra™ Multicolor High Range Protein Ladder	Invitrogen™ HiMark™ Prestained Protein Standard
Cat. No. 26625	Cat. No. LC5699

Good to know

Why do molecular weights change between the different gel chemistries?

Slight differences in protein mobilities will occur when the same proteins are run in different SDS-PAGE buffer systems (e.g., Bis-Tris vs. Tris-glycine). Each SDS-PAGE buffer system has a different pH, which affects the charge of a protein and its binding capacity for SDS. The degree of change in protein mobility is usually small in natural proteins but is more pronounced with atypical or chemically modified proteins, such as prestained standards. Apparent molecular weight values for prestained standards will vary between gel systems—it is important to use the apparent molecular weights that match your gel for the most accurate calibration of your sample proteins.

Good to know

How can protein transfer efficiency and consistency be assessed?

Transfer efficiency refers to the efficiency of transfer of protein out of the protein gel and onto the western blotting membrane. Specifically, the amount of protein that binds to the membrane will be the foundation for the immunoblotting steps. If very little protein transfers out of the gel and binds to the membrane, then the subsequent immunoblotting steps will be challenging.

Transfer efficiency is sometimes evaluated by visually assessing the amount of prestained protein ladder that is visible on the membrane post-transfer. However, this is not necessarily the best practice. Prestained ladder proteins are stained with dyes that allow for visualization during electrophoresis. These dyes affect the transfer of the ladder proteins out of the gel. Sample proteins, however, are not stained and thus can have a different mobility rate out of the gel.

Visual assessment of the transfer of ladder can be a useful quick process check for whether transfer occurred (sometimes electrodes may be reversed, or the transfer stack may not be assembled correctly). Not observing any prestained marker transfer to the membrane could indicate an issue with the transfer setup. A more favorable strategy to determine transfer efficiency is to use a reversible membrane stain, which will stain the total protein that successfully transferred to the membrane. Some choose to take this a step further and back-stain the gel, post-transfer, which can give an assessment of how much protein transferred out of the gel. It is not unusual to see ladder protein or sample protein remaining in the gel, as no transfer method is completely 100% efficient. What really matters is how much protein is bound to the membrane.

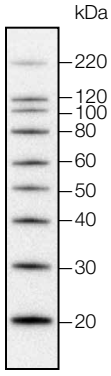
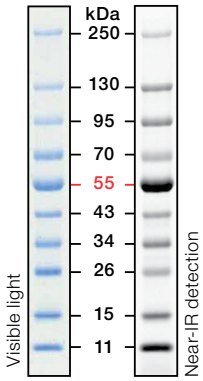
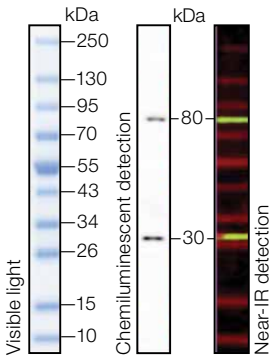
Transfer consistency refers to the evenness of protein transfer from gel to membrane. Ideally, protein is transferred evenly from side to side and top to bottom on the membrane. However, improper assembly of a transfer stack and failure to roll the transfer stack may lead to air bubbles, creases, and dead spots on the membrane. These artifacts make the subsequent immunoblotting steps challenging, as an uneven transfer of protein will lead to an uneven immunoblot and signal production. Transfer consistency cannot be assessed simply by the appearance of transferred prestained ladder proteins on the membrane. Instead, the use of a reversible membrane stain provides a more accurate picture of protein transfer efficiency across the entire membrane.

Western blot protein ladders

Western blot–specific protein ladders are designed for easy and convenient protein molecular weight estimation directly on western blots or indirectly by using various blotting detection systems (e.g., chemiluminescent, fluorescent, or chromogenic). The protein markers consist of recombinant proteins with an IgG binding site. The IgG binding site binds the primary or secondary antibody used for detection of the target protein, allowing visualization of the standard on the western blot.

Recommended for:

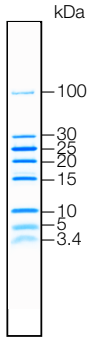
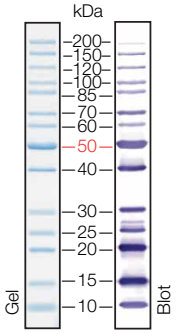
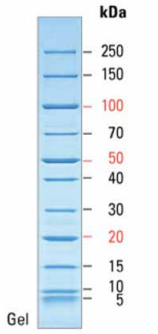
- Approximate molecular weight determination directly on western blots or indirectly through chemiluminescent, fluorescent, or chromogenic detection systems
- Qualitatively assessing transfer efficiency

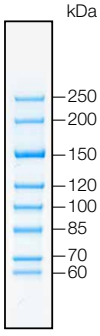
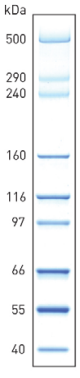
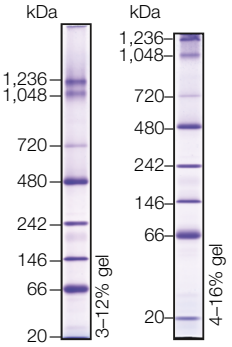
Indirect detection	Fluorescent detection	Indirect and direct detection
 NuPAGE 4–12% Bis-Tris Gel	 Gel: 4–20% Tris-glycine (SDS-PAGE)	 Novex 4–20% Tris-Glycine Gel
Invitrogen™ MagicMark™ XP Western Protein Standard	Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder	Invitrogen™ iBright™ Prestained Protein Ladder
Cat. No. LC5602	Cat. No. 26635	Cat. No. LC5615
Visualization: user-defined detection system (not recommended for RGB fluorescence systems)	**Visualization:** colorimetric, NIR fluorescence (670 nm red laser or 700 nm channel)	**Visualization:** colorimetric, NIR fluorescence (700 nm channel), user-defined detection system (2 bands)

Unstained protein ladders

Unstained protein ladders are useful when you need to accurately determine the size of your protein. However, the proteins can only be visualized after staining with a Coomassie™ stain or other nonspecific protein stain. The dyes in prestained protein ladders add additional molecular weight to the ladder proteins and thus affect their migration. Each dye molecule may not bind equally to a given protein or proteins, and some prestained protein ladders utilize multiple different types of colored dyes (for easier tracking of specific proteins in the ladder). Because of the dyes in prestained protein ladders, a given ladder protein's true molecular weight is altered and thus is an apparent molecular weight. Unstained protein ladders do not have this issue because the migration of the proteins in the ladder are not affected by added dye, and thus migrate as normal (although one can observe differences from one gel chemistry to the next), and as such are suitable for the most accurate determination of molecular weights in a protein sample.

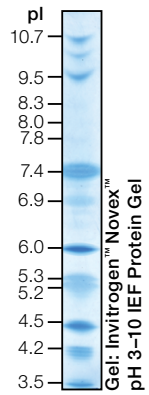
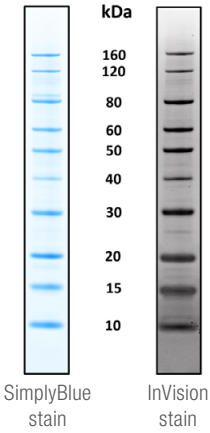
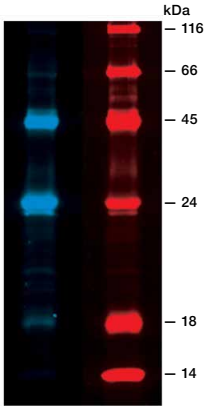
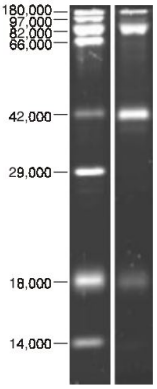
These ready-to-use ladders include proteins of known molecular weight. Visualize them on SDS-PAGE gels by staining with Coomassie Brilliant Blue R250 or after western transfer using Ponceau S or a similar stain.

Low range	Broad range	Broad range
 Novex 16% Tricine Gel	 8–16% Tris-glycine gel (SDS-PAGE)	
Thermo Scientific™ PageRuler™ Unstained Low Range Protein Ladder	Thermo Scientific™ PageRuler™ Unstained Protein Ladder	Thermo Scientific™ PageRuler™ Unstained Broad Range Protein Ladder
Cat. No. 26632	Cat. No. 26614	Cat. No. 26630

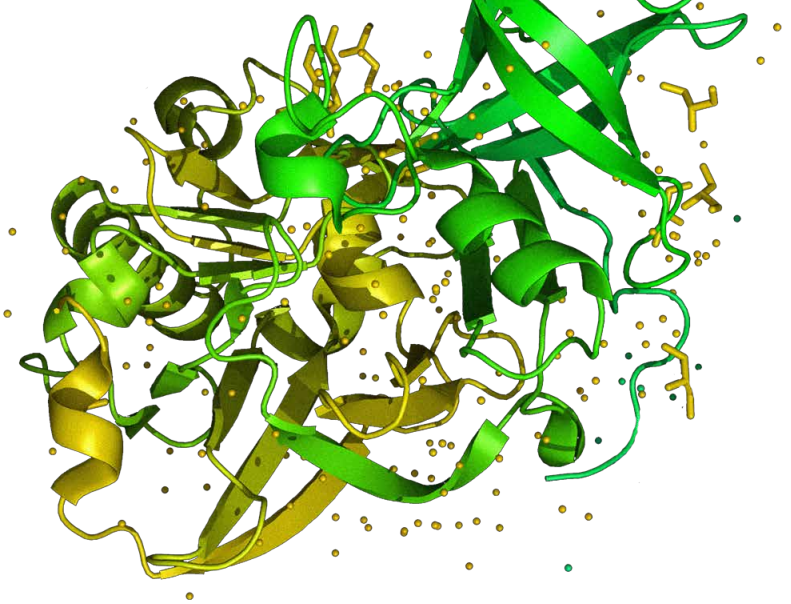
High range	High range	Native
 NuPAGE 3-8% Tris-Acetate Gel		 NativePAGE Bis-Tris Gel; stained with Coomassie R-250 dye
Thermo Scientific™ PageRuler™ Unstained High Range Protein Ladder	Invitrogen™ HiMark™ Unstained Protein Standard	Invitrogen™ NativeMark™ Unstained Protein Standard
Cat. No. 26637	Cat. No. LC5688	Cat. No. LC0725

IEF and specialty protein ladders

Protein ladders are available for use in isoelectric focusing (IEF) and for use when the experiment involves the detection of His-tagged, phosphorylated, or glycosylated proteins.

IEF	His-tagged proteins	Phosphorylated proteins	Glycosylated proteins
			
Invitrogen™ IEF Marker 3–10	Invitrogen™ BenchMark™ His-Tagged Protein Standard	Invitrogen™ PeppermintStick™ Phosphoprotein Molecular Weight Standards	Invitrogen™ CandyCane™ Glycoprotein Molecular Weight Standards
Cat. No. 39212-01	Cat. No. LC5606	Cat. No. P33350	Cat. No. C21852
Uses: IEF applications	**Uses:** positive control and for molecular weight sizing in His-tagged fusion protein detection	**Uses:** positive and negative controls for detection of phosphorylated proteins	**Uses:** positive and negative controls for detection of glycosylated proteins
Visualization: colorimetric stain	**Visualization:** Invitrogen™ InVision™ His-Tag In-Gel Stain or Invitrogen™ 6x-His Tag Monoclonal Antibody	**Visualization:** colorimetric stain or methods that detect phosphorylated proteins, such as Invitrogen™ Pro-Q™ Diamond phosphoprotein gel stains	**Visualization:** colorimetric stain or methods that detect glycosylated proteins, such as Invitrogen™ Pro-Q™ Emerald glycoprotein stain kits

Choosing the electrophoresis chamber and power supply



In electrical terms, the process of electrophoresis is closely associated with the following equations derived from Ohm's law:

Voltage = current × resistance (V = IR)

Wattage = current × voltage (W = IV)

Resistance

The electrical resistance of the assembled electrophoresis cell is dependent on buffer conductivity, gel thickness, temperature, and the number of gels being run. Although the resistance is determined by the gel system, the resistance varies over the course of the run.

- In discontinuous buffer systems (and to a lesser extent in continuous buffer systems) resistance increases over the course of electrophoresis. This occurs in the Tris-glycine buffer system as highly conductive chloride ions in the gel are replaced by less conductive glycine ions from the running buffer.
- Resistance decreases as the temperature increases.

Voltage

The velocity of an ion in an electric field varies in proportion to the field strength (volts per unit distance). The higher the voltage, the faster an ion moves. For most applications, **we recommend a constant voltage setting.**

- A constant voltage setting allows the current and power to decrease over the course of electrophoresis, providing a safety margin in case of a break in the system.
- The constant voltage setting does not need adjustment to account for differences in number or thickness of gels being electrophoresed.

Current

For a given gel/buffer system, at a given temperature, current varies in proportion to the field strength (voltage) and cross-sectional area (thickness and number of gels). When using a constant current setting, migration starts slow, and accelerates over time, thus favoring stacking in discontinuous gels.

When running under constant current, set a voltage limit on the power supply at or slightly above the maximum expected voltage to avoid unsafe conditions. At constant current, voltage increases as resistance increases. If a local fault condition occurs (e.g., a bad connection), high local resistance may cause the voltage to reach the maximum for the power supply, leading to overheating and damage of the electrophoresis cell.

Power

Wattage measures the rate of energy conversion, which is manifested as heat generated by the system. Using constant power ensures that the total amount of heat generated by the system remains constant throughout the run, but results in variable mobility since voltage increases and current decreases over the course of the run. Constant power is typically used when running IEF strips. When using constant power, set the voltage limit slightly above the maximum expected for the run. High local resistance can cause a large amount of heat to be generated over a small distance, damaging the electrophoresis cell and gels.

Electrophoresis chamber system selection guide

Our electrophoresis chamber systems are designed for compatibility with the full range of Invitrogen gel offerings. Refer to the table below to find which system is right for you.

