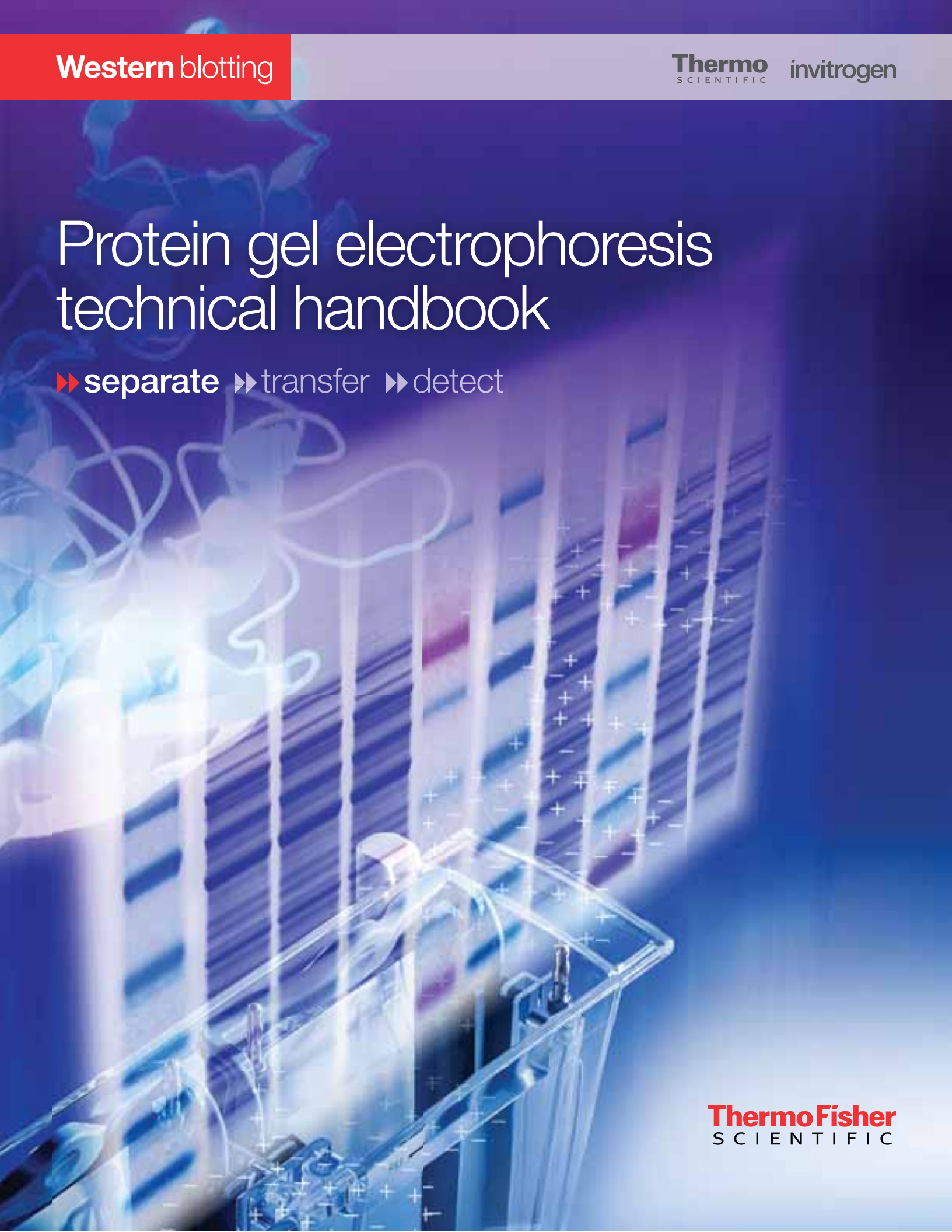
Western blotting

Thermo SCIENTIFIC invitrogen

Protein gel electrophoresis technical handbook

separate transfer detect



ThermoFisher SCIENTIFIC

Comprehensive solutions designed 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. We offer a complete array of products to support rapid, reliable protein electrophoresis for a variety of applications, whether it is the first or last step in your workflow. Our portfolio of high-quality protein electrophoresis products unites gels, stains, molecular weight markers, running buffers, and blotting products for your experiments.

For a complete listing of all available products and more, visit thermofisher.com/separate

Contents

Electrophoresis

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 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

Mini Gel Tank

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 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, minigels and midigels. Both gels have similar run lengths, but midigels are wider than minigels, allowing midigels to have more wells or larger wells. The additional wells in the midigels 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 and higher resolution.

Reference

1. Ornstein L (1964) Disc electrophoresis. 1. Background and theory. *Ann N Y Acad Sci* 121:321-349.

Electrophoresis conditions

The separation of molecules is dependent on the electrophoresis conditions. Electrophoresis can be performed under the following conditions:

Denaturing conditions

Electrophoresis is performed under denaturing conditions using an anionic detergent such as sodium dodecylsulfate (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 (native) conditions

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.

Did you know?

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

Comparison of discontinuous buffer systems

SDS-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. Both the Invitrogen™ NuPAGE™ systems (Bis-Tris and Tris-acetate gels) and the Laemmli (Tris-glycine) system are examples of discontinuous buffer systems and work in a similar fashion. However, the NuPAGE system operates at a lower pH as a result of the proprietary ions that are in the system.

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 (+), is 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.

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.

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 less gel-induced protein modifications.

The diagrams below (Figures 1, 2, and 3) summarize the migration differences in the stacking gel of each system.

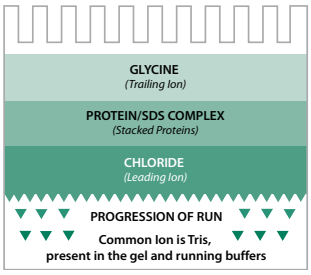


Figure 1. The Tris-glycine gel system.

- 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

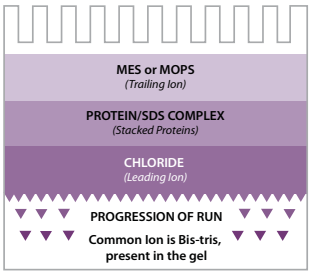


Figure 2. The Bis-Tris gel system.

- 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

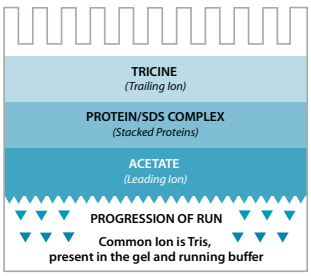


Figure 3. The Tris-acetate gel system.

- 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

Select precast gel

High-performance precast protein gels

If you are doing standard one-dimensional protein electrophoresis, we have a broad range of solutions to fit your research needs. Our selection of precast gels consists of several different chemistries, well formats, and gel sizes, so you can get the protein separation you need for accurate downstream results.

▶▶ Learn more at thermofisher.com/proteingels

Bolt Bis-Tris Plus gel.

Precast gels	
Popular gel chemistries	**Specialty gels**
• NuPAGE Bis-Tris gels	• Novex Tricine gels
• NuPAGE Tris-Acetate gels	• NativePAGE gels
• Bolt Bis-Tris Plus gels	• Novex IEF gels
• Novex Tris-Glycine gels	• Novex Zymogram gels
	• E-PAGE gels

Casting your own gels?

We offer preassembled empty cassettes, buffers, and reagents.

▶▶ Learn more at thermofisher.com/gelcastingaccessories

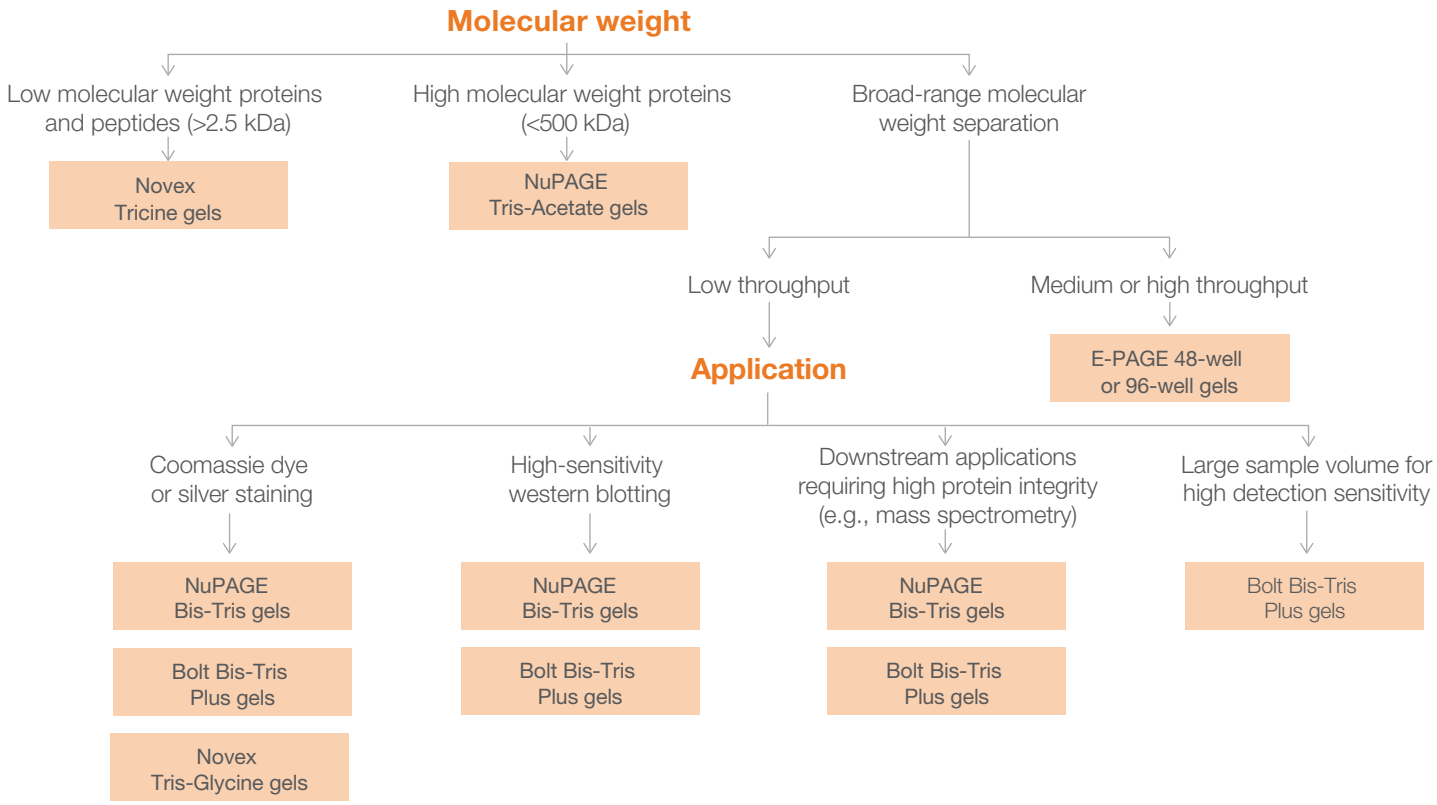
Did you know?

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

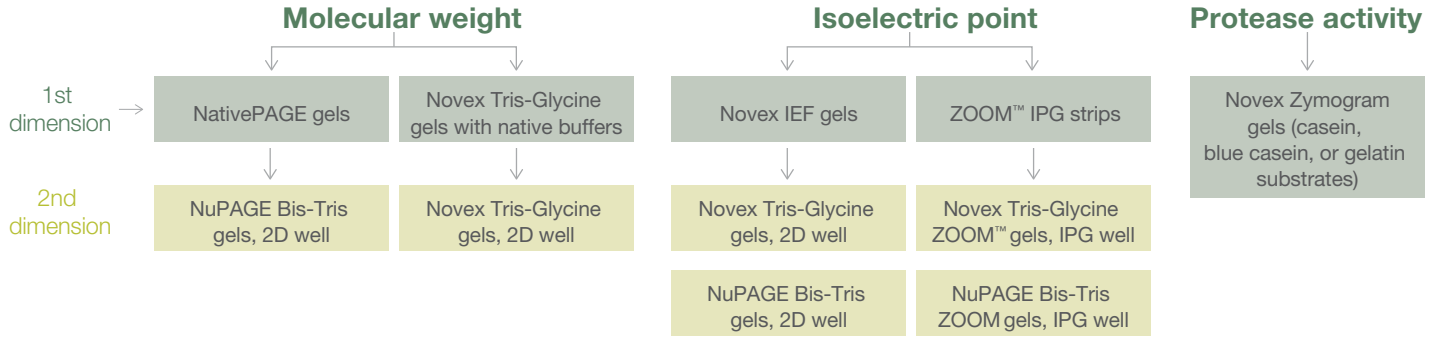
Gel selection guide

Find the right gel for your research needs based on molecular weight, downstream applications, and throughput requirements.

Denaturing separation



Native separation



▶▶ Find the right mini gel using our interactive gel selection tool at thermofisher.com/minigelselection

For ordering information refer to **pages 81–87**.

Choose the right gel chemistry for your research needs

Bis-Tris chemistry vs. Tris-glycine chemistry

The most widely used gel system for separating a broad range of proteins by SDS-PAGE is the Laemmli system, which uses Tris-glycine gels 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.

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

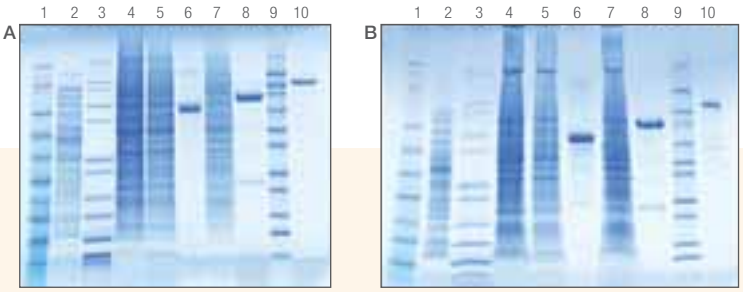


Figure 4. Protein separation using **(A)** a Bolt Bis-Tris Plus gel and **(B)** another manufacturer's traditional Tris-glycine gel.

Unlike traditional Tris-glycine gels, 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 systems 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 (Moos et al. 1998)
- 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

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

We offer most polyacrylamide gels in a choice of nine different well formats (17 well, 15 well, 12 well, 10 well, 9 well, 5 well, 1 well, 2D/preparative well, or IPG well). Two thicknesses (1.0 mm and 1.5 mm) are also 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) or a Bolt gel with its higher-capacity wedge wells is more appropriate. When blotting, remember that proteins will transfer more easily from a 1.0 mm thick gel than from a 1.5 mm thick gel.

Bolt Bis-Tris Plus mini gels

Neutral-pH gel system with a unique wedge well design

Invitrogen™ Bolt™ Bis-Tris Plus gels are precast polyacrylamide gels designed for optimal separation of a broad molecular weight range of proteins under denaturing conditions during gel electrophoresis (Figure 6 and 7). These gels help deliver consistent performance with a neutral-pH environment to minimize protein degradation. The unique wedge well design (Figure 5) allows loading of up to 2x more sample volume than other precast gels. Bolt gels are ideal for western blot transfer and analysis along with any other technique where protein integrity is crucial.

Bolt Bis-Tris Plus gels offer:

- **High sample volume capacity**—wedge well design allows detection of proteins in very dilute samples or measurement of low-abundance proteins
- **Preserved protein integrity**—neutral-pH formulation minimizes protein modifications
- **Superior band quality and band volume**—Invitrogen™ Novex™ Bis-Tris Plus chemistry is designed to deliver sharp, straight bands with higher band volume
- **Better protein resolution**—gels are 10% longer, allowing detection of more protein bands than standard mini gels
- **High lot-to-lot consistency**—coefficient of variation (CV) of only 2% for Rf values (migration)

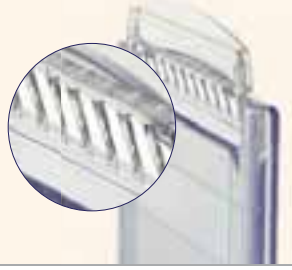


Figure 5. The unique wedge well design of Bolt Bis-Tris Plus gels.

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 12-well gel: ~40 µL, or two-thirds of the sample well volume

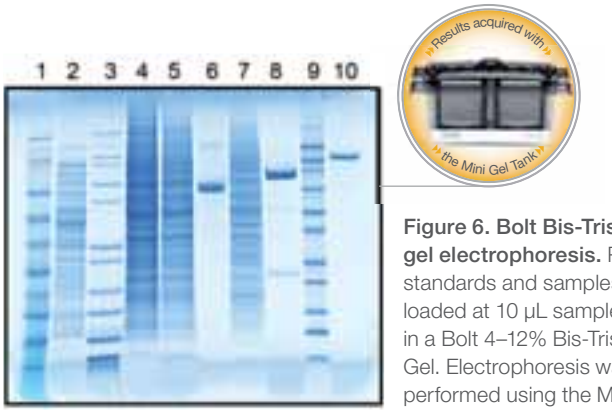


Figure 6. Bolt Bis-Tris Plus gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes in a Bolt 4–12% Bis-Tris Plus Gel. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands with consistent migration patterns were observed after 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.

Learn more at thermofisher.com/bolt

Bolt Bis-Tris Plus gels

Figure 7. Bolt Bis-Tris Plus gel migration chart. Optimal separation range is shown within the gray areas.

"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., Queen's University, Ontario, Canada*

"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., University of Pennsylvania, Philadelphia, US*

Did you know?

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

Recommended products

The Invitrogen™ Bolt™ Welcome Pack + iBlot™ 2 System offers a complete protein separation and western blot solution by combining our Mini Gel Tank, Invitrogen™ Bolt™ gels and buffers, SeeBlue Plus2 Prestained Standard, and Invitrogen™ iBlot™ 2 Gel Transfer Device with transfer stacks.

Thermo Scientific Pierce Power Stainer is recommended for fast Coomassie dye staining of Bolt Bis-Tris Plus Gels.

The Bolt Welcome Pack + iBlot 2 System.

NuPAGE gels

Revolutionary high-performance gels referenced in >20,000 publications

The Invitrogen™ NuPAGE™ SDS-PAGE gel system is a revolutionary high-performance polyacrylamide gel electrophoresis system that simulates the denaturing conditions of the traditional Laemmli system. NuPAGE™ gels use a unique buffer formulation to maintain a neutral operating pH during electrophoresis, which minimizes protein modifications that can result in poor band resolution.

Gels are available in two formulations— Invitrogen™ NuPAGE™ Bis-Tris gels are ideal for separating small to midsize proteins while Invitrogen™ NuPAGE™ Tris-Acetate gels are ideal for separating large proteins (Figure 8). A gel migration chart is shown in Figure 9.

NuPAGE gels are designed for:

- **Superior protein band resolution and stability**—neutral-pH environment during electrophoresis minimizes protein modifications
- **More efficient western blot transfer**—neutral pH prevents reoxidation of reduced samples during protein transfer
- **Fast sample run times**—typically 35–50 minutes
- **Long product shelf life**—stable for 8–16 months

Specifications

- Shelf life:
  - NuPAGE Bis-Tris gels: 16 months
  - NuPAGE Tris-Acetate gels: 8 months
- Average run time: ~35 minutes
- Separation range:
  - NuPAGE Bis-Tris gels: 1.5–300 kDa
  - NuPAGE Tris-Acetate gels: 30–400 kDa
- Polyacrylamide concentrations:
  - NuPAGE Bis-Tris gels: fixed 8%, 10%, and 12%; gradient 4–12%
  - NuPAGE Tris-Acetate gels: 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)

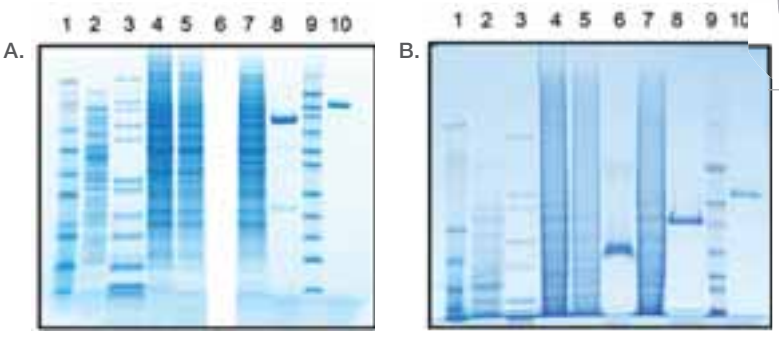


Figure 8. NuPAGE Bis-Tris and Tris-Acetate gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes in **(A)** Invitrogen™ NuPAGE™ 4–12% Bis-Tris and **(B)** Invitrogen™ NuPAGE™ 3–8% Tris-Acetate gels. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands were observed after staining with SimplyBlue SafeStain. Images were acquired using a flatbed scanner. **(A and B) 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:** (A) not used (B) 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.

