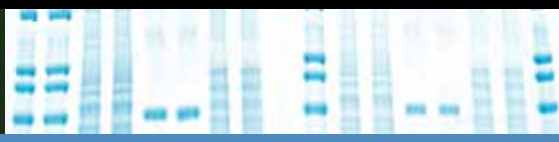
Thermo Scientific Pierce Electrophoresis Technical Handbook

Featuring Thermo Scientific GelCode Staining Kits

Version 2



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Thermo Scientific Pierce Products for Gel Electrophoresis of Proteins

Gel electrophoresis is a technique in which charged molecules, such as protein or DNA, are separated according to physical properties as they are forced through a gel by an electrical current. Proteins are commonly separated using polyacrylamide gel electrophoresis (PAGE) to characterize individual proteins in a complex sample or to examine multiple proteins within a single sample. PAGE can be used as a preparative tool to obtain a pure protein sample, or as an analytical tool to provide information on the mass, charge, purity or presence of a protein. Several forms of PAGE exist and can provide different types of information about the protein(s).

- **Nondenaturing PAGE**, also called native PAGE, separates proteins according to their mass:charge ratio
- **SDS-PAGE**, the most widely used electrophoresis technique, separates proteins primarily by mass
- **Two-dimensional PAGE (2-D PAGE)** separates proteins by isoelectric point in the first dimension and by mass in the second dimension

Homemade Gel Recipes

Acrylamide is the material of choice for preparing electrophoretic gels to separate proteins by size. Acrylamide mixed with bisacrylamide forms a crosslinked polymer network when the polymerizing agent ammonium persulfate is added (Figure 1). The ammonium persulfate produces free radicals faster in the presence of TEMED (N,N,N,N'-tetramethylenediamine). The size of the pores created in the gel is inversely related to the amount of acrylamide used. For example, a 7% polyacrylamide gel will have larger pores in the gel than a 12% polyacrylamide gel. Gels with a low percentage of acrylamide are typically used to resolve large proteins and gels with a high percentage of acrylamide are used to resolve small proteins. Table 1 provides recipes for preparing gels with different acrylamide concentrations. We offer many of the raw materials necessary for preparing PAGE gels, all of which are supplied at high purity grades. For example, Thermo Scientific SDS (Product # 28312) is a high-grade material, containing at least 98% of the C_{12} alkyl sulfate chain length, with minimal presence of C_{14} or C_{16} chain length. This results in more consistent SDS-PAGE separations and improved renaturation of proteins for *in situ* enzyme activity.[1]

Analysis of multiple samples is accomplished using a one-dimensional slab gel. Slab gel sizes commonly range from 15 cm x 18 cm down to 2 cm x 3 cm. Small gels typically require less time and reagents than their larger counterparts and are suited for rapid screening. However, larger gels provide better resolution and are needed for separating similar proteins or a large number of proteins. Samples are applied at the top of the slab gel in sample wells that span the width of the gel.

When the electrical current is applied, the proteins migrate down through the gel matrix, creating lanes of protein bands. In native PAGE, migration occurs because most proteins carry a net negative charge at slightly basic pH. The higher the negative charge density (more charges per molecule mass), the faster a protein will migrate. At the same time, the frictional force of the gel matrix creates a sieving effect, retarding the movement of proteins according to their size. Small proteins face only a small frictional force while large proteins face a larger frictional force. Thus native PAGE separates proteins based upon both their charge and mass.

In SDS-PAGE, proteins are treated with sodium dodecyl sulfate (SDS) before electrophoresis so that the charge density of all proteins is made roughly equal. When these samples are electrophoresed, proteins are separated according to mass. SDS-PAGE allows estimation of the molecular weight (MW) of proteins. In this application, a sample of unknown molecular weight is compared directly with proteins of known molecular weight (MW standards) in an adjacent lane. SDS-PAGE is also used for routine separation and analysis of proteins because of its speed, simplicity and resolving capability.

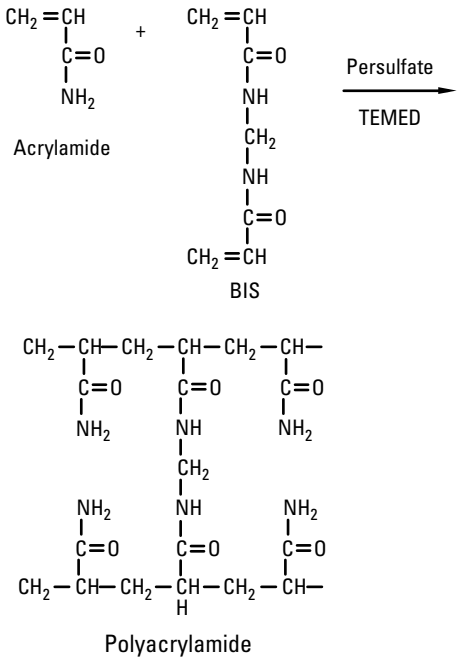


Figure 1. Polymerization and crosslinking of acrylamide.

Gel Electrophoresis of Proteins

Step 1 — **Prepare the gel**

Table 1. SDS-PAGE formulas for mini-gels (8.0 cm x 8.0 cm).

	Percent Acrylamide Gel			
Running Gel	**7%**	**10%**	**11%**	**12.5%**
40% Acrylamide Solution (w/v)	5.25 ml	7.5 ml	8.25 ml	9.375 ml
1% Bisacrylamide	4.8 ml	3.9 ml	3.6 ml	3.1 ml
1.5 M Tris•HCl, pH 8.7	7.5 ml	7.5 ml	7.5 ml	7.5 ml
********** Add distilled water to bring total volume to 30 ml **********				
10% Ammonium Persulfate (Product # 17874)	0.3 ml	0.3 ml	0.3 ml	0.3 ml
10% SDS, C12 grade (Product # 28312)	0.3 ml	0.3 ml	0.3 ml	0.3 ml
TEMED (Product # 17919)	0.03 ml	0.03 ml	0.03 ml	0.03 ml
Stacking Gel	**7% Acrylamide Gel**			
40% Acrylamide Solution (w/v)	0.75 ml			
1% Bisacrylamide	0.1 ml			
0.5 M Tris•HCl, pH 6.8	2.5 ml			
Deionized Water	5.6 ml			
10% Ammonium Persulfate (Product # 17874)	0.1 ml			
10% SDS, C12 grade (Product # 28312)	0.1 ml			
TEMED (Product # 17919)	0.01 ml			

Running Buffer:	25 mM Tris, 192 mM Glycine and 0.1% SDS, pH 8.3 Use: Thermo Scientific BupH Tris-Glycine-SDS Buffer (Product # 28378)
Sample Buffer:	0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% glycerol, bright pink tracking dye Use: Lane Marker Non-Reducing Sample Buffer (Product # 39001) For reducing gels use: Lane Marker Reducing Sample Buffer (Product # 39000) that contains 100 mM Dithiothreitol (Product # 20290) Add one volume of Product # 39001 or 39000 to four volumes of protein sample. Boil for 3-5 minutes then cool to room temperature before applying 15 µl-25 µl in the sample well.
Coomassie Stain:	0.125% Coomassie Brilliant Blue R-250 (Product # 20278) 50% Methanol 10% Acetic Acid
Coomassie Destaining Solution:	50% Methanol + 10% Acetic Acid

} Alternatively, stain directly with Thermo Scientific GelCode Blue Stain Reagent (Product # 24592), GelCode Blue Safe Protein Stain (Product # 24594) or Imperial Protein Stain (Product # 24617)

Multiple components of a single sample may be resolved most completely by 2-D PAGE. The first dimension separates proteins according to isoelectric point (pI) and the second dimension separates by mass. 2-D PAGE provides the highest resolution for protein analysis and is a key technique in proteomic research in which resolution of thousands of proteins on a single gel is necessary.

To obtain optimal resolution of proteins, a "stacking" gel is poured over the top of the "resolving" gel. The stacking gel has a lower concentration of acrylamide (larger pore size), lower pH and a different ionic content. This allows the proteins in a lane to be concentrated into a tight band before entering the running or resolving gel and produces a gel with tighter or better separated protein bands.

The resolving gel may consist of a constant acrylamide concentration or a gradient of acrylamide concentration (high percentage of acrylamide at the bottom of the gel and low percentage at the top). A gradient gel is prepared by mixing two different concentrations of acrylamide solution to form a gradient with decreasing concentrations of acrylamide. As the gradient forms, it is layered into a gel cassette. A gradient gel allows separation of a mixture of proteins with a greater molecular weight range than a gel with a fixed acrylamide concentration. If a sample contains proteins with large differences in molecular weights, then a gradient gel is recommended. A stacking gel is unnecessary when using a gradient gel because the continually decreasing pore size performs this function.

Precast Gels

While many researchers continue to pour acrylamide gels on a routine basis, a growing number have adopted some form of precast gel. Purchasing precast gels saves considerable time, and gels are available in a variety of percentages including difficult-to-pour gradient gels that provide excellent resolution and separate proteins over the widest range of molecular weights. Another reason to use precast gels is the reproducibility offered by the long shelf life versions of such gels that are poured consistently and that continue to perform consistently over time. Under the conditions normally used to pour polyacrylamide gels, hydrolysis occurs, resulting in the formation of acrylic acid from polyacrylamide. This indicates that the performance of the gel changes with time and places severe limits on the useful shelf life of the gel. In addition, precast polyacrylamide gels obviate the need to work with the acrylamide monomer – a known neurotoxin and suspected carcinogen.

Thermo Scientific Pierce Protein Gels are cast in a durable plastic cassette using a neutral pH buffer that inhibits hydrolysis of polyacrylamide and allows us to guarantee the performance of the gels for one year. They are compatible with standard mini-gel tanks so there is no need to purchase specialized equipment. The Tris-HEPES-SDS running buffer produces excellent resolution of protein bands and short run times of only 45 minutes. Pierce Gels can be stained using common methods or transferred efficiently for 60-90 minutes using wet tank methods.

Isoelectric Focusing and 2-D Gels

An isoelectric focusing gel (IEF gel) can be used to separate proteins according to charge and to determine the pH at which a protein has a net charge of zero. This pH is known as the pI of the protein and is a distinguishing characteristic of the protein that provides useful information for purifying and handling the protein. The pI of a protein is determined by the number of acidic and basic residues in the protein. At physiological pH, the carboxyl groups of acidic residues are predominantly deprotonated and impart a negative charge. In contrast, the amine groups of basic residues are protonated and carry a positive charge.

By identifying the pI value of a protein, buffer systems for large-scale purification can be designed. For example, a protein with a pI of 5.6 will have a net charge of zero in a solution at pH 5.6. As the pH of the buffer system is increased, this protein (pI 5.6) takes on an overall negative charge because the carboxyls and amines are both deprotonated. The protein can then be purified on an anion exchange (DEAE) column because the protein will be retained on the positively charged column.

To perform IEF, a pH gradient is established in a tube or strip gel using a specially formulated buffer system or ampholyte mixture. Ampholytes are a mixture of amino acid polymers that have surface charges corresponding to different pH ranges and are available as immobilized pH gradient (IPG) strips for consistency and convenience. A protein sample is loaded onto the gel and electrodes are attached (anode at the acidic end of the gradient and cathode at the basic end of the gradient). Proteins with a net positive charge on the surface will migrate to the cathode when an electrical current is applied. Negatively charged proteins will move toward the anode. When the protein in the pH gradient reaches a zone in which the net surface charge is zero, it will no longer move. At this point the protein becomes "focused" and a band is formed in the gel.

Following isoelectric focusing, a protein mixture may be separated in a second dimension by SDS-PAGE. This technique, known as 2-D PAGE, is used to resolve complex protein mixtures into the greatest number of individual protein "spots." The IEF gel is equilibrated with SDS and laid across the top of an SDS-PAGE gel. Current is applied and the proteins migrate into the gel where separation occurs according to mass. This two-dimensional separation of proteins according to pI and mass allows resolution of proteins that would not normally be separated by a one-dimensional method. Several thousand protein spots may be resolved on a single 2-D PAGE gel, making this technique suitable for proteomics analysis (Figure 2).

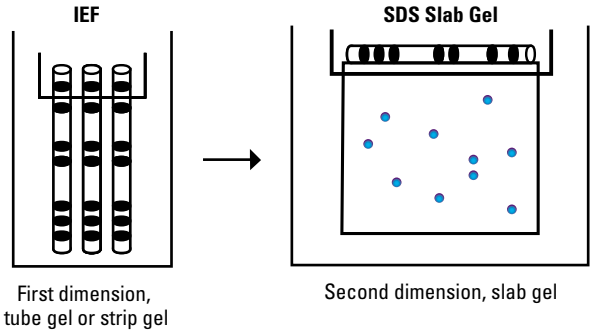


Figure 2. Schematic illustration of 2-D PAGE.

Native PAGE

Electrophoresis of a protein in its native state relies upon the intrinsic charge of the protein and its mass. For a protein to migrate into the gel toward the anode, it must have an overall negative charge at the pH of the gel/buffer system. For this reason, native PAGE is commonly performed at a slightly basic pH where proteins with a neutral or acidic pI will have the required net negative charge. Alternatively, the process can be done in reverse by using an acidic pH to impart a positive charge to most proteins. This method requires reversing the anode and cathode because positively charged proteins will migrate toward the cathode. In addition to the charge of a protein, other factors such as the size and shape of a protein also influence its mobility in native PAGE.

Because no denaturants are present in native PAGE, subunit interactions within a multimeric protein are generally retained and information may be gained about the quaternary structure. In addition, many proteins have been shown to be enzymatically active following separation by native PAGE. Thus, it may be used for preparation of purified, active proteins.[2] Following electrophoresis, proteins may be recovered from a native gel by passive diffusion or electroelution.[3] To maintain the integrity of proteins during electrophoresis, it is important to keep the apparatus cool and minimize the effects of denaturation and proteolysis. Extremes of pH should generally be avoided in native PAGE as they may lead to irreversible damage, such as denaturation or aggregation, to the protein of interest.

Step 1 — **Prepare the gel**

Thermo Scientific Products for SDS-PAGE

In SDS-PAGE applications, the sample applied to the slab gel has been treated with the detergent sodium dodecyl sulfate (SDS). This ionic detergent denatures the proteins in the sample and binds tightly to the uncoiled molecule. The SDS molecules mask the intrinsic charge of the protein and create a relatively uniform negative charge distribution caused by the sulfate groups on SDS. When an electric current is applied, all proteins will migrate through the gel toward the anode, which is placed at the bottom of the gel. The SDS-PAGE gel separates proteins primarily according to size because the SDS-coated proteins have a uniform charge: mass ratio. Proteins with less mass travel more quickly through the gel than those with greater mass because of the sieving effect of the gel matrix. Protein molecular weights can be estimated by running standard proteins of known molecular weights in a separate lane of the same gel.

References

1. Lacks, S.A., *et al.* (1979). *Anal. Biochem.* **100,** 357-363.
2. Rothe, G.M. and Maurer, W.D. (1986). In Gel Electrophoresis of Proteins. IOP Publishing Limited, Bristol, England. pp.55-56.
3. Bollag, D.M., *et al.* (2002). Protein Methods. Second Edition. New York, N.Y. Wiley-Liss, Inc. pp.149. (Product # 20001).

Ammonium Persulfate

Catalyst for acrylamide gel polymerization.

$(NH_4)_2S_2O_8$

Ammonium Persulfate
MW 228.20

Ordering Information

Product #	Description	Pkg. Size
17874	Ammonium Persulfate	4 x 25 g

TEMED

Greater than 99% pure!

$C_6H_{16}N_2$

TEMED
MW 116.21

Specifications:

- Purity: > 99.9%
- Refractive Index: 1.417-1.419
- Boiling Range: 119-121°C
- Acrylamide polymerization reagent

Ordering Information

Product #	Description	Pkg. Size
17919	TEMED (N,N,N,N-Tetramethylethylenediamine)	25 ml

Urea

A low UV-absorbing protein denaturant.

Highlights:

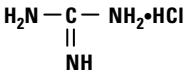
- Melting point: 132-136°C
- Specification: A_{280} < 0.100

Ordering Information

Product #	Description	Pkg. Size
29700	Urea	1 kg

8 M Guanidine•HCl Solution and Guanidine•HCl[4,5]

Ready-to-use, highly purified denaturants.



Guanidine Hydrochloride
MW 95.54

Highlights:

- Free of UV-absorbing materials in the range of 225-300 nm
- Sharp UV cut-off spectrum with OD_{260} less than 0.03
- Typical metals: Cu ≤ 1 ppm; Fe ≤ 0.1 ppm; Pb ≤ 0.1 ppm; Zn ≤ 0.1 ppm
- Particulate-free, crystal-clear, colorless solution
- Excellent stability
- Excellent for washing affinity ligand columns (nonprotein ligands)

8 M Guanidine•Hydrochloride Dilution Table

Beginning with 10 ml of Thermo Scientific 8 M Guanidine•HCl Solution (Product # 24115), dilution to the indicated final volume will give the stated molarity.

Desired Molarity	Final Volume
8 M	10 ml
7 M	11.4 ml
6 M	13.3 ml
5 M	16 ml
4 M	20 ml

Desired Molarity	Final Volume
3 M	26.7 ml
2 M	40 ml
1.5 M	52 ml
1 M	80 ml
0.5 M	160 ml

References

4. Tanaka, S., *et al.* (1985). *J. Biochem.* **97(5),** 1377-1384.
5. Wong, K.P., *et al.* (1971). *Anal. Biochem.* **40(2),** 459-464.