	Mini Gel Tank	XCell *SureLock* Mini-Cell	SureLock Tandem Midi Gel Tank	XCell4 *SureLock* Midi-Cell
				
Gel capacity	Up to 2 mini gels	Up to 2 mini gels	Up to 2 midi gels	Up to 4 midi gels
Cell dimensions (L x W x H; height with lid on)	32 x 11.5 x 16 cm	14 x 13 x 16 cm	25 × 17.9 × 17.3 cm	21 x 19 x 16 cm
Advantages	• The Mini Gel Tank is versatile and compatible with all Invitrogen precast and handcast mini gels; the unique tank design enables convenient side-by-side gel loading and enhanced viewing during use • Mini Blot Module is available for wet protein transfers	• Instrument incorporates a gel tension wedge in place of the rear wedge used on earlier models • XCell II Blot Module is available for semi-wet protein transfers	• Easy-to-use apparatus, with separate chambers for each gel, enabling scalable buffer usage • SureLock Tandem Midi Blot Module is available for wet protein transfers	• Advanced apparatus for easier, more reliable electrophoresis with midi gels

Mini Gel Tank

One tank, 181 gels

The Mini Gel Tank is designed for more intuitive use and greater convenience compared to traditional electrophoresis tanks. The unique, side-by-side tank design allows you to perform electrophoresis of 1 or 2 mini gels.

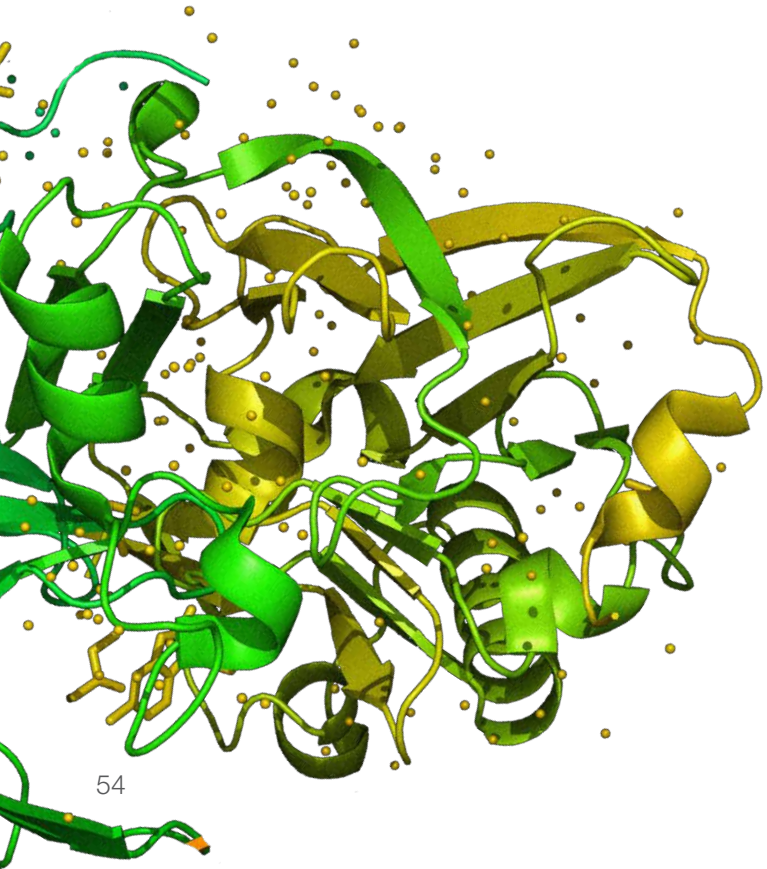
The Mini Gel Tank offers:

- **Versatility**—compatible with all of our mini gels, including Invitrogen™ NuPAGE™, Novex™, Bolt™, and specialty gels
- **Easy sample loading**—forward-facing well configuration
- **Simultaneous visualization of both gels**—streamlined, side-by-side tank configuration
- **Simple monitoring of gels**—white tank stand provides easy visualization of prestained markers
- **Less running buffer required**—gel chambers are separated, so you only need to load sufficient buffer for each gel to the specified fill line

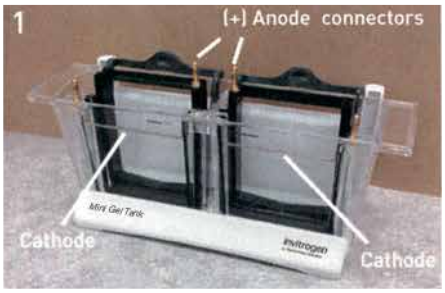
Specifications

- Gel capacity: up to 2 mini gels
- Cell size (L x W x H): 32 x 11.5 x 16 cm (height with lid on)
- Buffer requirement: 400 mL for each mini gel chamber
- Material: polycarbonate
- Chemical resistance: not compatible with acetone, chlorinated hydrocarbons (e.g., chloroform), or aromatic hydrocarbons (e.g., toluene, benzene)

Watch our Mini Gel Tank video at thermofisher.com/minigeltank.

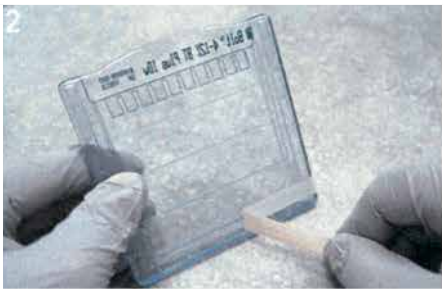


Learn more at
thermofisher.com/minigeltank



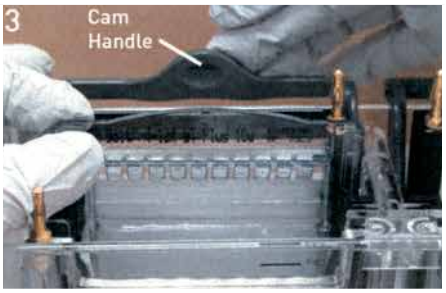
1. Snap the electrophoresis tank into the base, and place the cassette clamp(s) into the chamber(s) with the anode connector(s) (+) aligned to the center.

   Fill the chamber(s) with 1X buffer to the level of the cathode.



2. Remove the comb, and peel away the tape at the bottom of the gel cassette.

   Rinse the wells 3 times with 1X buffer.

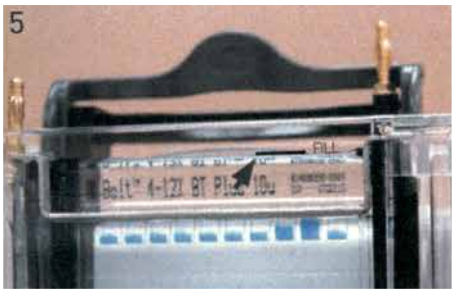


3. Place the cassette in the chamber with the wells facing towards you.

   Hold the cassette in a raised position and close the clamp by moving the cam handle forward.

4. Make sure the wells are completely filled with 1X buffer.

   Load your samples and markers.



5. Hold the cassette and release the cassette clamp.

   Gently lower the casette so that it rests on the bottom of the chamber, and close the cassette clamp.

   Add 1X buffer to the level of the fill line.

6. Make sure the power supply is off.

   If only running one gel, remove the cassette clamp from unused chamber.

   Place the lid on the tank and plug the electrode cords into the power supply.

   Turn the power supply on to begin electrophoresis.

Figure 25. How to use the Mini Gel Tank.

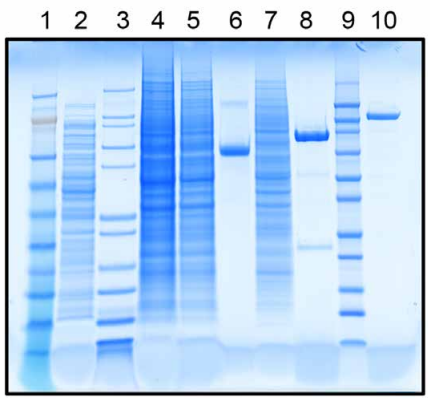


Figure 26. Electrophoresis of Bolt gel using the Mini Gel Tank. Protein standards and samples were loaded at 10 µL sample volumes on an Invitrogen™ Bolt™ 4–12% Bis-Tris Plus gel. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands with consistent migration patterns were observed after staining with Invitrogen™ SimplyBlue™ SafeStain. Images were acquired using a flatbed scanner. **Lane 1:** SeeBlue Plus2 Prestained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** Invitrogen™ Mark12™ Unstained Standard (blend of 12 purified proteins); **lane 4:** 40 µg HeLa cell lysate; **lane 5:** 20 µg HeLa cell lysate; **lane 6:** 5 µg BSA; **lane 7:** 40 µg Jurkat cell lysate; **lane 8:** 5 µg GST fusion protein; **lane 9:** Invitrogen™ Novex™ Sharp Unstained Protein Standard; **lane 10:** 5 µg β-galactosidase.

Recommended products

The **Invitrogen™ Mini Blot Module** is a wet transfer apparatus that conveniently fits into the chambers of the Mini Gel Tank to easily transfer proteins from mini gels to nitrocellulose or PVDF membranes.

XCell *SureLock* Mini-Cell

Simultaneous electrophoresis of up to 2 mini gels

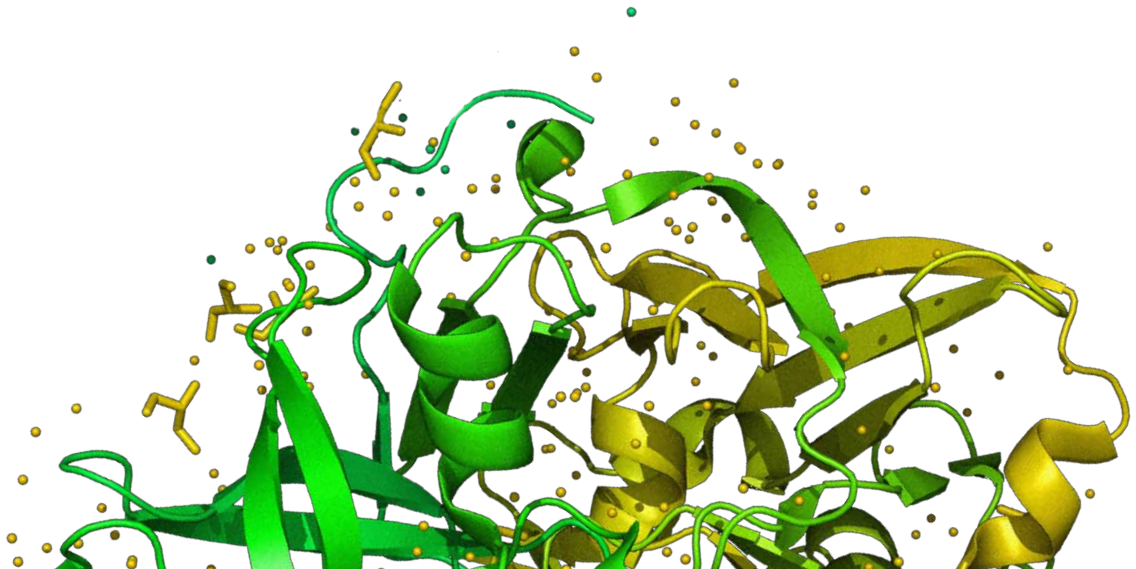
The unique design of the Invitrogen™ XCell *SureLock*™ Mini-Cell allows you to run mini gels quickly and easily without any clamps or grease (Figure 27). The tight seal provided by the gel tension wedge results in consistent performance. The XCell *SureLock* Mini-Cell is compatible with Bolt, NuPAGE, Novex, and specialty gels (Figure 28).

Key features of the XCell *Sure*Lock Mini-Cell:

- **User-friendly design**—uses single gel tension wedge with no clamps or grease
- **Flexibility**—perform electrophoresis of 2 mini gels simultaneously
- **Unique, heat dissipating design**—no need for a cooling device
- **Built-in usability features**—retractable plugs, recessed jacks, and a specially designed lid enhances user safety

Specifications

- Gel capacity: up to 2 mini gels
- Cell size (L x W x H): 14 x 13 x 16 cm (height with lid on)
- Buffer chamber requirement (Invitrogen mini gels):
  - Upper buffer chamber: 200 mL
  - Lower buffer chamber: 600 mL
- Chemical resistance: impervious to most alcohols but not compatible with acetone, chlorinated hydrocarbons (e.g., chloroform), or aromatic hydrocarbons (e.g., toluene, benzene)

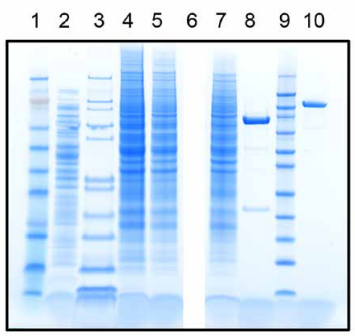


1. Drop buffer core into the lower buffer chamber of the XCell *SureLock* Mini-Cell. Insert one mini gel in front of the buffer core and a second mini gel or the buffer dam behind the buffer core.

2. Lock the gel tension wedge in place, load samples, and fill the buffer chambers with the appropriate running buffers.

3. Place the cell lid on the unit and you're ready to run.

Figure 27. How to use the XCell *SureLock* Mini-Cell.



NuPAGE Bis-Tris gel in
XCell *SureLock* Mini-Cell

Figure 28. Electrophoresis of NuPAGE Bis-Tris gel with the XCell *SureLock* Mini-Cell. Lane 1: SeeBlue Plus2 Prestained Standard; **lane 2:** 10 µg *E. coli* lysate; **lane 3:** Mark12 Unstained Standard (blend of 12 purified proteins); **lane 4:** 40 µg HeLa cell lysate; **lane 5:** 20 µg HeLa cell lysate; **lane 6:** not used; **lane 7:** 40 µg Jurkat cell lysate; **lane 8:** 5 µg of a GST fusion protein; **lane 9:** Novex Sharp Unstained Protein Standard; **lane 10:** 5 µg β-galactosidase. Gel electrophoresis was performed at 200 V (constant), and gels were stained using SimplyBlue SafeStain. Image was acquired using a flatbed scanner.

Recommended products

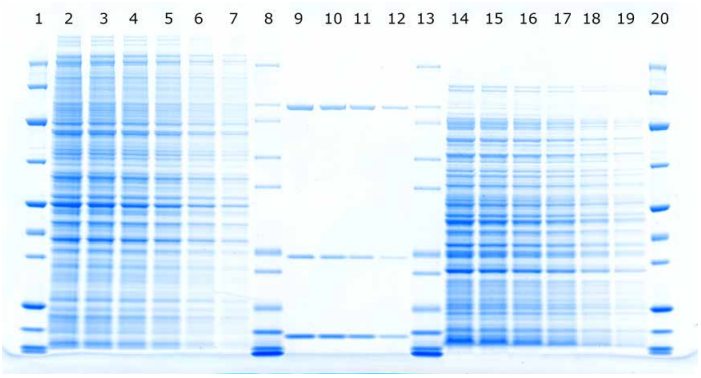
The XCell *SureLock* Mini-Cell can be easily adapted for transfer of proteins from mini gels to membranes by simply inserting the **Invitrogen™ XCell II™ Blot Module** into the lower buffer chamber.