Learn more at thermofisher.com/nupage

NuPAGE gels

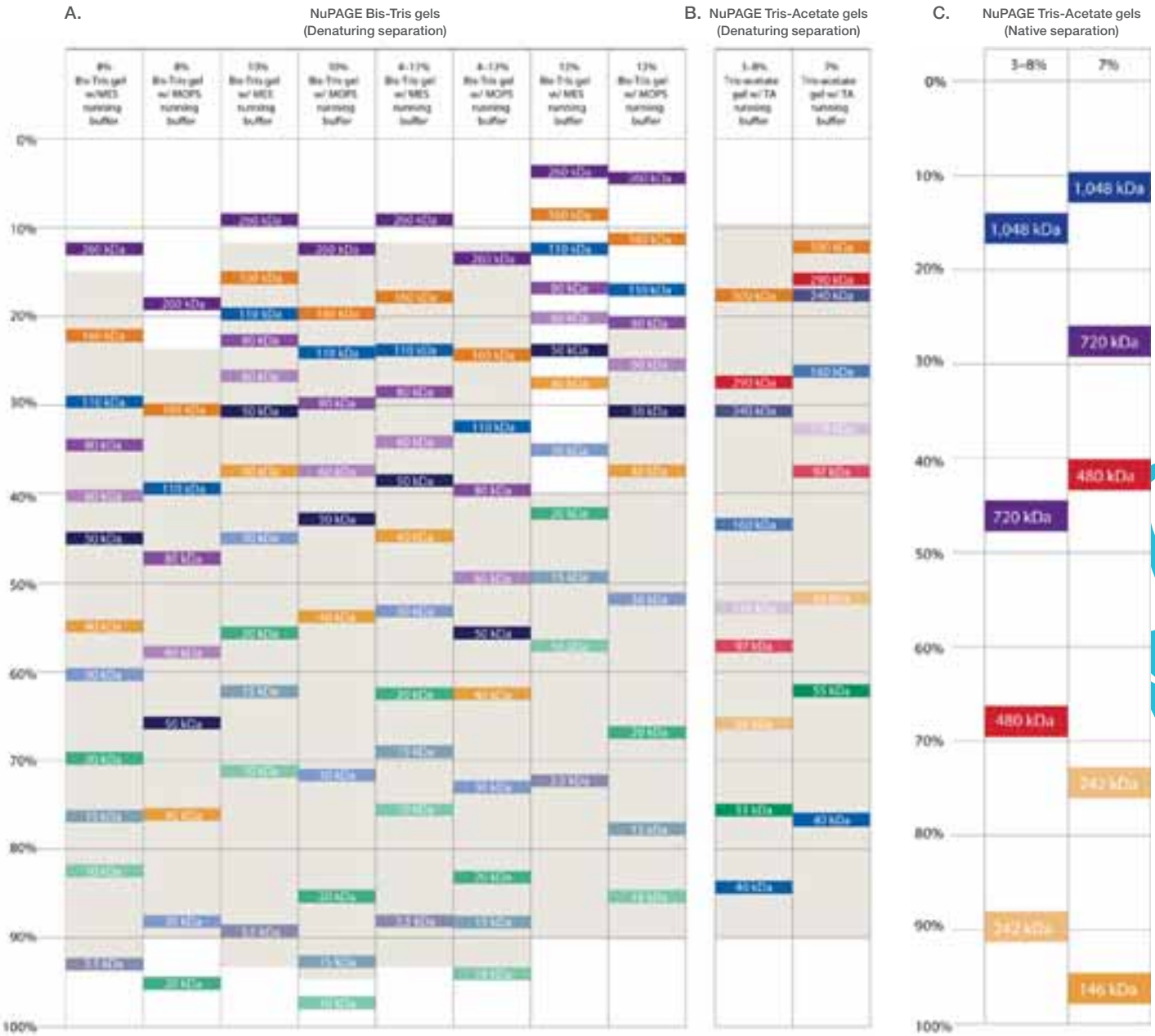


Figure 9. Migration patterns achieved in NuPAGE gels. For optimal results, protein bands should migrate within the gray shaded areas. **(A)** Migration patterns of Invitrogen™ Novex™ Sharp Prestained Protein Standard or Novex Sharp Unstained Protein Standard on NuPAGE Bis-Tris gels. **(B)** Migration patterns of HiMark Unstained Protein Standard on NuPAGE Tris-Acetate gels. **(C)** Migration pattern for Tris-acetate gel native separation is for the Invitrogen™ NativeMark™ Unstained Protein Standard.

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.

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 “Stain the gel”, **page 62**).

Novex Tris-Glycine gels

Laemmli-based precast gels for high efficiency, reproducibility, and performance

Invitrogen™ Novex™ Tris-Glycine gels are based on traditional Laemmli protein electrophoresis with minor modifications for maximum performance in the precast format. These gels provide reproducible separation of a wide range of proteins into well-resolved bands (Figure 10). A gel migration chart is shown in Figure 11.

Novex Tris-Glycine gels are:

- Individually packaged for convenience
- Compatible with most protein standards for accurate size determination
- Flexible for use with native or denatured protein samples, with specially formulated buffers for each condition

Specifications

- Shelf life: 1–2 months
- Run time: ~90 minutes
- Separation range: 6–500 kDa
- Polyacrylamide concentrations:
  - Fixed concentrations available from 4% to 18%
  - Gradient gels with ranges of 4–12%, 4–20%, 8–16%, and 10–20%
- 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 well: 25 µL (1 mm thick); 37 µL (1.5 mm thick)

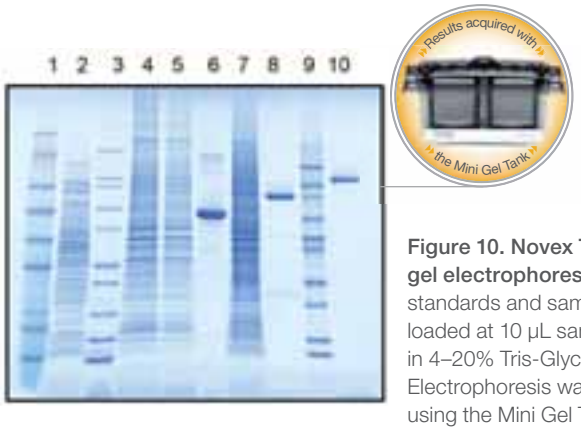


Figure 10. Novex Tris-Glycine gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes in 4–20% Tris-Glycine gels. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands were observed after staining with SimplyBlue SafeStain. Images 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.

Learn more at thermofisher.com/trisglycine

For ordering information refer to **page 82–83**. For quick reference on the protocol please refer to **page 77-78**.

Novex Tris-Glycine gels

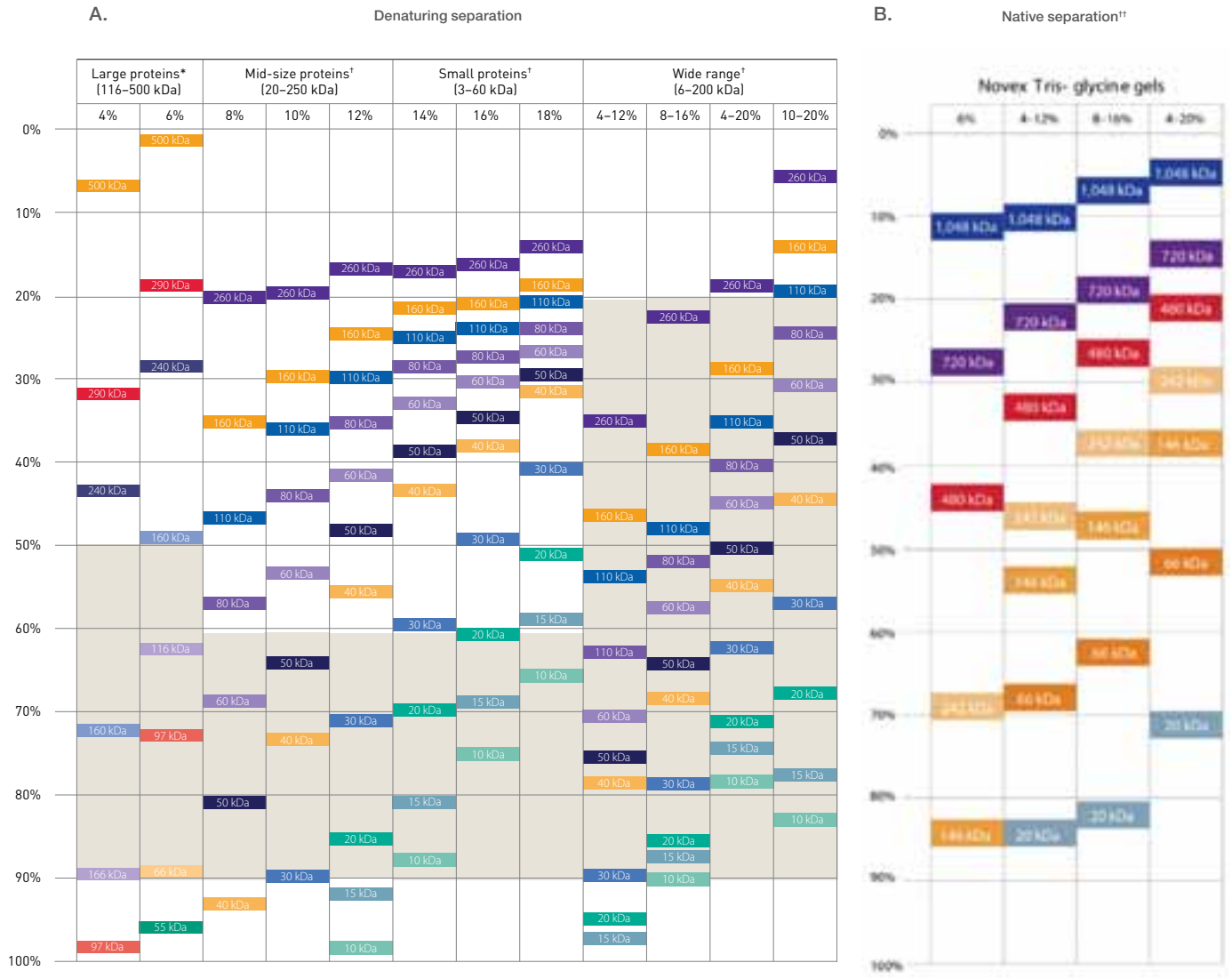


Figure 11. Migration patterns of protein molecular weight standards in Novex Tris-glycine gels. For optimal results, protein bands should migrate within the gray shaded areas. **(A)** *Migration patterns of HiMark™ Unstained Protein Standard. †Migration patterns of Novex Sharp Pre-Stained Protein Standard or Novex Sharp Unstained Protein Standard. **(B)** ††Migration pattern of NativeMARK Unstained Protein Standard.

Recommended products

For sample cleanup prior to electrophoresis, we recommend using the **Pierce SDS-PAGE Sample Prep Kit**.

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

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

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

NativePAGE 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 12). 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 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 13.

The NativePAGE gel system is designed for:

- **A wide resolving range**—from 15 kDa to over 10 MDa (Figure 12), 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

Advantages of the NativePAGE gel system over the Tris-glycine gel system include:

- **Reduced vertical streaking**—Coomassie G-250 dye binds to nonionic detergent molecules in the sample and carries them in the dye front, ahead of resolving proteins
- **Better separation of proteins**—positively charged proteins with high isoelectric points are converted to proteins with a net negative charge, allowing migration to the anode
- **Minimized protein aggregation**—Coomassie G-250 dye binding allows separation of membrane proteins and proteins with exposed hydrophobic areas

Specifications

- Shelf life: 6 months
- Average run time: 90 minutes
- Separation range: 15–10,000 kDa
- Polyacrylamide concentrations: gradient 3–12% and 4–16%
- Gel dimensions: 8 x 8 cm (1 mm thick)
- Maximum sample volume per 10-well gel: 25 µL

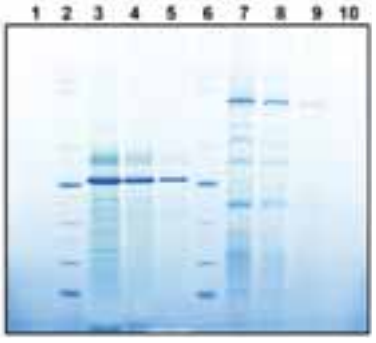


Figure 12. NativePAGE gel electrophoresis. Two-fold dilution series of protein extracts were run on an Invitrogen™ NativePAGE™ Novex™ 3–12% Bis-Tris Protein Gel using a Mini Gel Tank. Following electrophoresis, the gel was stained with Coomassie dye and imaged using a flatbed scanner. **Lanes 1 and 10:** blank; **Lanes 2 and 6:** 5 µL NativeMark Unstained Protein Standard; **Lanes 3, 4 and 5:** 10, 5, and 2.5 µg spinach chloroplast extract; **Lanes 7, 8 and 9:** 10, 5, and 2.5 µg bovine mitochondrial extract.

Learn more at thermofisher.com/nativepage

NativePAGE gel

Figure 13. NativePAGE gel migration chart. Migration patterns of the NativeMark Unstained Protein Standard on NativePAGE gels are shown.

Recommended products

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 band is visible as a red band after electrophoresis for reference (prior to staining). See page 40 for details.

Did you know?

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

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 14).

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

- **Increased resolution of proteins** with molecular weights as low as 2 kDa (Figure 15)
- **Improved compatibility** with direct protein sequencing applications after transferring to PVDF membranes
- **Minimized protein modification** due to the lower pH of the tricine buffering system

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.

Specifications

- Shelf life: 1–2 months
- Average run time: 90 minutes
- Separation range: 2–20 kDa
- Polyacrylamide concentrations: fixed 10% and 16%; gradient 10–20%
- Gel dimensions: 8 x 8 cm (1 mm thick)
- Maximum sample volume per 10-well gel: 25 µL

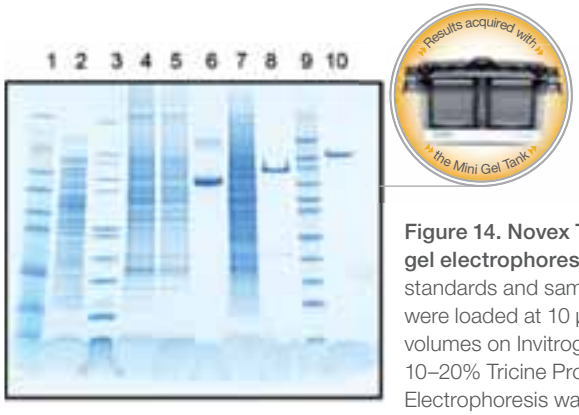


Figure 14. Novex Tricine gel electrophoresis. Protein standards and samples were loaded at 10 µL sample volumes on Invitrogen™ Novex™ 10–20% Tricine Protein Gels. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands were observed after staining with SimplyBlue SafeStain. 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.

Learn more at thermofisher.com/tricine

Novex Tricine gel

Figure 15. Novex Tricine gel migration chart. For optimal resolution, protein bands should migrate within the shaded areas.

Recommended products

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

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.

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 (Figure 16 and 17). 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, pre-optimized 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

Specifications

- Shelf life: 2 months
- Average run time: 2.5 hours
- Separation range:
  —pH 3–10 gels: pI performance range is 3.5–8
  —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

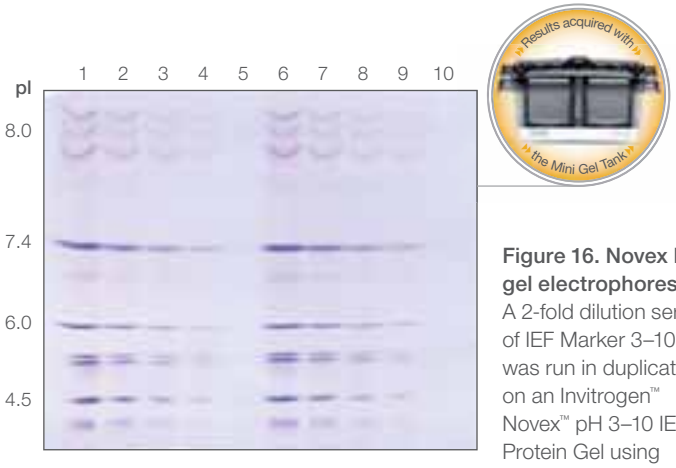


Figure 16. Novex IEF gel electrophoresis. A 2-fold dilution series of IEF Marker 3–10 was run in duplicate on an Invitrogen™ Novex™ pH 3–10 IEF Protein Gel using a Mini Gel Tank. The IEF Marker 3–10 consists of proteins with a variety of isoelectric points; these proteins include lectin (pI = 7.8, 8.0, 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. **Volume of IEF Marker 3–10 loaded: Lanes 1 and 6:** 20 µL; **Lanes 2 and 7:** 10 µL; **Lanes 3 and 8:** 5 µL; **Lanes 4 and 9:** 2.5 µL; **Lanes 5 and 10:** blank.

▸▸ Learn more at thermofisher.com/ief

For ordering information refer to **page 85**. For quick reference on the protocol please refer to **page 79**.

Novex IEF gel

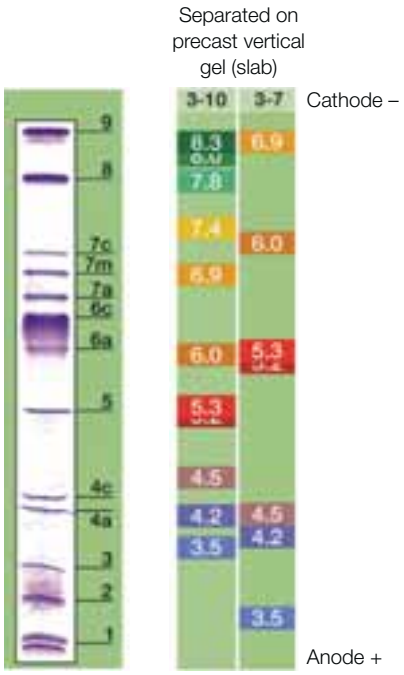


Figure 17. Novex IEF gel migration chart using the Novex IEF marker. Proteins shown are **1:** amyloglucosidase (*Aspergillus niger*), pI = 3.5; **2:** glucose oxidase (*Aspergillus niger*), pI = 4.2; **3:** trypsin inhibitor (soybean), pI = 4.5; **4a and 4c:** β-lactoglobulin (bovine, milk), pI = 5.2 and 5.3; **5:** carbonic anhydrase (bovine, erythrocytes), pI = 6.0; **6a and 6c:** myoglobin (horse, muscle), pI = 6.9 and 7.4; **7a, 7m and 7c:** lectin (*Lens culinaris*), pI = 7.8, 8.0 and 8.3; **8:** ribonuclease A (bovine, pancreas), pI = 9.5; and **9:** 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

Novex IEF buffer kits—includes optimized cathode, anode, and sample buffers to reduce variability and enable consistent results.

IEF Marker 3–10—ready to use, enables accurate results.

ZOOM™ IEF Fractionator Combo Kit—offers a fast, reliable method to reduce sample complexity, enrich low-abundance proteins, and increase the dynamic range of detection.

Novex Zymogram gels

Easy in-gel protease analysis

Invitrogen™ Novex™ Zymogram gels are excellent tools for detecting and characterizing proteases that utilize casein or gelatin as a substrate. Casein and gelatin are the most commonly used substrates for demonstrating the activity of proteases. Novex Zymogram gels are used to analyze a variety of enzymes, including matrix metalloproteinases, lipases, and other proteases (Figure 18). Available gel types are shown in Table 1.

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 gel using Novex Tris-Glycine SDS Running Buffer. After electrophoresis, the enzyme is renatured by incubating the gel in Invitrogen™ Novex™ Zymogram™ Renaturing Buffer that 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.

Table 1. Novex Zymogram gels available.

	Novex Zymogram gelatin gel	Novex Zymogram casein gel	Novex Zymogram blue casein gel
Gel composition	10% Tris-Glycine gel	12% Tris-Glycine gel	4–16% Tris-Glycine gel
Substrate	0.1% gelatin	0.05% casein	0.1% casein, with blue stain incorporated in gel
Sensitivity	10–6 units of collagenase	7 x 10^{-4} units of trypsin	1.5 x 10^{-3} units of trypsin
Post-staining required?	Yes	Yes	No
Separation range	20–120 kDa	30–150 kDa	10–220 kDa

Specifications

- Shelf life: 2 months
- Average run time: 90 minutes
- Separation range: 10–220 kDa (Figure 19)
- Polyacrylamide concentrations: fixed 10% (with gelatin), fixed 12% (with casein); gradient 4–16% (with blue casein)
- Gel dimensions: 8 x 8 cm (1 mm thick)
- Maximum sample volume per well: 20 µL

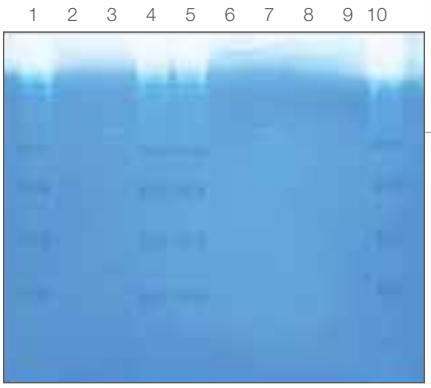


Figure 18. Novex Zymogram gel electrophoresis. Type I collagenase was run in duplicate on an Invitrogen™ Novex™ 10% Zymogram (Gelatin) Protein Gel using a Mini Gel Tank. The gel was developed using Novex Zymogram Renaturing Buffer and Novex Zymogram Developing Buffer and stained using SimplyBlue SafeStain. Images were acquired using a flatbed scanner. **Lanes 3 and 7**: 5 µL of 2.0 µU/mL type I collagenase; **Lanes 1, 4, 5, and 10:** 12 µL SeeBlue Prestained Protein Standard.