Ordering Information

Product #	Description	Pkg. Size
24115	8 Molar Guanidine•HCl Solution Sequencing Grade	200 ml
24110	Guanidine•HCl Crystalline, Sequencing Grade	500 g

SDS (Sodium Dodecyl Sulfate)[6-8]

When high resolution is the key, this is the ideal detergent.

$CH_3(CH_2)_{11}OSO_3Na$

SDS
MW 288.38

SDS (C_{12}) Highlights:

- Greater than 99% alkyl sulfate
- Greater than 98% C_{12} alkyl sulfate
- Contains a low level of hexadecyl sulfate C_{16}, which inhibits protein renaturation

SDS (Lauryl) Highlights:

- Unique distribution of carbon chain lengths is advantageous when resolving viral proteins during gel electrophoresis
- Can be used for renaturation after SDS-PAGE (if gels are treated according to the procedure of Blank, *et al.*[8] to remove C_{14} and C_{16} alkyl sulfates)

References

6. Matheka, H.D., *et al.* (1977). *Anal. Biochem.* **81(1)**, 9-17.
7. Swaney, J.B., *et al.* (1974). *Anal. Biochem.* **58(2)**, 337-346.
8. Blank, A., *et al.* (1980). *Federation Proceedings* **39(6)**, Abstracts ABSC/TBS, Abstract No. 1285, 1951.

Ordering Information

Product #	Description	Pkg. Size
28312	**SDS, C_{12} Grade** (Sodium Dodecyl Sulfate, > 98% C_{12})	500 g
28364	**SDS** (Sodium Dodecyl Sulfate, Lauryl) Typical Analysis: C_{12}: 63.5%. C_{14}: 29.5%, C_{16}: 7.0%	100 g
28365	**SDS** (Sodium Dodecyl Sulfate, Lauryl) Typical Analysis: C_{12}: 63.5%. C_{14}: 29.5%, C_{16}: 7.0%	1 kg

Thermo Scientific Pierce Protein Gels

Protein electrophoresis made easy.

Thermo Scientific Pierce Protein Gels take ease-of-use to new levels. The gels use a special formulation to produce stronger, more resilient gels, making handling after electrophoresis easier. The extra stability of Pierce Protein Gels combined with the Tris-HEPES-SDS Running Buffer offers both speed and excellent resolution of your proteins with the same size ranges as the Laemmli system.

Pierce® Protein Gels make gel loading easier than ever. Their novel red-dyed stacking gel makes the wells highly visible, helping you guide your pipette. The reinforced wells do not fall over and are resistant to damage when loading. The well fingers extend above the plate, decreasing the chances of spill over and well-to-well contamination.

Never ruin a gel again because there are no combs to pull out. All wells are supplied intact. The updated cassette design makes gel removal after electrophoresis a snap, with no special tools required.

The Pierce Protein Gels are available as SDS denaturing gels in 4-8%, 4-20% or 12% acrylamide. Select from either 12- or 17-well formats, with 20 µl or 35 µl capacity respectively. The gels have a long shelf life of one year from date of purchase.

Highlights:

- **Fast** – 45-minute run time
- **Convenient sample loading**
  - Dyed stacking gel allows for easy loading of samples up to 35 µl
  - Sample wells reinforced with plastic eliminate damage when loading
  - Sample well dividers do not deform or fall over
- **Resilient** – up to 10X stronger than regular gels
- **Ease of use** – easy-to-open cassette with no comb or tape to remove
- **Maintain sample purity** – gel fingers extend above lower plate to prevent well-to-well contamination
- **Longer shelf life** – gels are stable for 1 year from date of purchase
- **Flexible** – cassette compatible with 10 cm x 10 cm gel systems

Step 1 — **Prepare the gel**

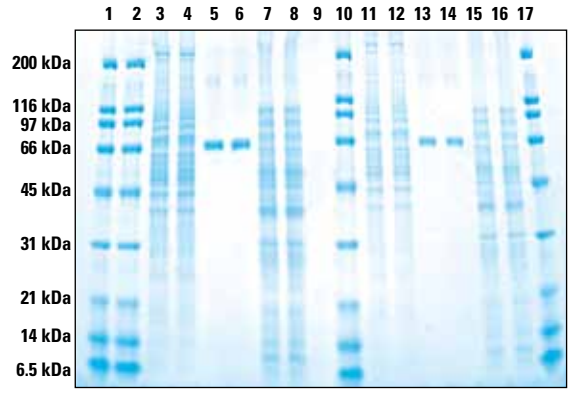


Figure 3. Thermo Scientific Pierce Protein Gels, 4-20%, stained with Thermo Scientific GelCode Blue Stain. Proteins were separated on 4-20% 17-well Pierce Protein Gel (Product # 84714), washed 30 minutes with water, stained for 60 minutes with GelCode Blue Stain (Product # 24592) and destained for 60 minutes (3 x 20-minute washes with laboratory tissues) with water. **Lane 1, 2:** MW marker; **Lane 3, 4:** HeLa cell lysate (1.88 µg); **Lane 5, 6:** Purified BSA (300 ng); **Lane 7, 8:** *E. coli* lysate (1.88 µg); **Lane 9:** No protein; **Lane 10:** MW marker; **Lane 11, 12:** HeLa cell lysate (0.88 µg); **Lane 13, 14:** Purified BSA (150 ng); **Lane 15, 16:** *E. coli* lysate (0.88 µg); and **Lane 17:** MW marker.

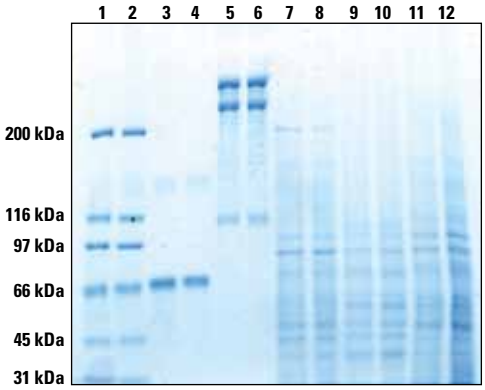


Figure 4. Thermo Scientific Pierce Protein Gel, 4-8%, stained with Thermo Scientific GelCode Blue Stain. Proteins were separated on 4-8% 12-well Pierce Protein Gel (Product # 84708), washed three times for 10 minutes each with water, stained for 60 minutes with GelCode Blue Stain (Product # 24592) and destained for 60 minutes (3 x 20-minute washes with laboratory tissues) with water. **Lane 1, 2:** MW marker; **Lane 3, 4:** Purified BSA (300 ng); **Lane 5, 6:** Blue carrier hemocyanin protein (300 ng); **Lane 7, 8:** Jurkat cell lysate (1.88 µg); **Lane 9, 10:** A549 cell lysate (1.88 µg); **Lane 11, 12:** MOPC cell lysate (1.88 µg).

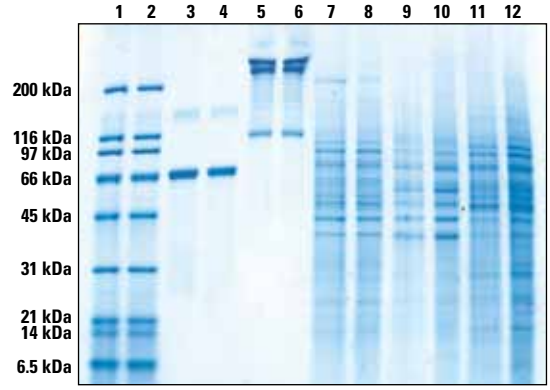


Figure 5. Thermo Scientific Pierce Protein Gel, 12%, stained with Thermo Scientific GelCode Blue Stain. Proteins were separated on 12% 12-well Pierce Protein Gel (Product # 84711), washed three times for 10 minutes each with water, stained for 60 minutes with GelCode Blue Stain (Product # 24592) and destained for 60 minutes (3 x 20-minute washes with laboratory tissues) with water. **Lane 1, 2:** MW marker; **Lane 3, 4:** Purified BSA (300 ng); **Lane 5, 6:** Blue carrier hemocyanin protein (300 ng); **Lane 7, 8:** Jurkat cell lysate (1.88 µg); **Lane 9, 10:** A549 cell lysate (1.88 µg); **Lane 11, 12:** MOPC cell lysate (1.88 µg).

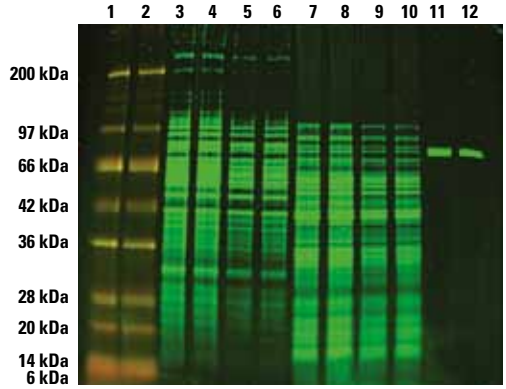


Figure 6. Thermo Scientific Pierce Protein Gel, 12%, stained with Thermo Scientific Krypton Protein Stain. Proteins were separated on 12% 12-well Pierce Protein Gel (Product # 84711) and stained with Krypton Protein Stain (Product # 46630) according to the product protocol. The multiplex gel image was captured on Typhoon® 9410 at 532 nm excitation / 580BP30 emission and 633 nm excitation / 670BP30 emission. **Lane 1, 2:** Thermo Scientific DyLight 549/649 Fluorescent Protein Molecular Weight Markers (5 µl); **Lane 3, 4:** *E. coli* lysate (3.75 µg); **Lane 5, 6:** E. coli lysate (1.88 µg); **Lane 7, 8:** HeLa cell lysate (3.75 µg); **Lane 9, 10:** HeLa cell lysate (1.88 µg); **Lane 11:** Purified BSA (600 ng); Lane 12: Purified BSA (300 ng).

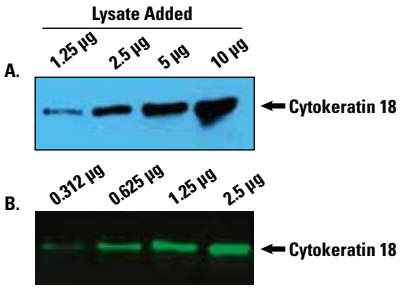
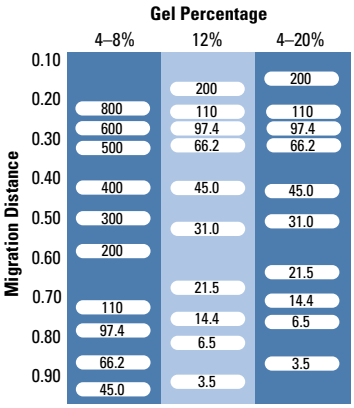


Figure 7. Pierce Protein Gels enable excellent protein transfer efficiency. Western blot detection of Cytokeratin 18. Protein lysate from transfected A549 cells **(A)** or HeLa cells **(B)** was separated using 4-20% (Product # 84713) and 12% (Product # 84711) Pierce Protein Gels, respectively. **Panel A:** The proteins were transferred to the nitrocellulose membrane for 12 minutes at 25V using Pierce Fast Semi-Dry Blotter (Product # 88217) and Fast Semi-Dry Transfer Buffer (Product # 35035). The blot was blocked overnight in 1X BSA / PBS-0.05% Tween®-20. After blocking, the membrane was incubated for 60 minutes with Rabbit Anti-Cytokeratin 18, washed 3 times 10 minutes each with PBS-0.05% Tween-20 followed by 60 minute incubation with HRP-conjugated Goat anti-Rabbit IgG (Product # 31460). After six 5-minute washes with PBS-0.05% Tween-20, the blot was incubated for 5 minutes in Pierce ECL Western Blotting Substrate (Product # 32106), placed in the plastic sheet and exposed to CL-XPosure Film for 1 minute. **Panel B:** The proteins were transferred to Low Fluorescence PVDF (Product # 22860) for 40 minutes at 20V (semi-dry transfer) using BupH Tris-Glycine Buffer (Product # 28380). The blot was blocked for 60 minutes in SEA Block Protein Blocker and then probed for 60 minutes with Rabbit Anti-Cytokeratin 18, washed 3 times 10 minutes each with PBS-0.05% Tween-20 followed by 60 minute incubation with DyLight 680B-Goat anti-Rabbit conjugate (Product # 35574). After the blot was washed 6 times 5 minutes with PBS-0.05% Tween-20, the image was captured on LI-COR Odyssey® at 700 Channel.



Gel Specifications

Cassette size: 10 cm x 10 cm x 7 mm
Gel size: 8 cm x 8.5 cm x 1 mm
Shelf life: 12 months at 4°C
Running buffer: Tris-HEPES-SDS
Sample buffer: Tris-HCl-LDS

Compatible Gel Tanks

Thermo Scientific Owl P82 System
Novex® XCell I, II™ and Surelock® Systems
C.B.S. Scientific CBDCX-700 Dual Cool System
PAGEr® Minigel Chamber

Ordering Information

Thermo Scientific Pierce Protein Gels

Product #	% Acrylamide	# Wells	Well Volume	Pkg. Size
84708	**4-8**	12	35 µl	10 gels
84711	**12**	12	35 µl	10 gels
84713	**4-20**	12	35 µl	10 gels
84710	**4-8**	17	20 µl	10 gels
84712	**12**	17	20 µl	10 gels
84714	**4-20**	17	20 µl	10 gels

**Choose a Pierce Protein Gel equivalent to the gel that is used in the Laemmli system.*
*** All cassettes are 10 cm x 10 cm x 7 mm.*

Thermo Scientific Precise Protein Gels

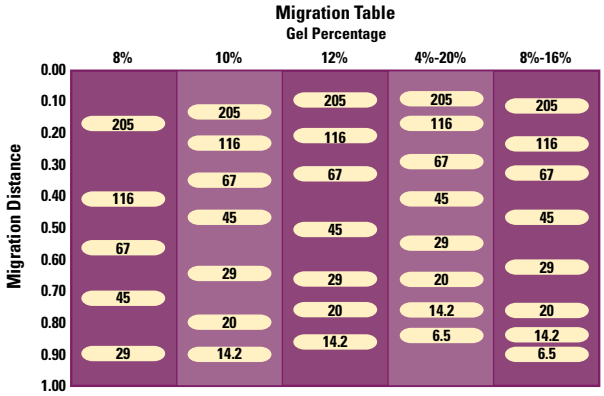
Long shelf life … short run time.

Thermo Scientific Precise Protein Gels are cast in a durable plastic cassette using a neutral pH buffer that prevents polyacrylamide breakdown and results in a long shelf life. High-resolution staining and transfer of proteins is accomplished quickly on these 1 mm thick gels. Gels are individually packaged in an easy-to-open plastic pouch and are ready to run with no comb or tape to remove. The gels are available in both gradient and fixed concentrations and in 10-, 12- and 15-well formats.

Highlights:

- 12-month guarantee ensures consistent performance
- 45-minute run time provides results quickly
- Sample wells hold up to twice the volume of Novex Brand gels (10-well=50 µl, 12-well=30 µl, 15-well=25 µl)
- Unique running buffer produces excellent separation and high-resolution protein bands
- Compatible with Laemmli sample buffer
- Compatible with standard mini-gel tanks so there is no need to purchase new equipment
- Stains quickly and with high sensitivity using coomassie and silver stains
- Transfers quickly and efficiently to nitrocellulose and PVDF membranes for Western blotting
- More resolving power than Novex Gels
- Plastic lane dividers prevent sample cross-contamination

Step 1 — **Prepare the gel**



Gel Specifications:

Cassette size	10 cm x 8.5 cm x 4.5 mm
Gel size	8 cm x 5.8 cm x 1 mm
Shelf life	12 months @ 4°C
Running buffer	Tris-HEPES-SDS
Sample buffer	Tris-HCl-SDS

Compatible Gel Tanks:

Thermo Scientific Owl P8 Systems
Hoefer® Tall Mighty Small (SE 280), Mighty Small (SE 260/SE 250) and miniVE (SE 300)
C.B.S. Scientific MGV 302/402
GradiGel Mini 4-Cell
IBI Universal Protein System EC 4-Cell
Bio-Rad Mini-PROTEAN™ II & 3
Daiichi Mini 2-Gel & 6-Gel
Novex XCell I and II Surelock

Ordering Information

Product #	Percent Acrylamide	# of Sample Wells	Sample Well Volume	Pkg. Size
25200	8%	10	50 µl	10 gels
25201	10%	10	50 µl	10 gels
25202	12%	10	50 µl	10 gels
25203	8-16%	10	50 µl	10 gels
25204	4-20%	10	50 µl	10 gels
25220	8%	12	30 µl	10 gels
25221	10%	12	30 µl	10 gels
25222	12%	12	30 µl	10 gels
25223	8-16%	12	30 µl	10 gels
25224	4-20%	12	30 µl	10 gels
25240	8%	15	25 µl	10 gels
25241	10%	15	25 µl	10 gels
25242	12%	15	25 µl	10 gels
25243	8-16%	15	25 µl	10 gels
25244	4-20%	15	25 µl	10 gels

Thermo Scientific Tris-HEPES-SDS Running Buffer

Required running buffer for use with Pierce and Precise Gels.

Both Pierce and Precise Protein Gels use a unique Tris-HEPES-SDS running buffer to improve band resolution and reduce run-time. The buffer can be made according to the recipe provided in the Pierce and Precise Gel product instructions or purchased premixed, as a dry powder or as a 20X liquid concentrate (BupH pack).