SureLock Tandem Midi Gel Tank

2-in-1 midi gel electrophoresis and transfer tank

The Invitrogen™ SureLock™ Tandem Midi Gel Tank enables rapid electrophoresis of midi gels using minimal buffer (~520 mL/gel) in a leak-free system. With a setup time of ~30 seconds, the tank efficiently runs midi gels while also providing consistent performance. The tank features two independent chambers, allowing electrophoresis of one or two gels at a time, which saves on buffer and limits waste.

The SureLock Tandem Midi Gel Tank can be used for wet tank transfers when paired with the SureLock Tandem Midi Blot Module. The SureLock Tandem Midi Blot Module performs efficient, room-temperature wet protein transfers for downstream western blot analysis. The tank accommodates two blot modules, allowing transfer of one or two gels at a time, using considerably less transfer buffer (only ~300 mL per transfer) than other wet transfer systems. This lower buffer requirement keeps the amount of methanol waste (a hazardous material) to a minimum.



NuPAGE Bis-Tris 4–12% gradient midi gel

Figure 29. Publication-quality protein electrophoresis gel results using a NuPAGE Bis-Tris 4–12% gradient midi gel and the SureLock Tandem Midi Gel Tank. The gel was loaded as follows: **Lanes 1, 20:** 5 µL PageRuler Broad Range Unstained Protein Ladder (Cat. No. 26630); **lanes 2–7:** 10 µg, 8 µg, 6 µg, 4 µg, 2 µg, 1 µg HeLa lysate; **lanes 8, 13:** 5 µL Novex Mark12 Unstained Standard (Cat. No. LC5677); **lanes 9–12:** 240 ng, 180 ng, 120 ng, 60 ng of protein mix containing β-galactosidase, lactate dehydrogenase, and lysozyme; **lanes 14–19:** 10 µg, 8 µg, 6 µg, 4 µg, 2 µg, 1 µg *E. coli* lysate. Electrophoresis was conducted with NuPAGE MOPS running buffer and stained with SimplyBlue Safe Stain.

- **Double duty instrument**—enables electrophoresis and transfer of high-performance Invitrogen™ midi gels using the same tank
- **Two separate chambers**—run 1 or 2 gels or transfers using only the necessary amount of buffer for each gel, minimizing buffer cost and waste
- **Room-temperature transfer**—eliminate the need to prechill buffers and the hassle and messiness of ice baths
- **User-friendly design**—easy setup, sample loading, and simple workflow, without dummy cassettes or buffer dams
- **Compatible**—fits all Invitrogen precast midi gels and Invitrogen midi gel cassettes

Specifications

- Gel capacity: up to 2 midi gels (8 x 13 mm)
- Cell size (L x W x H): 25 x 17.9 x 17.3 cm (height with lid on)
- Buffer chamber requirement:
  - Upper chamber: 170 mL (per gel)
  - Lower chamber: 350 mL (per gel)
- Chemical resistance: impervious to most alcohols but not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), acetone, or isopropyl alcohol

XCell4 *SureLock* Midi-Cell

Simultaneous electrophoresis of up to 4 midi gels

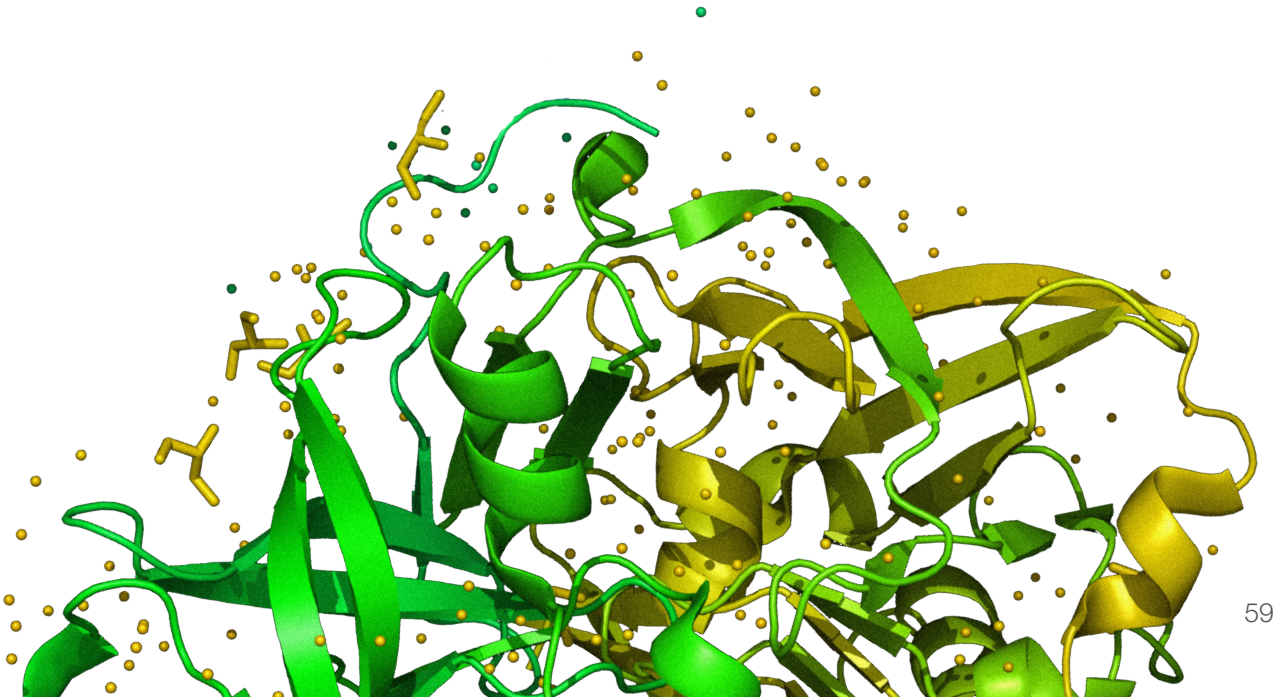
The Invitrogen™ XCell4 *SureLock*™ Midi-Cell allows simultaneous electrophoresis of 1–4 midi gels without leaking, enabling consistent performance. The system is designed to dissipate heat effectively and evenly, and enable high-resolution results when using Invitrogen™ Novex™ midi gels (Figures 30 and 31).

Key features of the XCell4 *SureLock* Midi-Cell:

- **User-friendly design**—electrophoresis without clamps or grease
- **Flexibility**—perform electrophoresis of 1–4 midi gels
- **Unique, heat-dissipating design**—no need for a cooling device
- **Built-in safety features**—specially designed lid enhances safety

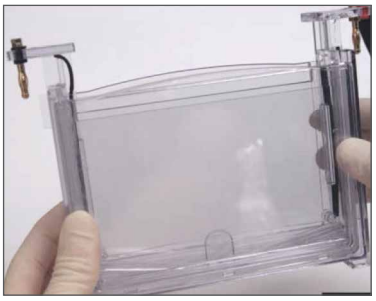
Specifications

- Gel capacity: up to 4 midi gels (8 x 13 cm)
- Cell size (L x W x H): 21 x 19 x 16 cm (height with lid on)
- Buffer chamber requirement:
  - Upper buffer chamber: 175 mL x 4
  - Lower buffer chamber: 540–700 mL
- Chemical resistance: not compatible with acetone, chlorinated hydrocarbons (e.g., chloroform), or aromatic hydrocarbons (e.g., toluene, benzene)

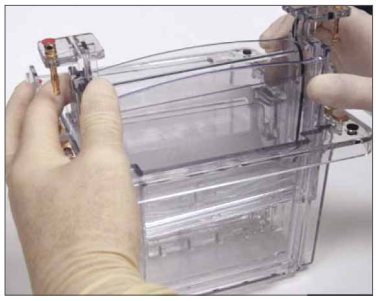


XCell4 *SureLock* Midi-Cell (continued)

A



B



1. Insert the XCell4 *SureLock* Midi-Cell assembly in its unlocked position into the center of the midi-cell base. The XCell4 *SureLock* assembly slides down over the protrusion in the midi-cell base.
2. Place one cassette on each side of the buffer core for each of the two cores. For each cassette, the shorter "well" side of the cassette must face out towards the lower buffer chamber.
3. While holding the assembly together with your hands **(A)**, insert the buffer cores with the gel cassettes into the lower buffer chamber such that the negative electrode fits into the opening in the gold plate on the lower buffer chamber **(B)**. Always hold the cassette assembly by its edges as shown in the figure.

Note: If you are having difficulty inserting the assembly into the lower buffer chamber, make sure the cathode (black polarity indicator) of the buffer core is aligned with the cathode (black polarity indicator) of the lower buffer chamber.

4. The upper buffer chamber (cathode) is the void formed between a gel and the buffer core at the center of each core.
5. Lock the XCell4 *SureLock* assembly by moving the tension lever to the locked position (indicated on the XCell4 *SureLock* Assembly). This will squeeze the gels and buffer cores together, creating leak-free seals.
6. Proceed to loading samples and buffers.

Figure 30. How to use the XCell4 *SureLock* Midi-Cell with 4 gels.

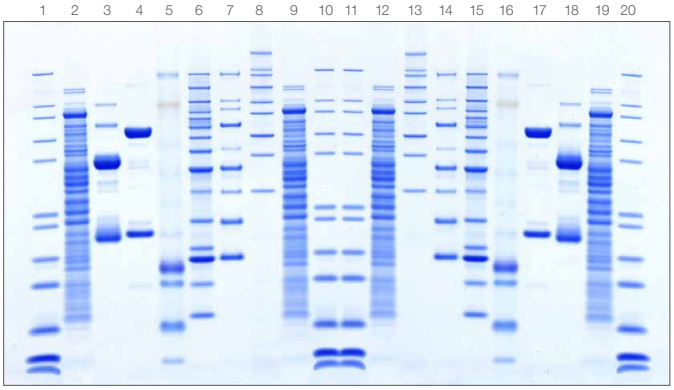


Figure 31. Quality of a precast Invitrogen™ NuPAGE™ 4–12% Bis-Tris Midi Gel with a variety of protein standards, lysates, and purified proteins. Electrophoresis was performed using MES running buffer and an XCell4 *SureLock* Midi Cell at 200 V (constant). Following electrophoresis, the gel was stained using SimplyBlue SafeStain, destained using water, and imaged using a flatbed scanner. Sharp, straight bands were observed. **Lanes 1, 10, 11, 20:** 5 µL of Mark12 Unstained Standard (blend of 12 purified proteins); **lanes 2, 9, 12, 19:** 10 µg of *E. coli* lysate; **lanes 3, 18:** 6 µg of human IgG; **lanes 4, 17:** 6 µg of human IgM; **lanes 5, 16:** 5 µL of SeeBlue Plus2 Prestained Protein Standard; **lanes 6, 15:** 5 µL of Invitrogen™ BenchMark™ Protein Ladder; **lanes 7, 14:** 15 µL of Invitrogen™ MagicMark™ XP Western Protein Standard; **lanes 8, 13:** 5 µL of Invitrogen™ HiMark™ Unstained Protein Standard.

PowerEase Touch Power Supplies

Easy touchscreen programming and operation

The Invitrogen™ PowerEase™ Touch Power Supplies make setting up custom protocols or selecting one of the several preprogrammed gel electrophoresis and transfer methods a breeze with an improved 4.3-inch backlit LCD touchscreen display and user interface. The power supplies are ideal for DNA or RNA electrophoresis, SDS-PAGE, and native PAGE. The PowerEase Touch Power Supplies offer four sets of output jacks that can be used simultaneously and three modes: constant voltage, constant current, and constant power for flexibility of use and efficiency. The sturdy polyurethane feet and stackable housing design allow stacking of power supplies for a reduced footprint on the lab bench.

- **Ease of use**—LCD touchscreen display and user interface show clear menu prompts for easy use by hand or stylus and convenient monitoring of run progress
- **Convenient**—four sets of output terminals allow running of multiple electrophoresis units
- **Customizable**—program up to 100 custom methods, 20 steps per method, 999 minutes per step, or select one of several preprogrammed Invitrogen gel electrophoresis and transfer methods
- **Safety**—features automatic No Load, Over Temperature, Over Voltage, Over Current, Load Change, and Ground Leak detection

The PowerEase Touch 120W Power Supply is a medium-throughput power supply, with a maximum output of 300 V, 500 mA, and 120 W. The PowerEase Touch 350W Power Supply is a high-throughput power supply, with a maximum output of 300 V, 3 A (3,000 mA), and 350 W.

Running gels

Mini gel running conditions in Invitrogen mini gel electrophoresis chamber systems.

	Voltage (V)	Approximate run time (min)
Bolt 4–12% Bis-Tris Plus gel (MES buffer)	200	20
Bolt 4–12% Bis-Tris Plus gel (MOPS buffer)	200	35
NuPAGE 4–12% Bis-Tris gel (MES buffer)	200	30
NuPAGE 4–12% Bis-Tris gel (MOPS buffer)	200	42
Novex Tris-Glycine gel, WedgeWell format (denatured)	225	25–40
Novex Tris-Glycine gel, WedgeWell format (native)	125	60–90
NuPAGE 3–8% Tris-Acetate gel (denatured)	150	50
NuPAGE 3–8% Tris-Acetate gel (native)	150	100
Novex 10–20% Tricine gel	125	65
NativePAGE 3–12% gel	150	80
Novex IEF gel, pH 3–10	100	60
	200	60
	500	30
Novex 10% Zymogram Plus gel (gelatin)	125	90

Note: Run times may vary depending on the power supply and gel percentage.

Midi gel running conditions in Invitrogen midi gel electrophoresis chamber systems.

	Voltage (V)	Approximate run time (min)
NuPAGE Bis-Tris gel (MES buffer)	200	30
NuPAGE Bis-Tris gel (MOPS buffer)	200	40
Novex Tris-Glycine Plus gel (denatured)	200	60
Novex Tris-Glycine Plus gel (native)	125	120
NuPAGE Tris-Acetate gel (denatured)	150	60
NuPAGE Tris-Acetate gel (native)	150	135

Note: Run times may vary depending on the power supply and gel percentage.

Staining gels

Protein stains

Once protein bands have been separated by electrophoresis, they can be directly visualized using different methods of in-gel detection. Over the past several decades, demand for improved sensitivity and compatibility with downstream applications and detection instrumentation has driven the development of several basic staining methods. Each method has particular advantages and disadvantages, and a number of specific formulations for each type of method provide optimal performance for various situations.