Learn more at thermofisher.com/zymogram

Novex Zymogram gel

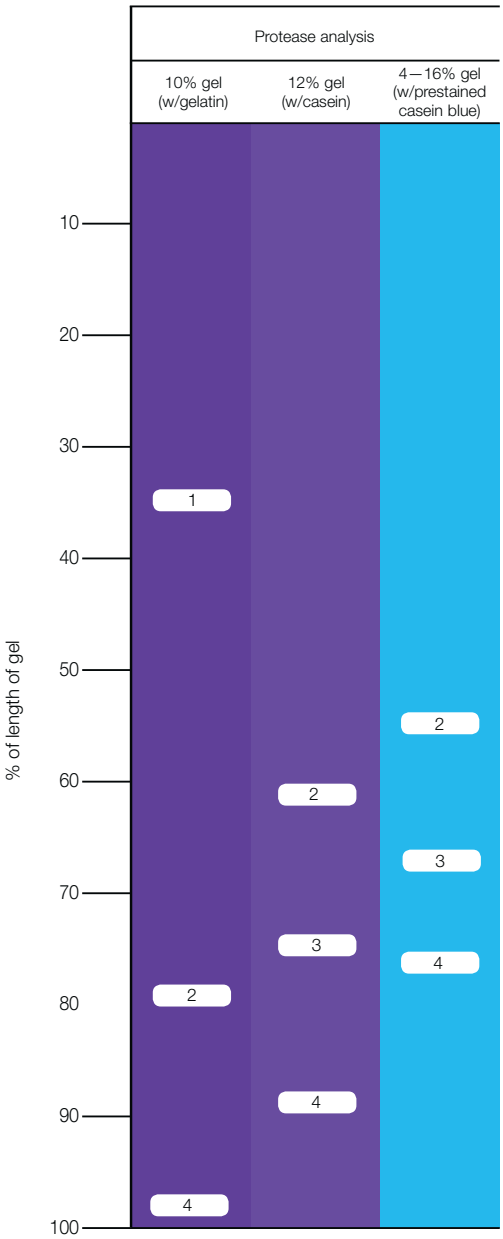


Figure 19. Novex Zymogram gel migration chart. The numbered bands refer to the following proteases:
Band 1: Collagenase Type I (140 kDa)
Band 2: Thermolysin (37 kDa)
Band 3: Chymotrypsin (30 kDa)
Band 4: Trypsin (19 kDa)

Recommended products

After electrophoresis, incubate the gel in **Zymogram Renaturing Buffer** to renature the enzyme. The gels are then equilibrated in **Zymogram Developing Buffer** 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 by multichannel pipettor or automated loading system. The E-PAGE system also includes 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

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.

Specifications

- Shelf life: 6 months
- Average run time: 14 minutes
- Separation range: 10–200 kDa
- 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

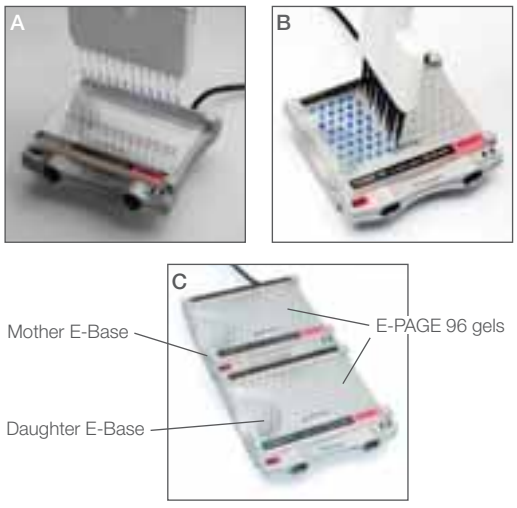


Figure 20. Loading and running E-PAGE gels. (A) Loading E-PAGE 48 gels using a multi-channel pipettor. **(B)** Loading E-PAGE 96 gels using a multi-channel pipettor. **(C)** The Mother/Daughter E-Base combination.

Learn more at thermofisher.com/epage

For ordering information refer to **page 85**.

E-PAGE gel

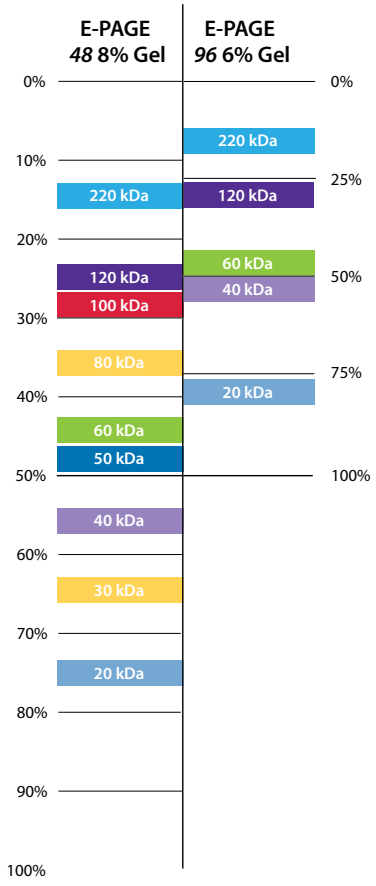


Figure 21. E-PAGE gel migration chart. Migration patterns of the Invitrogen™ E-PAGE™ MagicMark™ Unstained Protein Standard are shown.

Recommended products

The E-PAGE™ SeeBlue™ Prestained Protein Standard or **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.

Prepare the sample

Sample prep kits

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, adjusting the ionic strength, and removing interfering contaminants. General guidelines for preparing samples are provided below.

General guidelines for preparing samples:

Prepare your sample in the appropriate sample buffer such that the final concentration of the sample buffer is 1X. Recommended sample buffers are listed on page 29.

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

Heating samples: Heating the sample at 100°C in SDS-containing buffer results in proteolysis (Kubo, 1995). We recommend heating samples for denaturing electrophoresis (reduced or non-reduced) at 85°C for 2–5 minutes for optimal results. Do not heat the samples for non-denaturing (native) electrophoresis or Novex Zymogram Gels.

High salt concentration in samples: High salt concentrations result in increased conductivity that affects protein migration, and can result in gel artifacts in adjacent lanes containing samples with normal salt concentrations. Perform dialysis or precipitate and resuspend samples in lower-salt buffer prior to electrophoresis.

Guanidine-HCl in samples: Samples solubilized in guanidine-HCl have high ionic strength, and produce increased conductivity similar to high salt concentrations. In addition, guanidine precipitates in the presence of SDS leading to various types of gel artifacts. If possible, change the solubilization agent by dialysis prior to electrophoresis.

Cell lysates

Consider the following when performing electrophoresis of cell lysates:

- Genomic DNA in the cell lysate may cause the sample to become viscous and affect protein migration patterns and resolution. Shear genomic DNA to reduce viscosity before loading the sample.
- Cells lysates contain soluble and insoluble fractions. The size of each fraction depends on the type of sample being analyzed. The nature of the insoluble fraction may result in altered protein migration patterns and resolution. Separate the two fractions by centrifugation and load them on separate lanes for electrophoresis.
- If radioimmunoprecipitation assay (RIPA) buffer is used in cell lysis, subsequent blotting of proteins less than 40 kDa may be inhibited due to the presence of Triton™ X-100 in the buffer.

For quick protein clean-up and enrichment for SDS-PAGE we recommend using the Thermo Scientific Pierce SDS-PAGE Sample Prep Kit, which removes substances such as guanidine-HCL and ionic detergents that can result in protein bands that appear smeared or wavy in the gel or on a western blot.

For ordering information refer to **page 85**.

Pierce SDS-PAGE Sample Prep Kit

Quick protein clean-up and enrichment for SDS-PAGE

A protein sample can be purged of any contaminants typically in only 10 minutes using the Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit. This is much faster than dialysis or ultrafiltration and yields higher protein recoveries while concentrating the sample.

Advantages of using the Pierce SDS-PAGE Sample Prep Kit include:

- **Eliminates artifacts caused by incompatible contaminants**—removes dyes, reducing agents, detergents, sugars, glycerol, guanidine, urea, and ammonium sulfate to provide reproducible results for SDS-PAGE analysis (Figure 22)
- **Compatible with the Thermo Scientific™ Pierce™ BCA Assay**—allows quantification of the processed sample
- **Enriches dilute protein solutions**—concentrates protein sample by eight-fold in less than 20 minutes for SDS-PAGE analysis (Figure 22)
- **Fast and easy to use for up to 70 µg of protein per sample**—uses new spin cup format that allows higher amounts of protein to be processed than with the original procedure

Good to know

Our Pierce SDS-PAGE Sample Prep Kit uses a unique resin of modified diatomaceous earth that binds protein in DMSO. Simply combine 2–300 µL of sample containing up to 70 µg of protein with 20 µL of Pierce™ SDS Protein Binding Resin and DMSO. After the proteins bind to the resin, wash away the unbound contaminating chemicals. Finally, elute the sample in 50 µL of the Elution Buffer. The recovered protein sample is ready to mix with the supplied Sample Loading Buffer for gel loading.

Learn more at thermofisher.com/PAGEsampleprep

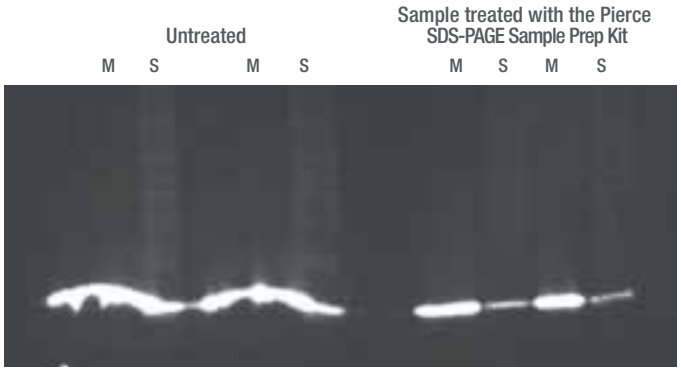


Figure 22. Minimize distortion caused by detergents. Rat C6 cells were lysed and a membrane protein fraction isolated using the Thermo Scientific™ MemPER™ Eukaryotic Membrane Protein Extraction Reagent. Membrane and hydrophilic cell fractions were separated by SDS-PAGE using 4–20% gradient gels with or without prior treatment using the Pierce SDS-PAGE Sample Prep Kit. Western blot analysis was performed using an antibody against cytochrome oxidase subunit 4 (COX4) and Thermo Scientific™ SuperSignal™ West Femto chemiluminescent substrate. Kit-treated samples exhibit better band straightness and resolution with low molecular weight proteins than samples that were untreated.
S = Soluble fraction (hydrophilic) M = Membrane fraction

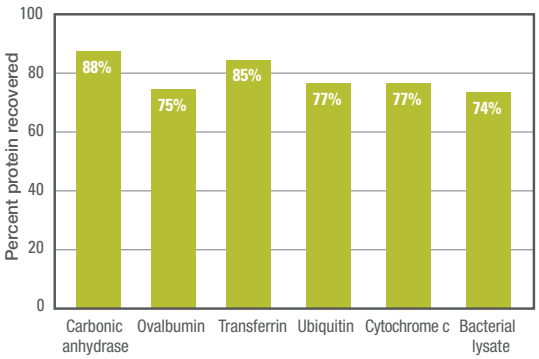


Figure 23. Consistent protein recovery is achieved using the Pierce SDS-PAGE Sample Prep Kit. Pure proteins (60 µg) of assorted molecular mass (30, 44, 80, 86, and 120 kDa) as well as a bacterial lysate were processed using this kit. Protein concentrations were determined with the Pierce BCA Protein Assay Kit and reported as percent protein recovered.

Table 2. Interfering substances effectively removed.

Interfering reagents	Percent protein recovered (Starting amount = 20 µg BSA)
Control (water)	75%
0.5 M Sodium chloride	80%
2 M Ammonium sulfate	76%
20% SDS	75%
10% Triton™ detergent	75%
6 M Urea:DMSO (1:3 ratio)	75%
1 M Sodium chloride	75%
6 M Urea	74%
10% CHAPS	80%
25% Glycerol	71%
10% OTG	71%
2 M Guanidinium•HCl	70%
40% Sucrose	70%

Select buffers

Buffers and reagents

Protein samples prepared for PAGE analysis are denatured by heating in the presence of a sample buffer with or without a reducing agent. The protein sample is mixed with the sample buffer and heated for 2–10 minutes, then cooled to room temperature before it is applied to the sample well on the gel. Loading buffers also contain glycerol so that they are heavier than water and sink neatly to the bottom of the buffer-submerged well when added to 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 loading buffers is bromophenol blue.

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

Learn more at thermofisher.com/electrophoresisbuffers

For ordering information refer to **page 85**.

Recommended SDS-PAGE buffers and reagents

Gel type	Sample buffer optimized for use with the gel	Other compatible sample buffers	Running buffer optimized for use with the gel	
Bolt Bis-Tris Plus Gel	• Bolt™ Sample Reducing Agent (10X) • Bolt™ LDS Sample Buffer (4X) (nonreducing) • Bolt Antioxidant	• Pierce™ LDS Sample Buffer (4X) for storage at RT • Pierce™ Lane Marker Non-Reducing Sample Buffer (5X)—storage at RT; when you desire to dilute your sample less and require transferable marker dye to nitrocellulose membranes • Pierce™ Lane Marker Reducing Sample Buffer (5X)—when you desire to dilute your sample less and require transferable marker dye to nitrocellulose membranes	• Bolt™ MES SDS Running Buffer (20X) • Bolt™ MOPS SDS Running Buffer (20X)	MES vs. MOPS Running Buffer: • Use MES SDS running buffers to resolve small molecular weight proteins. • Use MOPS running buffers to resolve mid-size proteins. MES has a lower pKa 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.
NuPAGE Bis-Tris Gel	• NuPAGE™ Sample Reducing Agent (10X) • NuPAGE Antioxidant • NuPAGE™ LDS Sample Buffer (4X) (nonreducing)		• NuPAGE™ MES SDS Running Buffer (20X) • NuPAGE™ MOPS SDS Running Buffer (20X)	
NuPAGE Tris-Acetate Gel	• Novex Tris-Glycine SDS Sample Buffer (2X) • 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)	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.
Novex Tris-Glycine Gel	• Novex Tris-Glycine SDS Sample Buffer (2X) • NuPAGE Sample Reducing Agent • 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-SDS 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) • Novex™ 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 Gels*	• Novex Tris-Glycine SDS Sample Buffer (2X)		• Novex Tris-Glycine SDS Running Buffer (10X)	

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

Buffer recipes

NuPAGE 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	0% 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™ 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 Tris-Acetate SDS Running Buffer	+4°–25°C	Tris base Tricine SDS	50 mM 50 mM 0.1% (pH 8.24)

* The pre-mixed 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.

For ordering information refer to **page 85**.

Tris-glycine buffer recipes

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

Buffer recipes

Tricine buffer recipes			
Buffer	**Storage**	**Component**	**Concentration (1X)**
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)
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)**
Zymogram Renaturing Buffer	Room temperature	Triton™ X-100 Deionized water	2.7% (w/v) in H_2O
Zymogram Developing Buffer	Room temperature	Tris HCl* NaCl $CaCl_2$•2 H_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.

For ordering information refer to **page 85**.

Isoelectric focusing buffer recipes

Buffer	Storage	Component	Concentration (1X)
IEF Sample Buffer pH 3-7	+4˚C	Lysine (free base) Glycerol Deionized water	40 mM 15% —
IEF Sample Buffer pH 3-10	+4˚C	Arginine (free base) Lysine (free base) Glycerol Deionized water	20 mM 20 mM 15% —
IEF Cathode Buffer pH 3-7 (upper buffer chamber)	+4˚C	Lysine (free base) Deionized water	40 mM —
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)
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

* For ZOOM™ Strip pH 9-12 use 1% ZOOM™ Focusing Buffer pH 7-12 instead of ampholytes.

Select the standard

Protein ladders and standards

To assess the relative molecular weights (sizes) of proteins in a sample, a mixture containing several proteins of known molecular mass are run alongside the test sample lane(s). Often these protein mixtures are run on the outer lanes of the gel, to maximize the number of remaining gel wells for test samples, but can also be useful in the middle wells of the gel when running a large gel with many wells. Such sets of known protein mixtures are called protein molecular weight markers or protein ladders. It is important to choose a protein ladder that consists of proteins with molecular weights that span the molecular weight range of the protein(s) of interest. A standard curve can be constructed from the distances each marker protein migrates through the gel. 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.

Several kinds of ready-to-use protein molecular weight (MW) markers are available that are labeled, prestained, or unstained for different modes of detection and downstream applications. We offer ladders suitable for both SDS-PAGE as well as native PAGE.

Unstained protein ladders	
Low range	PageRuler Unstained Low Range Protein Ladder
Broad range	PageRuler Unstained Protein Ladder
High range	NativeMark Unstained Protein Standard
Recommended for: • Precise determination of target protein molecular weight	

Prestained protein ladders	
Low range	PageRuler Prestained Protein Ladder
Broad range	PageRuler Plus Prestained Protein Ladder Spectra™ Multicolor Broad Range Protein Ladder
High range	HiMark Prestained Protein Standard Spectra Multicolor High Range Protein Ladder
Recommended for: • Approximate determination of molecular weight • Monitoring the progress of electrophoresis runs • Estimating the efficiency of protein transfer to the membrane during western blotting	

Other	
Western	MagicMark XP Western Protein Standard
Specialty	PageRuler Prestained NIR Protein Ladder BenchMark Fluorescent Protein Standard BenchMark His-tagged Protein Standard IEF Marker 3-10

Ready-to-use prestained and unstained protein ladders with exceptional lot-to-lot consistency

We offer a broad range of prestained and unstained protein ladders supplied in a ready-to-use format to facilitate easy protein analysis during gel electrophoresis and western blotting (Table 3). All of our protein ladders offer:

- **Performance**—sharp protein bands and consistent migration patterns provide easy molecular weight determination
- **Convenience**—protein ladders are ready to load, with no heating required
- **Reliability**—exceptional lot-to-lot consistency and reproducibility

Learn more at thermofisher.com/proteinstandards

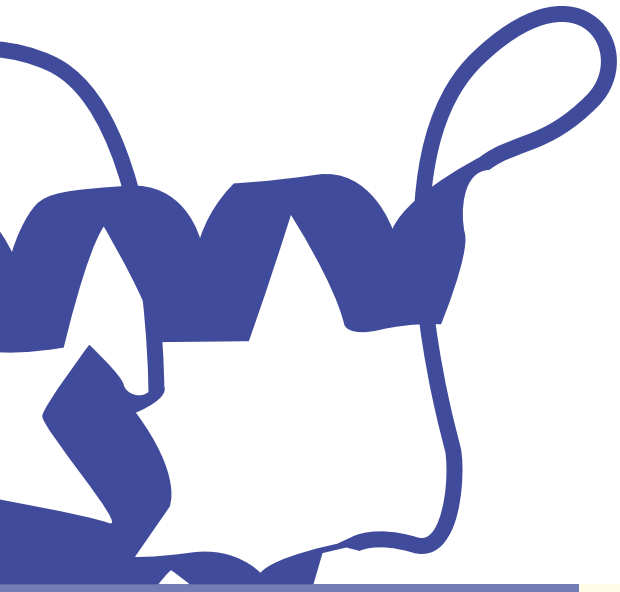
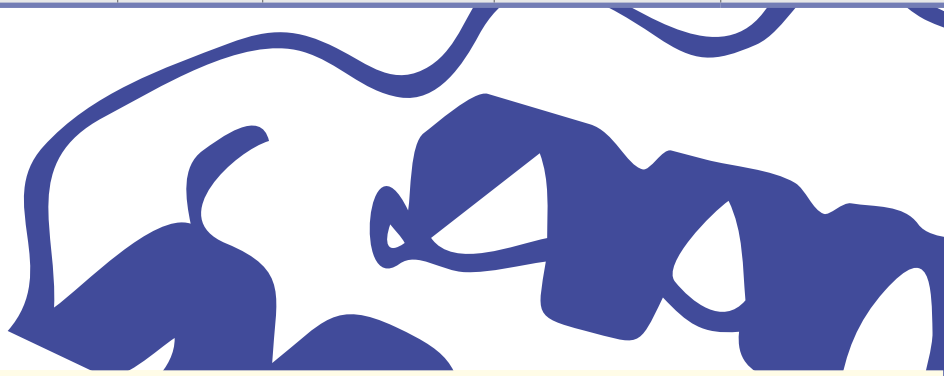


Table 3. Protein standard selection guide

Category	Product	Range	No. of bands	Reference bands	Protein MW determination	Protein band visualization
Unstained ladders and standards						
Unstained standards	PageRuler Unstained Low Range Protein Ladder	3.4–100 kDa	8	25 kDa	Best	NA
	PageRuler Unstained Protein Ladder	10–200 kDa	14	50 kDa	Good	NA
	NativeMark Unstained Protein Standard	20–1,200 kDa	8		Best for native electrophoresis	NA
Pretained protein ladders						
Prestained protein standards	PageRuler Prestained Protein Ladder	10–180 kDa	10	Green 10 kDa; orange 70 kDa	Good	Good
	PageRuler Plus Prestained Protein Ladder	10–250 kDa	9	Green 10 kDa; orange 25 and 70 kDa	Good	Good
	HiMark Prestained Protein Standard	30–460 kDa	9		Best for high MW proteins	Good
	Spectra Multicolor Broad Range Protein Ladder	10–260 kDa	10	Green 10 and 50 kDa; orange 40, 70, and 260 kDa; pink 140 kDa	Good	Best
	Spectra Multicolor High Range Protein Ladder	40–300 kDa	8	Green 50 kDa; orange 70 and 300 kDa	Good	Best
Other ladders and standards						
IEF	IEF Marker 3-10	pI 3.5–10.7	13		Best for pI estimation	NA
Chemiluminescent standard	MagicMark XP Western Protein Standard	20–220 kDa	9		Good	NA
Near infrared (NIR) standard	PageRuler Prestained NIR Protein Ladder	11–250 kDa	10	55 kDa	Good	NA
Fluorescent standard	BenchMark Fluorescent Protein Standard	11–155 kDa	7		Good	NA
His-tag standard	BenchMark His-tagged Protein Standard	10–160 kDa	10		Best	NA

▸▸ Learn more at thermofisher.com/proteinstandards



	Monitoring electrophoresis run	Coomassie dye, silver, or fluorescent staining	Monitoring protein transfer	Chemiluminescent band visualization
	NA	Best	NA	Good
	NA	Good	NA	Good
	NA	Best	NA	Good
	Good	NA	Good	Good
	Good	NA	Good	NA
	Good	NA	Best for high MW proteins	NA
	Best	NA	Best	NA
	Best	NA	Best	NA
	NA	Good	NA	NA
	NA	Good	NA	Best
	NA	NA	NA	NA
	NA	NA	NA	Good
	NA	Good	NA	Good for detection with anti-His antibody

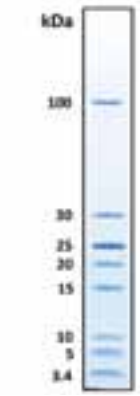
Unstained ladders and standards

PageRuler Unstained Low Range Protein Ladder

Sharp bands and precise molecular weight estimation for low molecular weight proteins

Thermo Scientific™ PageRuler™ Unstained Low Range Protein Ladder is a mixture of eight proteins and peptides for use as size standards that resolve into clearly identifiable sharp bands when analyzed by SDS-PAGE. The proteins (except for the 5 and 3.4 kDa peptides) contain an integral Strep-tag™ II Sequence and may be detected on western blots using Strep-Tactin™ Conjugates.