Ordering Information

Product #	Description	Pkg. Size
28398	**BupH Tris-HEPES-SDS Running Buffer** Each pack yields 500 ml of 100 mM Tris, 100 mM HEPES, 3 mM SDS, pH 8 ± 0.25 when dissolved in 500 ml distilled water (5 L total).	10 pack
28368	**20X Tris-HEPES-SDS Running Buffer** 20X Concentrate, 1X = 0.1 M Tris, 0.1 M HEPES, 3 mM SDS, pH 8 + 0.25	500 ml

Thermo Scientific LDS Sample Buffer

The LDS Sample Buffer, Non-Reducing (4X) is specifically formulated and recommended for use with Pierce Protein Gels. The solution is specifically formulated and recommended for use with Pierce Protein Gels. The solution is a convenient sample buffer for use in SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The buffer contains coomassie dye, enabling visualization of the electrophoresis progress by the location of the dye front. The LDS Sample Buffer, Non-Reducing (4X) may be used in denaturing gels and is compatible with coomassie dye and silver staining, and Western blotting procedures.

Ordering Information

Product #	Description	Pkg. Size
84788	**LDS Sample Buffer, Non-Reducing (4X)**	5 ml

Thermo Scientific Lane Marker Sample Buffers

The 5X concentration allows you to load more sample!

Highlights:

- Bright pink hydrophobic tracking dye (5X) for SDS-PAGE that transfers to nitrocellulose membranes
- Transfer of the dye front is an indicator of protein transfer efficiency
- Dye front is visible on both the gel and nitrocellulose membrane for determination of molecular weight (Rf values)

Note: *These products are not compatible with fluorescent detection systems. (The pink tracking dye fluoresces strongly.)*

Ordering Information

Product #	Description	Pkg. Size
39000	**Lane Marker Reducing Sample Buffer (5X)** 0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% Glycerol, 100 mM Dithiothreitol, Lane Marker Tracking Dye	5 ml
39001	**Lane Marker Non-Reducing Sample Buffer (5X)** 0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% Glycerol, Lane Marker Tracking Dye	5 ml

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.

Samples may contain substances that interfere with obtaining a well-resolved protein band in the gel. Substances such as guanidine hydrochloride and ionic detergents can result in protein bands that appear smeared or wavy in the gel or on a Western blot. The Thermo Scientific Pierce SDS-PAGE Sample Prep Kit (Product # 89888) removes these interfering components using an affinity resin that selectively binds then releases proteins. Using 20 µl of Pierce SDS-PAGE Protein Binding Resin, a protein sample (2-300 µl) can be purged of any contaminants in only 10 minutes. This is much faster than dialysis or ultrafiltration and yields higher protein recoveries while concentrating the sample.

Thermo Scientific Pierce SDS-PAGE Sample Prep Kit

Quick protein clean-up and enrichment for SDS-PAGE.

Numerous compounds interfere with typical sample buffers for polyacrylamide gel electrophoresis (SDS-PAGE). For example, protein samples containing 6 M guanidine•HCl will precipitate when mixed with Laemmli buffer for SDS-PAGE, causing the sample to run poorly in a gel. Fortunately, samples containing a wide range of interfering chemicals, such as chaotropic agents, detergents, lipids, pH extremes and salts, can be "cleaned-up" in minutes using the SDS-PAGE Sample Prep Kit. Even high concentrations of detergents that are difficult to remove by standard sample process methods can be treated easily with Pierce SDS-PAGE Sample Prep Kit to eliminate distortion of bands during analysis (Figure 1).

Sample concentration is also an important factor in SDS-PAGE when the gel sample well volume limits the amount of dilute protein that may be loaded. Fortunately, our SDS-PAGE Sample Prep Kit not only removes interfering substances but also can rapidly concentrate dilute protein samples up to 10 fold, enabling more protein to be loaded per gel lane (Figure 2).

Our 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 nonbound contaminating chemicals. Finally, elute the sample in 50 µl of the Elution Buffer.

The recovered protein sample is ready to mix with the supplied 5X Sample Loading Buffer for gel loading. In addition, the Thermo Scientific Pierce BCA Protein Assay (Product # 23225) is compatible with the elution buffer and may be used to determine final protein concentration before gel loading.

Highlights:

- Eliminates artifacts caused by incompatible contaminants – removes dyes, reducing agents, detergents, sugars, glycerol, guanidine, urea and ammonium sulfate to provide reproducible results on SDS-PAGE analysis
- Compatible with the 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
- 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

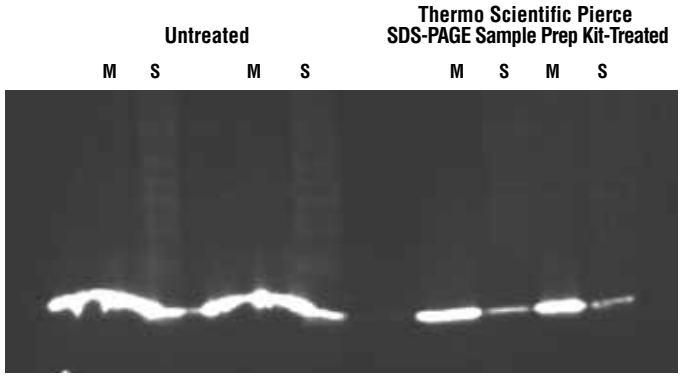


Figure 1. Eliminate distortion caused by detergents. Rat C6 cells were lysed and a membrane protein fraction isolated using Thermo Scientific Mem-PER Eukaryotic Membrane Protein Extraction Reagent (Product # 89826). Membrane and hydrophilic cell fractions were separated by SDS-PAGE using 4-20% gradient gels with or without prior treatment using the Pierce SDS-PAGE Protein Binding Resin. Western blot analysis was performed using an antibody against cytochrome oxidase subunit 4 (COX 4) and Thermo Scientific SuperSignal West Femto Chemiluminescent Substrate (Product # 34095). 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

Prepare samples for SDS-PAGE analysis from:

- Inclusion bodies solubilized in guanidine•HCl
- Samples containing low-pH buffers, thiocyanate or urea
- Proteins precipitated in ammonium sulfate
- Dilute protein solutions

Step 2 — **Prepare the sample**

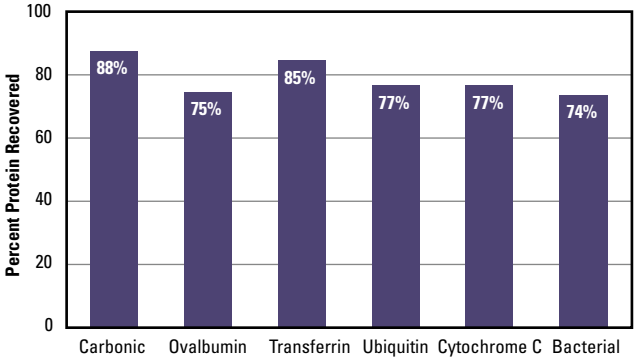


Figure 2. Consistent protein recovery is achieved using the Thermo Scientific Pierce SDS PAGE Sample Prep Kit. Pure proteins (60 µg) of assorted molecular weights: 30K, 44K, 80K, 86K and 12K and bacterial lysate at 27K were processed using this kit. Protein concentrations were determined with the Thermo Scientific Pierce BCA Protein Assay and reported as percent protein recovered.

Table 1. 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%
1M Sodium Chloride	75%
6M Urea	74%
10% CHAPS	80%
25% Glycerol	71%
10% OTG	71%
2 M Guanidinium•HCl	70%
40% Sucrose	70%

Ordering Information

Product #	Description	Pkg. Size
89888	**Pierce SDS-PAGE Sample Prep Kit** *Sufficient reagents to prepare 50 samples.* *This product replaces Product # 26800.* Includes: Pierce SDS-PAGE Protein Binding Resin Elution Buffer Purified DMSO Spin Cups Collection Tubes Lane Marker, Non-Reducing Sample Buffer (5X)	Kit 1 ml 5.0 ml 27 ml 50 72 5 ml

2-D Gels

Isolating and extracting proteins may result in charged buffer components that interfere with IEF in the first dimension of 2-D electrophoresis. To address this, we offer Thermo Scientific 2-D Sample Prep for Nuclear Proteins (Product # 89863).

Our 2-D Sample Preparation Kits contain mini-desalting spin-columns for exchanging small sample sizes (< 400 µl) directly into a 2-D sample buffer supplied. The protein sample is effectively concentrated as it is desalted. This sample can be directly applied to the IEF gel. This assures that the 2-D gel results are consistent and the proteins migrate properly in the second dimension.[9,10] In addition, Thermo Scientific 660 nm Protein Assay (Product # 22660) is compatible with 2-D sample buffers for accurate determination of protein before electrophoresis.

References

9. Rabilloud, T., *et al.* (1997). *Electrophoresis* **18,** 307-316.
10. Lanne, B., *et al.* (2001). *Proteomics* **1,** 819-828.

Thermo Scientific 2-D Sample Prep Kit for Nuclear Proteins

Suited for nuclear protein fractionation along with sample cleanup.

Streamlines nuclear protein extraction with 2-D sample preparation. Nuclear proteins are isolated, concentrated and exchanged into 2-D sample buffer without precipitation.

Highlights:

- Removes small charged contaminants that interfere with 2-D electrophoresis – reduces the time for isoelectric focusing and prevents loss of data on 2-D gels due to salt fronts
- Buffer exchanges nuclear proteins into 2-D Sample Buffer – "concentrates" protein by increasing amount of protein that can be applied to an IPG strip and maintains proteins in solution throughout the desalting process
- Uses Thermo Scientific NE-PER Nuclear and Cytoplasmic Reagents – prepares a highly purified nuclear protein extract
- Streamlines nuclear protein extraction with 2-D sample preparation – contains a faster and more efficient protocol than the two procedures performed separately
- Contains thiourea in sample buffer – increases protein solubility and improves protein resolution on 2-D gels
- Desalts faster than existing 2-D sample prep kits – allows multiple samples to be processed in less than 15 minutes instead of one plus hours required for precipitation and dialysis

Ordering Information

Product #	Description	Pkg. Size
89863	**2-D Sample Prep for Nuclear Proteins Kit** *Sufficient reagents for 25 applications.* Includes: NE-PER Nuclear and Cytoplasmic Extraction Reagents: Cytoplasmic Extraction Reagent I (CER I) Cytoplasmic Extraction Reagent II (CER II) Nuclear Extraction Reagent (NER) 2-D Sample Buffer for Nuclear Proteins: 2-D Sample Buffer for Nuclear Proteins, Component A 2-D Sample Buffer for Nuclear Proteins, Component B Protein Desalting Spin Columns	Kit 5 ml 0.275 ml 2.5 ml 18 ml 16.5 g 25 columns

SDS-PAGE Running and Transfer Buffers

Protein samples prepared for SDS-PAGE analysis are denatured by heating in the presence of a sample buffer containing 0.5% SDS with or without a reducing agent such as 50-100 mM DTT (Product # 20290 or 20291) or Mercaptoethanol (Product # 35602). TCEP (Product # 77720) is a stable, odorless and highly effective reducing agent alternative. The protein sample is mixed with the sample buffer and boiled for 3-5 minutes, then cooled to room temperature before it is applied to the sample well on the gel. As a protein sample passes through a gel, the buffer front can be visualized using small molecular weight dyes that migrate with the buffer front. The most commonly used tracking dye is bromophenol blue. This dye aids in loading the gel and shows the movement of the buffer front through the gel. The Thermo Scientific Lane Marker Sample Buffers contain an alternative bright pink tracking dye and are available in a reducing (Product # 39000) and a nonreducing (Product # 39001) formulation. The pink tracking dye also can be transferred onto nitrocellulose membranes to prepare immunoblots, thereby acting as an indicator to assure that the proteins have been successfully transferred from the gel to a blotting membrane. Thermo Scientific Piece 660 nm Protein Assay (Product # 22660) with Ionic Detergent Compatibility Reagent (Product # 22663) is compatible with samples directly lysed with Laemmli sample buffer containing bromophenol blue, enabling quick, yet accurate determination of protein.

Thermo Scientific Premade Buffers

For buffer recipes see the product description.

Tris-HEPES-SDS Buffers

A nonreducing buffer for use with Thermo Scientific Pierce and Precise Gels.

Ordering Information

Product #	Description	Pkg. Size
28398	**BupH Tris-HEPES-SDS Running Buffer** Each pack yields 500 ml of 100 mM Tris, 100 mM HEPES, 3 mM SDS, pH 8 ± 0.5 when dissolved in 500 ml distilled water (5 L total).	10 pack
28368	**20X Tris-HEPES-SDS Buffer** 20X Concentrate, 1X = 0.1 M Tris, 0.1 M HEPES, 3 mM SDS, pH 8 + 0.25	500 ml

Tris-Glycine-SDS Buffers

A ready-to-use nonreducing electrophoresis buffer.

Ordering Information

Product #	Description	Pkg. Size
28378	**BupH Tris-Glycine-SDS Buffer Packs** Each pack yields 500 ml of 25 mM Tris, 192 mM Glycine and 0.1% SDS, pH 8.3 when dissolved in 500 ml distilled water (20 L total). (Not for use with Precise Protein Gels and Pierce Protein Gels)	40 pack
28362	**10X Tris-Glycine-SDS Buffer** 10X Solution	1 L

Tris-Glycine Buffers

Ready-to-use transfer buffers.

Ordering Information

Product #	Description	Pkg. Size
28380	**BupH Tris-Glycine Buffer Packs** Each pack yields 500 ml of 25 mM Tris and 192 mM Glycine at a pH of approximately 8 when dissolved in 400 ml distilled water and 100 ml of methanol (20 L total).	40 pack
28363	**10X Tris-Glycine Buffer**	1 L
35040	**10X Pierce Western Blot Transfer Buffer, Methanol-free**	5 L
35035	**Fast Semi-Dry Transfer Buffer, 10X**	500 ml

Step 3 — **Prepare the buffers**

Lane Marker Sample Buffers

The 5X concentration allows you to load more sample!

Highlights:

- Bright pink hydrophobic tracking dye (5X) for SDS-PAGE that transfers to membranes
- Transfer of the dye front is an indicator of protein transfer efficiency
- Dye front is visible on both the gel and nitrocellulose membrane for determination of molecular weight (Rf values)

Note: *These products are not compatible with fluorescent detection systems. (The pink tracking dye fluoresces strongly.)*

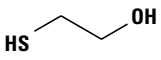
Ordering Information

Product #	Description	Pkg. Size
39000	Lane Marker Reducing Sample Buffer (5X) 0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% Glycerol, 100 mM Dithiothreitol, Lane Marker Tracking Dye	5 ml
39001	Lane Marker Non-Reducing Sample Buffer (5X) 0.3 M Tris•HCl, pH 6.8, 5% SDS, 50% Glycerol, Lane Marker Tracking Dye	5 ml

Thermo Scientific Solution and Solid-phase Reductants for Disulfide-containing Peptides and Proteins

2-Mercaptoethanol

A mild reducing agent for cleaving disulfide bonds to thiols.



2-Mercaptoethanol
MW 78.13

Highlights:

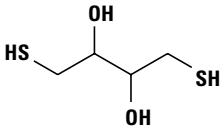
- Also known as β-Mercaptoethanol (BME)
- Often included in enzyme solutions to protect against catalytic site inactivation due to cysteine sulfhydryl oxidation

Ordering Information

Product #	Description	Pkg. Size
35602	2-Mercaptoethanol (2-ME)	10 x 1 ml ampules

DTT

A water-soluble reagent that reduces disulfide bonds.



DTT
MW 154.25

Applications:

- Maintains mono-thiols completely in the reduced state and reduces disulfide bonds quantitatively
- Specific and sensitive assay for disulfides using DTT with Ellman's Reagent (Product # 22582)

Ordering Information

Product #	Description	Pkg. Size
20290	DTT, Cleland's Reagent (Dithiothreitol)	5 g

No-Weigh™ DTT

Don't waste your talents at the balance!

Make a 500 mM solution of DTT in less than 30 seconds with our convenient No-Weigh Packaged DTT. The unique packaging ensures that the reducing agent is at full strength and able to protect proteins from oxidative damage or reduce any disulfides before electrophoresis.

Applications:

- Saves time – just pipette and use
- Eliminates waste – make 100 µl DTT solution
- Ensures a fresh solution with full reducing strength

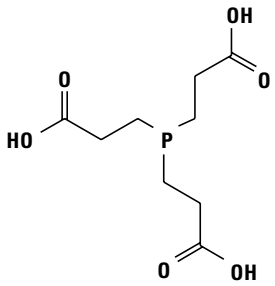
No-Weigh DTT is a pre-measured, dry, room temperature-stable aliquot of the reductant sealed in a microtube. All you do is puncture the seal with a pipette tip and add 100 µl of water or buffer. In seconds, you will have a fresh, 500 mM solution of DTT to use.

Ordering Information

Product #	Description	Pkg. Size
20291	No-Weigh Dithiothreitol (DTT) 7.7 mg DTT/Tube	48 micro-tubes

Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH[1]

The efficient, odor-free alternative to sample reduction prior to SDS-PAGE analysis.