Typically, these stains can be classified broadly based on the molecules that help visualize the proteins:

Coomassie stains

- Thermo Scientific™ PageBlue™ Protein Staining Solution
- Invitrogen™ SimplyBlue™ SafeStain
- Thermo Scientific™ Imperial™ Protein Stain

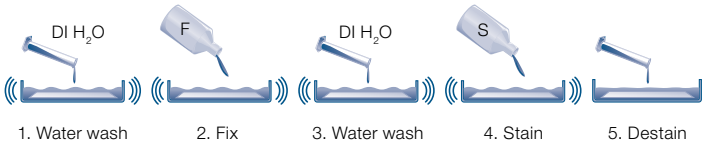
Silver stains

- Thermo Scientific™ Pierce™ Silver Stain Kit
- Invitrogen™ SilverXpress™ Silver Staining Kit
- Thermo Scientific™ Pierce™ Silver Stain for Mass Spectrometry

Fluorescent and specialty stains

- Invitrogen™ SYPRO™ Orange, SYPRO Red, SYPRO Ruby gel stains
- Thermo Scientific™ Pierce™ Reversible Protein Stain Kit for Nitrocellulose or PVDF Membranes
- Invitrogen™ Pro-Q™ Emerald glycoprotein stain
- Invitrogen™ Pro-Q™ Diamond phosphoprotein stain

To visualize the proteins, a protein-specific, dye-binding or color-producing chemical reaction must be performed on the proteins within the gel. Depending on the particular chemistry of the stain, various steps are necessary to hold the proteins in the matrix and to facilitate the necessary chemical reaction. Most staining methods involve some version of the same general incubation steps:



- A water wash to remove electrophoresis buffers from the gel matrix
- An acid or alcohol wash to condition or fix the gel to limit diffusion of protein bands from the matrix
- Treatment with the stain reagent to allow the reagent to diffuse into the gel and bind to (or react with) the proteins
- Destaining to remove excess dye from the background gel matrix

Depending on the particular staining method, two or more of these functions can be accomplished with one step. For example, a dye reagent that is formulated in an acidic buffer can effectively fix and stain in one step. Conversely, certain functions require several steps. For example, silver staining requires both a staining reagent step and a development step to produce the colored reaction product.

Coomassie dye–based protein gel stains

Convenient, ready-to-use reagents with no permanent chemical modification

The most common methods of in-gel protein detection use stains with Coomassie dye. These stains use either the G-250 (colloidal) or R-250 form of the dye. Colloidal Coomassie stain can be formulated to effectively stain proteins within one hour and require only water (no methanol or acetic acid) for destaining.

Key features:

- **Simple**—Coomassie dye–based formulations are easy to formulate and are widely used
- **Easy to use**—simply soak the gel in staining solution, and destain to observe protein bands
- **Economical**—Coomassie dye–based formulations are cost-effective
- **Flexible**—useful for qualitative visualization, quantitative densitometry, and gel excision and analysis by mass spectrometry

Our Coomassie stains provide sensitive protein detection along with simplified protocols. Staining protocols and example data are shown for SimplyBlue SafeStain (Figures 32, 35, 36), Imperial Protein Stain (Figures 33, 37), and PageBlue Protein Staining Solution (Figure 34).

Coomassie dye–based protein gel stains.

	SimplyBlue SafeStain	Imperial Protein Stain	PageBlue Protein Staining Solution
Type	G-250	R-250	G-250
Limit of detection	>7 ng	3 ng	5 ng
Time to stain*	12 min	12 min	30 min
Compatible with:			
PVDF membranes	Yes	Yes	Yes
Nitrocellulose membranes	No	No	No
Reusable	No	No	Yes (up to 3 times)
Mass spectrometry compatible	Yes	Yes	Yes
Color	Purple	Purple	Blue-green
Feature	Free of methanol and acetic acid	Photographs better than Coomassie G-250 dye	Free of methanol and acetic acid
Advantages	Rapid, sensitive, completely nonhazardous staining (does not require methanol or acetic acid fixatives or destains)	Fast, ultrasensitive protein detection	Cost-effective option for fast, sensitive staining

* Approximate staining time using microwave oven

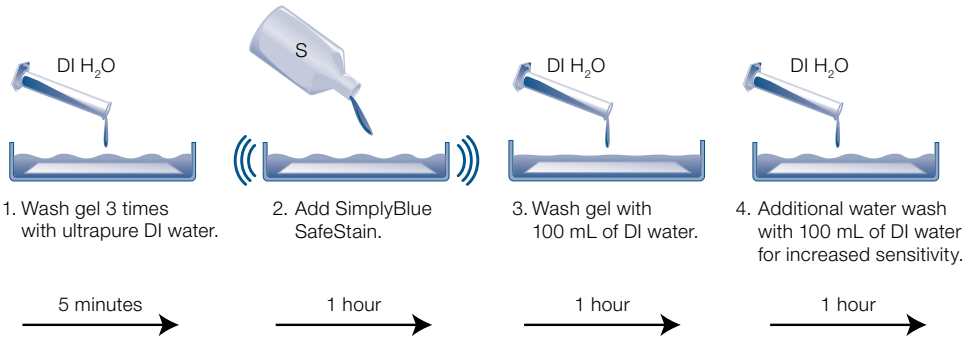


Figure 32. SimplyBlue SafeStain protocol.

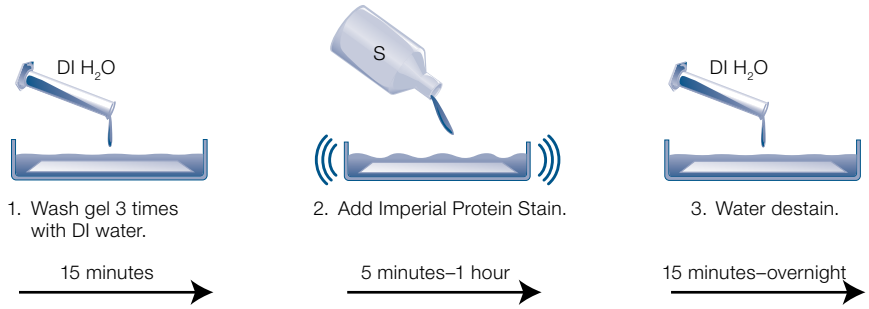


Figure 33. Imperial Protein Stain protocol.

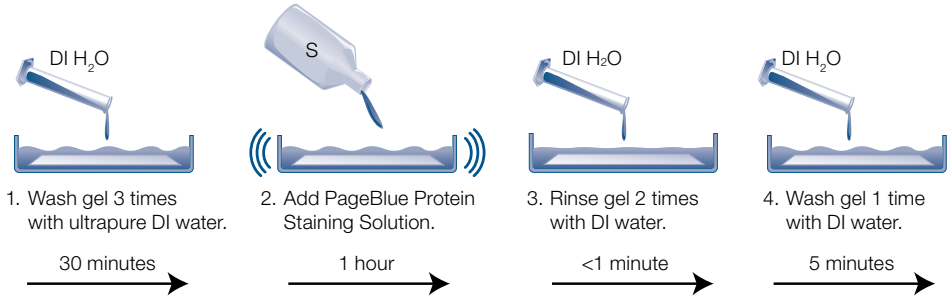


Figure 34. PageBlue Protein Staining Solution protocol.

Example data

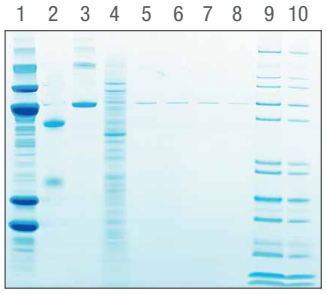


Figure 35. Sensitive staining results with SimplyBlue SafeStain. The following samples were separated on an Invitrogen™ NuPAGE™ 4–12% Bis-Tris gel and then stained with SimplyBlue SafeStain. **Lane 1:** 6 µg protein mix; **lane 2:** 1 µg rabbit IgG; **lane 3:** 1 µg reduced BSA; **lane 4:** 5 µg *E. coli* lysate; **lane 5:** 20 ng reduced BSA; **lane 6:** 10 ng reduced BSA; **lane 7:** 7 ng reduced BSA; **lane 8:** 3 ng reduced BSA; **lane 9:** 10 µL Invitrogen™ Mark12™ Unstained Standard (blend of 12 purified proteins); **lane 10:** 5 µL Mark12 Unstained Standard.

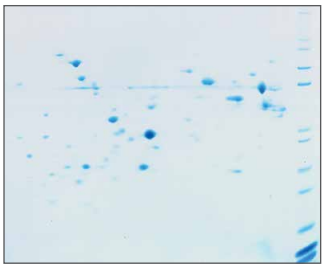


Figure 36. Two-dimensional electrophoresis analysis of spinach chloroplast extract; staining with SimplyBlue SafeStain. A spinach chloroplast extract was prefractionated in the Invitrogen™ ZOOM™ IEF Fractionator, and the individual fractions were then separated by 2D electrophoresis using narrow pH–range Invitrogen™ ZOOM™ IPG strips and an Invitrogen™ NuPAGE™ 4–12% Bis-Tris ZOOM™ gel. Gels were stained using SimplyBlue SafeStain.

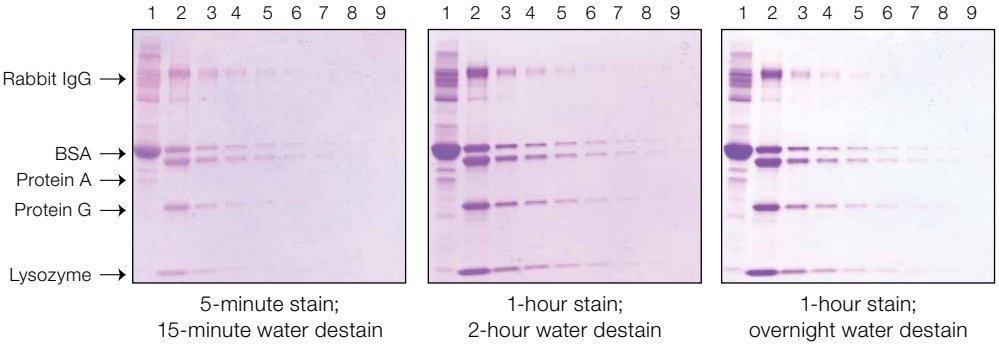


Figure 37. Enhanced sensitivity and clear background using Imperial Protein Stain. For even greater sensitivity and reduced background, gels can be stained with Imperial Protein Stain for 1 hour and washed with water from 1 hour to overnight. **Lane 1:** BSA only (6 µg); **lanes 2–9:** loaded with 1,000, 200, 100, 50, 25, 12, 6, and 3 ng protein.

Did you know

Staining with a Coomassie stain prior to silver staining allows for more uniform staining of certain proteins since silver ions can interact with certain functional groups such as carboxylic acid groups, imidazole, sulfhydryls, and amines.

Silver stains

Ultrasensitive stains with optimized protocols and manufactured for minimal variability

Silver staining is the most sensitive colorimetric method for detecting total protein, and functions by the deposition of metallic silver at the location of protein bands. Silver ions (from silver nitrate in the stain reagent) interact and bind with certain protein functional groups. The strongest interactions occur with carboxylic acid groups (Asp and Glu), imidazole (His), sulfhydryl groups (Cys), and amines (Lys). Various sensitizer and enhancer reagents are essential for controlling the specificity and efficiency of silver ion binding to proteins and effective conversion (development) of the bound silver to metallic silver.

Key features:

- **Sensitive**—silver stains are highly sensitive stains that allow for visualization of proteins at subnanogram levels
- **Easy to use and flexible**—silver stains are optimized for minimal steps and have the flexibility to accommodate shorter or longer protocols
- **Workflow compatible**—our mild chemical formulations help ensure compatibility with mass spectrometry and sequencing
- **Robust performance**—detailed protocol enables consistent results with clear background

We offer highly sensitive silver stains with short protocol times that are also compatible with mass spectrometry. The Invitrogen™ SilverXpress™ Silver Staining Kit provides nanogram-level sensitivity with minimal background (Figures 38, 39), while the Thermo Scientific™ Pierce™ Silver Stain Kit provides protocol flexibility (Figures 40, 41).

Silver stain kits.

	Pierce Silver Stain for Mass Spectrometry	Pierce Silver Stain Kit	SilverXpress Silver Staining Kit
Components (steps)	6 (7)	4 (7)	5 (9)
Time required	1 hr 13 min	1 hr 30 min	2 hr
Limit of detection	0.25 ng	0.25 ng	0.86 ng
Mass spectrometry compatible	Yes	Yes	Yes
Storage	Room temperature	Room temperature	4°C
Stability	1 year	1 year	6 months
Advantages	• Fast and sensitive staining and destaining of protein gels • Optimized for peptide recovery after in-gel trypsin digestion for mass spectrometry • Flexible gel fixation (15–30 min to overnight) and staining (1–30 min)	• Rapid, ultrasensitive, and versatile silver stain system • Flexible gel fixation (30 min–overnight) and staining (5 min–20 hr)	• Nanogram-level sensitivity for silver staining with minimal background

Protocols and example data

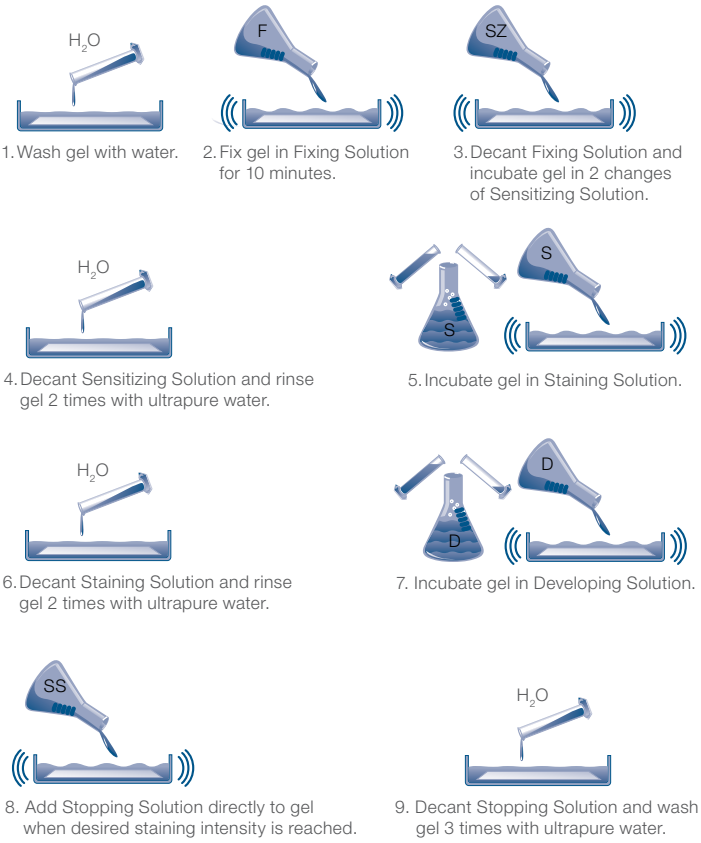


Figure 38. SilverXpress Silver Staining Kit protocol.