- **Comprehensive**—eight proteins and peptides spanning 3.4 to 100 kDa; the 25 kDa band is more intense than the other bands for easy orientation
- **Versatile**—compatible with western blots by staining with Ponceau S dye or Coomassie dye; compatible with Thermo Scientific™ Pierce™ Reversible Protein Stain Kit for Nitrocellulose Membranes or other protein stains



PageRuler Unstained Low Range Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

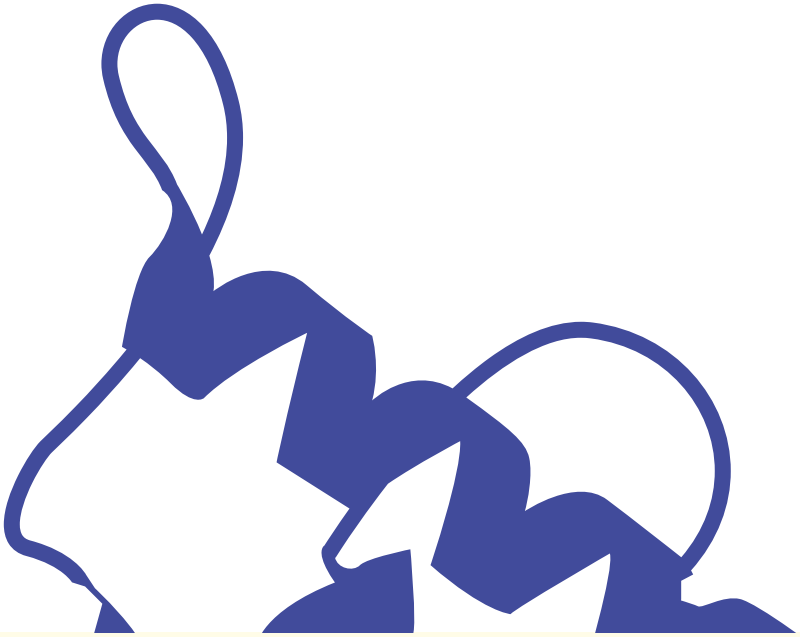
Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The PageRuler Unstained Protein Ladder is recommended for **Novex Tris-Glycine, Bis-Tris** or **Tris-Acetate gels**.

Learn more at thermofisher.com/unstainedstandards



For ordering information refer to **page 86**.

PageRuler Unstained Protein Ladder

Sharp bands and precise molecular weight estimation for a wide range of proteins

Thermo Scientific™ PageRuler™ Unstained Protein Ladder is a mixture of 14 recombinant, highly purified, unstained proteins for use as size standards in SDS-PAGE and western blotting. Each protein in the ladder contains an integral Strep-tag II Sequence, which can be detected directly on western blots using a Strep-Tactin Conjugate or an antibody against the Strep-tag II Sequence.

- **Comprehensive**—14 highly purified proteins with excellent accuracy spanning 10 to 200 kDa; the ladder contains one 50 kDa reference band of higher intensity
- **Versatile**—compatible with Coomassie dye; compatible with Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes, silver staining, or western blotting

PageRuler Unstained Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

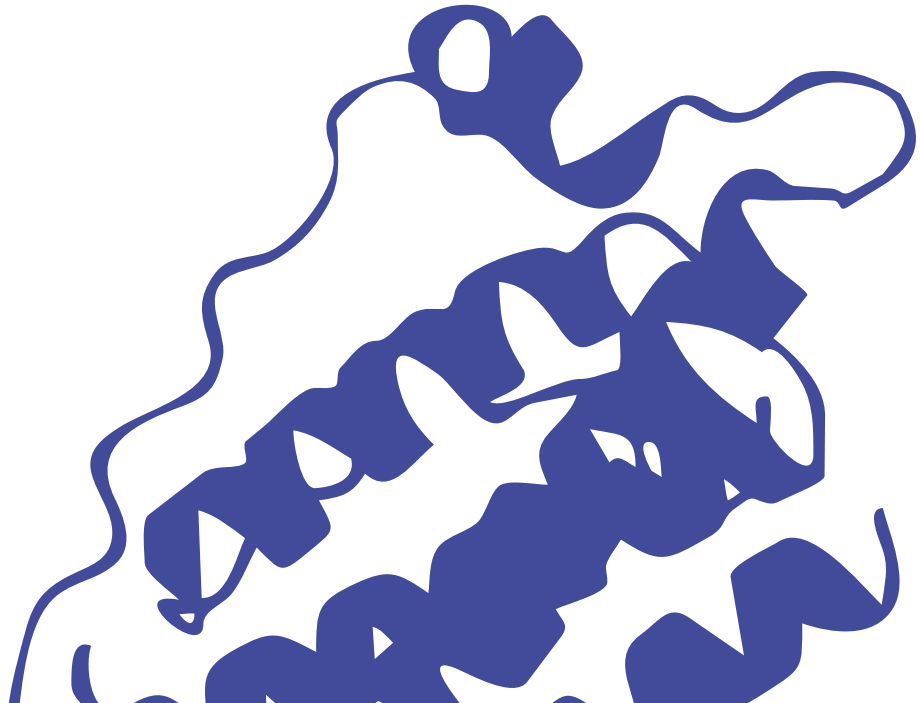
Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The PageRuler Unstained Protein Ladder is recommended for **Novex Tris-Glycine, Bis-Tris** or **Tris-Acetate gels**.

Learn more at thermofisher.com/unstainedstandards

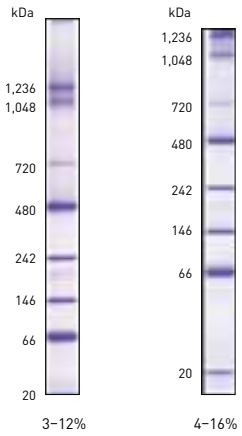


NativeMark Unstained Protein Standard

Convenient molecular weight estimation for native electrophoresis

The Invitrogen™ NativeMark™ Unstained Protein Standard is designed for molecular weight estimation of proteins using native gel electrophoresis.

- **Comprehensive**—contains a wide range of high molecular weight proteins, providing 8 protein bands in the range of 20–1,200 kDa
- **Versatile**—can be visualized using Coomassie, silver, or fluorescent stains after electrophoresis, or with Ponceau S, Coomassie, or other membrane stains after western transfer



NativeMark Unstained Protein Standard
NativePAGE Bis-Tris gels

Storage specifications

- Storage buffer: Bis/Tris-HCl (pH 7.0), NaCl, glycerol, and Ponceau S
- Storage conditions: upon receipt store at –20°C
- Stability: 6 months

Recommended products

The NativeMark Unstained Protein Standard is recommended for use with **NativePAGE Bis-Tris gels, Novex Tris-Glycine gels**, or **NuPAGE Tris-Acetate gels**.

▶▶ Learn more at thermofisher.com/unstainedstandards

For ordering information refer to **page 86**.

Prestained ladders

PageRuler Prestained Protein Ladder

Outstanding clarity for easy molecular weight determination of low molecular weight proteins

Thermo Scientific™ PageRuler™ Prestained Protein Ladder is a mixture of 10 blue-, orange-, and green-stained proteins for use as size standards in SDS-PAGE and western blotting. The mobility of prestained proteins can vary in different SDS-PAGE buffer systems; however, they are suitable for approximate molecular weight determination when calibrated against unstained standards in the same system.

- **Comprehensive**—contains 10 proteins with a range of 10 to 180 kDa; includes one 70 kDa reference protein colored with an orange dye and one 10 kDa reference protein colored with a green dye
- **Versatile**—compatible with Coomassie dye staining and western blotting

PageRuler Prestained Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

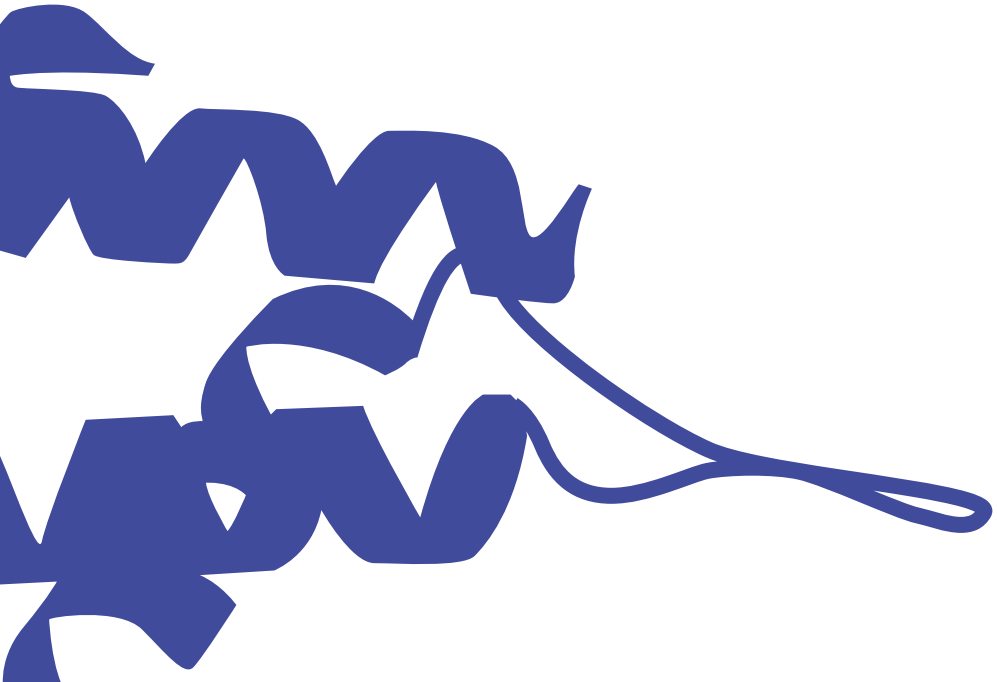
Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The PageRuler Prestained Protein Ladder is recommended for use with **Tris-glycine, Bis-Tris,** and **Tris-acetate gels**.

Learn more at thermofisher.com/prestainedstandards



PageRuler Plus Prestained Protein Ladder

Outstanding clarity for easy molecular weight determination of a broad range of proteins

Thermo Scientific™ PageRuler™ Plus Prestained Protein Ladder is a mixture of 9 blue-, orange-, and green-stained proteins for use as size standards in SDS-PAGE and western blotting. The mobility of prestained proteins can vary in different SDS-PAGE buffer systems; however, they are suitable for approximate molecular weight determination when calibrated against unstained standards in the same system.

- **Comprehensive**—9 proteins with a broad range of 10 to 250 kDa; includes 70 kDa and 25 kDa reference proteins that are colored with an orange dye and one 10 kDa reference protein that is colored with a green dye
- **Versatile**—compatible with Coomassie dye staining and western blotting

PageRuler Plus Prestained Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The PageRuler Plus Prestained Protein Ladder is recommended for **Tris-glycine, Bis-Tris,** and **Tris-acetate gels**.

Learn more at thermofisher.com/prestainedstandards

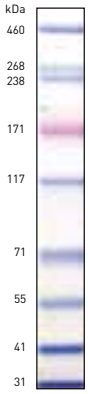
For ordering information refer to **page 86**.

HiMark Prestained Protein Standard

Superb analysis of high molecular weight proteins

The Invitrogen™ HiMark™ Prestained Protein Standard is designed for analysis of high molecular weight proteins on NuPAGE Tris-acetate gels.

- **Comprehensive**—contains a wide range of high molecular weight proteins, providing 9 protein bands in the range of 30–460 kDa
- **Versatile**—easy visualization of band migration during electrophoresis and rapid evaluation of western transfer efficiency



HiMark Prestained Protein Standard
NuPAGE 3–8% Tris-acetate SDS buffer

Storage specifications

- Storage buffer: Tris-HCl, formamide, SDS, and phenol red
- Storage conditions: upon receipt store at –20°C
- Stability: 6 months from date of receipt

Recommended products

The HiMark Prestained Protein Standard is recommended for use with **NuPAGE Tris-Acetate gels** under denaturing conditions. This standard can also be used with **NuPAGE 4–12% Bis-Tris gels** with **Invitrogen™ NuPAGE MOPS SDS Running Buffer** and **Novex 4% Tris-Glycine gels**. However, to obtain the best results with high molecular weight proteins, always use **NuPAGE Tris-Acetate gels**.

The HiMark Prestained Protein Standard is also available as part of the following kits that include gels, running and sample buffers, and stains or blotting materials:

- **Invitrogen™ NuPAGE™ Large Protein Staining Kit**
- **Invitrogen™ NuPAGE™ Large Protein Sensitive Staining Kit**
- **Invitrogen™ NuPAGE™ Large Protein Blotting Kit**

Learn more at thermofisher.com/prestainedstandards

Spectra Multicolor Broad Range Protein Ladder

Superior visualization and analysis of a broad range of proteins

Thermo Scientific™ Spectra™ Multicolor Broad Range Protein Ladder is a 4-color protein standard containing 10 prestained proteins for use in gel electrophoresis and western blotting. This standard is designed for monitoring the progress of gels during SDS-PAGE and for assessing western blot transfer efficiency. Four different chromophores (blue, orange, green, and pink) are bound to the different component proteins, producing a brightly colored ladder with an easy-to-remember pattern.

- **Comprehensive**—10 proteins with similar intensity spanning a broad range of 10 to 260 kDa
- **Versatile**—compatible with Coomassie dye staining and western blotting

Spectra Multicolor Broad Range Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

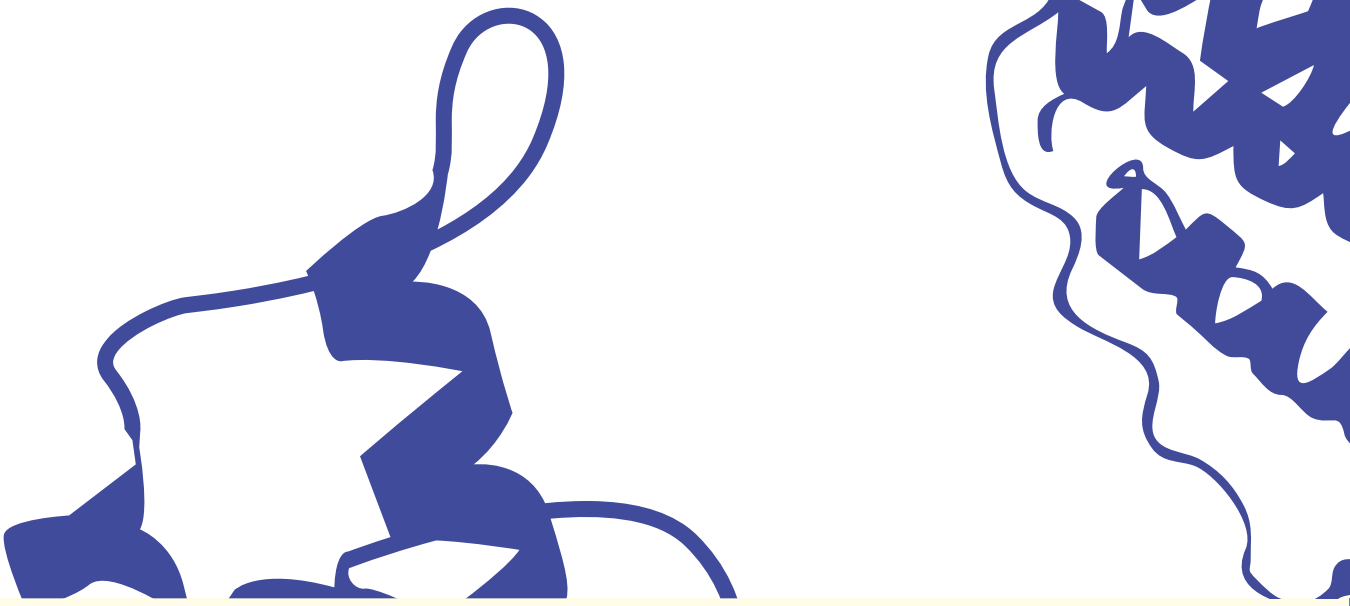
Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The Spectra Multicolor Broad Range Protein Ladder is recommended for **Tris-glycine, Bis-Tris,** and **Tris-acetate gels**.

Learn more at thermofisher.com/prestainedstandards



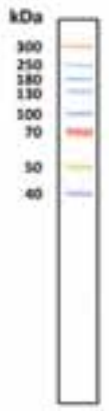
For ordering information refer to **page 86**.

Spectra Multicolor High Range Protein Ladder

Superior and convenient visualization of high molecular weight proteins

Thermo Scientific™ Spectra™ Multicolor High Range Protein Ladder is a mixture of 8 blue-, green-, and orange-stained proteins for use as size standards for high molecular weight proteins in gel electrophoresis and western blotting. This marker is designed for monitoring the progress of gels during SDS-PAGE, assessing western blot transfer efficiency, and estimating the approximate size of proteins after gel staining or western blotting.

- **Comprehensive**—8 proteins of similar intensity spanning a range of 40 to 300 kDa; 3 different chromophores (blue, orange, and green) are bound to the different component proteins, producing a brightly colored ladder with an easy-to-remember pattern
- **Versatile**—compatible with Coomassie dye staining and western blotting



Spectra Multicolor High Range Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The Spectra Multicolor High Range Protein Ladder is recommended for **Tris-glycine, Bis-Tris,** and **Tris-acetate gels**.

▶▶ Learn more at thermofisher.com/prestainedstandards

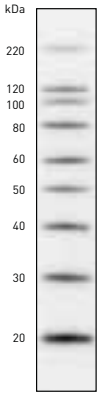
Other ladders and standards

MagicMark XP Western Protein Standard

Accurate molecular weight estimation directly on western blots

The MagicMark™ XP Western Protein Standard is specifically designed for easy and convenient protein molecular weight estimation directly on western blots. Each recombinant protein in the standard contains an IgG binding site, which binds the primary or secondary antibody used for detection of the target protein, allowing direct visualization of the standard on the western blot.

- **Comprehensive**—consists of 9 recombinant proteins from 20 to 220 kDa
- **Versatile**—compatible with chemiluminescent, chromogenic, and fluorescent detection



MagicMark XP Western Protein Standard
NuPAGE Bis-Tris gel, blotted to nitrocellulose, and detected with Invitrogen™ WesternBreeze™ Chemiluminescent Kit

Storage specifications

- Storage buffer: Tris-HCl (pH 6.8), DTT, glycerol, SDS, and bromophenol blue
- Storage conditions: upon receipt store at –20°C
- Stability: 4 months from date of receipt

Recommended products

The MagicMark XP Western Protein Standard is compatible with a broad range of gels—**NuPAGE Bis-Tris gels, Novex Tris-Glycine gels, Novex Tricine gels, NuPAGE Tris-Acetate gels,** and **Bolt Bis-Tris Plus gels.**

Learn more at thermofisher.com/westernblotstandard

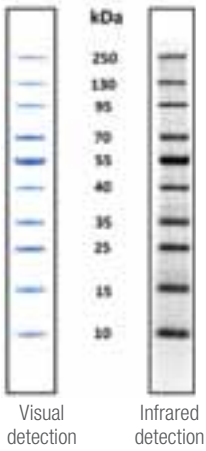
For ordering information refer to **page 86**.

PageRuler Prestained NIR Protein Ladder

Sharp prestained standard for near-IR fluorescent visualization and protein sizing

Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder is a mixture of 10 proteins that are stained blue and labeled with a fluorophore for near-infrared (NIR) fluorescent visualization and protein sizing following electrophoresis. The molecular weight markers in this ladder resolve into sharp bands when analyzed by SDS-PAGE. The 55 kDa band is of greater intensity and serves as a reference band.

- **Comprehensive**—10 protein bands spanning 11 to 250 kDa
- **Versatile**—visualize using instruments equipped for detection of near-infrared fluorescence such as certain Typhoon™ Imagers and the LI-COR Odyssey™ Infrared Imaging System; bands are directly visible because the proteins are prestained blue



PageRuler Prestained NIR Protein Ladder
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

Storage specifications

- Storage buffer: Tris-H_3PO_4, EDTA, SDS, DTT, sodium azide, bromophenol blue, and glycerol
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The PageRuler Prestained NIR Protein Ladder is recommended for visual detection, infrared imaging detection, and western blotting.

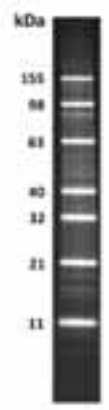
Learn more at thermofisher.com/specialtystandards

BenchMark Fluorescent Protein Standard

Efficient estimation of molecular weight by fluorescent detection

The Invitrogen™ BenchMark™ Fluorescent Protein Standard consists of Alexa Fluor™ 488 dye–conjugated proteins for molecular weight estimation of fluorescently labeled proteins.