TCEP
MW 250.15

Highlights:

- Ready-to-use, odor-free, stable and neutral 0.5 M TCEP (Tris[2-carboxyethyl]phosphine hydrochloride) solution
- More effective than β-mercaptoethanol or DTT in reducing disulfides for SDS-PAGE
- Eliminates TCEP•HCl stock solution preparation and neutralization
- Neutral pH minimizes possibility of amide bond cleavage during reduction
- Room temperature-stable, saves valuable refrigerator space
- Contributes to more pleasant, safer labratory environment

Reference

1. Huh, K. and Wenthold, R.J. (1999). *J. Biol. Chem.* **274,** 151-157.

Ordering Information

Product #	Description	Pkg. Size
77720	Bond-Breaker® TCEP Solution, Neutral pH	5 ml

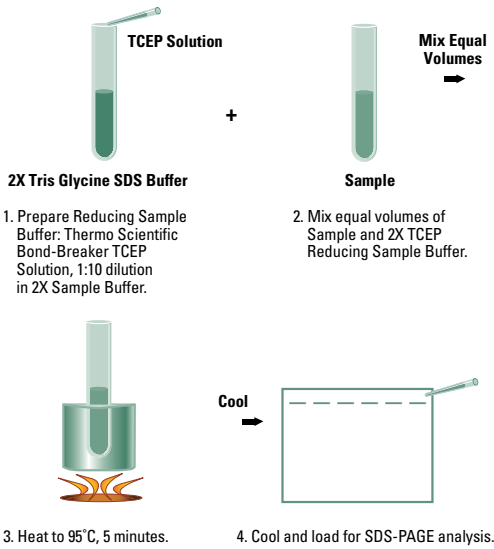


Figure 1. Thermo Scientific Bond-Breaker TCEP Solution procedure.

Thermo Scientific TCEP•HCl[2-3]

Potent, water-soluble, odorless reducing agent in a conventional solid format.

Highlights:

- Selective and complete reduction of even the most stable water-soluble alkyl disulfides
- Effective reduction at room temperature and pH 5 in less than five minutes
- Water solubility of 310 g/L
- Resistant to air oxidation; nonvolatile and nonreactive toward other functional groups found in proteins

References

2. Kirley, T.L. (1989). *Anal. Biochem.* **180,** 231-236.
3. Han, J. and Han, G. (1994). *Anal. Biochem.* **220,** 5-10.
4. Oda, Y., *et al.* (2001). *Nature Biotech.* **19,** 379-382.

Ordering Information

Product #	Description	Pkg. Size
20490	TCEP•HCl (Tris[2-carboxyethyl]phosphine hydrochloride)	1 g
20491	TCEP•HCl	10 g

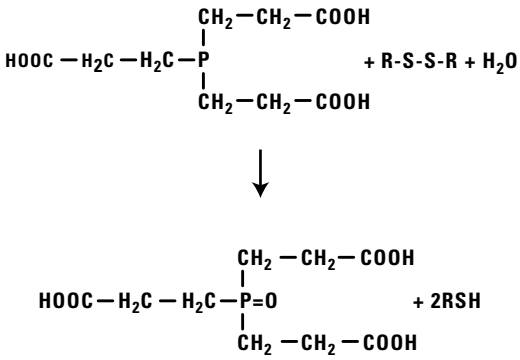


Figure 2. The reduction of disulfides by TCEP.

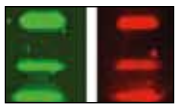
Step 4 — **Choose MW markers**

Molecular Weight Markers

To assess the relative molecular weight (MW) of a protein on a gel, protein MW markers are run in the outer lanes of the gel for comparison. A standard curve can be constructed from the distances migrated by each marker protein. The distance migrated by the unknown protein is then plotted, and the molecular weight is interpolated from the standard curve.

We offer a variety of MW markers for use with one- and two-dimensional protein gels and for various detection methods. Table 1 summarizes the different features of each marker mix. Of the five MW marker mixes for reducing SDS-PAGE, three contain proteins that are prestained for direct in-gel visualization during and after electrophoresis and upon transfer to membrane. These three prestained markers are provided as stabilized, pre-reduced and lyophilized aliquots in SDS-PAGE sample loading buffer. There is no need to heat the samples; simply puncture the protective foil layer, add running buffer to rehydrate the proteins and transfer 2-10 µl of the mix to a lane on the gel. As the name suggests, the Thermo Scientific Chemiluminescent Blue Marker was created for chemiluminescent detection on Western blots; the constituent prestained and peroxidase-labeled proteins are detectable on film or CCD camera when used with a chemiluminescent substrate for HRP. The Thermo Scientific DyLight Fluor- and IR-Labeled MW Markers produce their respective signal types with excellent uniformity; both markers also contain sufficient protein for detection by coomassie and silver staining, making them extremely versatile. The Thermo Scientific Pierce 2-D MW Marker Mix (Product # 26659) includes proteins with a broad range of isoelectric points (pI 4.5-8.7) and molecular weights (17K-80K).

Table 1. Thermo Scientific Protein Molecular Weight Markers products for electrophoresis*.

Protein	Pierce Blue (Product #26681) 	Pierce Three-Color (Product # 26691) 	Pierce Chemiluminescent Blue (Product # 26651) 	DyLight Dual Fluor-labeled (Product #26665) 	DyLight Dual IR-labeled (Product # 22859) 	Pierce 2-D MW Marker Mix (Product # 26659) 
Myosin	210K	210K	220K	200K	200K	-
Phosphorylase B	120K	110K	104K	97K	97K	-
Apotransferrin	-	-	-	-	-	80K, pI 6.2
Bovine Serum Albumin (BSA)	84K	80K	76K	66K	66K	-
Glutamic Dehydrogenase	-	-	-	-	-	56K, pI 6.5, 6.7, 6.9
Ovalbumin	60K	47K	45K	-	-	-
Actin	-	-	-	-	-	43K, pI 5.2
Protein A	-	-	-	42K	42K	-
Protein L	-	-	-	36K	36K	-
Carbonic Anhydrase	39.2K	32K	33K	-	-	29K, pI 6.3
Peanut Agglutinin	-	-	-	28K	28K	-
Myokinase	-	-	-	-	-	22.5K, pI 8.7
Soybean Trypsin Inhibitor	28K	25K	26K	20K	20K	20K, pI 4.5
Myoglobin	-	-	-	-	-	17K, pI 7.0, 7.4
Lysozyme	18.3K	16.5K	18K	14K	14K	-
Aprotinin	-	-	-	6K	6K	-
Staining Feature	Prestained (1 color)	Prestained (3 colors)	Peroxidase-labeled, prestained (1 color)	Fluorescent (2 channels), stainable	Infra-Red (IR) (2 channels), stainable	Unstained
Package	48 microtubes (48-96 gels)	48 microtubes (48-96 gels)	48 microtubes (48-96 gels)	250 µl (25-100 gels)	250 µl (25-100 gels)	500 µl (~250 gels)

**Actual molecular weights are lot-specific because the proteins are prestained. Lot-specific information is included in each package.*

Step 4 — **Choose MW markers**

Thermo Scientific Pierce Blue Prestained Molecular Weight Markers[1,2]

A fresh marker every time, not just the first time.

A totally new idea in how molecular weight markers are packaged!

- Innovative single-dose package
- Room temperature stable
- Excellent performance on wide range of gel compositions
- Efficient membrane transfer

Highlights:

- Single-dose packaging in a novel microtube plate format eliminates opportunities for contamination due to multiple marker withdrawals from the same vial
- Unique stabilized prestained markers can be stored at room temperature
- Compatible with a broad range of SDS-PAGE gel compositions and downstream applications
- Prestained proteins transfer well to both nitrocellulose and PVDF membrane
- Can be used with our GelCode Blue, Silver and Reversible Stains
- Formulated to yield prestained protein bands of equal intensity

Here's how it works:

These prestained markers are an individually dried and stabilized formulation of seven proteins spanning the range from 18.3K to 210K. The plate is covered with a foil that can be easily punctured with a pipette tip. Simply puncture the foil covering a single well containing the dried marker mix with a pipette tip and add 10 µl deionized water. The marker proteins are reconstituted instantly and ready for loading onto a gel lane.

The proteins listed, covering a broad molecular weight range, have been prestained and purified to give single bands on 4-20% SDS-PAGE gels. Each protein has been proportioned into the mix to yield uniform band intensity.

Markers are ready when you are and room temperature-stable.

1. Open the resealable plastic pouch and remove the Prestained Protein Molecular Weight Marker Mix. This prestained marker mix is packaged with a desiccant in a moisture-resistant, resealable pouch.

2. Load 10 µl of DI water into a pipette tip, puncture the foil over a single tube and dissolve the prestained markers.

3. Dispense 5-10 µl of the marker into a sample well of the gel to be run. Each tube can be used to prepare one or two lanes of a gel.

4. Return the prestained marker mix to its pouch and reseal. The markers are stable at room temperature and can be kept right on your bench-top ready for your next SDS-PAGE gel.

References

1. Foubert, T.R., *et al.* (2001). *J. Biol. Chem.* **276,** 38852-38861.
2. Prozialeck, W.C., *et al.* (2002). *Infect. Immun.* **70,** 2605-2613.

Thermo Scientific Pierce 3-Color Prestained Molecular Weight Markers[3,4]

Fresh marker every time, with reference bands too.

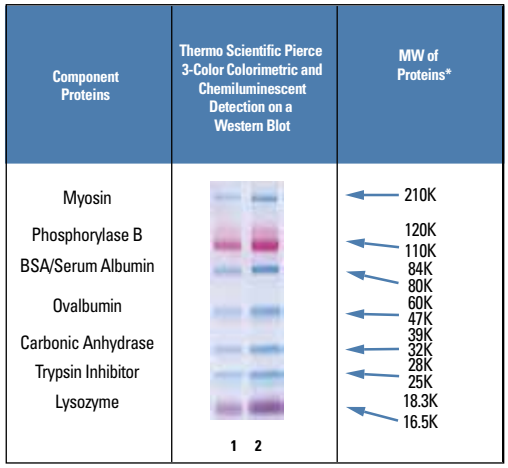


Figure 1. Thermo Scientific Pierce Prestained Marker Protein molecular weights.* Each tube of the Pierce Marker consists of a stabilized and lyophilized formulation of seven proteins, ranging from 16.5K to 210K. Each protein in the mixture is proportioned to yield uniform band intensities. Two specially modified bands (one red, one violet) serve as references for the order of the marker proteins.

**These are representative molecular weight values. The covalently bound dye and enzyme alter the apparent molecular weight (MW) of the component proteins relative to their unstained counterparts. Lot-specific MW values are provided with each package.*

Highlights:

- Innovative single-dose packaging allows you to dissolve only the marker you need exactly when you want it
- The single-dose packaging eliminates the possibility of contamination due to multiple withdrawals
- Room-temperature storage eliminates the need to expose protein markers to detrimental freeze-thaw cycles

References

3. Myers, C.R. and Myers, J.M. (2002). *Appl. Envir. Microbiol.* **68,** 5585-5594.
4. Cui, L., *et al.* (2002). *Am. J. Physiol. Cell Physiol.* **283,** C623-C630.

Ordering Information

Product #	Description	Pkg. Size
26681	**Pierce Blue Prestained Protein Molecular Weight Marker Mix** *Sufficient material for loading 48-96 gel lanes.*	1 x 48 microtube plate
26685	**Pierce Blue Prestained Protein Molecular Weight Marker Mix** *Sufficient material for loading 240-480 gel lanes.*	5 x 48 microtube plates
26691	**Pierce 3-Color Prestained Protein Molecular Weight Marker Mix** *Sufficient material for loading 48-96 gel lanes.*	1 x 48 microtube plate

Thermo Scientific Pierce Chemiluminescent Molecular Weight Markers

Protein MW standard looks and acts like a typical pre-stained marker for SDS-PAGE and can also "light up" after transfer or in-gel.

Our Chemiluminescent Marker consists of seven proteins spanning the molecular weight range from 18K to 220K. Each marker component is covalently linked to a blue dye and chemically modified to impart peroxidase capability. Unlike any other chemiluminescent detection-compatible marker for Western blot applications, Pierce Chemiluminescent Marker does not need an HRP-antibody conjugate to yield a chemiluminescent signal.

Highlights:

- Colorimetric and chemiluminescent – two detection options are available: on-membrane or in-gel
- Visual detection in-gel – already prestained; does not require staining to detect in-gel
- Universal compatibility with HRP conjugates – self-contained peroxidase activity, does not require an HRP-antibody conjugate for chemiluminescence and no variability due to host animal or antibody class
- Compatible with streptavidin-HRP conjugates
- Room temperature stable
- Convenient packaging – single dose in 48-well microtube plate

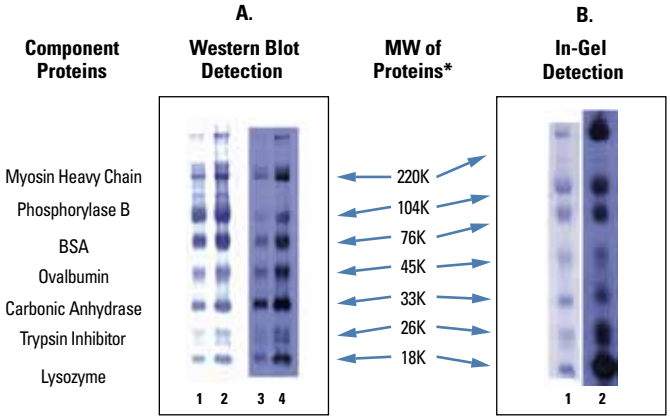


Figure 2. A. Western blot detection. Lanes 1-4 show the Thermo Scientific Pierce Chemiluminescent marker run on a 4-20% Tris-Glycine SDS-polyacrylamide gel and transferred to nitrocellulose. Lanes 1 and 3 were loaded with 2 µl of marker. Lanes 2 and 4 were loaded with 5 µl of marker. Lanes 1 and 2 show the marker colorimetrically after transfer to the membrane. Lanes 3 and 4 were treated with Thermo Scientific SuperSignal West Pico Chemiluminescent Substrate (Product # 34080) and exposed to X-ray film for 1 minute. **B. In-gel detection (Thermo Scientific Pierce In-Gel Detection Technology).** Lanes 1 and 2 were each loaded with 10 µl of marker before electrophoresis on a 4-20% Tris-Glycine gel. Lane 1 shows the marker bands colorimetrically in-gel. Lane 2 shows the marker proteins detected in-gel using Pierce In-Gel Detection Technology with Pierce In-Gel Detection Chemiluminescent Substrate (Product # 33550) and exposure of the gel to X-ray film for one minute.

**These are representative molecular weight values. The covalently bound dye and enzyme alter the apparent molecular weight (MW) of the component proteins relative to their unstained counterparts. Lot-specific MW values are provided with each package.*

Step 4 — **Choose MW markers**

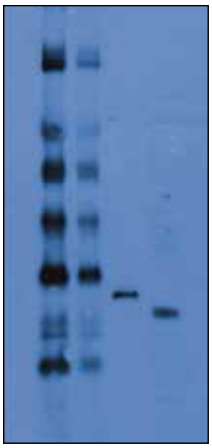


Figure 3. Thermo Scientific Pierce Chemiluminescent Molecular Weight Markers (5 µl and 2 µl loading) (Lanes 1 and 2) and two 6xHis proteins (~10 ng) (Lanes 3 and 4) were separated by electrophoresis using a 4-20% Tris-Glycine gradient gel. The proteins were transferred to nitrocellulose and detected for the 6xHis tag using the Thermo Scientific SuperSignal West HisProbe Kit (Product # 15168). The blot was exposed to X-ray film for 1 minute to capture the chemiluminescent signal. (The blot was scanned to document the color.)

Ordering Information

Product #	Description	Pkg. Size
26651	Pierce Chemiluminescent Molecular Weight Marker Mix	1 x 48 microtube plate

CAUTION: These chemiluminescent markers are prelabeled with a peroxidase enzyme. This means they must be handled more gently than traditional prestained markers. These markers can be overheated to the point of inactivation during the transfer from the gel to the membrane. They can also be inactivated by other conditions that are detrimental to peroxidases such as too much EDTA/EGTA, azide or acidic membrane stains such as Ponceau S. In most systems, inactivation is unlikely to occur, but if it does occur in your system, please return the remaining product for a full refund.

Thermo Scientific DyLight Fluorescent Protein Molecular Weight Markers

One- or two-color fluorescent detection with one protein molecular weight marker.

The DyLight Fluorescent Protein Molecular Weight Markers are optimized for direct visualization of marker proteins after SDS-PAGE. Each protein in the mixture is labeled with two fluorescent dyes to provide flexible one- or two-color detection with the LI-COR Odyssey® (infrared markers only) or common CCD instruments (Figure 4). The markers are compatible with Western blotting (Figure 5) and can be detected by virtually any in-gel staining method (Figure 6). The DyLight Fluorescent Protein Molecular Weight Markers consists of nine proteins with molecular weights in the range of 6K to 200K.

Highlights:

- **Easily multiplexed** – two excitation and emission maxima enable one- or two-color fluorescent detection
- **Easy to use and convenient** – eliminate the need for awkward marking or overlay procedures
- **Fluorescent and colorimetric** – two detection options available: in-gel or on-membrane
- **Instrument-compatible** – DyLight Dye spectra are compatible with common imaging systems
- **Photostable** – allows long exposure times for maximum sensitivity

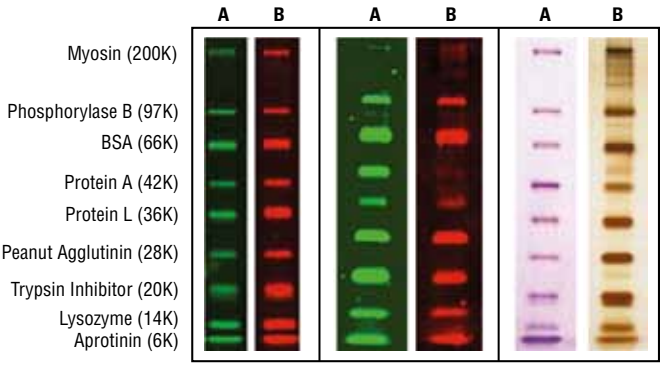


Figure 4 **Figure 5** **Figure 6**

Figure 4-6. Excellent marker detection in several formats. Figure 4. Direct in-gel fluorescent detection, 550/568 nm (A) and 646/674 nm (B). **Figure 5.** Western blot detection on nitrocellulose (A) and PVDF (B). **Figure 6.** In-gel colorimetric staining detection, coomassie dye (A) and silver stain (B).