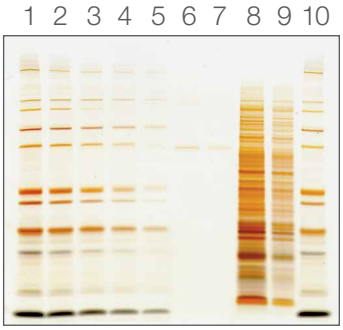


Figure 39. Crystal clear background with the SilverXpress Silver Staining Kit. Samples were separated on a NuPAGE 4–12% Bis-Tris gel and stained using the SilverXpress kit. **Lanes 1, 10:** Mark12 Unstained Standard (blend of 12 purified proteins) diluted 1:4; **lane 2:** Mark12 Unstained Standard diluted 1:8; **lane 3:** Mark12 Unstained Standard diluted 1:16; **lane 4:** Mark12 Unstained Standard diluted 1:32; **lane 5:** Mark12 Unstained Standard diluted 1:64; **lane 6:** 1.6 ng BSA; **lane 7:** 0.8 ng BSA; **lane 8:** *E. coli* lysate diluted 1:20; **lane 9:** *E. coli* lysate diluted 1:80.

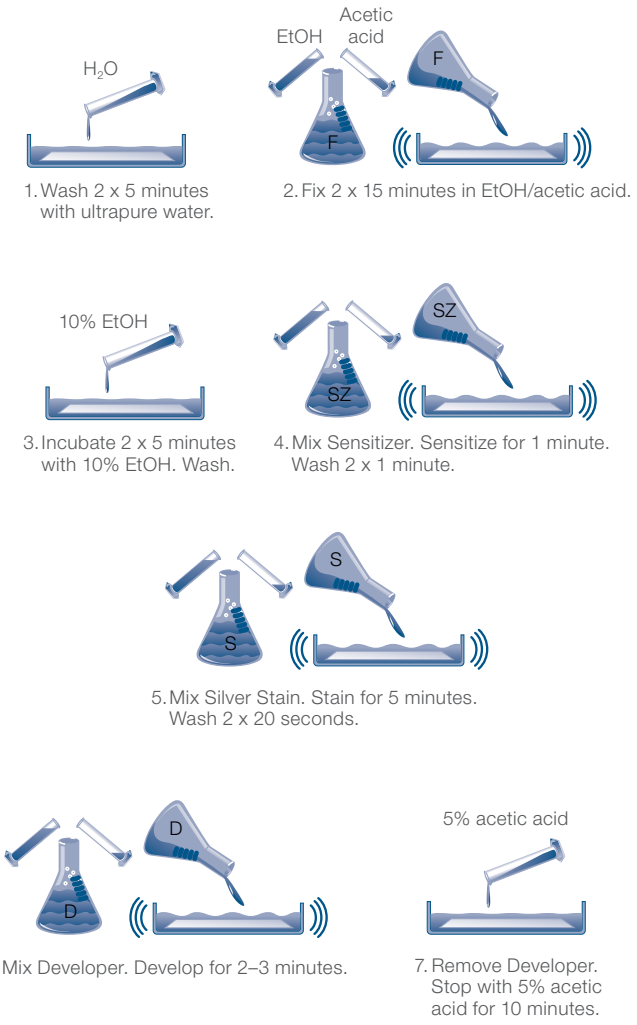


Figure 40. Pierce Silver Stain Kit protocol.

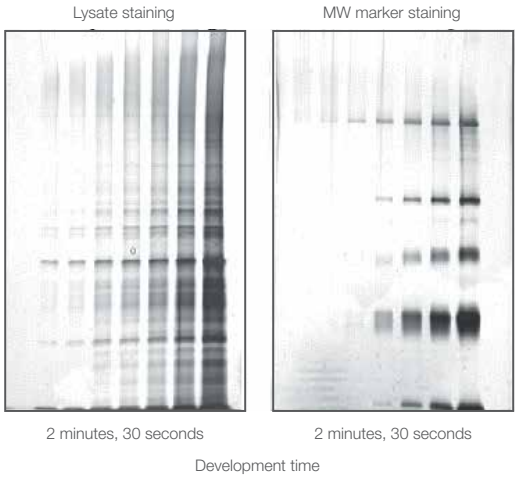
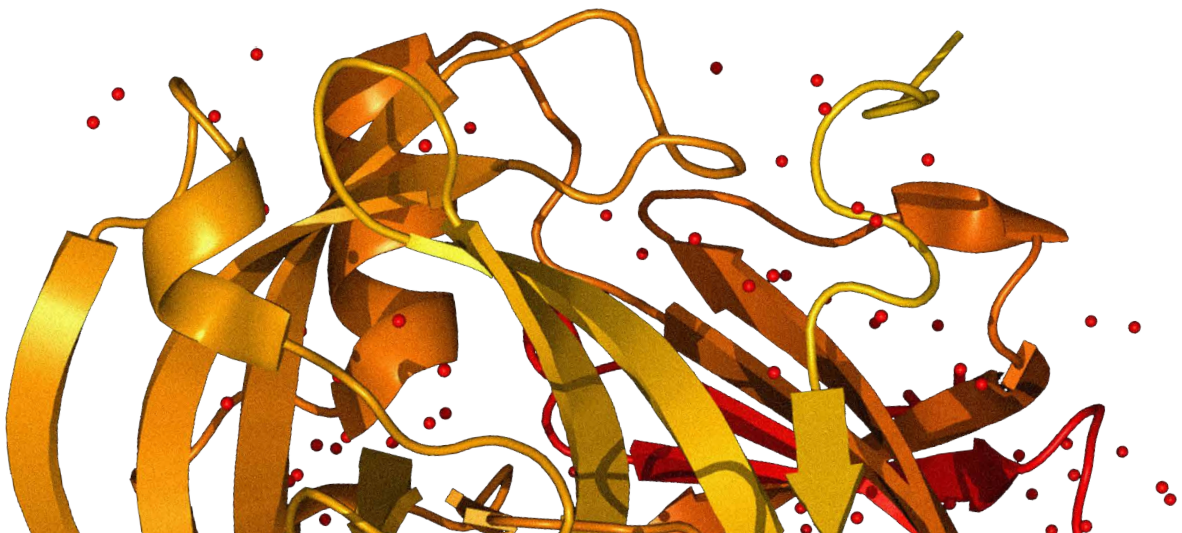


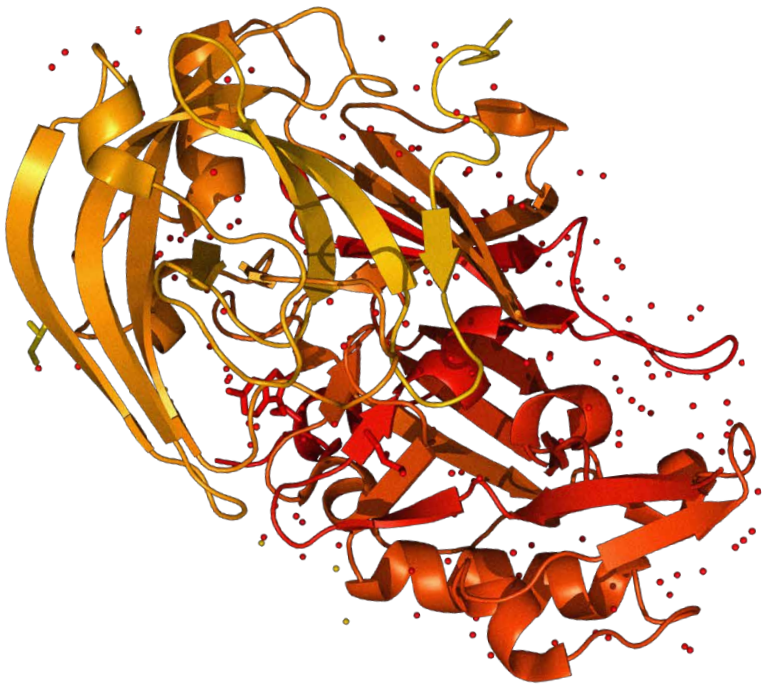
Figure 41. The Pierce Silver Stain Kit exhibits excellent sensitivity. In standard mini gels, proteins are detectable at greater than 0.25 ng per band or spot.



Fluorescent protein gel stains

Rapid, highly sensitive fluorescent stains for total protein detection after electrophoresis

Fluorescent gel stains are designed for use in 1D and 2D PAGE and offer sensitivities similar to those of silver staining techniques. Invitrogen™ SYPRO™ protein stains are easy-to-use fluorescent stains for the detection of proteins separated by PAGE. Stained proteins can be viewed with a standard UV or blue-light transilluminator or with a laser scanner.



Features:

- **Simple**—no destaining or timed steps required; minimal hands-on time
- **Quantitative**—linear quantitation range over two orders of magnitude with low protein-to-protein variability
- **Highly sensitive**—typically more sensitive than Coomassie dye–based stains and equivalent to silver stains

SYPRO protein stains.

	SYPRO Ruby stain	SYPRO Orange stain	SYPRO Red stain
Limit of detection	0.25 ng	4–8 ng	4–8 ng
Stain and destain time	90 min microwave; 18 hr standard	~1 hr	~1 hr
Ex/Em	280 nm, 450/610 nm	300 nm, 470/510 nm	300 nm, 550/630 nm
Ease of use	Ready to use	Supplied as stock solution	Supplied as stock solution
Compatible applications	Mass spectrometry, IEF, 2D gels, on-membrane staining	Mass spectrometry, IEF, 2D gels, on-membrane staining	Mass spectrometry, IEF, 2D gels, on-membrane staining

Specialty protein stains

Our specialty protein stains include in-gel phosphoprotein and glycoprotein detection staining kits.

Specialty protein stains.

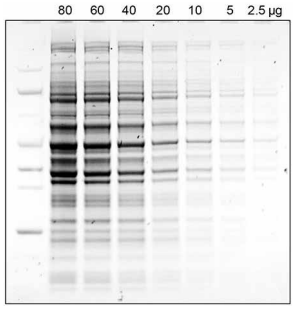
	Pro-Q Emerald 488 Glycoprotein Gel and Blot Stain Kit	Pro-Q Emerald 300 Glycoprotein Gel and Blot Stain Kit	Pro-Q Diamond Phosphoprotein Gel Staining Kit
Detects	Glycoproteins	Glycoproteins	Phosphoproteins
Sensitivity	4 ng glycoprotein per band	0.5 ng glycoprotein per band	1–16 ng phosphoprotein per band
Stain and destain time	~6 hr	~5 hr	4–5 hr
Ex/Em	510/520 nm	280/530 nm	555/580 nm
Advantages	Selective staining of glycoproteins	Selective staining of glycoproteins	Selective staining of phosphoproteins

Learn more at **thermofisher.com/specialtystains**

No-Stain Labeling Reagent

Instant visualization of proteins in gels

The Invitrogen™ No-Stain™ Protein Labeling Reagent provides a flexible, accurate, rapid, and reliable method to visualize and normalize proteins in a gel or on a membrane (post-transfer). It forms covalent bonds with lysine residues in proteins in gels or on membranes within 10 minutes without the need for destaining, making it much faster than Coomassie or other gel staining techniques. The results can be instantly visualized using any commonly available imager, with nanogram-level sensitivity (Figure 42). No-Stain reagent does not require any particular gels or other reagents and is compatible with gel stains and western workflows.



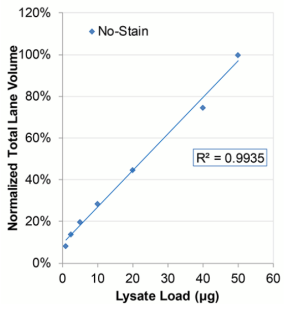


Figure 42. Quantitative gel staining with No-Stain Protein Labeling Reagent. A Bolt 4–12% Bis-Tris Plus gel was loaded with HeLa cell lysate containing 2.5 to 80 µg of protein, and electrophoresed with MES running buffer. After electrophoresis, the proteins were labeled in-gel with No-Stain Protein Labeling Reagent, and the gel was imaged using an Invitrogen™ iBright™ imager with the transilluminator for excitation (490–520 nm) and the 565–615 nm emission filter.

Western blotting

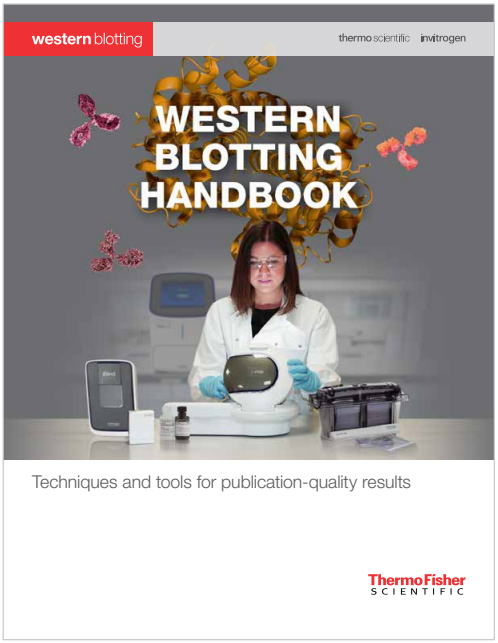
Transfer and detection

After electrophoresis, the separated proteins are transferred or blotted onto a second matrix, generally a nitrocellulose or polyvinylidene difluoride (PVDF) membrane. Next, the membrane is blocked to minimize potential nonspecific binding of antibodies to the surface of the membrane.

Detailed procedures vary widely for the detection steps of the western blot workflow. One common variation involves direct vs. indirect detection methods. In both the direct and indirect detection methods, the blocked membrane is probed with an antibody (primary antibody) specific to the protein of interest (antigen). In direct detection techniques, this primary antibody is enzyme conjugated or labeled with a fluorophore. However, in indirect detection techniques, the blocked membrane is probed first with an antibody (primary antibody) that is specific to the antigen followed by another antibody (secondary antibody) raised against the host species of the primary antibody. This secondary antibody is often enzyme conjugated or labeled with a fluorophore. The direct method is not widely used, as most researchers prefer the indirect detection method for a variety of reasons, including workflow flexibility, broader choice of conjugates, and the potential for cost savings and increased sensitivity.