- **Comprehensive**—consists of 7 distinct protein bands in the range of ~11–155 kDa
- **Versatile**—visualize on a UV transilluminator or laser-based scanning instrument after SDS-PAGE



BenchMark Fluorescent Protein Standard
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

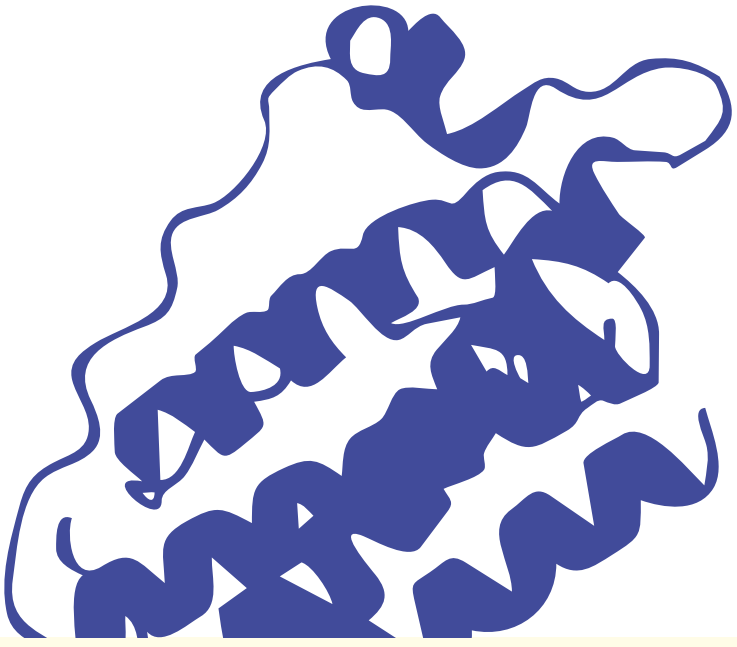
Storage specifications

- Storage buffer: Tris-HCl, SDS, glycerol, and Coomassie Blue G-250
- Storage conditions: upon receipt store at –20°C
- Stability: 6 months from date of receipt

Recommended products

The BenchMark Fluorescent Protein Standard is recommended for use with **NuPAGE gels** or **Novex Tris-Glycine gels**.

Learn more at thermofisher.com/specialtystandards



For ordering information refer to **page 86**.

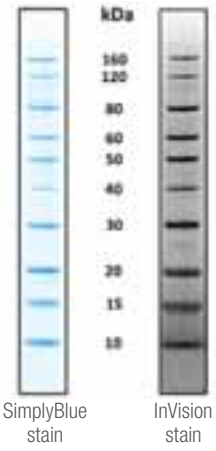
BenchMark His-tagged Protein Standard

Convenient detection and protein sizing of His-tagged proteins

The Invitrogen™ BenchMark™ His-tagged Protein Standard can be used as a positive control and for molecular weight sizing in His-tagged fusion protein detection. Each protein in the standard has a 6xHis tag.

- **Comprehensive**—10 sharp and clear bands from 10 to 160 kDa for molecular weight estimation of His-tagged proteins
- **Versatile**—can be visualized with Invitrogen™ InVision™ His-Tag In-Gel Stain or Coomassie R-250 stain on SDS-PAGE gels, or with Anti-His (C-term) Antibody using chromogenic or chemiluminescent detection systems

Learn more at thermofisher.com/specialtystandards



BenchMark His-tagged Protein Standard
NuPAGE 4–12% Bis-Tris Gel with MES SDS buffer

Storage specifications

- Storage buffer: Tris-HCl, SDS, glycerol, DTT, and Coomassie Blue G-250
- Storage conditions: upon receipt store at –20°C
- Stability: 6 months from date of receipt

Recommended products

The BenchMark His-tagged Protein Standard is recommended for use with **NuPAGE gels** and **Novex Tris-Glycine gels**.

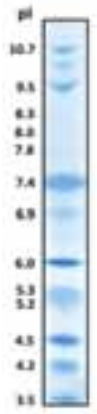
IEF Marker 3-10

Accurate determination of protein isoelectric points

The IEF Marker 3-10 is a ready-to-use protein standard developed for IEF applications. This marker can be used for monitoring of protein separation on IEF gels and pI determination of unknown protein samples.

- **Comprehensive**—13 purified isoforms from pI 3.5–10.7; no additional high range or low range markers are required
- **Versatile**—can be used for both native and denaturing conditions

Learn more at thermofisher.com/iefstandards



IEF Marker 3-10
Novex pH 3–10 Gel

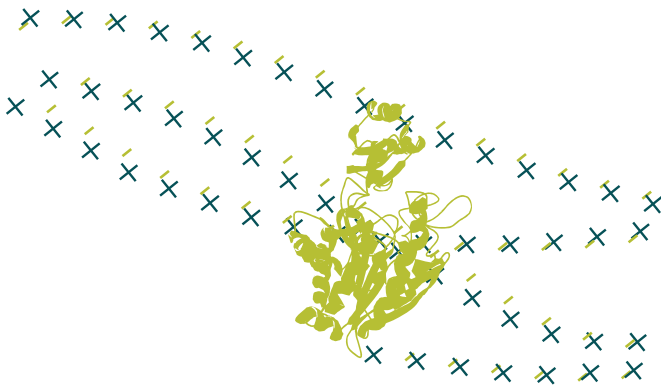
Storage specifications

- Storage buffer: 10% glycerol containing bromophenol blue (0.01%) and methyl red (0.01%)
- Storage conditions: upon receipt store at –20°C
- Stability: 1 year from date of receipt

Recommended products

The IEF Marker 3-10 is applicable to **all IEF gels** (vertical or horizontal).

Electrophoresis chambers and power supplies



Electrophoresis run considerations:

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 using 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.

For ordering information refer to **page 87**.

Which electrophoresis chamber system is right for you?

	Mini Gel Tank	XCell SureLock Mini-Cell	XCell4 SureLock Midi-Cell
			
Gel capacity	Up to 2 minigels	Up to 2 minigels (8 x 8 cm)	Up to 4 midigels (8 x 13 cm)
Cell dimensions (L x W x H)	32 x 11.5 x 16 cm (height with lid on)	14 x 13 x 16 cm (height with lid on)	21 x 19 x 16 cm (height with lid on)
Advantages	• The Mini Gel Tank is versatile and compatible with NuPAGE, Bolt, or Novex minigels. 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.	• XCell II Blot Module is available for semi-wet protein transfers • Instrument incorporates a gel tension wedge in place of the rear wedge used on earlier models	• Advanced apparatus for easier, more reliable electrophoresis with midigels

Learn more at thermofisher.com/electrophoresischambers

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 (Figure 24). The unique, side-by-side tank design allows you to perform electrophoresis of 1 or 2 minigels.

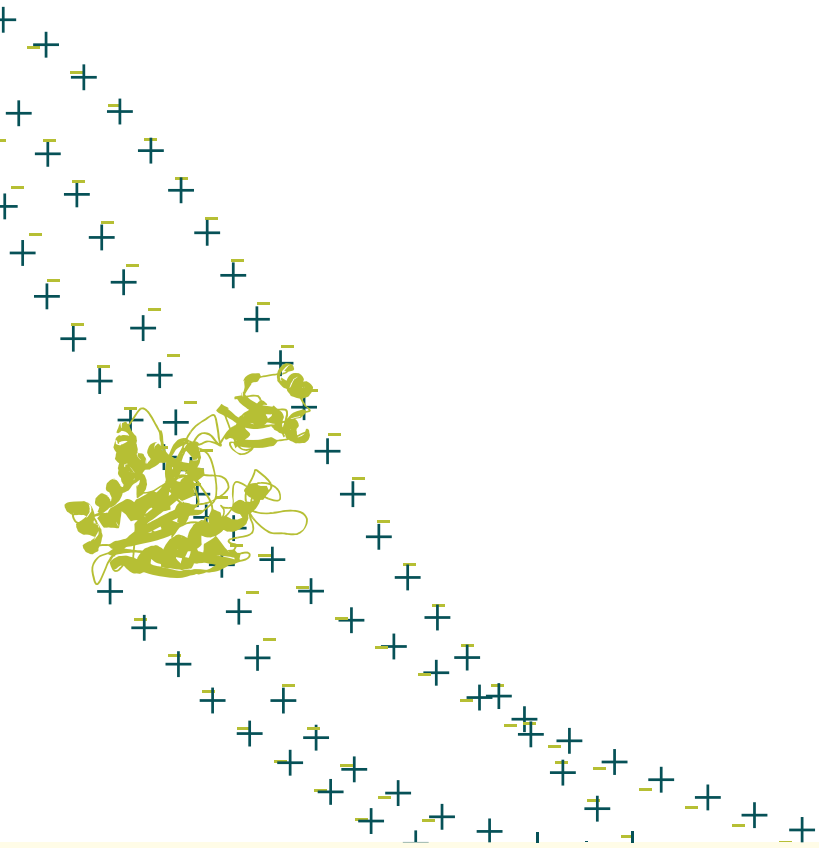
The Mini Gel Tank offers:

- **Versatility**—compatible with all of our minigels, including 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 minigels
- Cell size (L x W x H): 32 x 11.5 x 16 cm (height with lid on)
- Buffer requirement: 400 mL for each minigel chamber
- Material: polycarbonate
- Chemical resistance: not compatible with acetone, chlorinated hydrocarbons, or aromatic hydrocarbons

Watch our Mini Gel Tank video. 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 24. How to use the Mini Gel Tank.

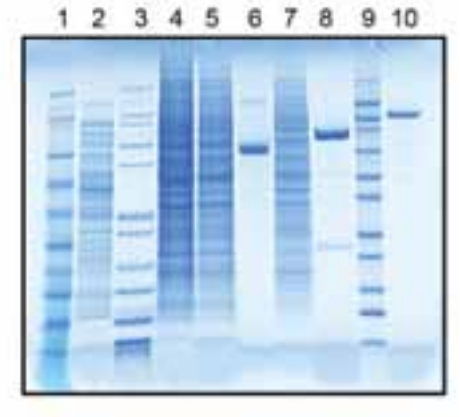


Figure 25. Electrophoresis of Bolt gel using the Mini Gel Tank. Protein standards and samples were loaded at 10 µL sample volumes in an Invitrogen™ Bolt™ 4–12% Bis-Tris Plus Gel. Electrophoresis was performed using the Mini Gel Tank at 200 V (constant). Sharp, straight bands with consistent migration patterns were observed after staining with 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.

Recommended products

The **Mini Blot Module** is a wet transfer device that conveniently fits into the chambers of the Mini Gel Tank to easily transfer proteins from minigels to nitrocellulose or PVDF membranes.

XCell SureLock Mini-Cell

Simultaneous electrophoresis of up to 2 minigels

The unique design of the Invitrogen™ XCell™ SureLock Mini-Cell allows you to run minigels quickly and easily without any clamps or grease (Figure 26). The tight seal provided by the gel tension wedge results in leak-free, consistent performance. The XCell SureLock Mini-Cell is compatible with NuPAGE, Novex, and specialty gels (Figure 27).

Key features of the XCell SureLock Mini-Cell:

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

Specifications

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

▶▶ Learn more at thermofisher.com/surelockmini

For ordering information refer to **page 87**.

1. Drop buffer core into the lower buffer chamber of the XCell SureLock Mini-Cell. Insert one minigel in front of the buffer core and a second minigel 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 26. How to use the XCell SureLock Mini-Cell.

NuPAGE Bis-Tris gel in XCell SureLock Mini-Cell

Figure 27. Electrophoresis of NuPAGE Bis-Tris gels 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:** Invitrogen™ Novex™ Sharp Protein Standard; and **Lane 10:** 5 µg β-galactosidase. Gel electrophoresis was performed at 200 V (constant) and gels were stained using SimplyBlue SafeStain. Images were acquired using a flatbed scanner.

Recommended products

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

XCell4 SureLock Midi-Cell

Simultaneous electrophoresis of up to 4 midigels

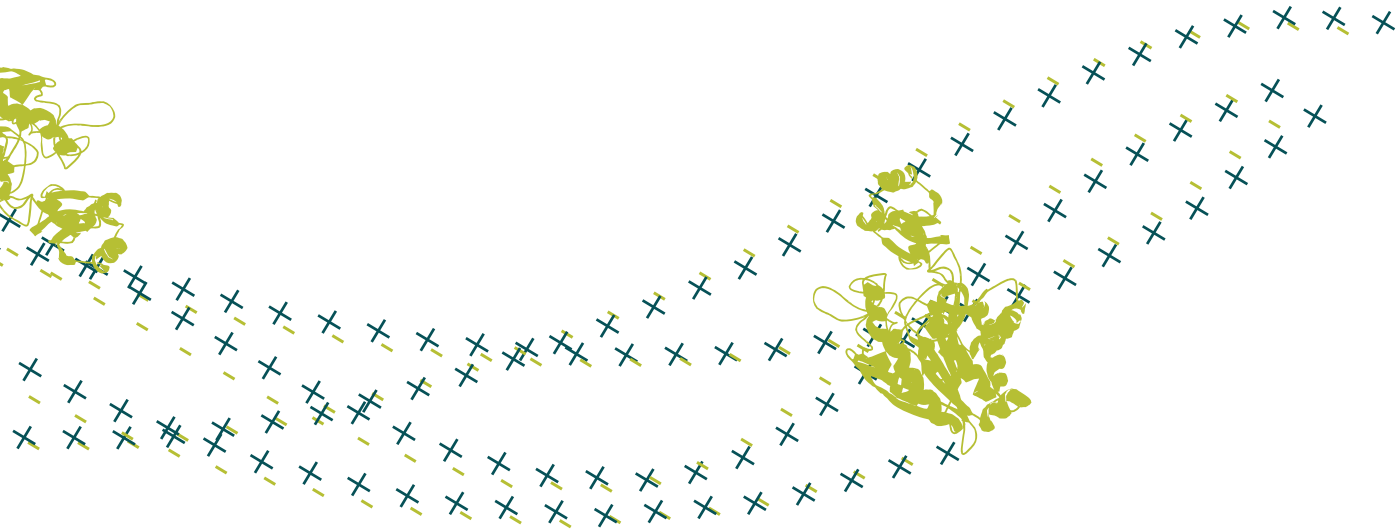
The Invitrogen™ XCell4 SureLock™ Midi-Cell allows simultaneous vertical electrophoresis of 1–4 midigels without leaking, enabling consistent performance. The system is designed to dissipate heat effectively and evenly, and enable high-resolution results when using Novex midigels (Figure 29).

Key features of the XCell4 SureLock Midi-Cell:

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

Specifications

- Gel capacity: up to 4 midigels (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, or aromatic hydrocarbons



▶▶ Learn more at thermofisher.com/surelockmidi

A.

B.

1. Insert the XCell4 SureLock 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 28. How to use the XCell4 SureLock Midi-Cell with 4 gels.

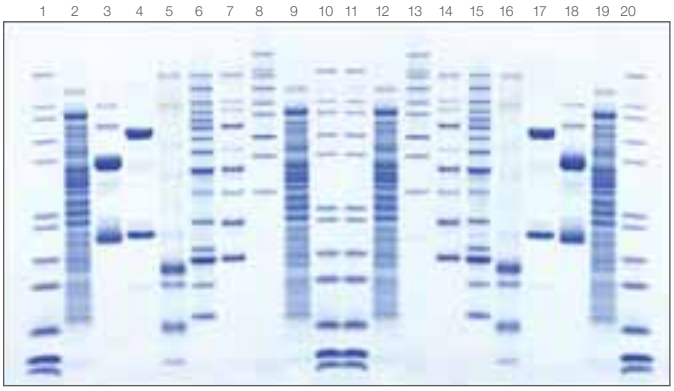


Figure 29. Quality of a precast NuPAGE Novex 4–12% Bis-Tris Midi Gel with a variety of protein standards, lysates and purified proteins. Electrophoresis was performed using MES running buffer and an XCell4 SureLock Midi Cell at 200 V (constant). Following electrophoresis, the gel was stained using SimplyBlue SafeStain, destained using water, and imaged using a flatbed scanner. Sharp, straight bands were observed. **Lanes 1, 10, 11, and 20** were each loaded with 5 µL of Mark12 Unstained Standard (blend of 12 purified proteins). **Lanes 2, 9, 12, and 19** were each loaded with 10 µg of *E. coli* lysate. **Lanes 3 and 18** were each loaded with 6 µg of human IgG. **Lanes 4 and 17** were each loaded with 6 µg of human IgM. **Lanes 5 and 16** were each loaded with 5 µL of SeeBlue Plus2 Prestained Protein Standard. **Lanes 6 and 15** were each loaded with 5 µL of BenchMark Protein Ladder. **Lanes 7 and 14** were each loaded with 15 µL of MagicMark XP Western Protein Standard. **Lanes 8 and 13** were each loaded with 5 µL of HiMark Unstained Protein Standard.

PowerEase 90W Power Supply

Simple, affordable power supply specifically for minigel electrophoresis

The Invitrogen™ PowerEase™ 90W Power Supply is designed specifically for minigel electrophoresis. The straightforward, intuitive interface makes the powering of gel runs a simple and easy process. In addition, the PowerEase 90W Power Supply features:

- **Constant voltage** or current settings
- **Built-in timer** for walk-away gel electrophoresis
- **Output jacks** that are compatible with most electrophoresis devices

Learn more at thermofisher.com/powerease

PowerEase 300W Power Supply

Programmable power supply designed for high-throughput gel electrophoresis

The Invitrogen™ PowerEase™ 300W Power Supply is a fully programmable power supply designed for high-throughput gel electrophoresis. The straightforward, intuitive interface makes the powering of gel runs a simple and easy process. In addition, the PowerEase 300W Power Supply features:

- **Constant voltage,** current, or power settings
- **Built-in timer** for walk-away gel electrophoresis
- **Up to 10 custom programs** with 10 steps each
- **Four sets of output jacks** that are compatible with most electrophoresis devices

For ordering information refer to **page 87**.

Run the gel

Table 4. Gel running conditions in electrophoresis chamber systems.

	Running conditions in XCell Surelock Mini-Cell				Running conditions in Mini Gel Tank			
	Voltage (V)	Starting current (mA)*	End current (mA)*	Approximate run time (minutes)	Voltage (V)	Starting current (mA)*	End current (mA)*	Approximate run time (minutes)
Bolt 4–12% (MES)	NA	NA	NA	NA	200	160	70	20
Bolt 4–12% (MOPS)	NA	NA	NA	NA	200	160	50	35
NuPAGE 4–12% Bis-Tris (MES)	200	100 to 125	60 to 80	35	200	160	90	30
NuPAGE 4–12% Bis-Tris (MOPS)	200	100 to 125	60 to 80	50	200	140	50	42
Novex 4–20% Tris-Glycine (denatured)	125	30 to 40	8 to 12	90	125	40	10	100
Novex 4–20% Tris-Glycine (native)	125	6 to 12	3 to 6	1 to 12 hours	125	30	10	90
NuPAGE 3–8% Tris-Acetate (denatured)	150	40 to 55	25 to 40	60	150	60	20	50
NuPAGE 3–8% Tris-Acetate (native)	150	18	7	2 to 3 hours	150	40	10	100
Novex 10–20% Tricine	125	80	40	90	125	110	40	65
NativePAGE 3–12%	150	12 to 16	2 to 4	90 to 115	150	10	<10	80
pH 3-10 IEF	100	7	NA	60	100	8	NA	60
	200	NA	NA	60	200	NA	NA	60
	500	NA	5	30	500	NA	5	30
10% Zymogram (Gelatin)	125	30 to 40	8 to 12	90	125	40	10	90

* Per gel.

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

Troubleshooting tips

XCell SureLock Mini-Cell troubleshooting

Observation	Cause	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 re-make if necessary.
	Voltage, current, or wattage is set at a higher limit	Decrease power conditions to recommended running conditions (see page 59).
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.

Mini Gel Tank troubleshooting

Observation	Cause	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 re-make if necessary.
	Current is set at a higher limit	Decrease current to recommended running conditions (see page 59).
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.

Electrophoresis troubleshooting

Problem	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.

Stain the gel

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, demands for improved sensitivity for small sample sizes and compatibility with downstream applications and detection instrumentation have driven the development of several basic staining methods. Each method has particular advantages and disadvantages, and a number of specific formulations of each type of method provide optimal performance for various situations.

Typically these stains can be classified broadly based on the dye or molecule that helps visualize the protein stains:

Coomassie stains

- Thermo Scientific™ PageBlue™ stain
- SimplyBlue SafeStain
- Thermo Scientific™ Imperial™ Protein Stain

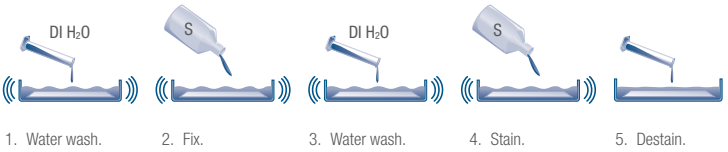
Silver stains

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

Fluorescent/specialty stains

- Invitrogen™ SYPRO™ Orange, Red, or Ruby gel stain
- Pierce Reversible stain for nitrocellulose or PVDF membranes
- Pro-Q™ Emerald Glycoprotein stain
- 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:



1. Water wash. 2. Fix. 3. Water wash. 4. Stain. 5. Destain.

- 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 dye or chemical to diffuse into the gel and bind (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 developer step to produce the colored reaction product.

Learn more at thermofisher.com/proteinstains

For ordering information refer to **page 87**.

Coomassie dye protein gel stains

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

The most common methods for in-gel protein detection use stains with Coomassie dye. These stains use either the G-250 (colloidal) or R-250 form of the dye (Table 6). 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 stain solution and destain to observe protein bands
- **Economical**—Coomassie dye–based stain formulations are cost effective
- **Flexible**—useful for qualitative visualization, quantitative densitometry, and gel excision and analysis by mass spectrometry

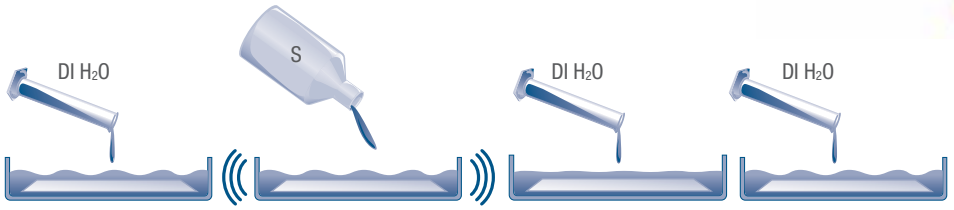
Our Coomassie stains provide sensitive protein detection along with simplified protocols. Example data and staining protocols are shown for SimplyBlue SafeStain (Figure 30, 33 and 34), PageBlue Protein Staining Solution (Figure 32), and Imperial Protein Stain (Figure 31 and 35).

Learn more at thermofisher.com/coomassiestains

Table 6. Coomassie dye–based protein gel stains.