Table 2. Special characteristics of Thermo Scientific DyLight Fluorescent Protein Molecular Weight Markers.

	Excitation (nm)	Emission (nm)	Extinction Coefficient (min)
DyLight 549 Dye	560	574	150,000 M^{-1} cm^{-1}
DyLight 649 Dye	654	673	250,000 M^{-1} cm^{-1}
DyLight 680 Dye	692	712	140,000 M^{-1} cm^{-1}
DyLight 800 Dye	777	790	270,000 M^{-1} cm^{-1}

Ordering Information

Product #	Description	Pkg. Size
26665	DyLight Fluorescent Protein Molecular Weight Markers *Sufficient material for loading 50 gel lanes*	250 µl
22859	DyLight Dual IR-labeled Protein Molecular Weight Markers *Sufficient material for loading 50 gel lanes*	250 µl

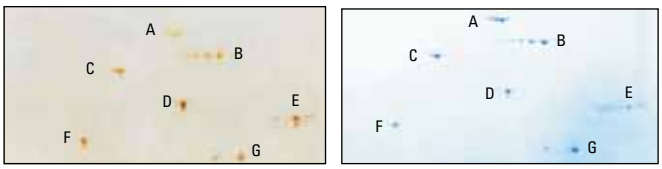
Thermo Scientific Pierce 2-D Protein Molecular Weight Markers

A 2-D gel marker mix that offers a broad range of pI and MW.

The new ready-to-use 2-D Gel Marker Mix is designed specifically to aid the proteome analyst. This 2-D Gel Marker Mix contains a complement of seven reduced and denatured proteins. When performing protein 2-D separation and analysis, each protein in the mix provides important features for assessing system performance or estimating pI and molecular weight values.

A unique complement of proteins

Each protein in this marker mix was carefully selected to give a useful range of molecular weight and pI coverage. Proteins were selected that give a variety of features from tight single spots to characteristic charge trains to aid the analyst in gel orientation. Molecular weights range from 17K to 80K, with pI values ranging from 4.5 to 8.7.



A. Stained with Silver **B. Coomassie Blue Dye**

Spot	2-D Marker Protein	MW	pI Value
A	Apo-Transferrin (human plasma)	80K	6.2
B	Glutamic Dehydrogenase (bovine liver)	56K	6.5, 6.7, 6.9
C	Actin (bovine muscle)	43K	5.2
D	Carbonic Anhydrase (bovine erythrocytes)	29K	6.3
E	Myokinase (chicken muscle)	22.5K	8.7
F	Trypsin Inhibitor (soybean)	20K	4.5
G	Myoglobin (equine skeletal muscle)	17K	7.0, 7.4

Figure 7. Thermo Scientific Pierce 2-D Markers shown stained with silver (left) and coomassie blue dye (right).

Table 3. Thermo Scientific Pierce 2-D Marker Mix is sufficient for the following number of applications, depending on gel size and staining method.

Gel Size	Stain Method	Marker Volume	# of Gels/ Vial
Mini-Gels	Coomassie Blue Dye	2.5 µl	200
Mini-Gels	Silver	0.5-1.0 µl	500-1000
Large Format Gels	Coomassie Blue Dye	5-7.5 µl	66-100
Large Format Gels	Silver	1.0-2.5 µl	200-500

The Thermo Scientific Pierce 2-D Protein Molecular Weight Marker Mix is supplied frozen. For optimal long-term stability, aliquot into sample vials upon receipt and refreeze.

Ordering Information

Product #	Description	Pkg. Size
26659	Pierce 2-D Protein Molecular Weight Marker Mix	500 µl

Step 6 — **Stain the gel**

General In-Gel Detection of Protein Bands

Once protein bands have been separated on a gel (1-D or 2-D), they can be visualized using different methods of in-gel detection. One method, called autoradiography, involves radiolabeling proteins before electrophoresis and then exposing the resulting gel to X-ray film. As radiation is emitted, it produces metallic silver within the silver halide crystals on the film and can be seen as gray bands once the film is developed. For this method, proteins in a sample can be radioiodinated using ^{125}I and Thermo Scientific Pierce Iodination Tubes (Product # 28601). Other radioisotopes such as ^{14}C, ^{35}S and ^{3}H also can be used in autoradiography.

The most common method for in-gel protein detection is staining with coomassie dye. Although the mechanism is not completely understood, binding of coomassie dye to proteins depends in part on basic and hydrophobic residues. Therefore, binding (i.e., staining intensity) varies among proteins whose amino acid compositions differ with respect to these residues. Upon incubation in coomassie staining solution, most protein gels become entirely blue and must be destained with a methanol/acetic acid mixture to remove stain from the background gel matrix and see the protein bands. The combination of alcohol and acid in the stain, destain and/or wash solutions also helps to fix proteins so that they do not diffuse from the gel matrix during the procedure.

Several coomassie gel stain recipes exist in the literature and use either the G-250 ("colloidal"; Product # 20278) or R-250 (Product # 20279) form of the dye. We offer three exceptional coomassie-based gel stains. Thermo Scientific GelCode Blue Stain Reagent (Product # 24590, 24592) is a colloidal coomassie reagent that stains effectively in an hour and requires only water (no methanol or acetic acid) for destaining. GelCode Blue Safe Stain (Product # 24594, 24596) is a colloidal coomassie reagent formulation that does not require costly hazardous shipping charges. The Thermo Scientific Imperial Protein Stain (Product # 24615, 24617) uses coomassie R-250 dye in a novel formulation that produces intense, purple bands and extends staining sensitivity two-fold over traditional coomassie dye stains. All three stains are compatible with subsequent trypsin digestion and mass spectrometry,[1-4] N-terminal sequence analysis,[5] and enable detection of bands containing less than 10 ng of protein.

Another popular method for detecting protein bands within a gel is silver staining, which deposits metallic silver onto the surface of a gel at the location of protein bands. With the Thermo Scientific Pierce Color Silver Stain (Product # 24597), silver ions bind to proteins and are then reduced with formaldehyde and base (sodium hydroxide) to yield protein bands that stained black, blue-brown, red or yellow, depending on charge and other characteristics of the particular proteins.[6,7] This is particularly useful for differentiating overlapping spots on 2-D gels. Our Color Silver Stain (Product # 24597) can detect protein concentrations as low as 0.1 ng/mm. This sensitivity is comparable to ^{35}S-methionine autoradiography. The Thermo Scientific Pierce Silver Stain II Staining System (Product # 24602) offers slightly less sensitivity but results can be obtained in less than 40 minutes.[8,9] Thermo Scientific Pierce Silver Stain (Product # 24612) uses a similar formaldehyde-enhanced method to obtain monochromatic brown-gray staining of protein bands. Our Silver Stain is exceptionally robust and easy to use, detecting most less than 0.5 nanograms of protein in typical gels.[8,9] Unlike many other silver stains whose glutaraldehyde or formaldehyde enhancers irreversibly crosslink proteins in the gel matrix, Silver Stain is fully compatible with destaining and elution methods for analysis by mass spectrometry. The Thermo Scienific Pierce Silver Stain for Mass Spectrometry (Product # 24600) includes the silver stain reagents with additional components needed to process stained bands for this application with a monochromatic silver staining system or a coomassie-based stain. In 1985 Slisz and Van Frank improved the sensitivity and shortened the time needed to fix the gel before staining with the GelCode System.[7]

Often protein bands from SDS-PAGE gels must be recovered for sequencing and mass spectral analysis. Silver stains and coomassie-based stains are not easily removed to allow this type of analysis. Thermo Scientific Pierce Zinc Reversible Stain (Product # 24582) does not stain the protein directly, but instead results in an opaque background with clear, unstained protein bands in just 15 minutes. The bands can be photographed by placing a dark background behind the gel.[10] The protocol does not require a fixing step as other stains do (silver and coomassie-based) and is, therefore, ideal for further characterization by mass spec analysis or Western blotting. Refer to Table 1 for a review of GelCode Stain Products for detecting proteins. Zinc staining is as sensitive as typical silver staining (detects < 1 ng of protein), includes no fixing steps and is easily reversed (erased), allowing trouble-free downstream analysis by mass spectrometry (MS) or Western blotting.

In recent years, improvements in fluorescence imagers have resulted in greater demand for fluorescent stains. We offer two fluorescent stains. Thermo Scientific Krypton Protein Stain (Product # 46629, 46630) provides exceptional fluorescent staining performance with a fast (30-160 minutes) and easy procedure. Excitation and emission maxima (520/580 nm) correspond to common filter sets and laser settings of most fluorescence imagers. The stain is competitively priced and as compatible with mass spectrometry and other downstream applications as other fluorescent stains. Thermo Scientific Krypton Infrared Protein Stain (Product # 53071, 53070) provides many of the same features as Krypton Protein Stain but produces signal in the infrared range (excitation/emission: 690/720 nm).

Specific Functional Group Stains

It is often desirable to detect a subset of proteins in a gel rather than all proteins. The Thermo Scientific Pierce Glycoprotein Stain (Product # 24562) allows detection of proteins that have been post-translationally modified with carbohydrate.[10,11] After fixing and washing the gel in 50% methanol and acetic acid, sugar residues in the glycoproteins are oxidized with sodium meta-periodate to form aldehyde groups that are then reacted with an amine-containing dye. Subsequent reduction stabilizes the dye-protein bond, resulting in bright magenta bands. The Krypton Glycoprotein Staining Kit (Product # 53074) involves a similar method but detects using a fluorescent dye (excitation/emission: 646/674 nm).

Thermo Scientific Pierce Phosphoprotein Staining Kit (Product # 24550) is for detection of abundant phosphorylated proteins. Phosphate groups are cleaved from phosphoserine and phosphothreonine and precipitated with calcium. The precipitate is detected with molybdate and methyl green, yielding a green to green-blue colored band. The same gel can then be stained for total protein content with GelCode Blue Stain for comparison. Detection limits are variable and must be determined empirically.

Fusion proteins containing a polyhistidine tag can be stained with Thermo Scientific Pierce 6xHis Protein Tag Staining Kit (Product # 24575). The staining process requires less than two hours and eliminates the need to perform a Western transfer and antibody-based detection when the tagged protein is in abundance. The 6xHis-tagged proteins fluoresce as yellow bands in the gel when exposed to UV light (300 nm) and photographed with a CCD camera.[12] Less than 0.25 µg of His-tagged protein can be detected, providing sufficient sensitivity to verify expression of tagged protein in small amounts of bacterial cell lysate. This staining method may also be followed by total protein staining with GelCode Blue or Imperial Protein Stain.

References

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Step 6 — **Stain the gel**

Table 1. Thermo Scientific Stain Products comparison.

Thermo Scientific Stain Description	Number of Components	Number of Steps	Starting Time[1]	Type of Detection[5]	Sensitivity	Mass Spec. Compatible
Krypton Fluorescent Protein Stain	1	3[3]	30-160 min	F	0.25 ng	Yes
Krypton Infrared Fluorescent Protein Stain	1	3[3]	30-160 min	F	0.25 ng	Yes
Krypton Fluorescent Glycoprotein Staining Kit	3[2]	6[3]	3-4 hours	F	15 ng	U[6]
GelCode 6xHis Protein Tag Stain	2[2]	6	1 hour, 35 min	F	0.2 µg of a 35 kDa fusion protein	U[6]
GelCode Blue Safe Protein Stain	2	2	15-60 min	C	9 ng	Yes
GelCode Blue Stain Reagent	1	2	60 min	C	8 ng	Yes
Imperial Protein Stain	1	4	60 min	C	3 ng	Yes
GelCode Glycoprotein Stain	3[2]	6[3]	~ 2 hours	C	0.16 µg[4]	U[6]
GelCode Phosphoprotein Stain	7[2]	10	3 hours-overnight	C	80 ng phosvitin, 160 ng β-casein	U[6]
Pierce Silver Stain for Mass Spectrometry	6	7[3]	30 min[3]	C	0.25 ng	Yes
Pierce Silver Stain II	4	4	50 min	C	0.25 ng	Yes
Pierce Color Silver Stain	4	4	65 min[1]	C	0.1 ng	Yes
Pierce Zinc Reversible Stain	3	2	15 min	C	0.25 ng	Yes

Notes

1. 0.75 mm gel thickness, does not include fixing and washing.
2. After the initial gel fixation and wash protocol.
3. Horseradish peroxidase (sensitivity will vary with extent of glycosylation of the protein under analysis).
4. Includes destain step.
5. Not tried.
6. U = unknown

Commercial vs Homemade Coomassie Stains

Most assume that making coomassie gel stain saves money over using a premade stain. However, when you calculate the costs for the stain and destain reagents, Thermo Scientific Commassie-based Gel Stains are a more cost-effective option than making your own gel stain (Table 2).

Unlike traditional coomassie stains, our coomassie stains require no tedious methanol or acetic acid protein fixation before staining, nor do they require special destaining solutions, saving time and minimizing reagent costs. Simply follow the easy three-step method – wash, stain, wash – to directly stain proteins in your gel. Whether you are looking for more sensitivity or for an eco-friendly gel stain, look to Thermo Scientific Coomassie Stains for your specific gel staining needs.

Table 2. Thermo Scientific Coomassie Stains are more cost-effective than homemade stains.

Coomassie Stain	Sensitivity	Cost to stain mini gel (20 ml stain)	Comments
GelCode Blue Safe Stain	9 ng	$0.97	• Saves preparation time and reagent costs • Lot-to-lot reproducibility • Reduces solvent disposal costs • No destain step required • Better sensitivity
GelCode Blue Stain	8 ng	$1.33	
Imperial Protein Stain	3 ng	$1.36	
Homemade coomassie stain	100 ng	$1.91*	• Hazardous disposal • No lot-to-lot reproducibility • Tedious: long prep time

** Based on the average price of Fisher Brand methanol, acetic acid, coomassie dye R-250 to major academic institutions in the U.S.*

Thermo Scientific Imperial Protein Stain

Give your gel the royal treatment with this fast, sensitive and consistent coomassie stain.

Our Imperial Protein Stain is a ready-to-use coomassie stain for the detection of protein bands in SDS-PAGE and 2-D gels. The stain is a unique formulation of coomassie R-250 that delivers substantial improvements in protein-staining performance compared to homemade or other commercial stains.

Achieve levels of sensitivity and crystal-clear background through increased staining time and destaining in water (Figure 1). Imperial Protein Stain eliminates problems associated with coomassie G-250 stain preparations, such as inconsistent staining. In addition to faster protein band development and more sensitivity than standard coomassie G-250 stains, Imperial Protein Stain does not require methanol/acetic acid fixation and destaining, saving valuable preparation time and minimizing reagent cost.

Multiple staining protocols are provided to meet demanding time and sensitivity requirements (Figure 2). For fast results, a five-minute stain combined with a 15-minute water destain easily detects 6 ng protein bands. (Figure 3).

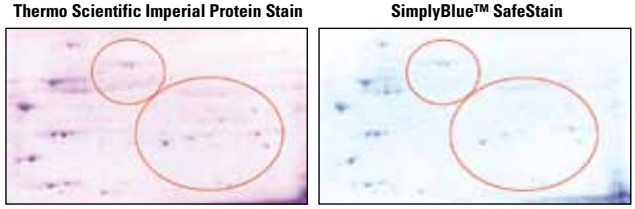


Figure 1. Thermo Scientific Imperial Protein Stain reveals spots that are faint or not detected with other coomassie stains. Mitochondrial protein extract was prepared from heart tissue of six-week-old Sprague-Dawley rat. Processed protein extract (72 µg) was focused on a pH 5-8 IPG strip followed by 8-16% SDS-PAGE. The gels were stained for 1 hour and destained overnight following manufacturer-recommended protocols.

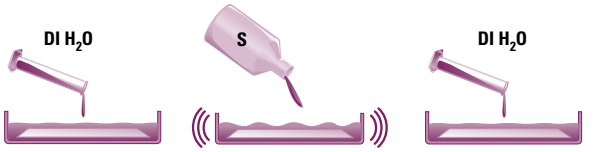
Highlights:

Outstanding Performance

- **Sensitive** – 3 ng protein/band and less can be detected with the enhanced protocol (3 hours)
- **Fast** – detect as little as 6 ng protein/band in just 20 minutes
- **Robust** – highly consistent, reproducible protein staining
- **Excellent photo-documentation** – photographs/scans better than other coomassie stains
- **Mass spectrometry-compatible**

Convenience:

- Destain with water
- No fixation step required
- Ready-to-use reagent
- Stable – store on your bench top for up to one year
- Flexible – multiple protocols to meet demanding time/sensitivity requirements



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

Figure 2. Thermo Scientific Imperial Protein Stain protocol.