Horseradish peroxidase (HRP) or alkaline phosphatase (AP) are the most popular enzymes conjugated to antibodies used in the western blot workflow. After incubating the membrane with the detection antibody or antibodies, if an enzyme-conjugated antibody was utilized, an appropriate substrate (chromogenic or chemiluminescent) is added and that results in a detectable product. A popular substrate of choice is a chemiluminescent substrate that, when combined with the enzyme, produces light as a by-product. With the chemiluminescent substrate, the light output can be captured on X-ray film or an imaging instrument. In recent years, fluorescent detection became a popular alternative to enzymatic detection since it allows for more quantitative data analysis. Fluorescent detection utilizes dye-labeled primary antibodies or dye-labeled secondary antibodies, and the signal output is captured on an appropriate imaging system. Regardless of the detection system used, the intensity of the signal should correlate with the abundance of the antigen on the blotting membrane.

We offer a wide range of reagents, kits, equipment, and antibodies to facilitate every step of western blot analysis.



Key products for western blot transfer:

Wet	Semi-dry	Dry
		
Mini Blot Module	Invitrogen™ Power Blotter	Invitrogen™ iBlot™ 2 Dry Blotting System

Key products for western blot detection:

Automated detection		
		
Invitrogen™ iBind™ Flex Western Device	Invitrogen™ Bandmate™ Automated Western Blot Processor	Invitrogen™ iBright™ Imaging Systems

Manual detection
Blocking buffers Wash buffers Detergents Enhancers Substrates Stripping buffers X-ray film

Appendix

Protocol quick references

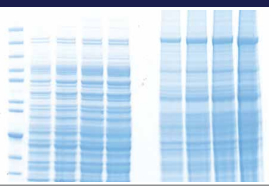
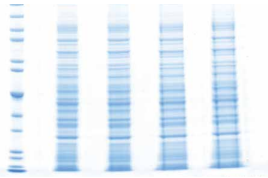
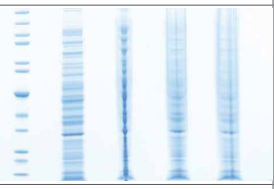
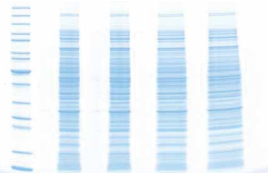
To learn more about our various gel products and their uses, check out these links to protocols that describe how to use the products.

- **Bolt Bis-Tris Plus mini gels**
- **NuPAGE Bis-Tris mini gels**
- **NuPAGE Bis-Tris midi gels**
- **NuPAGE Tris-Acetate mini gels**
- **NuPAGE Tris-Acetate midi gels**
- **Novex Tris-Glycine mini gels, WedgeWell format**
- **Novex Tris-Glycine Plus midi gels**
- **NativePAGE Bis-Tris gels**
- **Novex Tricine gels**
- **Novex IEF gels**
- **Zymogram Plus gels**

Troubleshooting tips

Sample preparation

Protein gel electrophoresis

Observation		Possible cause	Suggested solution
Protein bands lose resolution, lanes have streaks and are not straight		Too much protein loaded per lane	Reduce the sample loads. The maximum recommended sample load for optimal resolution in mini gels with 10, 12, 15, or 17 wells is 0.5 µg per band or about 10–15 µg of cell lysate per lane.
Viscous samples, streaks at sample lane edges, dumbbell-shaped bands, lane widening		Excess salt (ammonium sulfate) in sample during gel electrophoresis	Perform dialysis to decrease salt concentration. Use a small dialysis device such as the Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Device, 0.5 mL (Cat. No. 88401). Concentrate and resuspend samples in lower-salt buffer prior to electrophoresis. Use small-volume concentrators such as Thermo Scientific™ Pierce™ Protein Concentrators PES, 0.5 mL (Cat. No. 88513). Make sure that the salt concentration does not exceed 100 mM.
Protein aggregation resulting in narrow lanes that cannot be interpreted		DNA contamination—genomic DNA in the cell lysate may cause the sample to become viscous, resulting in protein aggregation, which can affect protein migration patterns and resolution	Shear genomic DNA to reduce viscosity before loading the sample.
Uneven sample lanes, lane widening		Excess salt (sodium chloride) in sample during gel electrophoresis. High salt concentrations result in increased conductivity, which affects protein migration and can result in protein bands spreading into adjacent lanes containing samples with normal salt concentrations	Perform dialysis to decrease salt concentration. Use a small dialysis device such as the Slide-A-Lyzer MINI Dialysis Device, 0.5 mL (Cat. No. 88401). Concentrate and resuspend samples in lower-salt buffer prior to electrophoresis. Use small-volume concentrators such as Pierce Protein Concentrators PES, 0.5 mL (Cat. No. 88513). Make sure that the salt concentration does not exceed 100 mM.
		High detergent concentration (e.g., SDS or Triton X-100 detergent) in gel electrophoresis. Detergents form mixed micelles with the anionic detergent SDS in the gel and migrate down into the gel; they interfere with the SDS–protein binding equilibrium	Most of the nonionic detergents (e.g., Triton X-100, NP-40, and Tween 20 detergents) interfere with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Keep the ratio of SDS to nonionic detergent at 10:1 or greater to minimize these effects. Use detergent removal columns or the Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit (Cat. No. 89888) to remove excess detergent.
		High concentration of RIPA (radioimmunoprecipitation assay) buffer results in widening of lanes and significant streaking during electrophoresis	Dilute samples before electrophoresis to lower the final concentration of lysis buffer to prevent buffer-related defects.
Shadow at lane edges		Excess reducing agent in the lysis or sample buffer	The final concentration of reducing agents for SDS-PAGE should be less than 50 mM for DTT (dithiothreitol) and TCEP (tris(2-carboxyethyl) phosphine), and less than 2.5% for β-ME (β-mercaptoethanol).

Electrophoresis

Observation	Possible cause	Suggested solution
Run taking longer time with recommended voltage	Running buffer too dilute	Make fresh running buffer and use a 1X dilution.
Current too high and excessive heat generated with recommended voltage	Running buffer too concentrated	Make fresh running buffer and use a 1X dilution.
Current too low or no current with recommended voltage	Incomplete circuit	Remove the tape from the bottom of the gel cassette prior to electrophoresis. Make sure the buffer covers sample wells; check the wire connections on the buffer core.
Streaking of proteins	Sample overload	Load less protein.
	High salt concentration in sample	Decrease the sample salt concentration by dialysis or gel filtration.
	Sample precipitates	Increase the concentration of SDS in the sample.
	Contaminants such as lipids or DNA complexes in sample	Centrifuge or clarify the sample to remove particulate contaminants. Treat sample with nuclease(s).
	Poorly poured gel	Make sure the gel is poured evenly and all at once.
Fuzzy bands	Protein sample only partially denatured	Fully denature the protein.
	Protein sample only partially reduced	Make sure a sufficient amount of DTT or β-mercaptoethanol is added.
	Gel runs for too long	Watch the dye front as an indicator for proper running time.
Dumbbell-shaped bands or “smiling” bands	Loading a large volume of sample causes incomplete stacking	Load appropriate volume of sample. If the sample is too dilute, concentrate it using ultrafiltration.
	Uneven electric field during run	Try to make sure the loading is symmetrical if the protein concentration is known.
	Uneven surface of the resolving gel	Try to make the resolving gel surface even while pouring the gel.
	Expired gels	Use the gels before the specified expiration date. Note: NuPAGE gels have an extended 12-month shelf life, minimizing the risk of having expired gels.

Protein gel electrophoresis chamber systems

Mini Gel Tank troubleshooting

Observation	Possible cause	Suggested solution
Run taking longer than usual	Buffers are too dilute	Check buffer recipe; dilute from concentrate or remake if necessary.
	Buffer chamber is leaking	Make sure the cassette clamp is firmly seated, the gaskets are in place, and the cassette clamp is locked.
	Current is set too low	Set correct current.
Current reading on power supply is zero or very low	Tape left on the bottom of the cassette	Remove tape from bottom of cassette.
	Connection to power supply not complete	Check all connections with a voltmeter for conductance.
	Insufficient buffer level	Make sure there is sufficient buffer in the electrophoresis tank to cover the wells of the gel.
Run is faster than normal with poor resolution	Buffers are too concentrated or incorrect	Check buffer recipe; dilute or remake if necessary.
	Current is set at a higher limit	Decrease current to recommended running conditions (see **page 62**).
Cannot see the sample wells to load sample	There is little contrast between the sample well and the rest of the gel	Mark cassette at the bottom of the wells with a marker pen prior to placing the cassette in the electrophoresis tank.

XCell *SureLock* Mini-Cell troubleshooting

Observation	Possible cause	Suggested solution
Run taking longer than usual	Buffers are too dilute	Check if buffer was diluted properly. Check buffer recipe; dilute from concentrate or remake if necessary.
	Upper buffer chamber is leaking	Make sure the buffer core is firmly seated, the gaskets are in place, and the gel tension lever is locked.
	Voltage is set too low	Set correct voltage.
Current reading on power supply is zero or very low	Tape left on the bottom of the cassette	Remove tape from bottom of cassette.
	Connection to power supply not complete	Check all connections with a voltmeter for conductance.
	Insufficient buffer level	Make sure the upper buffer (cathode) is covering the wells of the gel. Be sure there is sufficient buffer in the lower buffer chamber to cover the slot at the bottom of the gel.
Run is faster than normal with poor resolution	Buffers are too concentrated or incorrect	Check buffer recipe; dilute or remake if necessary.
	Voltage, current, or wattage is set at a higher limit	Decrease power conditions to recommended running conditions (see **page 62**).
Cannot see the sample wells to load sample	There is little contrast between the sample well and the rest of the gel	Mark cassette at the bottom of the wells with a marker pen prior to assembling the upper buffer chamber. Illuminate the bench area with a light source placed directly behind the XCell SureLock unit.

SureLock Tandem Midi Gel Tank troubleshooting

Observation	Possible cause	Suggested solution
Run takes longer than normal	Buffers were too dilute	Check buffer recipes. Do not reuse buffers. Remake buffer if necessary.
	Buffer chamber was leaking	Make sure the cassette clamp is firmly seated, the gaskets are in place, and the cassette clamps are locked.
	Voltage and/or current was set too low	Set the correct voltage and/or current. See "Running conditions" in the "**Gel electrophoresis protocol**" (p. 10) of the user guide for more details.
Run is faster than normal, with poor resolution	Incorrect running buffer was used or buffer was too concentrated	Check buffer recipe. Dilute or remake buffer if necessary.
	Voltage and/or current was set too high	Decrease voltage and/or current to recommended running conditions. See "Running conditions" in the "**Gel electrophoresis protocol**" (p. 10) of the user guide for more details.
Current reading on power supply is zero or very low	Tape was left on the bottom of the cassette	Remove the tape from the cassette.
	Connection to power supply was not complete	Check conductance on all connections using a voltmeter. **Note:** Do not check connections if not trained in this procedure. Contact Technical Support for further help.
	Insufficient buffer level	Make sure there is sufficient buffer in the tank. The cathode (inside) chamber needs to be filled to above the wells (~170 mL), and the anode (outside) chamber needs to be filled to the red fill line (~350 mL). See "Fill tank with running buffer and load samples" in the "**Gel electrophoresis protocol**" (p. 12) of the user guide for more details.
Cannot see the sample wells to load the sample	Difficult to see contrast between the sample well and the rest of the gel	Mark cassette at the bottom of the wells with a marker pen prior to placing the cassette in the electrophoresis tank.
Current reading on power supply is much higher than expected, or maxed out	Too many gels were being run at once using one power supply	Check the power limits of the power supply being used and use additional power supplies if needed.
	Cassette was not properly clamped	Ensure that the cassette clamp is firmly seated, the gaskets are in place, and the cassette clamps are in the locked position. See "Insert cassette" in the "**Gel electrophoresis protocol**" (p. 11) of the user guide for details.
	Tank was overfilled with running buffer	If the buffer level is above the height of the gel, the cathode and anode chambers are in direct contact, resulting in an electrical short circuit. Ensure correct buffer volumes. The cathode buffer level should be covering the wells but not above the height of the gel (~170 mL). The anode buffer level should be filled to the red fill line marked on the outside of the tank (~350 mL). See "Fill tank with running buffer and load samples" in the "**Gel electrophoresis protocol**" (p. 12) of the user guide for more details.
Proteins are not migrating into the gel	The cassette is installed backwards in the tank	Install the cassette in the correct orientation, with the well opening facing toward the cathode (center of the tank).
	The power cable is installed backwards into the power supply	Install the power cable in the correct orientation (red to red, black to black).

Ordering information

Product	Quantity	Cat. No.
Gel welcome packs		
Novex WedgeWell Welcome Pack, 6%, 10-well	1 kit	**XP0006A**
Novex WedgeWell Welcome Pack, 6%, 12-well	1 kit	**XP0006B**
Novex WedgeWell Welcome Pack, 6%, 15-well	1 kit	**XP0006C**
Novex WedgeWell Welcome Pack, 8%, 10-well	1 kit	**XP0008A**
Novex WedgeWell Welcome Pack, 8%, 12-well	1 kit	**XP0008B**
Novex WedgeWell Welcome Pack, 8%, 15-well	1 kit	**XP0008C**
Novex WedgeWell Welcome Pack, 10%, 10-well	1 kit	**XP0010A**
Novex WedgeWell Welcome Pack, 10%, 12-well	1 kit	**XP0010B**
Novex WedgeWell Welcome Pack, 10%, 15-well	1 kit	**XP0010C**
Novex WedgeWell Welcome Pack, 12%, 10-well	1 kit	**XP0012A**
Novex WedgeWell Welcome Pack, 12%, 12-well	1 kit	**XP0012B**
Novex WedgeWell Welcome Pack, 12%, 15-well	1 kit	**XP0012C**
Novex WedgeWell Welcome Pack, 14%, 10-well	1 kit	**XP0014A**
Novex WedgeWell Welcome Pack, 14%, 12-well	1 kit	**XP0014B**
Novex WedgeWell Welcome Pack, 14%, 15-well	1 kit	**XP0014C**
Novex WedgeWell Welcome Pack, 16%, 10-well	1 kit	**XP0016A**
Novex WedgeWell Welcome Pack, 16%, 12-well	1 kit	**XP0016B**
Novex WedgeWell Welcome Pack, 16%, 15-well	1 kit	**XP0016C**
Novex WedgeWell Welcome Pack, 4–12%, 10-well	1 kit	**XP0412A**
Novex WedgeWell Welcome Pack, 4–12%, 12-well	1 kit	**XP0412B**
Novex WedgeWell Welcome Pack, 4–12%, 15-well	1 kit	**XP0412C**
Novex WedgeWell Welcome Pack, 4–20%, 10-well	1 kit	**XP0420A**
Novex WedgeWell Welcome Pack, 4–20%, 12-well	1 kit	**XP0420B**
Novex WedgeWell Welcome Pack, 4–20%, 15-well	1 kit	**XP0420C**
Novex WedgeWell Welcome Pack, 8–16%, 10-well	1 kit	**XP0816A**
Novex WedgeWell Welcome Pack, 8–16%, 12-well	1 kit	**XP0816B**
Novex WedgeWell Welcome Pack, 8–16%, 15-well	1 kit	**XP0816C**
Novex WedgeWell Welcome Pack, 10–20%, 10-well	1 kit	**XP1020A**
Novex WedgeWell Welcome Pack, 10–20%, 12-well	1 kit	**XP1020B**
Novex WedgeWell Welcome Pack, 10–20%, 15-well	1 kit	**XP1020C**
Bolt Welcome Pack A (4–12%, 10-well)	1 kit	**NW0412A**
Bolt Welcome Pack B (4–12%, 15-well)	1 kit	**NW0412B**
Bolt Welcome Pack, 4–12%, 12-well	1 kit	**NW0412C**
Bolt Welcome Pack, 10%, 10-well	1 kit	**NW0010A**
Bolt Welcome Pack, 10%, 12-well	1 kit	**NW0010B**
Bolt Welcome Pack, 10%, 15-well	1 kit	**NW0010C**
Bolt Welcome Pack, 12%, 10-well	1 kit	**NW0012A**
Bolt Welcome Pack, 12%, 12-well	1 kit	**NW0012B**
Bolt Welcome Pack, 12%, 15-well	1 kit	**NW0012C**
Bolt Welcome Pack, 8%, 10-well	1 kit	**NW0008A**
Bolt Welcome Pack, 8%, 12-well	1 kit	**NW0008B**
Bolt Welcome Pack, 8%, 15-well	1 kit	**NW0008C**