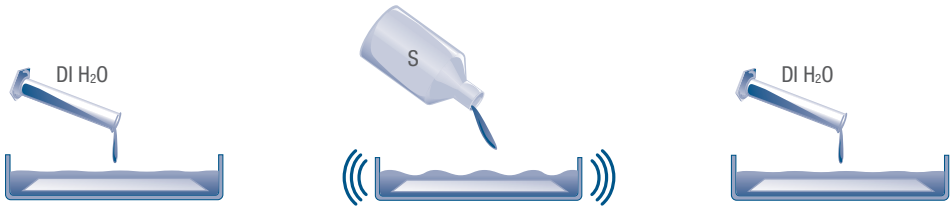
	SimplyBlue SafeStain	Imperial Protein Stain	PageBlue Protein Staining Solution
Type	G-250	R-250	G-250
Limit of detection	>7 ng	3 ng	5 ng
Time to stain (min)	12	60	60
Compatible with: PVDF membranes Nitrocellulose membranes	 Yes No	 Yes No	 Yes No
Reusable	No	No	Yes (up to 3x)
Mass spectrometry compatible	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 non-hazardous (does not require methanol or acetic acid fixatives or destains) staining	Fast, ultrasensitive protein detection	Cost-effective option for fast, sensitive staining

Protocols



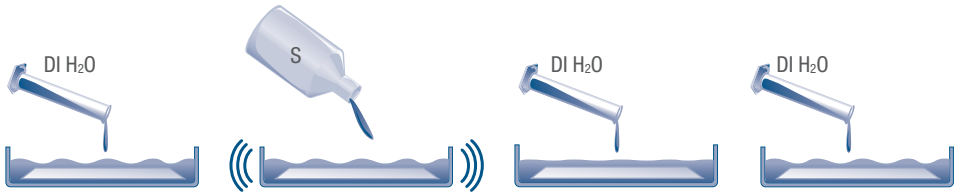
1. Wash the gel three times (5 minutes) with ultrapure water.
2. Add SimplyBlue SafeStain (1 hour).
3. Wash gel with 100 mL of DI water for 1 hour.
4. Additional water wash with 100 mL of DI water (1 hour) for increased sensitivity.

Figure 30. SimplyBlue SafeStain protocol.



1. Wash the gel three times with deionized water (15 minutes).
2. Add Imperial Protein Stain (5 minutes–1 hour).
3. Water destain (15 minutes–overnight).

Figure 31. Imperial Protein Stain protocol.



1. Wash the gel three times with ultrapure water (30 minutes).
2. Add PageBlue Protein Staining Solution (1 hour).
3. Rinse gel two times with ultrapure water (<1 minute)
4. Wash gel one time with ultrapure water (5 minutes)

Figure 32. PageBlue Protein Staining Solution protocol.

For ordering information refer to **page 87**.

Example data

Figure 33. Sensitive staining results with SimplyBlue SafeStain. The following samples were separated on a NuPAGE Novex 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 Mark12 Unstained Standard (blend of 12 purified proteins); **Lane 10:** 5 µL Mark12 Unstained Standard.

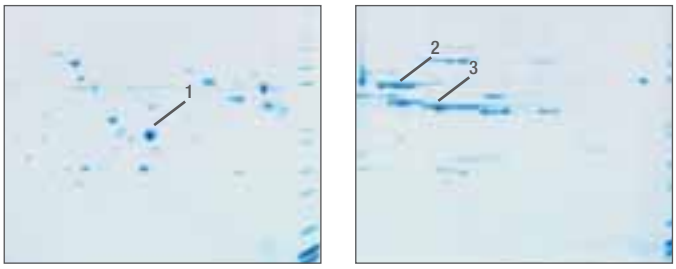


Figure 34. Two-dimensional electrophoresis (2DE) analysis of spinach chloroplast extract; staining with SimplyBlue SafeStain. Spinach chloroplast extract was prefractionated in the ZOOM™ IEF Fractionator and the individual fractions were then separated by 2DE using narrow pH range ZOOM™ Strips and NuPAGE™ Novex 4–12% Bis-Tris ZOOM™ Gels. Gels were Coomassie stained using SimplyBlue SafeStain.

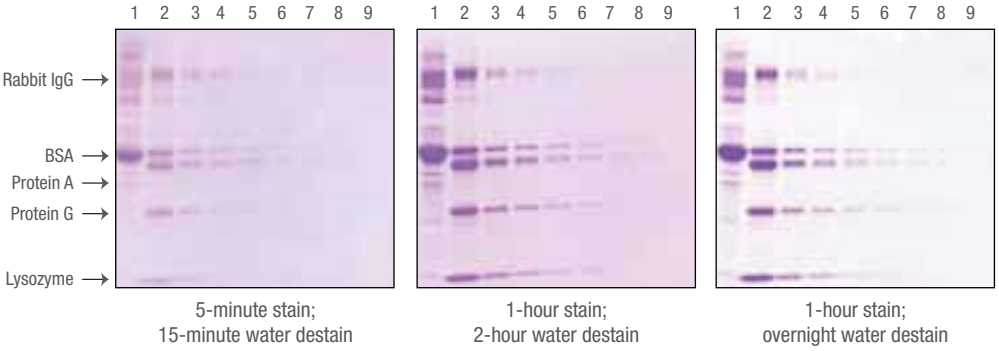


Figure 35. Enhanced sensitivity and clear background using Imperial Protein Stain. For even greater sensitivity and reduced background, gels can be stained with Imperial Protein Stain for 1 hour and washed with water from 1 hour to overnight. **Lane 1:** BSA only (6 µg); **Lane 2–9:** loaded left to right with 1,000, 200, 100, 50, 25, 12, 6, and 3 ng protein sample.

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.

Recommended products

The **Thermo Scientific Pierce Power Stainer** is designed for rapid Coomassie dye staining of proteins in up to two minigels and subsequent removal of unbound stain from the gel in a single step. Refer to page 72 of this brochure for more information.

Silver stains

Ultra-sensitive stains with optimized protocols, 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 sub-nanogram levels
- **Easy to use and flexible**—optimized for minimal steps and 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 (Table 7). The SilverXpress™ Silver Staining Kit provides nanogram-level sensitivity with minimal background (Figure 37), while the Pierce™ Silver Stain Kit provides protocol flexibility (Figure 38 and 39).

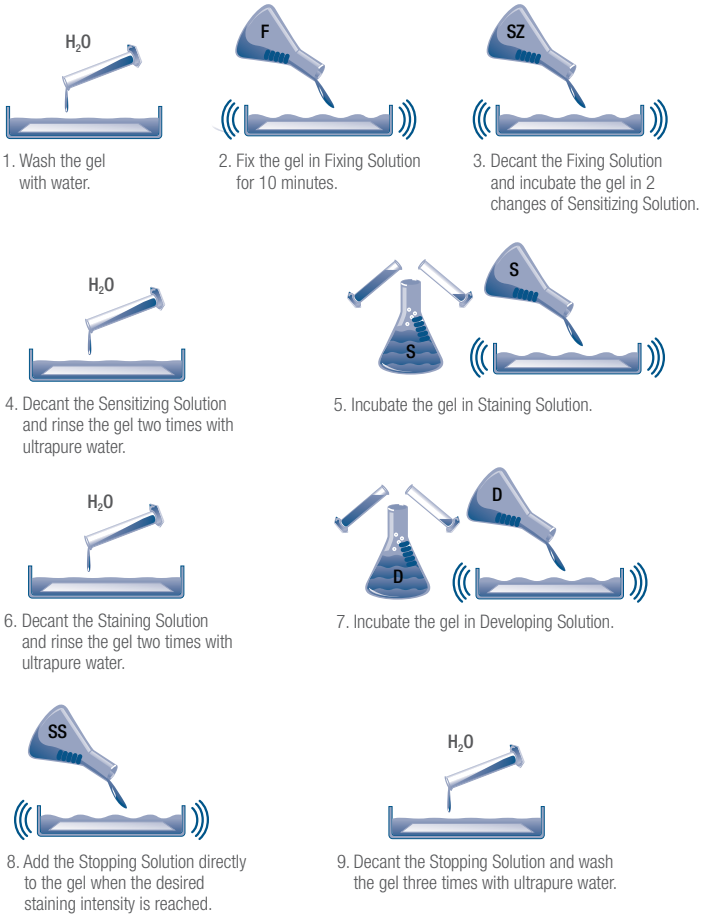
Learn more at thermofisher.com/silverstains

For ordering information refer to **page 87**.

Table 7. Silver stain kits.

	Pierce Silver Stain for Mass Spectrometry	Pierce Silver Stain Kit	SilverXpress Silver Staining Kit
Components (steps)	6 (17)	4 (15)	5 (13)
Time required	1 hr 13 min	2 hr 25 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 typsin 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 to overnight) and staining (5 min to 20 hours)	• Nanogram-level sensitivity for silver staining with minimal background

Protocols and example data



1. Wash the gel with water.
2. Fix the gel in Fixing Solution for 10 minutes.
3. Decant the Fixing Solution and incubate the gel in 2 changes of Sensitizing Solution.
4. Decant the Sensitizing Solution and rinse the gel two times with ultrapure water.
5. Incubate the gel in Staining Solution.
6. Decant the Staining Solution and rinse the gel two times with ultrapure water.
7. Incubate the gel in Developing Solution.
8. Add the Stopping Solution directly to the gel when the desired staining intensity is reached.
9. Decant the Stopping Solution and wash the gel three times with ultrapure water.

Figure 36. SilverXpress Silver Staining Kit protocol.

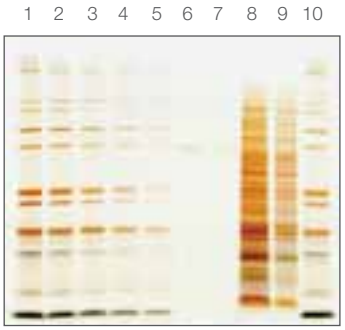


Figure 37. Crystal clear background with the SilverXpress Kit. Samples were separated on a NuPAGE Novex 4-12% Bis-Tris gel and stained with the SilverXpress Kit. **Lanes 1, 10:** Mark12 Unstained Standard (blend of 12 purified proteins) diluted 1:4; **Lane 2:** Mark12 Unstained Standard diluted 1:8; **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.

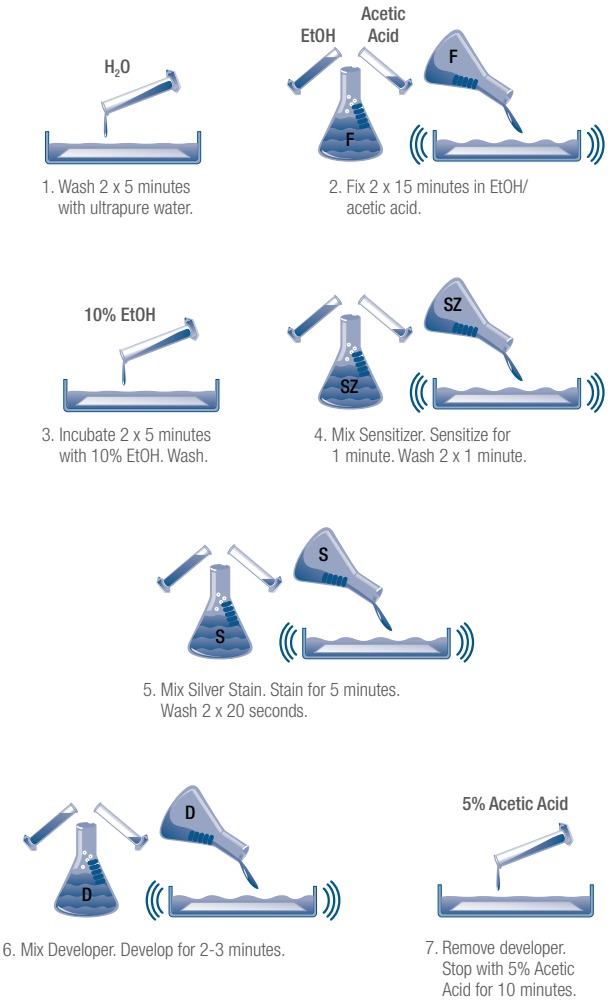


Figure 38. Pierce Silver Stain Kit protocol.

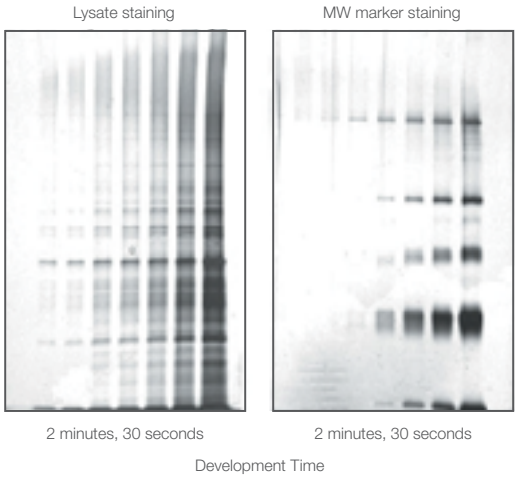


Figure 39. Pierce Silver Stain Kit exhibits excellent senstivity. In standard minigels, proteins are detectable at greater than 0.25 ng per band or spot.

For ordering information refer to **page 87**.

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 that obtained with silver staining techniques. Invitrogen™ SYPRO™ protein stains are easy-to-use fluorescent stains for the detection of proteins separated by PAGE (Table 8). 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

Learn more at thermofisher.com/fluorescentstains

Recommended products

For optimal sensitivity with Polaroid™ film, **SYPRO™ Photographic Filter** is recommended.

Table 8. 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 and on-membrane reversible protein staining kits (Table 9).

Learn more at thermofisher.com/specialtystains

Table 9. 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

For ordering information refer to **page 87**.

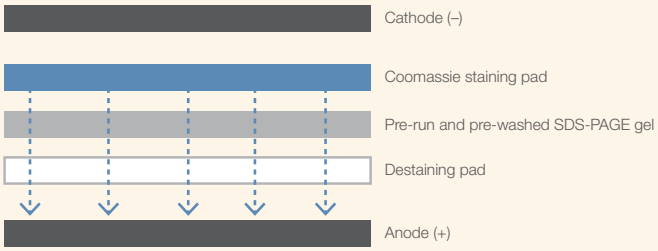
Stain the gel

Electrophoretic staining technology—Pierce Power Stainer

The Thermo Scientific Pierce Power Stainer consists of a Thermo Scientific™ Pierce™ Power Station with activated Staining Software and a Thermo Scientific™ Pierce™ Power Stain Cassette. It is designed for rapid Coomassie staining and destaining of proteins in polyacrylamide gels. Traditional Coomassie staining techniques require one hour to overnight staining and destaining to achieve desired results. When used in conjunction with Thermo Scientific™ Pierce™ Midi and Mini Gel Power Staining Kits, the Pierce Power Stainer is designed to provide staining efficiency in as few as 6 minutes that is equivalent to, or better than, traditional Coomassie staining techniques.

Good to know

How does electrostaining work?



The significant reduction in protein staining time is accomplished by utilizing inonic Power Stain Solution and Destain Solution to electrophoretically drive the negatively charged Coomassie R-250 dye out of the top gel pad, through the polyacrylamide gel matrix and the bottom gel pad, and toward the positively charged anode.

Watch our Pierce Power Stainer video.
thermofisher.com/powerstainer

Pierce Power Stainer

Rapid Coomassie dye staining and destaining in approximately 10 minutes

The Thermo Scientific™ Pierce™ Power Stainer is designed for rapid Coomassie dye staining of proteins in polyacrylamide gels and subsequent removal of unbound stains to give sharply stained protein bands with minimal or no background.

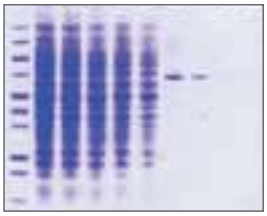
The Pierce Power Stainer offers:

- **Speed**—Coomassie dye staining and destaining of proteins in about 10 minutes
- **Convenience**—simultaneously stain and destain 1–2 minigels or 1 midigel
- **Reliable performance**—enables staining results that are equivalent to traditional staining techniques
- **Easy touch programming**—intuitive LCD touch-screen interface includes preprogrammed protocols

Specifications

- Mode of transfer: semi-dry blotting
- Gel compatibility: SDS-PAGE gels
- Running dimension: horizontal
- Platform: Pierce™ Power System

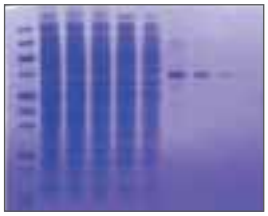
Pierce Power Stainer



Total time: 11 minutes

1. Wash gel 1 × 5 minutes in water
2. Power Stain/Destain gel, 6 minutes

Conventional Coomassie stain



Total time: 230 minutes to overnight

1. Wash gel 3 × 10 minutes in water
2. Incubate gel in Coomassie stain solution* for 60 minutes
3. Wash gel 2 × 10 minutes in water
4. Destain gel in destaining solution** for 3 × 20 minutes
5. Incubate gel in water for 60 minutes to overnight

*Coomassie stain solution: 45% methanol, 10% acetic acid, 0.25% Coomassie R-250

**Destain solution: 30% ethanol, 5% acetic acid

Figure 40. Pierce Power Stainer saves time and maintains sensitivity.

Learn more at thermofisher.com/powerstainer

Recommended products

The **Pierce Power System** can be used both for fast Coomassie dye staining of protein gels and for rapid semi-dry transfer of proteins from gel to membrane. The Pierce Power Stainer can be upgraded by adding the **Pierce™ Power Blot Cassette** to make a fully functional **Pierce Power System** with blotting and staining capabilities.

Thermo Scientific Pierce Power Stainer

Thermo Scientific Pierce Power Blotter

Did you know?

Conventional Coomassie dye–based staining techniques require 1 hour to overnight incubation.

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 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) which 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.

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 byproduct. With the chemiluminescent substrate, the light output can be captured on film or CCD camera. In recent years fluorescent detection became a popular alternative to the 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. Whatever substrate is 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	Thermo Scientific Pierce Power Blotter	iBlot™ 2 Dry Blotting System

Key products for western blot detection include:

Automated detection	Manual detection
	Blocking buffers Wash buffers Detergents Enhancers Substrates Stripping buffers X-ray film
iBind™ Flex Western Device	

Learn more at thermofisher.com/western

Protocol quick reference

QUICK REFERENCE

Bolt™ Mini Gels

Instructions for performing electrophoresis using Bolt™ Mini Gels are described below.

Prepare samples

Reagent	Reduced Sample	Non-reduced Sample
Sample	x µL	x µL
Bolt™ LDS Sample Buffer (4X)	10 µL	10 µL
Bolt™ Reducing Agent (10X)	4 µL	—
Deionized Water	to 26 µL	to 30 µL
Total Volume	40 µL*	40 µL*

Heat samples at 70˚C for 10 minutes.
* Scale samples up or down by adjusting all volumes proportionally.

Prepare 1X Buffer

Each chamber of the tank requires 400 mL of 1X SDS Running Buffer (mix 20 mL of 20X Bolt™ MES or MOPS SDS Running Buffer with 380 mL of deionized water). The same buffer type must be used for both chambers.

Run conditions

Run Bolt™ Mini Gels at constant voltage (1 or 2 mini gels).

Running Buffer	Standard Run	Run Time*
MES	200 V	22 min
MOPS	200 V	32 min

* Run times may vary depending upon gel type and power supply.

For research use only. Not for use in diagnostic procedures.

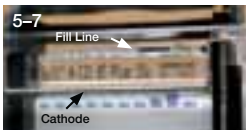
Bolt™ Mini Gels

Prepare gel and tank

1. Cut open the gel cassette pouch and remove the cassette.
2. Remove the gel comb and rinse wells 3 times with 1X Running Buffer.
3. Remove the tape covering the slot at the lower portion of the cassette.

Load samples

1. Pre-fill the chamber with 1X Running Buffer to the level of the cathode.
2. Place the cassette in the chamber with the wells facing towards you. Hold the cassette in a raised position and close the cassette clamp.
3. Fill all wells with 1X Running Buffer.
4. Load your samples and markers.
5. Hold the cassette and release the cassette clamp.
6. Gently lower the cassette to the bottom of the chamber, and close the cassette clamp
7. Add 1X buffer to the level of the fill line.



QUICK REFERENCE

NuPAGE Bis-Tris Mini Gels

Instructions for electrophoresis using the XCell SureLock Mini-Cell are described below.

Prepare Samples

Reagent	Reduced Sample	Non-reduced Sample
Sample	x µL	x µL
NuPAGE LDS Sample Buffer (4X)	2.5 µL	2.5 µL
NuPAGE Reducing Agent (10X)	1 µL	--
Deionized Water	to 6.5 µL	to 7.5 µL
Total Volume	10 µL	10 µL

Heat samples at 70˚C for 10 minutes.

Prepare 1X Buffer

Add 50 mL 20X NuPAGE MES or MOPS SDS Running Buffer to 950 mL deionized water to prepare 1X SDS Running Buffer.

Load Sample

Load the appropriate concentration of your protein sample on the gel.

Load Buffer

Fill the Upper (200 mL) and Lower (600 mL) Buffer Chambers with the appropriate 1X Running Buffer. **For reduced samples**, use 200 mL 1X Running Buffer with 500 µL NuPAGE Antioxidant in the Upper Buffer Chamber.

Run Conditions

Voltage:	200 V constant
Run Time:	35 minutes (MES Buffer), 50 minutes (MOPS Buffer)
Expected Current:	100–125 mA/gel (start); 60–80 mA/gel (end)

Intended Use: For research use only. Not for human or animal therapeutic or diagnostic use.

NuPAGE Tris-Acetate Mini Gels

Prepare Samples

Reagent	Denaturing Sample*	Native Sample
Sample	x µL	x µL
NuPAGE LDS Sample Buffer (4X)	2.5 µL	--
Tris-Glycine Native Sample Buffer (2X)	--	5 µL
Deionized Water	to 7.5 µL	to 5 µL
Total Volume	10 µL	10 µL
Samples	Heat samples at 70˚C for 10 minutes	**Do not heat**

*For reduced samples, add NuPAGE Reducing Agent (10X) to 1X.