Figure 3. Thermo Scientific Imperial Protein Stain is fast and sensitive. Proteins were separated on Novex 4-20% Tris-glycine gels, stained for 5 minutes and destained 3 x 5 minutes in water. **Lane 1**. BSA only (6 µg), **Lanes 2-9** contained the indicated proteins at the following concentrations: **Lane 2**. 1,000 ng, **Lane 3**. 200 ng, **Lane 4**. 100 ng, **Lane 5**. 50 ng, **Lane 6**. 25 ng, **Lane 7**. 12 ng, **Lane 8**. 6 ng and **Lane 9**. 3 ng.

Ordering Information

Product #	Description	Pkg. Size
24615	**Imperial Protein Stain** *Sufficient reagent to stain up to 50 mini gels (8 cm x 10 cm).*	1 L
24617	**Imperial Protein Stain** *Sufficient reagent to stain up to 150 mini gels (8 cm x 10 cm).*	3 x 1 L

Thermo Scientific GelCode Blue Safe Protein Stain

A safe, reliable and cost-effective stain for proteins.

GelCode Blue Safe Protein Stain is a Coomassie Brilliant Blue G-250-based stain that is non-hazardous, odorless, non-corrosive to skin and nonflammable. It does not require hazardous shipping per U.S. Department of Transportation (DOT) guidelines, thus minimizing product shipping costs.

Highlights:

- **Sensitive** – detect down to 9 ng of protein/band using a standard protocol (Figures 4 and 5)
- **Fast** – standard protocol provides results in ~15 minutes; a quick microwave protocol provides excellent results in 5 minutes
- **Outstanding signal-to-noise ratios**
- **Versatile** – compatible with mass spectrometry (Figure 6), 2-D gel staining, nitrocellulose and PVDF membrane staining, and quantitative densitometry
- **Safe** – noncorrosive to skin, nonflammable and safe to ship and store
- **Convenient** – no fixation step necessary; destain with water
- **Easy to use** – add activator crystals, shake and stain
- **Stable** – store stain at room temperature for up to one year
- **Flexible** – multiple protocols to meet your needs

1. Wash gel three times with deionized water (15 minutes).
2. Add Thermo Scientific GelCode Blue Stain Reagent (1 hour).
3. For increased clarity, use the optional Water Wash Enhancement Step (1 hour).

Figure 4. Thermo Scientific GelCode Blue Safe Protein Stain protocol.

Step 6 — **Stain the gel**

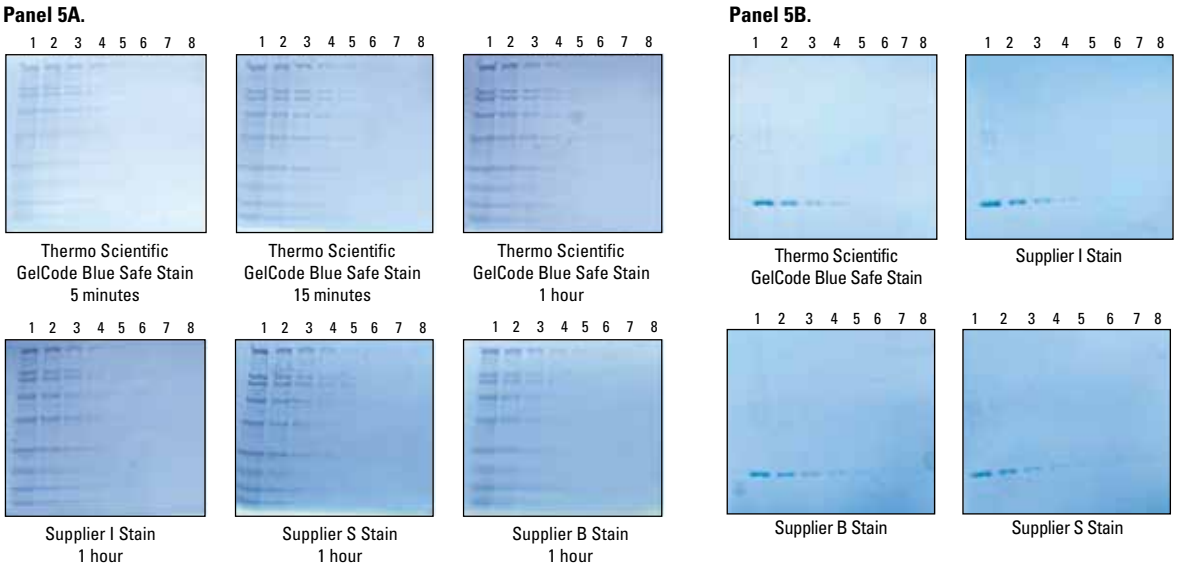


Figure 5. Thermo Scientific GelCode Blue Safe Protein Stain is fast and sensitive. Panel 5A. A mixture of proteins (myosin, β-galactosidase, phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme and aprotinin) was electrophoresed on 4-20% Thermo Scientific Precise Protein Gels (Product # 25224). The gels were stained with GelCode Blue Safe Protein Stain for five minutes, 15 minutes and one hour and with competitors' stains for one hour. All gels were destained for one hour in ultrapure water. **Lane 1.** 1,000 ng, **Lane 2.** 500 ng, **Lane 3.** 250 ng, **Lane 4.** 125 ng, **Lane 5.** 63 ng, **Lane 6.** 31 ng, **Lane 7.** 16 ng, and **Lane 8.** 8 ng. **Panel 5B.** Reduced HeLa cell lysate was electrophoresed on 4-20% Precise Protein Gels (Product # 25224). The gels were stained for one hour with GelCode Blue Safe Protein Stain or with stains from other suppliers. The gels were destained overnight in ultrapure water after staining. **Lane 1.** 40 µg, **Lane 2.** 20 µg, **Lane 3.** 10 µg, **Lane 4.** 5 µg, **Lane 5.** 2.5 µg, **Lane 6.** 1.25 µg, **Lane 7.** 0.625 µg, **Lane 8.** 0.312 µg and **Lane 9.** 0.156 µg.

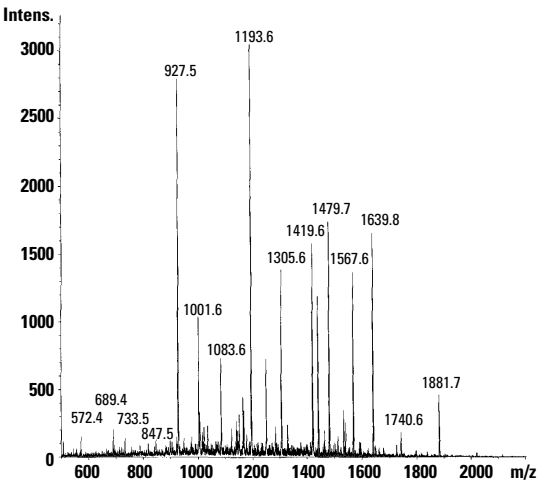


Figure 6. Thermo Scientific Gelcode Blue Safe Stain is Mass spectrometry (MS)-compatible. BSA (2 µg) was electrophoresed on a 4-20% Precise Protein Gel (Product # 25224) and stained with GelCode Blue Safe Stain and GelCode Blue Stain Reagent for one hour. BSA bands were excised from the gel and prepared for MALDI-MS analysis using the Thermo Scientific In-Gel Tryptic Digestion Kit (Product # 89871). Samples were purified using ZipTip® Pipette Tips (Millipore) before MS analysis on an LC/MSD Trap XCT (Agilent Technologies).

Ordering Information

Product #	Description	Pkg. Size
24594	GelCode Blue Safe Protein Stain	1 L
24596	GelCode Blue Safe Protein Stain	3.5 L

Thermo Scientific GelCode Blue Stain Reagent

Eliminates the pain of having to destain.

Unlike other Coomassie-based stains, GelCode Blue Stain Reagent doesn't require an organic solvent for destaining. Ready-to-use GelCode Blue Stain Reagent (Figure 7) uses the colloidal properties of coomassie G-250 dye for protein staining on polyacrylamide gels. This unique reagent stains only protein and allows bands to be viewed directly on the gel during the staining process. After staining, a water equilibration (Water Wash Enhancement) step further enhances staining sensitivity and yields a clear background.

Highlights:

No tedious, pungent methanol/acetic acid destaining step required

- Saves preparation time and reagent costs, and reduces solvent disposal problems and associated costs
- No destaining step necessary – no losses from overdestaining

Fast, one-step, one-hour staining

- Bands develop quickly and can be viewed directly in the staining tray
- No increased background from overnight staining
- Completely flexible fixing and washing protocols can be used
- No gel shrinkage – original gel dimensions maintained
- Stained gel can be dried

More sensitive than standard coomassie gel stain formulations

- Some protein bands visible to 8 ng
- Wide linear range for densitometric gel analysis[13]
- Allows gel staining after Western transfer
- Compatible with MALDI-TOF analysis[14-16] and sequence analysis[17]

Optional Water Wash Enhancement Step further increases staining sensitivity

- Crystal-clear gel background; even the weakly stained bands become easily visible

References

13. Mateer, S.C., *et al.* (2002). *J. Biol. Chem.* **227,** 12324-12333.
14. Aulak, K.S., *et al.* (2001). *Proc. Natl. Acad. Sci. USA* **98,** 12056-12061.
15. Lim, J., *et al.* (2002). *J. Biol. Chem.* **277,** 20774-20782.
16. Hilton, J.M., *et al.* (2001). *J. Biol. Chem.* **276,** 16341-16347.
17. Tani, M., *et al.* (2002). *J. Biol. Chem.* **275,** 3462-3468.

1. Wash gel three times with deionized water (15 minutes).
2. Add Thermo Scientific GelCode Blue Stain Reagent (1 hour).
3. For increased clarity, use the optional Water Wash Enhancement Step (1 hour).

Figure 7. Thermo Scientific GelCode Blue Staining protocol.

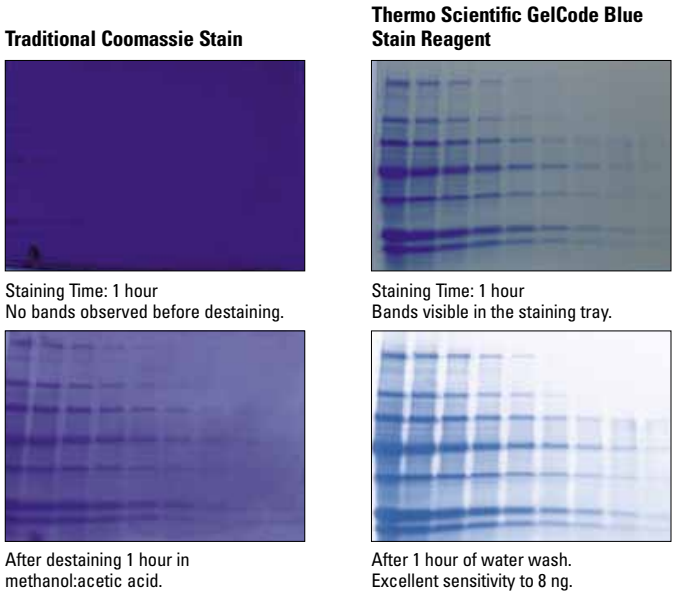


Figure 8. An optional Water Wash Enhancement Step increases Thermo Scientific GelCode Blue Stain Reagent sensitivity. An optional one-hour soak in deionized water provides a crystal-clear gel background. Even weakly stained bands become easily visible.

Ordering Information

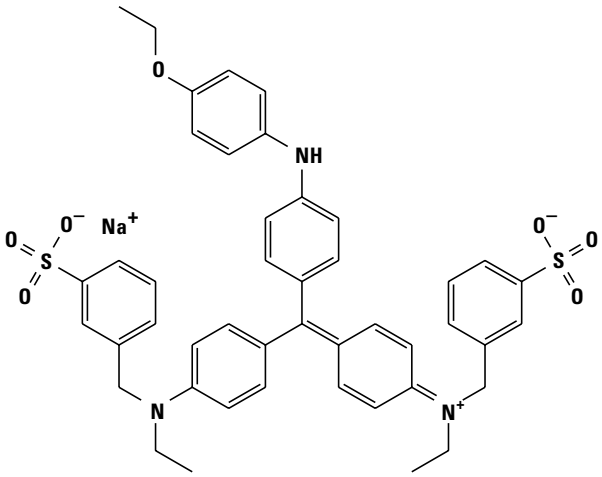
Product #	Description	Pkg. Size
24592	**GelCode Blue Stain Reagent** *Sufficient reagent to stain 175 mini (8 cm x 10 cm) gels.*	3.5 L
72300	**Pump (for 3.5 L package only)**	1 pump
24590	**GelCode Blue Stain Reagent** *Sufficient reagent to stain 25 mini (8 cm x 10 cm) gels.*	500 ml

** A reagent dispensing pump attachment is available free upon request for Product # 24592. Specify Product # 72300 when you place your order.*

Step 6 — **Stain the gel**

Thermo Scientific Coomassie Brilliant Blue R-250 and G-250 Dyes[18]

Ideal as a protein stain following electrophoresis.



Coomassie Brilliant Blue R-250 Dye
MW 825.97

Highlights:

- Develops intensely colored complexes with proteins
- Can determine as little as 0.5 µg/cm^2 of protein present in a gel matrix
- Anion of coomassie brilliant blue formed in the acidic staining medium combines with the protonated amino groups of proteins by electrostatic interaction; resulting complex is reversible under the proper conditions
- When dissolved in 0.01 M citrate buffer at pH 3.0, has an absorption maximum at 555 nm; protein-dye complex is characterized by a peak slightly broader than that of the free dye with a maximum at 549 nm

Reference

18. Syrovy, I. and Hodny, Z. (1991). *J. Chromatogr.* **569,** 175-196.

Ordering Information

Product #	Description	Pkg. Size
20278	Coomassie Brilliant Blue R-250 Dye	50 g
20279	Coomassie Brilliant Blue G-250 Dye (Colloidal)	50 g

Thermo Scientific Krypton Fluorescent Protein Stain

A faster, affordable fluorescent stain that provides excellent performance.

Krypton Protein Stain is a fluorescent stain for detecting proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2-D gels. The stain's unique formulation reduces the time and cost associated with typical fluorescent staining (Figures 9-10, Table 3). Krypton Protein Stain is a decisive improvement in fluorescent stain technology, providing sensitivity equivalent to, or greater than, other fluorescent stains, while minimizing protein quantitation problems associated with differential protein staining (Figure 11). The stain provides high signal intensity with a linear quantitative range of three to four orders of magnitude across a broad range of protein types, which maximizes the detection of low-abundant proteins (Figure 12).

Highlights:

- **Excitation/emission maxima** – 520/580 nm
- **Compatibility** – works with all SDS-polyacrylamide and 2-D gel types and with MS analysis
- **Linear quantitative range** – three to four orders of magnitude
- **Sensitive** – detects down to 0.25 ng protein with the basic 2.7-hour protocol
- **Fast** – using the rapid protocol, detects down to 2 ng protein in 30 minutes
- **Comparative** – minimal differential staining of proteins

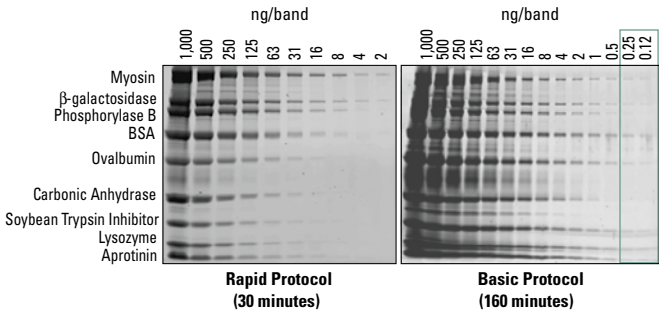


Figure 9. Thermo Scientific Krypton Protein Stain is fast and sensitive. Proteins were separated in 4-20% Tris-glycine gels and stained using the indicated protocols. The gels were imaged with the Typhoon® 9410 at 532 nm excitation and 580 BP30 emission.

Table 3. Thermo Scientific Krypton Protein Stain costs up to 53% less than other fluorescent stains.

	Flamingo™ Fluorescent Gel Stain	Deep Purple™ Total Protein Stain	SYPRO® Ruby Protein Gel Stain	Thermo Scientific Krypton Protein Stain	Cost Savings
Stain cost per **20 mini-gels**	$166	$188	$218	$114	$52–$104
Stain cost per **100 mini-gels**	$794	$791	$930	$412	$379–$518

Source: Online Catalogs (1/13/2010). All prices listed are US$.

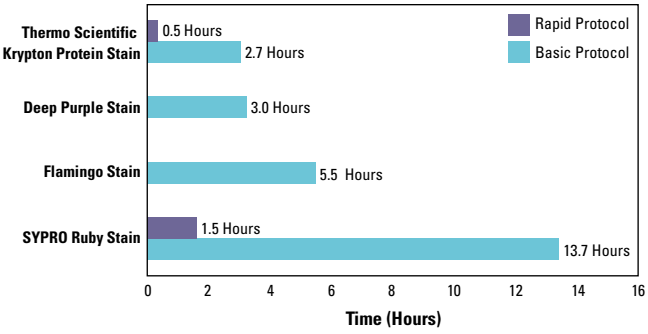


Figure 10. Thermo Scientific Krypton Protein Stain works up to five times faster than other fluorescent stain protocols.