Product	Quantity	Cat. No.
Gel welcome packs (continued)		
NuPAGE Tris-Acetate Welcome Pack, 3–8%, 10-well	1 kit	**EA0375A**
NuPAGE Tris-Acetate Welcome Pack, 3–8%, 12-well	1 kit	**EA0375B**
NuPAGE Tris-Acetate Welcome Pack, 3–8%, 15-well	1 kit	**EA0375C**
NuPAGE Tris-Acetate Welcome Pack, 7%, 10-well	1 kit	**EA0355A**
NuPAGE Tris-Acetate Welcome Pack, 7%, 12-well	1 kit	**EA0355B**
NuPAGE Tris-Acetate Welcome Pack, 7%, 15-well	1 kit	**EA0355C**
NuPAGE Bis-Tris Welcome Pack, 10%, 10-well	1 kit	**NP030A**
NuPAGE Bis-Tris Welcome Pack, 10%, 12-well	1 kit	**NP030B**
NuPAGE Bis-Tris Welcome Pack, 10%, 15-well	1 kit	**NP030C**
NuPAGE Bis-Tris Welcome Pack, 12%, 10-well	1 kit	**NP034A**
NuPAGE Bis-Tris Welcome Pack, 12%, 12-well	1 kit	**NP034B**
NuPAGE Bis-Tris Welcome Pack, 12%, 15-well	1 kit	**NP034C**
NuPAGE Bis-Tris Welcome Pack, 4–12%, 10-well	1 kit	**NP032A**
NuPAGE Bis-Tris Welcome Pack, 4–12%, 12-well	1 kit	**NP032B**
NuPAGE Bis-Tris Welcome Pack, 4–12%, 15-well	1 kit	**NP032C**
Novex Tricine Welcome Pack, 10%, 10-well	1 kit	**EC6675A**
Novex Tricine Welcome Pack, 10%, 12-well	1 kit	**EC6675B**
Novex Tricine Welcome Pack, 16%, 10-well	1 kit	**EC6695A**
Novex Tricine Welcome Pack, 16%, 12-well	1 kit	**EC6695B**
Novex Tricine Welcome Pack, 16%, 15-well	1 kit	**EC6695C**
Novex Tricine Welcome Pack, 10–20%, 10-well	1 kit	**EC6625A**
Novex Tricine Welcome Pack, 10–20%, 12-well	1 kit	**EC6625B**
Novex Tricine Welcome Pack, 10–20%, 15-well	1 kit	**EC6625C**

Learn more at
thermofisher.com/proteingelwelcome

Product	Quantity	Cat. No.
Bolt Bis-Tris Plus gels (8 x 8 cm)		
Bolt 8% Bis-Tris Plus Gels, 10-well	10 gels	**NW00080BOX**
Bolt 8% Bis-Tris Plus Gels, 12-well	10 gels	**NW00082BOX**
Bolt 8% Bis-Tris Plus Gels, 15-well	10 gels	**NW00085BOX**
Bolt 8% Bis-Tris Plus Gels, 17-well	10 gels	**NW00087BOX**
Bolt 10% Bis-Tris Plus Gels, 10-well	10 gels	**NW00100BOX**
Bolt 10% Bis-Tris Plus Gels, 12-well	10 gels	**NW00102BOX**
Bolt 10% Bis-Tris Plus Gels, 15-well	10 gels	**NW00105BOX**
Bolt 10% Bis-Tris Plus Gels, 17-well	10 gels	**NW00107BOX**
Bolt 12% Bis-Tris Plus Gels, 10-well	10 gels	**NW00120BOX**
Bolt 12% Bis-Tris Plus Gels, 12-well	10 gels	**NW00122BOX**
Bolt 12% Bis-Tris Plus Gels, 15-well	10 gels	**NW00125BOX**
Bolt 12% Bis-Tris Plus Gels, 17-well	10 gels	**NW00127BOX**
Bolt 4–12% Bis-Tris Plus Gels, 10-well	10 gels	**NW04120BOX**
Bolt 4–12% Bis-Tris Plus Gels, 12-well	10 gels	**NW04122BOX**
Bolt 4–12% Bis-Tris Plus Gels, 15-well	10 gels	**NW04125BOX**
Bolt 4–12% Bis-Tris Plus Gels, 17-well	10 gels	**NW04127BOX**
Bolt Empty Mini Gel Cassettes	20 cassettes	**NW2010**
Bolt Empty Mini Gel Cassette Combs, 10-well	20 combs	**NW3010**
Bolt Empty Mini Gel Cassette Combs, 12-well	20 combs	**NW3012**

Product	Quantity	Cat. No.
NuPAGE Bis-Tris mini gels (8 x 8 cm)		
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 1-well	10 gels	**NP0304BOX**
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	**NP0301BOX**
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 10-well	2 gels	**NP0301PK2**
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 12-well	10 gels	**NP0302BOX**
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 12-well	2 gels	**NP0302PK2**
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 15-well	10 gels	**NP0303BOX**
NuPAGE 10% Bis-Tris Protein Gels, 1.0 mm, 9-well	10 gels	**NP0307BOX**
NuPAGE 10% Bis-Tris Protein Gels, 1.5 mm, 10-well	10 gels	**NP0315BOX**
NuPAGE 10% Bis-Tris Protein Gels, 1.5 mm, 15-well	10 gels	**NP0316BOX**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 1-well	10 gels	**NP0344BOX**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	**NP0341BOX**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 10-well	2 gels	**NP0341PK2**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 12-well	10 gels	**NP0342BOX**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 12-well	2 gels	**NP0342PK2**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 15-well	10 gels	**NP0343BOX**
NuPAGE 12% Bis-Tris Protein Gels, 1.0 mm, 17-well	10 gels	**NP0349BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 1-well	10 gels	**NP0324BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	**NP0321BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 10-well	2 gels	**NP0321PK2**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 12-well	10 gels	**NP0322BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 12-well	2 gels	**NP0322PK2**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 15-well	10 gels	**NP0323BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 15-well	2 gels	**NP0323PK2**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 17-well	10 gels	**NP0329BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 17-well	2 gels	**NP0329PK2**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.0 mm, 9-well	10 gels	**NP0327BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.5 mm, 10-well	10 gels	**NP0335BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.5 mm, 10-well	2 gels	**NP0335PK2**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.5 mm, 15-well	10 gels	**NP0336BOX**
NuPAGE 4–12% Bis-Tris Protein Gels, 1.5 mm, 15-well	2 gels	**NP0336PK2**

Product	Quantity	Cat. No.
NuPAGE Bis-Tris midi gels (8 x 13 cm)		
NuPAGE 10% Bis-Tris Midi Protein Gels, 12+2 well	10 gels	**WG1201BOX**
NuPAGE 10% Bis-Tris Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WG1201A**
NuPAGE 10% Bis-Tris Midi Protein Gels, 20-well	10 gels	**WG1202BOX**
NuPAGE 10% Bis-Tris Midi Protein Gels, 20-well, w/ adapters	10 gels	**WG1202A**
NuPAGE 10% Bis-Tris Midi Protein Gels, 26-well	10 gels	**WG1203BOX**
NuPAGE 10% Bis-Tris Midi Protein Gels, 26-well, w/ adapters	10 gels	**WG1203A**
NuPAGE 4–12% Bis-Tris Midi Protein Gels, 12+2-well	10 gels	**WG1401BOX**
NuPAGE 4–12% Bis-Tris Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WG1401A**
NuPAGE 4–12% Bis-Tris Midi Protein Gels, 20-well	10 gels	**WG1402BOX**
NuPAGE 4–12% Bis-Tris Midi Protein Gels, 20-well, w/ adapters	10 gels	**WG1402A**
NuPAGE 4–12% Bis-Tris Midi Protein Gels, 26-well	10 gels	**WG1403BOX**
NuPAGE 4–12% Bis-Tris Midi Protein Gels, 26-well, w/ adapters	10 gels	**WG1403A**
NuPAGE 8% Bis-Tris Midi Protein Gels, 12+2-well	10 gels	**WG1001BOX**
NuPAGE 8% Bis-Tris Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WG1001A**
NuPAGE 8% Bis-Tris Midi Protein Gels, 20-well	10 gels	**WG1002BOX**
NuPAGE 8% Bis-Tris Midi Protein Gels, 20-well, w/ adapters	10 gels	**WG1002A**
NuPAGE 8% Bis-Tris Midi Protein Gels, 26-well	10 gels	**WG1003BOX**
NuPAGE 8% Bis-Tris Midi Protein Gels, 26-well, w/ adapters	10 gels	**WG1003A**

Product	Quantity	Cat. No.
Novex Tris-Glycine mini gels, WedgeWell format		
Novex 6% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP00060BOX**
Novex 6% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP00062BOX**
Novex 6% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP00065BOX**
Novex 8% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP00080BOX**
Novex 8% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP00082BOX**
Novex 8% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP00085BOX**
Novex 10% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP00100BOX**
Novex 10% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP00102BOX**
Novex 10% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP00105BOX**
Novex 10% Tris-Glycine Mini Gel, WedgeWell format, 10-well	2 gels	**XP00100PK2**
Novex 12% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP00120BOX**
Novex 12% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP00122BOX**
Novex 12% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP00125BOX**
Novex 14% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP00140BOX**
Novex 14% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP00142BOX**
Novex 14% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP00145BOX**
Novex 16% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP00160BOX**
Novex 16% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP00162BOX**
Novex 16% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP00165BOX**
Novex 4–12% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP04120BOX**
Novex 4–12% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP04122BOX**
Novex 4–12% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP04125BOX**
Novex 4–12% Tris-Glycine Mini Gel, WedgeWell format, 10-well	2 gels	**XP04120PK2**
Novex 4–20% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP04200BOX**
Novex 4–20% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP04202BOX**
Novex 4–20% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP04205BOX**
Novex 4–20% Tris-Glycine Mini Gel, WedgeWell format, 10-well	2 gels	**XP04200PK2**
Novex 8–16% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP08160BOX**
Novex 8–16% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP08162BOX**
Novex 8–16% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP08165BOX**
Novex 10–20% Tris-Glycine Mini Gel, WedgeWell format, 10-well	10 gels	**XP10200BOX**
Novex 10–20% Tris-Glycine Mini Gel, WedgeWell format, 12-well	10 gels	**XP10202BOX**
Novex 10–20% Tris-Glycine Mini Gel, WedgeWell format, 15-well	10 gels	**XP10205BOX**

Product	Quantity	Cat. No.
Novex Tris-Glycine Plus midi gels (8 x 13 cm)		
Novex 10% Tris-Glycine Plus Midi Protein Gels, 12+2-well	10 gels	**WPX01012BOX**
Novex 10% Tris-Glycine Plus Midi Protein Gels, 20-well	10 gels	**WXP01020BOX**
Novex 10% Tris-Glycine Plus Midi Protein Gels, 26-well	10 gels	**WXP01026BOX**
Novex 10% Tris-Glycine Plus Midi Protein Gels, 26-well, w/ adapters	10 gels	**WXP01026BOXA**
Novex 12% Tris-Glycine Plus Midi Protein Gels, 12+2-well	10 gels	**WXP01212BOX**
Novex 12% Tris-Glycine Plus Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WXP01212BOXA**
Novex 12% Tris-Glycine Plus Midi Protein Gels, 20-well	10 gels	**WXP01220BOX**
Novex 12% Tris-Glycine Plus Midi Protein Gels, 20-well, w/ adapters	10 gels	**WXP01220BOXA**
Novex 12% Tris-Glycine Plus Midi Protein Gels, 26-well	10 gels	**WXP01226BOX**
Novex 12% Tris-Glycine Plus Midi Protein Gels, 26-well, w/ adapters	10 gels	**WXP01226BOXA**
Novex 4–12% Tris-Glycine Plus Midi Protein Gels, 12+2-well	10 gels	**WXP41212BOX**
Novex 4–12% Tris-Glycine Plus Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WXP41212BOXA**
Novex 4–12% Tris-Glycine Plus Midi Protein Gels, 20-well	10 gels	**WXP41220BOX**
Novex 4–12% Tris-Glycine Plus Midi Protein Gels, 20-well, w/ adapters	10 gels	**WXP41220BOXA**
Novex 4–12% Tris-Glycine Plus Midi Protein Gels, 26-well	10 gels	**WXP41226BOX**
Novex 4–12% Tris-Glycine Plus Midi Protein Gels, 26-well, w/ adapters	10 gels	**WXP41226BOXA**
Novex 4–20% Tris-Glycine Plus Midi Protein Gels, 12+2-well	10 gels	**WXP42012BOX**
Novex 4–20% Tris-Glycine Plus Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WXP42012BOXA**
Novex 4–20% Tris-Glycine Plus Midi Protein Gels, 20-well	10 gels	**WXP42020BOX**
Novex 4–20% Tris-Glycine Plus Midi Protein Gels, 20-well, w/ adapters	10 gels	**WXP42020BOXA**
Novex 4–20% Tris-Glycine Plus Midi Protein Gels, 26-well	10 gels	**WXP42026BOX**
Novex 4–20% Tris-Glycine Plus Midi Protein Gels, 26-well, w/ adapters	10 gels	**WXP42026BOXA**
Novex 8–16% Tris-Glycine Plus Midi Protein Gels, 12+2-well	10 gels	**WXP81612BOX**
Novex 8–16% Tris-Glycine Plus Midi Protein Gels, 12+2-well, w/ adapters	10 gels	**WXP81612BOXA**
Novex 8–16% Tris-Glycine Plus Midi Protein Gels, 20-well	10 gels	**WXP81620BOX**
Novex 8–16% Tris-Glycine Plus Midi Protein Gels, 20-well, w/ adapters	10 gels	**WXP81620BOXA**
Novex 8–16% Tris-Glycine Plus Midi Protein Gels, 26-well	10 gels	**WXP81626BOX**
Novex 8–16% Tris-Glycine Plus Midi Protein Gels, 26-well, w/ adapters	10 gels	**WXP81626BOXA**