Prepare 1X Buffer

Denaturing Samples: Add 50 mL 20X NuPAGE Tris-Acetate SDS Running Buffer to 950 mL deionized water. **Native Samples:** Add 100 mL 10X Tris-Glycine Native Running Buffer to 900 mL deionized water.

Load Sample

Load the appropriate concentration of your protein sample on the gel.

Load Buffer

Fill the Upper (200 mL) and Lower (600 mL) Buffer Chambers with the appropriate 1X Running Buffer. **For reduced samples**, use 200 mL 1X Running Buffer with 500 µL NuPAGE Antioxidant in the Upper Buffer Chamber.

Run Conditions

Voltage:	150 V constant
Run Time:	1 hour (Denaturing gel), 2–3 hours (Native gel)
Expected Current:	40–55 mA/gel (start); 25–40 mA/gel (end) for denaturing gel 18 mA/gel (start); 7 mA/gel (end) for native gel

QUICK REFERENCE

NuPAGE Bis-Tris Midi Gels

Instructions for electrophoresis of Bis-Tris Gels using the XCell4 SureLock Midi-Cell are described below.

Prepare Samples

Reagent	Reduced sample	Non-reduced sample
Sample	x µL	x µL
NuPAGE LDS Sample Buffer (4X)	2.5 µL	2.5 µL
NuPAGE Reducing Agent (10X)	1 µL	—
Deionized Water	to 10 µL final	to 10 µL final

Heat samples at 70°C for 10 minutes.

Prepare 1X Buffer
Add 50 mL 20X NuPAGE MES or MOPS SDS Running Buffer to 950 mL deionized water to prepare 1X SDS Running Buffer.

Load Sample
Load the appropriate concentration of your protein sample on the gel.

Add Buffer
Fill Upper Buffer Chamber with 175 mL 1X NuPAGE SDS Running Buffer.
For reduced samples, use 175 mL 1X NuPAGE SDS Running Buffer with 435 µL NuPAGE Antioxidant in the Upper Buffer Chamber. Add a sufficient volume of 1X NuPAGE SDS Running Buffer to the Lower Buffer Chamber.

Run Conditions

Voltage:	200 V constant
Run Time:	40 min (MES Buffer), 55 min (MOPS Buffer)
Expected Current:	160-200 mA/gel (start); 120-170 mA/gel (end)

For Research Use Only. Not for use in diagnostic procedures.

NuPAGE Tris-Acetate Midi Gels

Prepare Samples

Reagent	Denaturing Sample	Native Sample
Sample	x µL	x µL
NuPAGE LDS Sample Buffer (4X)	2.5 µL	—
Tris-Glycine Native Sample Buffer (2X)	—	5 µL
NuPAGE Reducing Agent (10X)*	1 µL	—
Deionized Water	to 10 µL final	to 10 µL final

*For reduced samples only.

Heat denaturing samples at 70°C for 10 minutes. **Do not heat native samples.**

Prepare 1X Buffer
Denaturing samples: Add 50 mL 20X NuPAGE Tris-Acetate SDS Running Buffer to 950 mL deionized water. **Native samples**: Add 100 mL 10X Tris-Glycine Native Running Buffer to 900 mL deionized water.

Load Sample
Load the appropriate concentration of your protein sample on the gel.

Add Buffer
Fill Upper Buffer Chamber with 175 mL of the appropriate 1X Running Buffer.
For reduced samples, use 175 mL 1X Running Buffer with 435 µL NuPAGE Antioxidant in the Upper Buffer Chamber. Add a sufficient volume of Running Buffer the Lower Buffer Chamber.

Run Conditions

Voltage:	150 V constant
Run Time:	70 min (denaturing gel), 2–3 hours (native gel)
Expected Current:	70-90 mA/gel (start); 50-60 mA/gel (end); (denaturing gel)
	40-45 mA/gel (start); 15-20 mA/gel (end); (native gel)

QUICK REFERENCE

Novex Tris-Glycine Mini Gels

Instructions are provided below for electrophoresis of Novex Tris-Glycine Gels using the XCellSureLock Mini-Cell.

Denaturing Electrophoresis

Prepare Samples

Reagent	Reduced Sample	Non-reduced Sample
Sample	x µL	x µL
Tris-Glycine SDS Sample Buffer (2X)	5 µL	5 µL
NuPAGE Reducing Agent (10X)	1 µL	--
Deionized Water	to 4 µL	to 5 µL
Total Volume	10 µL	10 µL

Heat samples at 85°C for 2 minutes.

Prepare 1X Buffer
Add 100 mL 10X Novex Tris-Glycine SDS Running Buffer to 900 mL de-ionized water to prepare 1X Tris-Glycine SDS Running Buffer.

Load Sample
Load the appropriate concentration of your protein sample on the gel.

Load Buffer
Fill the Upper Buffer Chamber with 200 mL and the Lower Buffer Chamber with 600 mL of 1X Tris-Glycine SDS Running Buffer.

Run Conditions

Voltage:	125 V constant
Run Time:	90 minutes (dependent on gel percentage)
Expected Current:	30–40 mA/gel (start); 8–12 mA/gel (end)

Intended Use: For research use only. Not for human or animal therapeutic or diagnostic use.

Novex Tris-Glycine Mini Gels

Non-Denaturing (Native) Electrophoresis

Prepare Samples

Reagent	Sample
Sample	x µL
Tris-Glycine Native Sample Buffer (2X)	5 µL
Deionized Water	to 5 µL
Total Volume	10 µL

Do not heat samples for native electrophoresis.

Prepare 1X Buffer
Add 100 mL 10X Tris-Glycine Native Running Buffer to 900 mL deionized water to prepare 1X Tris-Glycine Native Running Buffer.

Load Sample
Load the appropriate concentration of your protein sample on the gel.

Load Buffer
Fill the Upper Buffer Chamber with 200 mL and the Lower Buffer Chamber with 600 mL of 1X Tris-Glycine Native Running Buffer.

Run Conditions

Voltage:	125 V constant
Run Time:	1–12 hours
Expected Current:	6–12 mA/gel (start); 3–6 mA/gel (end)

Blot Gel
For blotting denaturing and native gels, use 1X Tris-Glycine Transfer Buffer with 20% methanol. Perform blotting at 25 V constant for 1–2 hours using the XCell II Blot Module. The expected start current is 100 mA.

Protocol quick reference

QUICK REFERENCE

Tris-Glycine Midi Gels

Instructions for electrophoresis using the XCell4 SureLock Midi-Cell are described below.

Prepare Samples

Reagent	Reduced sample	Non-reduced sample
Sample	x µL	x µL
Tris-Glycine SDS Sample Buffer (2X)	5 µL	5 µL
NuPAGE Reducing Agent (10X)	1 µL	—
Deionized Water	to 10 µL final	to 10 µL final

Heat samples at 85°C for 2 minutes.

Prepare 1X Buffer

Add 100 mL 10X Tris-Glycine SDS Running Buffer to 900 mL deionized water to prepare 1X Tris-Glycine SDS Running Buffer.

Load Sample

Load the appropriate concentration of your protein sample on the gel.

Add Buffer

Fill each Upper Buffer Chamber with 175 mL 1X Tris-Glycine SDS Running Buffer. Fill the Lower Buffer Chamber up to the fill line mark with 1X Tris-Glycine SDS Running Buffer.

Run Conditions

Voltage:	125 V constant
Run Time:	105 min (dependent on gel percentage)
Expected Current:	40–50 mA/gel (start); 20–25 mA/gel (end)

For Research Use Only. Not for use in diagnostic procedures.

Non-denaturing (Native) Electrophoresis

Prepare Samples

Reagent	Native Sample
Sample	x µL
Tris-Glycine Native Sample Buffer (2X)	5 µL
Deionized Water	to 10 µL final

Do not heat native samples

Prepare 1X Buffer

Add 100 mL 10X Tris-Glycine SDS Running Buffer to 900 mL deionized water to prepare 1X Tris-Glycine SDS Running Buffer.

Load Sample

Load the appropriate concentration of your protein sample on the gel.

Add Buffer

Fill each Upper Buffer Chamber with 175 mL of 1X Tris-Glycine Native Running Buffer. Fill the Lower Buffer Chamber up to the fill line mark with 1X Tris-Glycine Native Running Buffer.

Run Conditions

Voltage:	125V constant
Run Time:	1–12 hours
Expected Current:	35–40 mA/gel (start); 15–20 mA/gel

QUICK REFERENCE

NativePAGE™ Bis-Tris Gels

Instructions are provided below for electrophoresis of NativePAGE™ Bis-Tris Gels using the XCell SureLock Mini-Cell.

Prepare Samples

Reagent	Sample with detergent	Detergent-free sample
Sample	*x* µL	*x* µL
NativePAGE™ Sample Buffer (4X)	2.5 µL	2.5 µL
NativePAGE™ 5% G-250 Additive	0.25–1 µL*	—
Deionized Water	to 10 µL	to 10 µL

Do not heat samples for native gel electrophoresis.

*Ensure the final G-250 concentration is ¼th the detergent concentration.

Prepare 1X Running Buffer

1X NativePAGE™ Anode Buffer: Add 50 mL 20X NativePAGE™ Running Buffer to 950 mL deionized water.

1X NativePAGE™ Cathode Buffer: Add 50 mL 20X NativePAGE™ Running Buffer and 50 mL 20X NativePAGE™ Cathode Additive to 900 mL deionized water.

Load Sample

Fill wells with 1X NativePAGE™ Cathode Buffer and load samples prior to filling the cathode chamber. Load an appropriate amount of your sample on the gel.

Add Buffer

Fill the Upper Buffer Chamber with ~200 mL 1X NativePAGE™ Cathode Buffer. Fill the Lower Buffer Chamber with ~550 mL 1X NativePAGE™ Anode Buffer.

Run Conditions

Voltage:	150 V constant
Run Time:	90–115 min (3–12% gel); 105–120 min (4–16% gel)
Expected Current:	12–16 mA/gel (start); 2–4 mA/gel (end)

For Research Use Only. Not for use in diagnostic procedures.

Staining Protocol

A quick staining protocol for NativePAGE Gels using the Coomassie G-250 from the sample additive is described below. The total staining time is ~2–3 hours. Sensitivity is ~60 ng BSA.

Step	Action	Time
1	Place the gel in 100 mL fixing solution (40% methanol, 10% acetic acid) and microwave on high (950–1100 watts).	45 seconds
2	Shake the gel on an orbital shaker.	15 minutes
3	Discard fixing solution.	—
4	Place the gel in 100 mL destain solution (8% acetic acid) and microwave on high (950–1100 watts).	45 seconds
5	Shake the gel on an orbital shaker until the desired background is obtained.	—

QUICK REFERENCE

Tricine Gels

Instructions are provided below for electrophoresis of Tricine Gels using the XCell SureLock Mini-Cell.

Prepare Samples

Reagent	Reduced Sample	Non-reduced Sample
Sample	x µL	x µL
Tricine SDS Sample Buffer (2X)	5 µL	5 µL
NuPAGE Reducing Agent (10X)	1 µL	--
Deionized Water	to 4 µL	to 5 µL
Total Volume	10 µL	10 µL

Heat samples at 85°C for 2 minutes.

Prepare 1X Buffer
Add 100 mL 10X Novex Tricine SDS Running Buffer to 900 mL deionized water to prepare 1X Tricine SDS Running Buffer.

Load Sample
Load the appropriate concentration of your protein sample on the gel.

Load Buffer
Fill the Upper Buffer Chamber with 200 mL and the Lower Buffer Chamber with 600 mL of 1X Tricine SDS Running Buffer.

Run Conditions

Voltage:	125 V constant
Run Time:	90 minutes (dependent on gel percentage)
Expected Current:	80 mA/gel (start); 40 mA/gel (end)

For research use only. Not for human or animal therapeutic or diagnostic use.

Tricine Gels

Blotting Conditions
For blotting Tricine gels, use 1X Tris-Glycine Transfer Buffer with 20% methanol. Perform transfer with nitrocellulose or PVDF membranes at 25 V constant for 1–2 hours using the XCell II Blot Module. The expected start current is 100 mA.

Alternate Transfer Buffers
The Tris-Glycine Transfer Buffer interferes with protein sequencing. If you are performing protein sequencing, use 1X NuPAGE Transfer Buffer or 0.5X TBE Transfer Buffer for blotting.
The NuPAGE Transfer Buffer protects against modification of the amino acid side chains and is compatible with N-terminal protein sequencing using Edman degradation.

QUICK REFERENCE

IEF Gels

Instructions are provided below for electrophoresis of IEF Gels using the XCell SureLock Mini-Cell.

Prepare Samples

Reagent	Sample
Sample	x µL
IEF Sample Buffer pH 3–10 or pH 3–7 (2X)	5 µL
Deionized Water	to 10 µL final

Prepare 1X Buffer
1X IEF Anode Buffer: Add 20 mL 50X IEF Anode Buffer to 980 mL deionized water. Chill to 4°C to 10°C.
1X IEF Cathode Buffer: Add 20 mL IEF Cathode Buffer pH 3–10 (10X) or pH 3–7 (10X) to 180 mL deionized water. Chill to 4°C to 10°C.

Load Sample
Load the appropriate concentration and volume of your protein on the gel.

Add Buffer
Fill the Upper Buffer Chamber with **chilled** 200 mL 1X IEF Cathode Buffer and the Lower Buffer Chamber with **chilled** 600 mL 1X IEF Anode Buffer.

Run Conditions

Voltage:	100 V constant for 1 hour 200 V constant for 1 hour 500 V constant for 30 minutes
Expected Current:	7 mA/gel (start); 5 mA/gel (end)

Stain Gel
Fix the IEF gel in 12% TCA or 12% TCA containing 3.5% sulfosalicylic acid for 30 minutes. Stain the IEF gel with method of choice.

For Research Use Only. Not for use in diagnostic procedures.

IEF Gels

Prepare for 2D SDS/ PAGE

1. Stain and destain the IEF gel. Incubate the IEF gel in 100 mL 20% ethanol for 10 minutes.
2. Cut out the desired lane (strip) from the gel for transfer to a SDS gel.
3. Incubate the gel strip in 2 mL 2X SDS sample buffer and 0.5 mL ethanol for 3–5 minutes. Aspirate the sample buffer and rinse the gel strip with 1X SDS Running Buffer.
4. Fill the SDS gel cassette with 1X SDS Running Buffer.
5. Trim the IEF gel strip to a length of 5.8–5.9 cm.

6a. Transfer the gel strip into a **1.0 mm** SDS gel by sliding the strip into the 2D-well using a gel loading tip. Avoid trapping air-bubbles between the strip and the SDS gel. Wet a piece of thick filter paper (5.8 cm × 4 cm) in SDS Running Buffer and insert the long edge of the paper into the 2D-well to push the gel strip into contact with the SDS gel.

6b. If transferring the gel strip into a **1.5 mm** SDS gel, wet 2 pieces of thin filter paper (5.8 cm × 4 cm) in 1X SDS Running Buffer. Sandwich the gel strip between the two filter papers along the long edge with the edge of the strip protruding ~0.5 mm beyond the paper. Insert the sandwich into the 2D-well of the SDS gel without trapping air bubbles and push the strip down so it is in contact with the SDS gel.

7. Insert gel into the mini-cell, fill the buffer chambers with 1X SDS Running Buffer, and perform SDS-PAGE.
8. After the dye front has moved into the stacking gel (~10 minutes), disconnect power, remove the paper, and resume electrophoresis.

QUICK REFERENCE

Zymogram Gels

Instructions are provided below for electrophoresis of Zymogram Gels using the XCell *SureLock* Mini-Cell.

Prepare Samples

Reagent	Sample
Sample	*x* µL
Tris-Glycine SDS Sample Buffer (2X)	5 µL
Deionized Water	to 10 µL final

Do not heat or reduce samples for Zymogram gels.

Prepare 1X Buffer

1X Tris-Glycine SDS Running Buffer: Add 100 mL 10X Tris-Glycine SDS Running Buffer to 900 mL deionized water.

Load Sample

Load the appropriate concentration and volume of your protein on the gel.

Add Buffer

Fill the Upper Buffer Chamber with 200 mL, and the Lower Buffer Chamber with 600 mL of 1X Tris-Glycine SDS Running Buffer.

Run Conditions

Voltage:	125 V constant
Run Time:	90 minutes (dependent on gel percentage)
Expected Current:	30–40 mA/gel (start); 8–12 mA/gel (end)

For Research Use Only. Not for use in diagnostic procedures.

Zymogram Gels

Develop Gel

1. Dilute Zymogram Renaturing Buffer (10X) and Zymogram Developing Buffer (10X) 1:9 with deionized water. Prepare 100 mL of each 1X buffer per 1–2 mini-gels.
2. After electrophoresis, incubate the gel in 1X Zymogram Renaturing Buffer for 30 minutes at room temperature with gentle agitation.
3. Decant Zymogram Renaturing Buffer and add 1X Zymogram Developing Buffer to the gel.
4. Equilibrate the gel for 30 minutes at room temperature with gentle agitation.
5. Decant buffer and add fresh 1X Zymogram Developing Buffer to the gel.
6. Incubate the gel at 37°C for at least 4 hours or overnight for maximum sensitivity. Optimize results empirically by varying the sample load or incubation time.

Stain Gel

Zymogram (Blue Casein) 4–16% gels do not require staining. For non-pre-stained Zymogram gels, stain the gels with Colloidal Blue Staining Kit or the SimplyBlue Safestain. Areas of protease activity appear as clear bands against a dark background.

Sensitivity Level

10% Zymogram (Gelatin) Gel:	10^{-6} units of collagenase
12% Zymogram (Casein) Gel:	7×10^{-4} units of trypsin
4–16% Zymogram (Blue Casein) Gel:	1.5×10^{-3} units of trypsin

Ordering information

Product	Quantity	Cat. No.
Select precast gel Bolt Bis-Tris Plus Gels		
Bolt™ 8% Bis-Tris Plus Gels, 10-well	1 box	NW00080BOX
Bolt™ 8% Bis-Tris Plus Gels, 12-well	1 box	NW00082BOX
Bolt™ 8% Bis-Tris Plus Gels, 15-well	1 box	NW00085BOX
Bolt™ 8% Bis-Tris Plus Gels, 17-well	1 box	NW00087BOX
Bolt™ 10% Bis-Tris Plus Gels, 10-well	1 box	NW00100BOX
Bolt™ 10% Bis-Tris Plus Gels, 12-well	1 box	NW00102BOX
Bolt™ 10% Bis-Tris Plus Gels, 15-well	1 box	NW00105BOX
Bolt™ 10% Bis-Tris Plus Gels, 17-well	1 box	NW00107BOX
Bolt™ 12% Bis-Tris Plus Gels, 10-well	1 box	NW00120BOX
Bolt™ 12% Bis-Tris Plus Gels, 12-well	1 box	NW00122BOX
Bolt™ 12% Bis-Tris Plus Gels, 15-well	1 box	NW00125BOX
Bolt™ 12% Bis-Tris Plus Gels, 17-well	1 box	NW00127BOX
Bolt™ 4–12% Bis-Tris Plus Gels, 10-well	1 box	NW04120BOX
Bolt™ 4–12% Bis-Tris Plus Gels, 12-well	1 box	NW04122BOX
Bolt™ 4–12% Bis-Tris Plus Gels, 15-well	1 box	NW04125BOX
Bolt™ 4–12% Bis-Tris Plus Gels, 17-well	1 box	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
Bolt™ Welcome Pack B, 4–12%, 15-well	1 kit**	NW0412B
Bolt™ Welcome Pack A, 4–12%, 10-well	1 kit**	NW0412A

One box contains 10 gels.
** Bolt Welcome Pack kit includes Mini Gel Tank, 2 boxes of Bolt 4–12% Gels (10-well/15-well), Bolt MES Running Buffer (20X), Bolt LDS Sample Buffer (4X), Bolt Sample Reducing Agent (10X) and SeeBlue™ Plus2 Prestained Standard

Product	Quantity	Cat. No.
NuPAGE Bis-Tris Mini Gels (8 cm x 8 cm)		
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 1-well	1 box	NP0304BOX
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	NP0301BOX
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 10-well	2 gels	NP0301PK2
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 12-well	10 gels	NP0302BOX
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 12-well	2 gels	NP0302PK2
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 15-well	1 box	NP0303BOX

Product	Quantity	Cat. No.
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.0 mm, 9-well	1 box	NP0307BOX
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.5 mm, 10-well	1 box	NP0315BOX
NuPAGE™ Novex™ 10% Bis-Tris Protein Gels, 1.5 mm, 15-well	1 box	NP0316BOX
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 1-well	1 box	NP0344BOX
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	NP0341BOX
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 10-well	2 gels	NP0341PK2
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 12-well	10 gels	NP0342BOX
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 12-well	2 gels	NP0342PK2
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 15-well	1 box	NP0343BOX
NuPAGE™ Novex™ 12% Bis-Tris Protein Gels, 1.0 mm, 17-well	1 box	NP0349BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 1-well	1 box	NP0324BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 10-well	10 gels	NP0321BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 10-well	2 gels	NP0321PK2
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 12-well	10 gels	NP0322BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 12-well	2 gels	NP0322PK2
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 15-well	10 gels	NP0323BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 15-well	2 gels	NP0323PK2
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 17-well	10 gels	NP0329BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 17-well	2 gels	NP0329PK2
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.0 mm, 9-well	1 box	NP0327BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.5 mm, 10-well	10 gels	NP0335BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.5 mm, 10-well	2 gels	NP0335PK2