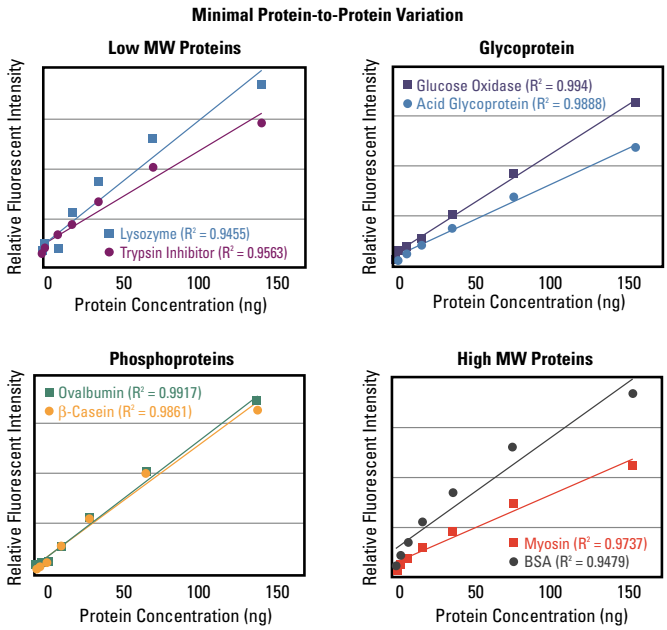


Figure 11. Thermo Scientific Krypton Protein Stain produces a linear response to staining with minimal protein-to-protein variation. Relative fluorescent intensity was plotted as a function of protein quantity for proteins of various sizes and containing post-translational modifications.

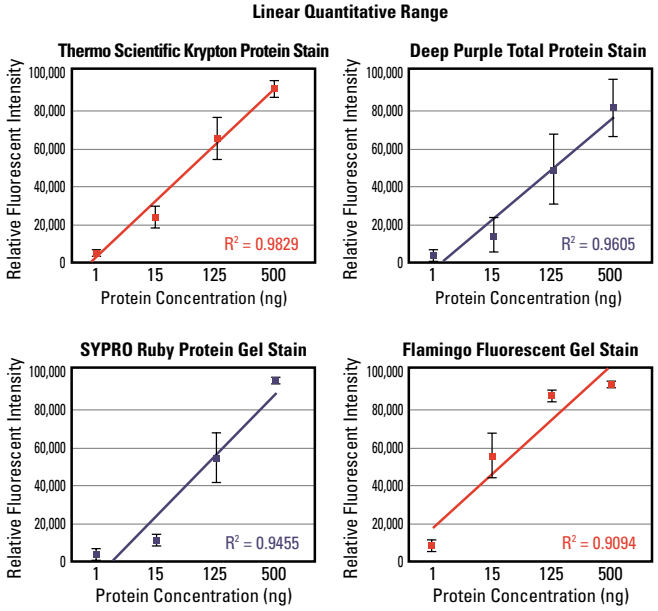


Figure 12. Thermo Scientific Krypton Protein Stain exhibits a more linear quantitative range than other fluorescent stains. Relative fluorescent intensity for each protein concentration is the average of nine different proteins separated in 4-20% Tris-glycine and Precise Gels. Error bars represent the standard deviation for triplicate gels. The gels were stained and imaged according to the manufacturer's basic protocols.

Ordering Information

Product #	Description	Pkg. Size
46628	**Krypton Protein Stain (10X)** *Sufficient reagent to stain four mini gels (8 cm x 10 cm).*	20 ml
46629	**Krypton Protein Stain (10X)** *Sufficient reagent to stain 20 mini gels (8 cm x 10 cm) or two to four large-format gels.*	100 ml
46630	**Krypton Protein Stain (10X)** *Sufficient reagent to stain 100 mini gels (8 cm x 10 cm) or 10 to 20 large-format gels.*	500 ml

Step 6 — **Stain the gel**

Thermo Scientific Krypton Infrared Protein Stain

Fluorescence detection that is compatible with LI-COR Odyssey and other infrared imaging systems.

Krypton Infrared Protein Stain (patent pending) is a fluorescent stain for detecting proteins in SDS-PAGE and 2-D gels. Researchers now have an easy-to-use, high-performance fluorescent protein stain for the near-infrared region of the spectrum compatible with the LI-COR Odyssey Infrared Imaging System and other commonly available CCD instruments (Figure 13). The stain delivers substantial improvements in protein-staining performance compared to coomassie stains (Figure 14). Krypton Infrared Protein Stain exhibits minimal protein-to-protein variation and provides high signal intensity with a linear response to staining (Figure 15-16).

Highlights:

- **Excitation/emission maxima** – 690/718 nm
- **Instrument-compatible** – ideal for LI-COR Odyssey Instruments and other CCD instrumentation
- **Inexpensive** – a fluorescent stain similar in price to coomassie stain
- **Versatile** – compatible with membrane staining and mass spectrometry
- **Wide quantitative range** – three to four orders of magnitude
- **Sensitive** – detect down to 0.25 ng protein with the basic protocol (~2 hours)
- **Fast** – detect down to 2 ng protein with the rapid protocol (~1 hour)

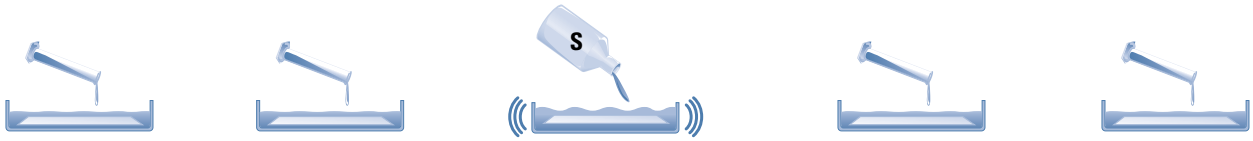


Figure 13. Thermo Scientific Krypton Infrared Protein Stain protocol.

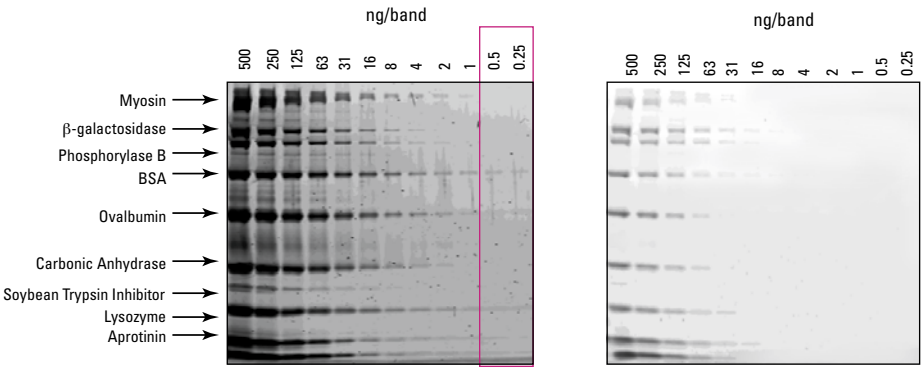


Figure 14. Thermo Scientific Krypton Infrared Protein Stain is up to 130 times more sensitive than coomassie stain. Proteins were separated in 4-20% Tris-glycine gels and stained with Krypton Infrared Protein Stain or coomassie stain (Thermo Scientific GelCode Blue Stain Reagent, Product # 24592). The gels were imaged with the Odyssey Infrared Imaging System at 680 nm excitation and 720 nm emission.

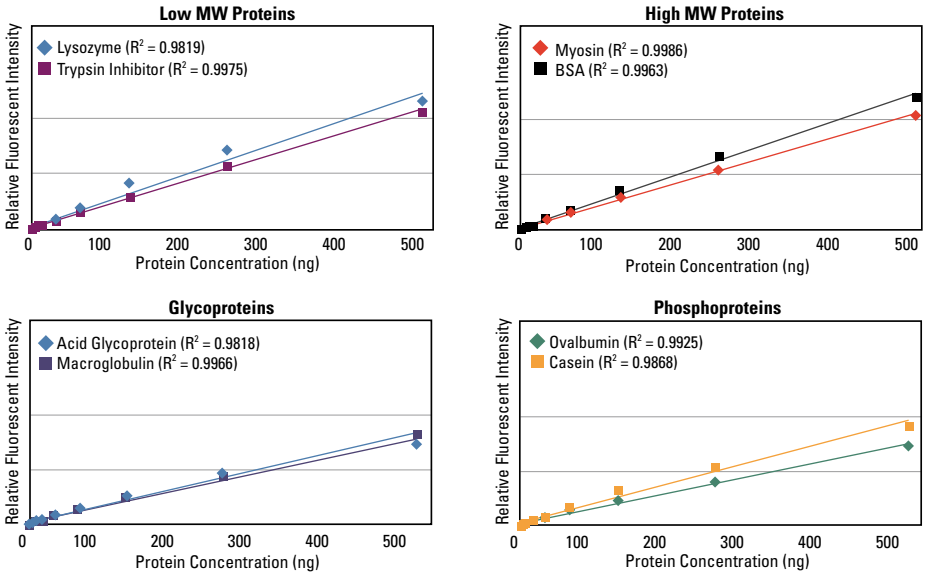


Figure 15. Thermo Scientific Krypton Infrared Protein Stain produces a linear response to staining with minimal protein-to-protein variation. Relative fluorescent intensity was plotted as a function of protein quantity for proteins of various sizes and containing post-translational modifications. The relative fluorescent intensity for each data point is the average value for triplicate gels.

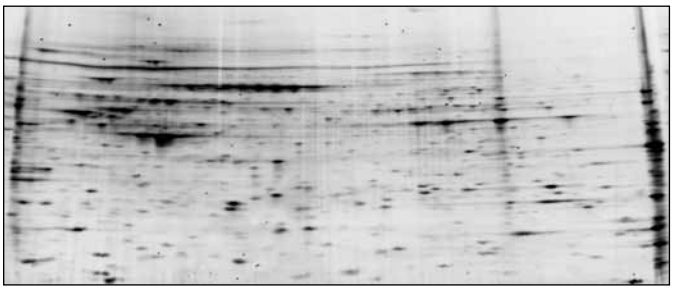


Figure 16. Thermo Scientific Krypton Infrared Protein Stain provides exceptional sensitivity and low background in 2-D analysis. Processed HeLa cell protein extract (28 µg) was focused on a pH 5-8 IPG strip followed by 4-20% SDS-PAGE. The gel was stained using the basic protocol (~2 hours) and imaged with the Odyssey Infrared Imaging System using the 700 nm channel.

Ordering Information

Product #	Description	Pkg. Size
53070	**Krypton Infrared Protein Stain (10X)** *Sufficient reagent to stain 4 mini gels (8 cm x 10 cm).*	20 ml
53071	**Krypton Infrared Protein Stain (10X)** *Sufficient reagent to stain 20 mini gels (8 cm x 10 cm) or two to four large-format gels.*	100 ml
53072	**Krypton Infrared Protein Stain (10X)** *Sufficient reagent to stain 100 mini gels (8 cm x 10 cm) or 10 to 20 large-format gels.*	500 ml

Step 6 — **Stain the gel**

Thermo Scientific Pierce Silver Stain Kit for Mass Spectrometry

Optimized for MS-based applications!

We recognize the need for a silver stain that is not only compatible with mass spectrometry (MS) applications, but truly optimized to provide the best results. Our researchers fine-tuned the chemistry of our Silver Stain and made adjustments to the protocol to provide peak kit performance, including flexibility, reliability and robustness in MS-targeted applications. Our Silver Stain Kit for Mass Spectrometry bundles a high-performance stain with an efficient gel-destaining chemistry and an optimized protocol. The result is an MS-compatible product that delivers outstanding sensitivity and maintains favorable conditions for the recovery and identification of protein by MS (Table 4).

Highlights:

- **Sensitivity** – this low-background, easy-to-use silver stain provides sub-nanogram sensitivity, detecting down to 0.25 ng protein/spot in 30 minutes after fixing; spots are de-stained and ready for tryptic digestion in one hour
- **MS compatibility** – provides excellent MS performance on 1-D and 2-D gels; MALDI-MS results are superior to other MS-compatible stains
- **Complete and ready to use** – turnkey kit contains all reagents for staining and destaining process before MS analysis; contains sufficient destain reagents for 500 excised spots, removing deposited silver from gel before tryptic digestion and MS sample preparation
- **Flexibility** – fix in 15-30 minutes or, for convenience, overnight; stain in 1-30 minutes (typically 2-3 minutes)
- **Robust** – effective for difficult-to-stain basic proteins, including low pI proteins such as lysozyme (pI 10) and chymotrypsinogen A (pI 9.2), detectable at 0.2 ng and 0.5 ng, respectively
- **Convenience** – room temperature-stable kit components eliminate the need to occupy refrigerator space

Table 4. Sequence coverage comparison.

Fifty (50) ng each of BSA, ovalbumin, chymotrypsinogen A and myoglobin preparations were loaded onto separate SDS-PAGE gels. After electrophoresis, the respective gels were stained with Thermo Scientific Pierce Silver Stain for Mass Spectrometry; Competitor I, a competing MS-compatible stain; and Thermo Scientific GelCode Blue Stain Reagent. The resultant bands were excised and destained, subjected to in-gel tryptic digestion (Product # 89871), and prepared for analysis by MALDI/MS. In all cases, Thermo Scientific Pierce Silver Stain for Mass Spectrometry performed better than the alternative silver staining method.

		Thermo Scientific Pierce Silver Stain for MS			Competitor I MS Stain			Thermo Scientific GelCode Blue Stain Reagent		
Protein	**Amount (ng)**	**# of Peptides**	**# of Protein-Specific Peptides**	**% Coverage**	**# of Peptides**	**# of Protein-Specific Peptides**	**% Coverage**	**# of Peptides**	**# of Protein-Specific Peptides**	**% Coverage**
BSA	50	63	13	21	53	6	11	40	7	18
Ovalbumin	50	40	5	13	44	1	2	42	1	2
Chymotrypsinogen A	50	47	4	9	41	2	5	41	1	2
Myoglobin	50	32	6	19	31	3	10	38	1	3

Table 5. Peptide mass fingerprinting bioinformatics data for 2-D rat mitochondrial protein analysis.

Ten spots that stained well with the Thermo Scientific Pierce Silver Stain Kit for Mass Spectrometry, Competitor I MS-compatible Stain and Thermo Scientific GelCode Blue Stain Reagent were selected and prepared for subsequent MS analysis. All proteins identified by peptide fragment mapping are known mitochondrial proteins. All gels were run in a pH 5-8 gradient.

2-D Spot	Proteins Identified	Methods
1	ATP synthase, H^+ transporting, mitochondrial F1 complex, beta subunit)	All
2	AJ18 protein	Thermo Scientific Silver Stain for MS and Thermo Scientific GelCode Blue Stain Reagent
	ATP synthase, H^+ transporting, mitochondrial F1 complex, subunit Δ	Competitor I
3	Electron transfer flavo protein (ETF protein)	All
4	H^+ transporting two-sector ATPase (EC 3.6.3.14), alpha chain precursor	Thermo Scientific Silver Stain for MS and Thermo Scientific GelCode Blue Stain Reagent
	Unknown protein for MGC:93808	Competitor I
5	Mitochondrial aldehyde dehydrogenase precursor	All
6	Glutamate dehydrogenase 1	All
7	Glucose-regulated protein, ER-60 protease	All
8	Enoyl coenzyme A hydratase short chain mitochondrial	Thermo Scientific Silver Stain for MS and Thermo Scientific GelCode Blue Stain Reagent
	Translocase of inner mitochondrial membrane homolog 44	Competitor I
9	Enoyl coenzyme A hydratase short chain mitochondrial	All
10	ATP synthase, H^+ transporting, mitochondrial F1 complex, beta subunit	All

Ordering Information

Product #	Description	Pkg. Size
24600	**Pierce Silver Stain Kit for Mass Spectrometry** *Sufficient reagents to stain up to 20 SDS-PAGE mini-gels (8 cm x 8 cm) and to destain more than 500 gel plugs for subsequent elution and analysis by mass spectrometry.*	Kit
	Includes: Sensitizer	2 ml
	Stain	500 ml
	Developer	500 ml
	Enhancer	25 ml
	Silver Destain Reagent A	4 ml
	Silver Destain Reagent B	14 ml

Step 6 — **Stain the gel**

Thermo Scientific Pierce Silver Stain II

A fast, flexible silver stain.

Silver Stain II is a fast, more flexible and forgiving stain that yields results competitive with – and often better than – any homemade or commercially available silver stain. Most importantly, our improved silver stain delivers consistently reliable staining performance each and every time at a very economical price.

Highlights:

- Remarkably low, uniform background
- Detect down to 0.25 ng of protein
- Complete staining in < 50 minutes
- Compatible with a wide assortment of homemade and precast gels
- Ideal for use with one- or two-dimensional PAGE and IEF gels (Figures 17 and 18)
- Can also be used to stain DNA or RNA following electrophoresis
- Flexible protocol (Figure 19) without altering sensitivity or background
  - – Fixing can be completed in 30 minutes or left overnight
  - – Staining can be performed in 5 minutes or left for up to 20 hours.

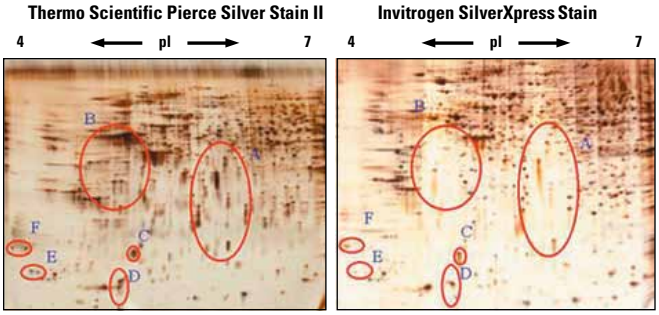


Figure 17. Comparison of identical 2-D gels stained with Thermo Scientific Pierce Silver Stain II with another popular brand. Circled regions indicate difference in staining intensity.