Product	Quantity	Cat. No.
NativePAGE gels		
NativePAGE 3–12% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	**BN1001BOX**
NativePAGE 4–16% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	**BN1002BOX**
NativePAGE 3–12% Bis-Tris Protein Gels, 1.0 mm, 15-well	10 gels	**BN1003BOX**
NativePAGE 4–16% Bis-Tris Protein Gels, 1.0 mm, 15-well	10 gels	**BN1004BOX**
Novex Tricine gels		
Novex 10% Tricine Protein Gels, 1.0 mm, 10-well	10 gels	**EC6675BOX**
Novex 10% Tricine Protein Gels, 1.0 mm, 12-well	10 gels	**EC66752BOX**
Novex 16% Tricine Protein Gels, 1.0 mm, 10-well	10 gels	**EC6695BOX**
Novex 16% Tricine Protein Gels, 1.0 mm, 12-well	10 gels	**EC66952BOX**
Novex 16% Tricine Protein Gels, 1.0 mm, 15-well	10 gels	**EC66955BOX**
Novex 10–20% Tricine Protein Gels, 1.0 mm, 10-well	10 gels	**EC6625BOX**
Novex 10–20% Tricine Protein Gels, 1.0 mm, 12-well	10 gels	**EC66252BOX**
Novex 10–20% Tricine Protein Gels, 1.0 mm, 15-well	10 gels	**EC66255BOX**
Novex IEF gels		
Novex pH 3–7 IEF Protein Gels, 1.0 mm, 12-well	5 gels	**EC66452BOX**
Novex pH 3–7 IEF Protein Gels, 1.0 mm, 10-well	5 gels	**EC6645BOX**
Novex pH 3–10 IEF Protein Gels, 1.0 mm, 10-well	5 gels	**EC6655BOX**

Product	Quantity	Cat. No.
Novex Zymogram Plus gels		
Novex 10% Zymogram Plus (Gelatin) Protein Gels, 1.0 mm, 15-well	10 gels	**ZY00105BOX**
Novex 10% Zymogram Plus (Gelatin) Protein Gels, 1.0 mm, 12-well	10 gels	**ZY00102BOX**
Novex 10% Zymogram Plus (Gelatin) Protein Gels, 1.0 mm, 10-well	10 gels	**ZY00100BOX**
E-PAGE High-Throughput Gel System		
E-PAGE 8% Protein Gels, 48-well	8 gels	**EP04808**
E-Holder Platform	2 units	**EH03**
E-PAGE Loading Buffer 1	4.5 mL	**EPBUF01**
E-PAGE 6% Protein Gels, 96-well	8 gels	**EP09606**
Daughter E-Base Device	1 unit	**EBD03**
Mother E-Base Device	1 unit	**EBM03**
Handcast polyacrylamide gels		
SureCast Gel Handcast Bundle A	Multiple	**HC1000SR**
SureCast Gel Handcast Bundle B	Multiple	**HC1000S**
SureCast Gel Handcast Station	1 casting station	**HC1000**
SureCast Glass Plates	2 sets	**HC1001**
SureCast Sealing Pads	2 pads	**HC1002**
SureCast Multi-Use Tool, 10-well	1 unit	**HC1010**
SureCast Multi-Use Tool, 12-well	1 unit	**HC1012**
SureCast Multi-Use Tool, 15-well	1 unit	**HC1015**
SureCast Gel Spacers	10 spacers	**HC1003**
SureCast Stacking Buffer	2 x 500 mL dry packs	**HC2112**
SureCast Stacking Buffer	5 x 500 mL dry packs	**HC2115**
SureCast Resolving Buffer	2 x 500 mL dry packs	**HC2212**
SureCast Resolving Buffer	5 x 500 mL dry packs	**HC2215**
SureCast APS	25 g	**HC2005**
SureCast Acrylamide Solution, 40%	450 mL	**HC2040**
SureCast TEMED	30 mL	**HC2006**

Product	Quantity	Cat. No.
SDS-PAGE buffers		
Pierce SDS-PAGE Sample Prep Kit	50 reactions	**89888**
Bolt Transfer Buffer (20X)	125 mL	**BT0006**
Bolt Transfer Buffer (20X)	1 L	**BT00061**
4X Bolt LDS Sample Buffer	10 mL	**B0007**
20X Bolt MES SDS Running Buffer	500 mL	**B0002**
20X Bolt MES SDS Running Buffer	5 L	**B0002-02**
20X Bolt MOPS SDS Running Buffer	500 mL	**B0001**
20X Bolt MOPS SDS Running Buffer	5 L	**B0001-02**
Bolt Antioxidant	15 mL	**BT0005**
NuPAGE Tris-Acetate SDS Running Buffer (20X)	500 mL	**LA0041**
NuPAGE MOPS SDS Running Buffer (20X)	500 mL	**NP0001**
NuPAGE MOPS SDS Running Buffer (20X)	5 L	**NP000102**
NuPAGE MES SDS Running Buffer (20X)	5 L	**NP000202**
NuPAGE MES SDS Running Buffer (20X)	500 mL	**NP0002**
Novex Tris-Glycine SDS Running Buffer (10X)	4 x 1 L	**LC26754**
Novex Tris-Glycine SDS Running Buffer (10X)	500 mL	**LC2675**
Novex Tris-Glycine SDS Running Buffer (10X)	5 L	**LC26755**
Novex Tricine SDS Running Buffer (10X)	500 mL	**LC1675**
NuPAGE LDS Sample Buffer (4X)	10 mL	**NP0007**
Novex Tricine SDS Sample Buffer (2X)	20 mL	**LC1676**
Novex Tris-Glycine SDS Sample Buffer (2X)	20 mL	**LC2676**
Novex Tris-Glycine Transfer Buffer (25X)	500 mL	**LC3675**
NuPAGE Transfer Buffer (20X)	125 mL	**NP0006**
NuPAGE Transfer Buffer (20X)	1 L	**NP00061**
NuPAGE Antioxidant	15 mL	**NP0005**
Novex Tris-Glycine SDS Buffer Kit	1 kit	**LC2677**
NuPAGE MOPS SDS Buffer Kit (for Bis-Tris Gels)	1 kit	**NP0050**
NuPAGE MES SDS Buffer Kit (for Bis-Tris Gels)	1 kit	**NP0060**
NuPAGE Tris-Acetate SDS Buffer Kit (for Tris-Acetate Gels), contains 1 each LA0041, NP0004, NP0005, and NP0007	1 kit	**LA0050**
Novex Tricine SDS Buffer Kit, includes LC1676 and LC1675	1 kit	**LC1677**
Pierce LDS Sample Buffer, Nonreducing (4X)	5 mL	**84788**
Pierce Lane Marker Nonreducing Sample Buffer	5 mL	**39001**
Pierce 10X Tris-Glycine SDS Buffer	1 L	**28362**
BupH Tris-Glycine Buffer Packs	40 packs	**28380**

Product	Quantity	Cat. No.
Native electrophoresis buffers		
Novex Tris-Glycine Native Running Buffer (10X)	500 mL	**LC2672**
Novex Tris-Glycine Native Sample Buffer (2X)	20 mL	**LC2673**
NativePAGE Running Buffer (20X)	1 L	**BN2001**
NativePAGE Running Buffer Kit	1 kit	**BN2007**
NativePAGE Cathode Buffer Additive (20X)	250 mL	**BN2002**
NativePAGE Sample Buffer (4X)	10 mL	**BN2003**
NativePAGE 5% G-250 Sample Additive	0.5 mL	**BN2004**
NativePAGE Sample Prep Kit	1 kit	**BN2008**
DDM (*n*-dodecyl β-D-maltoside) (10%)	1 mL	**BN2005**
Digitonin (5%)	1 mL	**BN2006**
Zymography buffers		
Novex Zymogram Developing Buffer (10X)	500 mL	**LC2671**
Novex Zymogram Renaturing Buffer (10X)	500 mL	**LC2670**
IEF buffers		
Novex IEF Anode Buffer (50X)	100 mL	**LC5300**
Novex IEF Cathode Buffer pH 3–10 (10X)	125 mL	**LC5310**
Novex IEF Cathode Buffer pH 3–7 (10X)	125 mL	**LC5370**
Novex pH 3–10 IEF Buffer Kit, includes LC5300, LC5310, LC5311	1 kit	**LC5317**
Novex pH 3–7 IEF Buffer Kit, includes LC5300, LC5370, LC5371	1 kit	**LC5377**
Novex IEF Sample Buffer pH 3–10 (2X)	25 mL	**LC5311**
Novex IEF Sample Buffer pH 3–7 (2X)	25 mL	**LC5371**

Product	Quantity	Cat. No.
Unstained standards		
HiMark Unstained Protein Standard	250 µL	**LC5688**
PageRuler Unstained Low Range Protein Ladder	2 x 250 µL	**26632**
PageRuler Unstained High Range Protein Ladder	2 x 250 µL	**26637**
PageRuler Unstained Protein Ladder	2 x 250 µL	**26614**
NativeMark Unstained Protein Standard	5 x 50 µL	**LC0725**
Prestained standards		
PageRuler Prestained Protein Ladder, 10–180 kDa	2 x 250 µL	**26616**
PageRuler Prestained Protein Ladder, 10–180 kDa	10 x 250 µL	**26617**
PageRuler Plus Prestained Protein Ladder, 10–250 kDa	2 x 250 µL	**26619**
PageRuler Plus Prestained Protein Ladder, 10–250 kDa	10 x 250 µL	**26620**
Spectra Multicolor Broad Range Protein Ladder	2 x 250 µL	**26634**
Spectra Multicolor Broad Range Protein Ladder	10 x 250 µL	**26623**
HiMark Prestained Protein Standard	250 µL	**LC5699**
Spectra Multicolor Low Range Protein Ladder	250 µL	**26628**
Spectra Multicolor High Range Protein Ladder	2 x 250 µL	**26625**
SeeBlue Prestained Protein Standard	500 µL	**LC5625**
SeeBlue Plus2 Prestained Protein Standard	500 µL	**LC5925**
Western standards		
iBright Prestained Protein Ladder	2 x 250 mL	**LC5615**
PageRuler Prestained NIR Protein Ladder	2 x 250 µL	**26635**
MagicMark XP Western Protein Standard	250 µL	**LC5602**
MagicMark XP Western Protein Standard	50 µL	**LC5603**
Specialty standards		
BenchMark Fluorescent Protein Standard	125 µL	**LC5928**
BenchMark His-Tagged Protein Standard	125 µL	**LC5606**
IEF Marker 3–10	500 µL	**3921201**
CandyCane Glycoprotein Molecular Weight Standards	400 µL	**C21852**
PeppermintStick Phosphoprotein Molecular Weight Standards	400 µL	**P33350**

Product	Quantity	Cat. No.
Electrophoresis chamber systems and power supplies		
Mini Gel Tank	1 unit	**A25977**
XCell *SureLock* Mini-Cell	1 unit	**EI0001**
SureLock Tandem Midi Gel Tank	1 each	**STM1001**
XCell4 *SureLock* Midi-Cell	1 each	**WR0100**
PowerEase Touch 350W Power Supply (115 VAC)	1 each	**PS0350**
PowerEase Touch 350W Power Supply (230 VAC)	1 each	**PS0351**
PowerEase Touch 120W Power Supply (115 VAC)	1 each	**PS0120**
PowerEase Touch 120W Power Supply (230 VAC)	1 each	**PS0121**

Product	Quantity	Cat. No.
Coomassie stains		
PageBlue Protein Staining Solution	1 L	**24620**
SimplyBlue SafeStain	1 L	**LC6060**
SimplyBlue SafeStain	3.5 L	**LC6065**
Imperial Protein Stain	1 L	**24615**
Imperial Protein Stain	3 x 1 L	**24617**
Silver stains		
Pierce Silver Stain Kit	1 L	**24612**
SilverXpress Silver Staining Kit	1 kit	**LC6100**
Pierce Silver Stain for Mass Spectrometry	1 L	**24600**
Fluorescent and specialty stains		
SYPRO Orange Protein Gel Stain	500 µL	**S6650**
SYPRO Orange Protein Gel Stain	10 x 50 µL	**S6651**
SYPRO Red Protein Gel Stain	500 µL	**S6653**
SYPRO Red Protein Gel Stain	10 x 50 µL	**S6654**
SYPRO Ruby Protein Gel Stain	1 L	**S12000**
SYPRO Ruby Protein Gel Stain	200 mL	**S12001**
SYPRO Ruby Protein Gel Stain	5 L	**S21900**
Pro-Q Emerald 488 Glycoprotein Gel and Blot Stain	1 kit	**P21875**
Pro-Q Diamond Phosphoprotein Gel Stain	1 L	**P33300**
Pro-Q Diamond Phosphoprotein Gel Stain	200 mL	**P33301**
Pro-Q Diamond Phosphoprotein Gel Stain	5 L	**P33302**
No-Stain Protein Labeling Reagent	10 reactions	**A44717**
No-Stain Protein Labeling Reagent	40 reactions	**A44449**

References

1. Ornstein L (1964) Disc electrophoresis. 1. Background and theory. *Ann N Y Acad Sci* 121:321–349.
2. Moos M Jr, Nguye NY, Liu TY (1988) Reproducible high yield sequencing of proteins electrophoretically separated and transferred to an inert support. *J Biol Chem* 263:6005–6008.
3. Kubo K (1995) Effect of incubation of solutions of proteins containing dodecyl sulfate on the cleavage of peptide bonds by boiling. *Anal Biochem* 225:351–353.