Ordering information

Product	Quantity	Cat. No.
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.5 mm, 15-well	10 gels	NP0336BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Protein Gels, 1.5 mm, 15-well	2 gels	NP0336PK2
NuPAGE Bis-Tris Midi Gels (8 cm x 13 cm)		
NuPAGE™ Novex™ 10% Bis-Tris Midi Protein Gels, 12+2-well	1 box	WG1201BOX
NuPAGE™ Novex™ 10% Bis-Tris Midi Protein Gels, 12+2-well, w/adapters	1 box	WG1201A
NuPAGE™ Novex™ 10% Bis-Tris Midi Protein Gels, 20-well	1 box	WG1202BOX
NuPAGE™ Novex™ 10% Bis-Tris Midi Protein Gels, 20-well, w/adapters	1 box	WG1202A
NuPAGE™ Novex™ 10% Bis-Tris Midi Protein Gels, 26-well	1 box	WG1203BOX
NuPAGE™ Novex™ 10% Bis-Tris Midi Protein Gels, 26-well, w/adapters	1 box	WG1203A
NuPAGE™ Novex™ 4–12% Bis-Tris Midi Protein Gels, 12+2-well	1 box	WG1401BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Midi Protein Gels, 12+2-well, w/adapters	1 box	WG1401A
NuPAGE™ Novex™ 4–12% Bis-Tris Midi Protein Gels, 20-well	1 box	WG1402BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Midi Protein Gels, 20-well, w/adapters	1 box	WG1402A
NuPAGE™ Novex™ 4–12% Bis-Tris Midi Protein Gels, 26-well	1 box	WG1403BOX
NuPAGE™ Novex™ 4–12% Bis-Tris Midi Protein Gels, 26-well, w/adapters	1 box	WG1403A
NuPAGE™ Novex™ 8% Bis-Tris Midi Protein Gels, 12+2-well	1 box	WG1001BOX
NuPAGE™ Novex™ 8% Bis-Tris Midi Protein Gels, 12+2-well, w/adapters	1 box	WG1001A
NuPAGE™ Novex™ 8% Bis-Tris Midi Protein Gels, 20-well	1 box	WG1002BOX
NuPAGE™ Novex™ 8% Bis-Tris Midi Protein Gels, 20-well, w/adapters	1 box	WG1002A
NuPAGE™ Novex™ 8% Bis-Tris Midi Protein Gels, 26-well	1 box	WG1003BOX
NuPAGE™ Novex™ 8% Bis-Tris Midi Protein Gels, 26-well, w/adapters	1 box	WG1003A
NuPAGE Tris-Acetate Mini Gels (8 cm x 8 cm)		
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.0 mm, 10-well	10 gels	EA0375BOX

Product	Quantity	Cat. No.
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.0 mm, 10-well	2 gels	EA0375PK2
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.0 mm, 12-well	10 gels	EA03752BOX
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.0 mm, 12-well	2 gels	EA03752PK2
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.0 mm, 15-well	1 box	EA03755BOX
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.5 mm, 10-well	1 box	EA0378BOX
NuPAGE™ Novex™ 3–8% Tris-Acetate Protein Gels, 1.5 mm, 15-well	1 box	EA03785BOX
NuPAGE™ Novex™ 7% Tris-Acetate Protein Gels, 1.0 mm, 10-well	1 box	EA0355BOX
NuPAGE™ Novex™ 7% Tris-Acetate Protein Gels, 1.0 mm, 12-well	1 box	EA03552BOX
NuPAGE™ Novex™ 7% Tris-Acetate Protein Gels, 1.0 mm, 15-well	1 box	EA03555BOX
NuPAGE™ Novex™ 7% Tris-Acetate Protein Gels, 1.5 mm, 10-well	1 box	EA0358BOX
NuPAGE™ Novex™ 7% Tris-Acetate Protein Gels, 1.5 mm, 15-well	1 box	EA03585BOX
NuPAGE Tris-Acetate Midi Gels (8 cm x 8 cm)		
NuPAGE™ Novex™ 3–8% Tris-Acetate Midi Protein Gels, 12+2W	1 box	WG1601BOX
NuPAGE™ Novex™ 3–8% Tris-Acetate Midi Protein Gels, 12+2W, w/adapters	1 box	WG1601A
NuPAGE™ Novex™ 3–8% Tris-Acetate Midi Protein Gels, 20W	1 box	WG1602BOX
NuPAGE™ Novex™ 3–8% Tris-Acetate Midi Protein Gels, 20W, w/adapters	1 box	WG1602A
NuPAGE™ Novex™ 3–8% Tris-Acetate Midi Protein Gels, 26W	1 box	WG1603BOX
NuPAGE™ Novex™ 3–8% Tris-Acetate Midi Protein Gels, 26W, w/adapters	1 box	WG1603A
Novex Tris-Glycine Mini Gels (8 cm x 8 cm)		
Novex™ 10% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6075BOX
Novex™ 10% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well - Value Pack	3 boxes	EC6075BX30
Novex™ 10% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC60752BOX
Novex™ 10% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC60755BOX

Product	Quantity	Cat. No.
Novex™ 10% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6078BOX
Novex™ 10% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC60785BOX
Novex™ 10–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6135BOX
Novex™ 10–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC61352BOX
Novex™ 10–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC61355BOX
Novex™ 10–20% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC61385BOX
Novex™ 12% Tris-Glycine Mini Protein Gels, 1.0 mm, 1-well	1 box	EC6001BOX
Novex™ 12% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6005BOX
Novex™ 12% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC60052BOX
Novex™ 12% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC60055BOX
Novex™ 12% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6008BOX
Novex™ 12% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC60085BOX
Novex™ 14% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6485BOX
Novex™ 14% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC64852BOX
Novex™ 14% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC64855BOX
Novex™ 14% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6488BOX
Novex™ 14% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC64885BOX
Novex™ 16% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6495BOX
Novex™ 16% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC64952BOX
Novex™ 16% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC64955BOX
Novex™ 16% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6498BOX
Novex™ 16% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC64985BOX

Product	Quantity	Cat. No.
Novex™ 18% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6505BOX
Novex™ 18% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC65052BOX
Novex™ 18% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC65055BOX
Novex™ 18% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6508BOX
Novex™ 18% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC65085BOX
Novex™ 4% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6055BOX
Novex™ 4% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC60552BOX
Novex™ 4% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6058BOX
Novex™ 4% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC60585BOX
Novex™ 4–12% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6035BOX
Novex™ 4–12% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC60352BOX
Novex™ 4–12% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC60355BOX
Novex™ 4–12% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6038BOX
Novex™ 4–12% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC60385BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 1-well	1 box	EC6021BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6025BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC60252BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC60255BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.0 mm, 9-well	1 box	EC60249BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.5 mm, 10-well	1 box	EC6028BOX
Novex™ 4–20% Tris-Glycine Mini Protein Gels, 1.5 mm, 15-well	1 box	EC60285BOX
Novex™ 6% Tris-Glycine Mini Protein Gels, 1.0 mm, 10-well	1 box	EC6065BOX

Ordering information

Product	Quantity	Cat. No.
Novex™ 6% Tris-Glycine Mini Protein Gels, 1.0 mm, 12-well	1 box	EC60652BOX
Novex™ 6% Tris-Glycine Mini Protein Gels, 1.0 mm, 15-well	1 box	EC60655BOX
Novex Tris-Glycine Midi Gels (8 cm x 13 cm)		
Novex™ 10% Tris-Glycine Midi Protein Gels, 12+2-well	1 box	WT0101BOX
Novex™ 10% Tris-Glycine Midi Protein Gels, 12+2-well, w/adapters	1 box	WT0101A
Novex™ 10% Tris-Glycine Midi Protein Gels, 20-well	1 box	WT0102BOX
Novex™ 10% Tris-Glycine Midi Protein Gels, 20-well, w/adapters	1 box	WT0102A
Novex™ 10% Tris-Glycine Midi Protein Gels, 26-well	1 box	WT0103BOX
Novex™ 10% Tris-Glycine Midi Protein Gels, 26-well, w/adapters	1 box	WT0103A
Novex™ 12% Tris-Glycine Midi Protein Gels, 12+2-well	1 box	WT0121BOX
Novex™ 12% Tris-Glycine Midi Protein Gels, 12+2-well, w/adapters	1 box	WT0121A
Novex™ 12% Tris-Glycine Midi Protein Gels, 20-well	1 box	WT0122BOX
Novex™ 12% Tris-Glycine Midi Protein Gels, 20-well, w/adapters	1 box	WT0122A
Novex™ 12% Tris-Glycine Midi Protein Gels, 26-well	1 box	WT0123BOX
Novex™ 12% Tris-Glycine Midi Protein Gels, 26-well, w/adapters	1 box	WT0123A
Novex™ 4–12% Tris-Glycine Midi Protein Gels, 12+2-well	1 box	WT4121BOX
Novex™ 4–12% Tris-Glycine Midi Protein Gels, 12+2-well, w/adapters	1 box	WT4121A
Novex™ 4–12% Tris-Glycine Midi Protein Gels, 20-well	1 box	WT4122BOX
Novex™ 4–12% Tris-Glycine Midi Protein Gels, 20-well, w/adapters	1 box	WT4122A
Novex™ 4–12% Tris-Glycine Midi Protein Gels, 26-well	1 box	WT4123BOX
Novex™ 4–12% Tris-Glycine Midi Protein Gels, 26-well, w/adapters	1 box	WT4123A
Novex™ 4–20% Tris-Glycine Midi Protein Gels, 12+2-well	1 box	WT4201BOX
Novex™ 4–20% Tris-Glycine Midi Protein Gels, 12+2-well, w/adapters	1 box	WT4201A
Novex™ 4–20% Tris-Glycine Midi Protein Gels, 20-well	1 box	WT4202BOX
Novex™ 4–20% Tris-Glycine Midi Protein Gels, 20-well, w/adapters	1 box	WT4202A
Novex™ 4–20% Tris-Glycine Midi Protein Gels, 26-well	1 box	WT4203BOX
Novex™ 4–20% Tris-Glycine Midi Protein Gels, 26-well, w/adapters	1 box	WT4203A
Novex™ 8% Tris-Glycine Midi Protein Gels, 12+2-well	1 box	WT0081BOX
Novex™ 8% Tris-Glycine Midi Protein Gels, 12+2-well, w/adapters	1 box	WT0081A
Novex™ 8% Tris-Glycine Midi Protein Gels, 20-well	1 box	WT0082BOX
Novex™ 8% Tris-Glycine Midi Protein Gels, 20-well, w/adapters	1 box	WT0082A
Novex™ 8% Tris-Glycine Midi Protein Gels, 26-well	1 box	WT0083BOX
Novex™ 8% Tris-Glycine Midi Protein Gels, 26-well, w/adapters	1 box	WT0083A
Novex™ 8–16% Tris-Glycine Midi Protein Gels, 12+2-well	1 box	WT8161BOX
Novex™ 8–16% Tris-Glycine Midi Protein Gels, 12+2-well, w/adapters	1 box	WT8161A
Novex™ 8–16% Tris-Glycine Midi Protein Gels, 20-well	1 box	WT8162BOX
Novex™ 8–16% Tris-Glycine Midi Protein Gels, 20-well, w/adapters	1 box	WT8162A
Novex™ 8–16% Tris-Glycine Midi Protein Gels, 26-well	1 box	WT8163BOX
Novex™ 8–16% Tris-Glycine Midi Protein Gels, 26-well, w/adapters	1 box	WT8163A
NativePAGE Gels		
NativePAGE™ Novex™ 3–12% Bis-Tris Protein Gels, 1.0 mm, 10-well	1 box	BN1001BOX
NativePAGE™ Novex™ 3–12% Bis-Tris Protein Gels, 1.0 mm, 15-well	1 box	BN1003BOX
NativePAGE™ Novex™ 4–16% Bis-Tris Protein Gels, 1.0 mm, 10-well	1 box	BN1002BOX
NativePAGE™ Novex™ 4–16% Bis-Tris Protein Gels, 1.0 mm, 15-well	1 box	BN1004BOX

Product	Quantity	Cat. No.
Novex Tricine Gels		
Novex™ 10% Tricine Protein Gels, 1.0 mm, 10-well	1 box	EC6675BOX
Novex™ 10% Tricine Protein Gels, 1.0 mm, 12-well	1 box	EC66752BOX
Novex™ 16% Tricine Protein Gels, 1.0 mm, 10-well	1 box	EC6695BOX
Novex™ 16% Tricine Protein Gels, 1.0 mm, 12-well	1 box	EC66952BOX
Novex™ 16% Tricine Protein Gels, 1.0 mm, 15-well	1 box	EC66955BOX
Novex™ 10–20% Tricine Protein Gels, 1.0 mm, 10-well	1 box	EC6625BOX
Novex™ 10–20% Tricine Protein Gels, 1.0 mm, 12-well	1 box	EC66252BOX
Novex™ 10–20% Tricine Protein Gels, 1.0 mm, 15-well	1 box	EC66255BOX
Novex IEF Gels		
Novex™ pH 3–7 IEF Protein Gels, 1.0 mm, 12-well	5 gels/box	EC66452BOX
Novex™ pH 3–7 IEF Protein Gels, 1.0 mm, 10-well	5 gels/box	EC6645BOX
Novex™ pH 3–10 IEF Protein Gels, 1.0 mm, 10-well	5 gels/box	EC6655BOX
Novex Zymogram Gels		
Novex™ 12% Zymogram (Casein) Protein Gels, 1.0 mm, 12-well	1 box	EC64052BOX
Novex™ 4–16% Zymogram (Blue Casein) Protein Gels, 1.0 mm, 10-well	1 box	EC6415BOX
Novex™ 12% Zymogram (Casein) Protein Gels, 1.0 mm, 10-well	1 box	EC6405BOX
Novex™ 10% Zymogram (Gelatin) Protein Gels, 1.0 mm, 15-well	1 box	EC61755BOX
Novex™ 10% Zymogram (Gelatin) Protein Gels, 1.0 mm, 12-well	1 box	EC61752BOX
Novex™ 10% Zymogram (Gelatin) Protein Gels, 1.0 mm, 10-well	1 box	EC6175BOX
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

Product	Quantity	Cat. No.
Prepare samples and select buffers: SDS-PAGE		
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

Ordering information

Product	Quantity	Cat. No.
NuPAGE™ Tris-Acetate SDS Buffer Kit (for Tris-Acetate gels), *Contains 1 ea. LA0041, NP0004, NP0005, NP0007*	1 kit	LA0050
Novex™ Tricine SDS Buffer Kit, *includes LC1676 & LC1675*	1 kit	LC1677
Pierce LDS Sample Buffer, Non-Reducing (4X)	5 mL	84788
Pierce Lane Marker Non-Reducing Sample Buffer	5 mL	39001
Pierce 10X Tris-Glycine SDS Buffer	1 L	28362
BupH™ Tris-Glycine-SDS Buffer Packs	40 packs	28378
Native Electrophoresis		
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		
Novex™ Zymogram Developing Buffer (10X)	500 mL	LC2671
Novex™ Zymogram Renaturing Buffer (10X)	500 mL	LC2670
IEF		
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

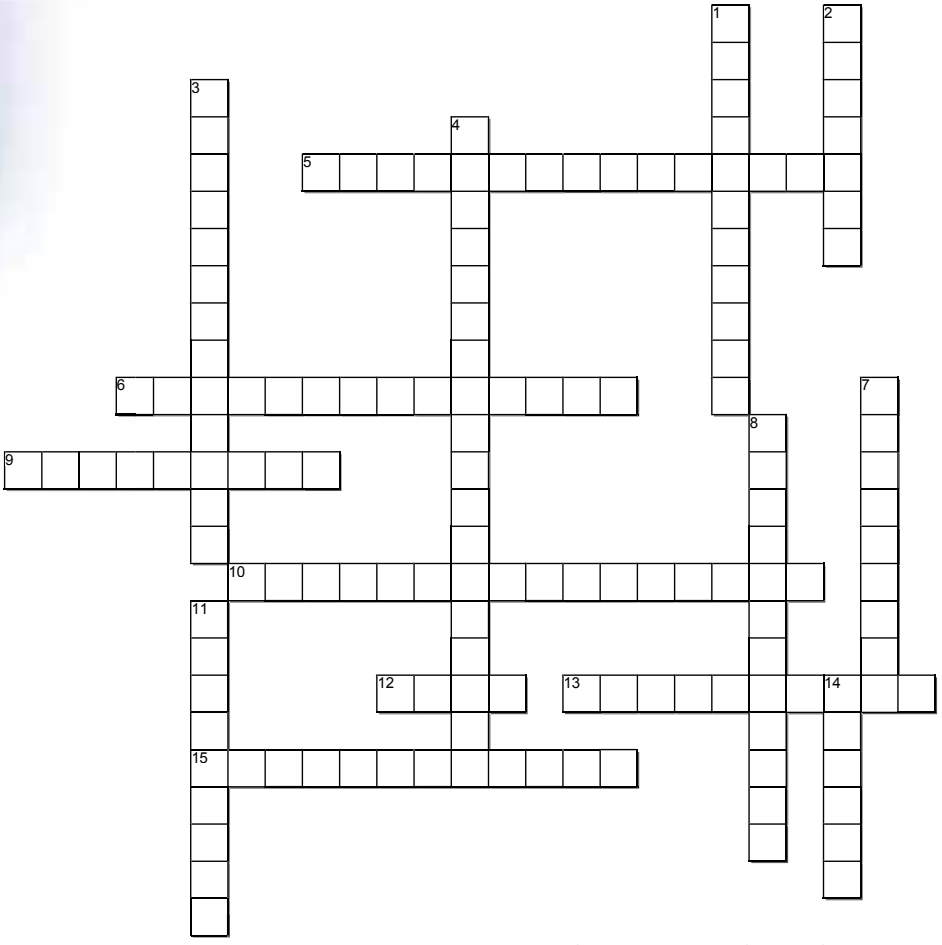
Product	Quantity	Cat. No.
Select Protein Standards: Unstained		
PageRuler™ Unstained Low Range Protein Ladder	2 x 250 µL	26632
PageRuler™ Unstained Protein Ladder	2 x 250 µL	26614
NativeMark Unstained Protein Standard	5 x 50 µL	LC0725
Prestained		
PageRuler™ Prestained Protein Ladder	2 x 250 µL	26616
PageRuler™ Prestained Protein Ladder	10 x 250 µL	26617
PageRuler™ Plus Prestained Protein Ladder	2 x 250 µL	26619
PageRuler™ Plus Prestained Protein Ladder	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 High Range Protein Ladder	2 x 250 µL	26625
Western		
MagicMark™ XP Western Protein Standard	250 µL	LC5602
MagicMark™ XP Western Protein Standard	50 µL	LC5603
Specialty		
PageRuler™ Prestained NIR Protein Ladder	2 x 250 µL	26635
BenchMark™ Fluorescent Protein Standard	125 µL	LC5928
BenchMark™ His-tagged Protein Standard	125 µL	LC5606
IEF Marker 3–10	500 µL	39212-01

Product	Quantity	Cat. No.
Electrophoresis chamber systems and power supplies		
Mini Gel Tank	1 unit	A25977
XCell SureLock Mini-Cell	1 unit	EI0001
XCell4 SureLock™ Midi-Cell	1 each	WR0100
PowerEase™ 90W Power Supply (115 VAC)	1 each	PS0090
PowerEase™ 90W Power Supply (230 VAC)	1 each	PS0091
PowerEase™ 300W Power Supply (115 VAC)	1 each	PS0300
PowerEase™ 300W Power Supply (230 VAC)	1 each	PS0301

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
*1 kit contains sufficient reagents to stain 25 mini gels		
Fluorescent and specialty stains		
SYPRO™ Orange Protein Gel Stain	500 µL	S-6650
SYPRO™ Orange Protein Gel Stain	10 x 50 µL	S-6651
SYPRO™ Red Protein Gel Stain	500 µL	S-6653
SYPRO™ Red Protein Gel Stain	10 x 50 µL	S-6654
SYPRO™ Ruby Protein Gel Stain	1 L	S-12000
SYPRO™ Ruby Protein Gel Stain	200 mL	S-12001
SYPRO™ Ruby Protein Gel Stain	5 L	S-21900
Pro-Q™ Emerald 488 Glycoprotein Gel Stain Kit	1 kit	P-21875
Pro-Q™ Diamond Phosophoprotein Gel Stain Kit	1 L	P-33300
Pro-Q™ Diamond Phosophoprotein Gel Stain Kit	200 mL	P-33301
Pro-Q™ Diamond Phosophoprotein Gel Stain Kit	5 L	P-33302
Pierce Power Stainer		
Pierce Power Stainer	1 unit	22833
Pierce™ Power Stainer Welcome Pack	1 unit	22833SPCL*
*Welcome pack includes Pierce Power Station, Pierce Power Stain Cassette, Western Blot Roller, Power Cord with C/13 Connector, Quick Start Guide, Pierce Mini Gel Power Staining Kit		

Crossword puzzle challenge

To participate in the crossword puzzle challenge, go to thermofisher.com/pagecrossword



Across

5. Can you name one of the scientists who developed blue native polyacrylamide gel electrophoresis? pg. 17
6. Which tank is compatible with midigels? pg. 56
9. Which ladder can be used for accurate molecular weight estimation directly on western blots? pg. 46
10. Can you name one of the scientists who filed a patent for the netural-pH Bis-Tris system in 1996? pg. 11
12. Which gel has a unique wedge well design that allows you to load 2x the sample volume? pg. 10
13. Which protein ladder would you use for approximate determination of molecular weight? pg. 35
15. Who won the Nobel Prize for analysis of serum proteins by electrophoresis in 1948? pg. 5

Down

1. Which tank is compatible with >180 mini gels? pg. 52
2. Which gel chemistry minimizes protein degradation? pg. 9
3. Who first published SDS-PAGE as a method for the analysis of cleavage of structural proteins in bacteriophage? pg. 7
4. Can you name one of the scientists who first described the theory of separation of amphoteric proteins along a pH gradient in the 1960s? pg. 21
7. What power supply is available for use with the Mini Gel Tank? pg. 58
8. What is a fast alternative to traditional Coomassie staining? pg. 72
11. Which ladder would you use for precise determination of molecular weight? pg. 35
14. Which type of gel has been referenced in >20,000 publications? pg. 12

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