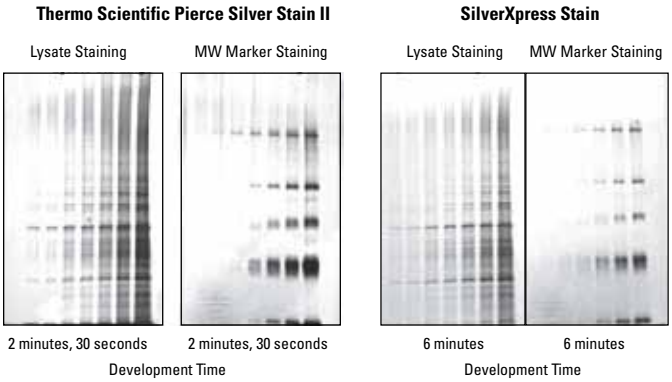


Figure 18. Thermo Scientific Pierce Silver Stain II provides more sensitive staining of a 1-D polyacrylamide gel than SilverXpress® Stain.

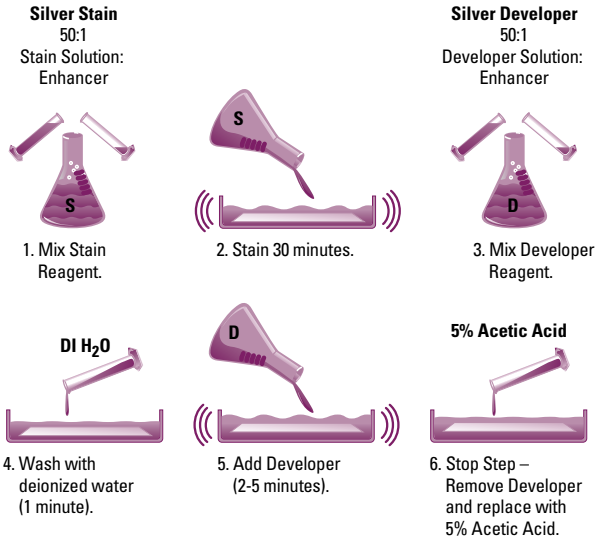


Figure 19. Thermo Scientific Pierce Silver Stain II protocol.

Precast Gel Supplier	Gel Buffer System	Thermo Scientific Pierce Silver Stain II Performance Rating
Invitrogen (Novex)	Tris-Glycine (homogeneous and gradient)	*****
	Bis-Tris	*****
	Tris-Tricine	***
Thermo Scientific Pierce and Precise Gels	Tris-HEPES (gradient and homogeneous)	*****
Bio-Rad	Tris-HCl	***
	Tris-Tricine	**
	Criterion® (1D and 2-D gels)	****
Homemade Gels	12% Tris-Glycine	*****

Ordering Information

Product #	Description	Pkg. Size
24612	**Pierce Silver Stain II** *This product replaces Product # 24602.* *Sufficient reagent to stain 20 mini gels.* Includes: Sensitizer Stain Enhancer Developer	Kit 2 ml 500 ml 25 ml 500 ml

Thermo Scientific Pierce Color Silver Stain

Brighten up your silver-stained gels by adding color and increased protein detection.

Highlights:

- Detects proteins that do not bind silver as yellow spots
- Quantitative
- Designed for one- and two-dimensional (Figure 20) PAGE gel staining
- Total time, after fixing, varies from 11-90 minutes (depending on gel thickness)
- Detect down to 0.1 ng/mm^2 protein in the gel
- Proteins stain in five basic colors: black, blue, brown, red and yellow
- Five simple staining steps (Figure 21)
- Also stains DNA[20]

Color aids in protein mapping by:

- Distinguishing overlapping spots
- Identifying post translationally modified proteins
- Tracking proteins in biological fluids
- Monitoring the alteration of proteins in disease states
- Monitoring the subcellular fractions of cells

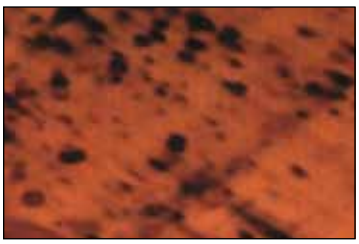


Figure 20. Thermo Scientific Pierce Color Silver Stain provides sensitive staining of 2-D gels.

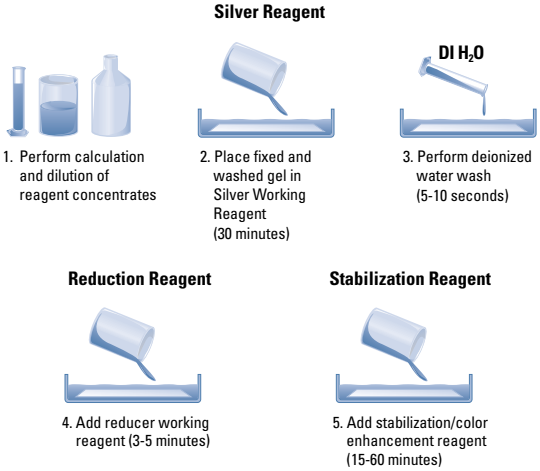


Figure 21. Thermo Scientific Pierce Color Silver Staining protocol. Basic protocol, after gel fixing step, based on 0.75 mm gel.

Ordering Information

Product #	Description	Pkg. Size
24597	**Pierce Color Silver Stain** *Sufficient reagent to stain up to 25 (18 cm x 18 cm) 2-D gels or 40 (10 cm x 13 cm) 2-D gels.*	Kit
	Includes: Silver Concentrate	500 ml
	Reducer Base	500 ml
	Reducer Aldehyde	500 ml
	Stabilizer Base	500 ml

References

19. Sammons, D.W., *et al.*, (1981). Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. *Electrophoresis* **2,** 135-141.
20. Stoppler, H., *et al* (1997). The human papillomavirus type 16 E6 and E7 oncoproteins dissociate cellular telomerase activity from the maintenance of telomere length. *J. Biol. Chem.* **272,** 13332-13337. Staining of DNA.

Step 6 — **Stain the gel**

Thermo Scientific Pierce Silver Stain Rescue Reagent

This new rescue reagent saves you from the dreaded "silver stain do-over."

Have you ever been frustrated by a silver-stained gel that did not turn out just right for that big meeting or publication deadline? The new Silver Stain Rescue Reagent can recover an out-of-control gel, while saving you the time and frustration of reloading the sample, running the gel and repeating the silver staining protocol. With Silver Stain Rescue Reagent, most mini gels are rescued in less than one hour, depending on the working concentration selected.

Highlights:

- **Easy to use** – mix the reagents at the recommended dilution and rescue a gel with an overdeveloped or non-uniform background (Figure 22)
- **Quick** – attenuates background in minutes, allowing removal of the appropriate amount of background to meet your objective; complete the entire process in ~1 hour
- **Economical and efficient** – costs US $0.56-US $1.12 per mini gel, coupled with a short process time
- **Preserves data** – removes silver from the gel uniformly, enabling improved band visibility without altering the data (Figure 23)
- **Compatible with any commercial or homemade silver stain** – removes high background or non-uniform staining, regardless of the silver stain formulation

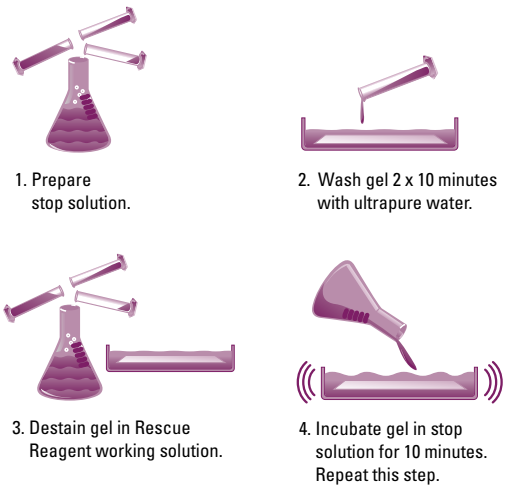


Figure 22. Thermo Scientific Pierce Silver Stain Rescue Reagent protocol.

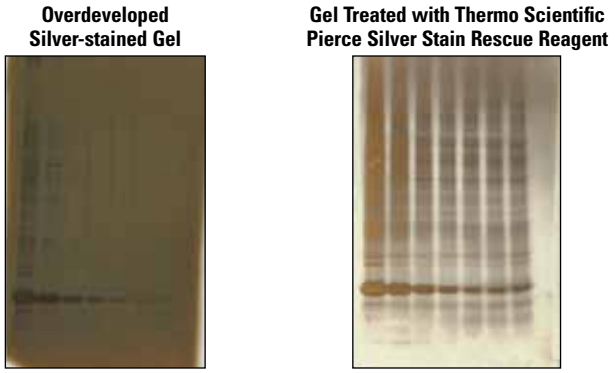


Figure 23. High background removed by Thermo Scientific Pierce Silver Stain Rescue Reagent. Dilutions of *Escherichia coli* cell lysate were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 4-12% gradient gels and stained with Thermo Scientific Silver Stain Kit II (Product # 24612).

Ordering Information

Product #	Description	Pkg. Size
24614	**Pierce Silver Stain Rescue Reagent** *Sufficient reagents to treat 100-200 mini-gels. Used with silver-stained gels to salvage results from gel-staining irregularities common to the method, including high background and non-uniform staining.*	40 ml
	Includes: Rescue Reagent A	20 ml
	Rescue Reagent B	20 ml

Thermo Scientific Pierce Zinc Reversible Stain

Reversible staining feature allows versatility other stains cannot offer.

When you need results quickly, our Zinc Stain can be used to stain a gel in just 15 minutes. The sensitivity is similar to silver staining – but without the tedious staining process. Best of all, the Pierce Zinc Stain doesn't require the proteins to be fixed in the gel, so the proteins are not altered. That means you can stain a gel before transferring for Western blot or analyzing by mass spectrometry.

Highlights:

- **Sensitive** – detect down to 0.25 ng of protein (Figure 24)
- **Fast** – results in 15 minutes
- **Convenient** – all components are ready to use (Figure 25)
- **Saves time** – no fixing of gel required
- Develops opaque white background while protein bands remain clear

Useful staining strategy for:

- Protein recovery for antibody generation or immunological detection
- Protein/peptide recovery from gel for sequencing purposes
- Protein digest sequencing by mass spectrometry
- Biological enzyme activity assays
- Western blots (pre- or post-transfer)
- Quick purity checks

Reversibility of stain allows:

- Alternative staining of same gel
- Protein elution or transfer after gel staining and destaining

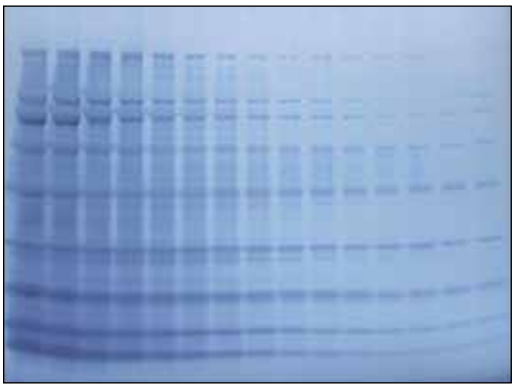
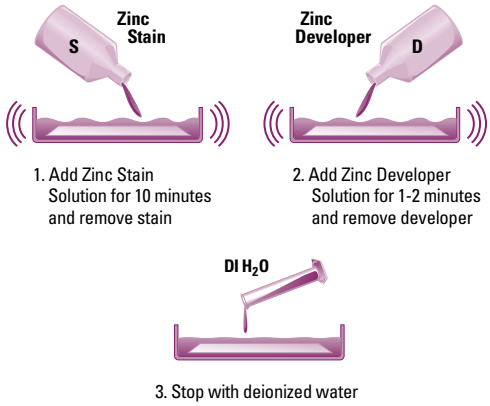


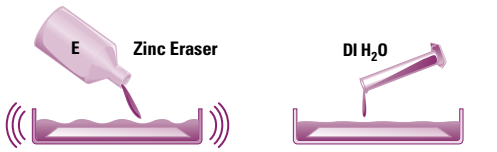
Figure 24. Thermo Scientific Pierce Zinc Reversible Stain is more sensitive than competing stains.

Staining Protocol



1. Add Zinc Stain Solution for 10 minutes and remove stain
2. Add Zinc Developer Solution for 1-2 minutes and remove developer
3. Stop with deionized water

Erasing Protocol



1. Add Zinc Eraser Solution
2. Wash with deionized water

Figure 25. Thermo Scientific Pierce Zinc Reversible Stain protocol.

References

21. Amano, S., *et al.* (2000). *J. Biol. Chem.* **275,** 22728-22735.
22. Steiglitz, B.M., *et al.* (2002). *J. Biol. Chem.* **277,** 49820-29830.

Ordering Information

Product #	Description	Pkg. Size
24582	**Pierce Zinc Reversible Stain Kit** *Sufficient reagent to stain up to 20 SDS-PAGE mini (8 cm x 10 cm) gels.*	Kit
	Includes: Zinc Stain	500 ml
	Zinc Developer	500 ml
	Zinc Eraser	500 ml

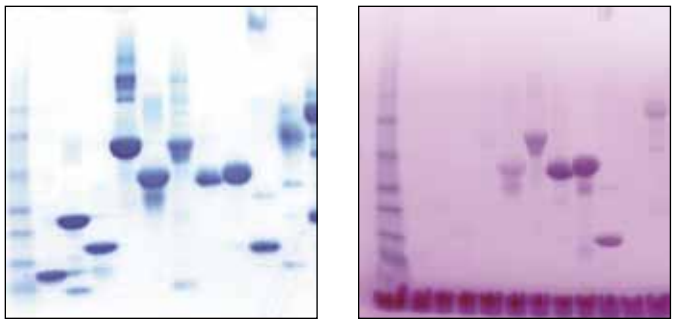
Step 6 — **Stain the gel**

Thermo Scientific Pierce Glycoprotein Stain

A fast and specific staining protocol for glycoprotein detection on gels or membranes.

Highlights:

- Detects glycoproteins on SDS-polyacrylamide gels or Western blotting membranes (Figure 26)
- Three-reagent protocol yields results in less than two hours vs. four to five hours for other staining methods (Figure 27)
- Glycoproteins are detected as magenta bands with light pink or colorless background
- Detects glycoproteins, such as avidin and horseradish peroxidase, down to 0.625 ng and 0.16 µg, respectively
- Kit includes one positive and one negative control standard
- Compact, easy-to-store kit



A. **B.**

Figure 26. Sensitive staining of glycoproteins. A. Glycoprotein-containing gel stained with Thermo Scientific GelCode Blue Stain Reagent. **B.** Glycoprotein-containing gel stained with Thermo Scientific Pierce Glycoprotein Staining Kit.

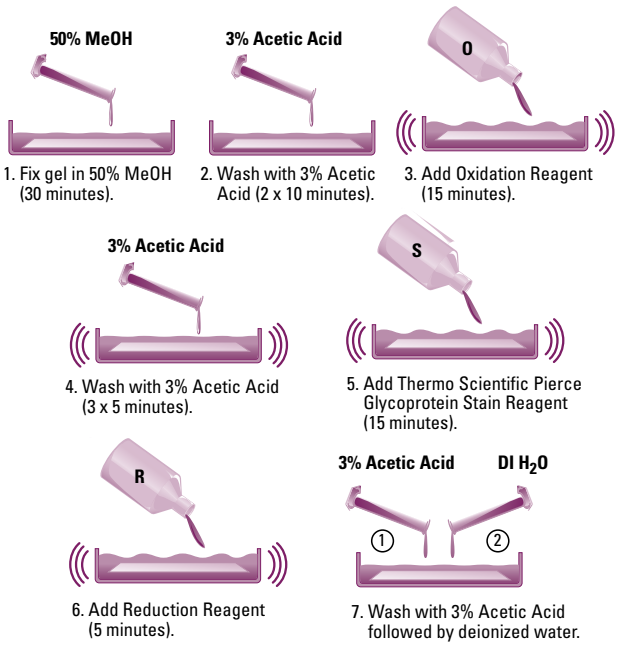


Figure 27. Thermo Scientific Pierce Glycoprotein Staining protocol.

Ordering Information

Product #	Description	Pkg. Size
24562	**Pierce Glycoprotein Staining Kit** *Sufficient for staining up to 10 SDS-PAGE mini (8 cm x 10 cm) gels.*	Kit
	Includes: Glycoprotein Oxidation Reagent	Makes 250 ml
	Glycoprotein Stain Reagent	250 ml
	Reduction Reagent	Makes 250 ml
	Standards: Horseradish Peroxidase (positive control)]	1 mg
	Soybean Trypsin Inhibitor (negative control)	1 mg

Also available:

Ordering Information

Product #	Description	Pkg. Size
23260	**Glycoprotein Carbohydrate Estimation Kit**	Kit
89804	**Glycoprotein Isolation Kit, ConA** *Sufficient reagents for the isolation of glycoproteins with strong affinity for ConA from 10 samples of up to 640 µl (1-1.5 mg total protein) each.*	Kit
	Includes: ConA Lectin Resin, 1.1 ml settled resin supplied as a 50% slurry containing a hapten buffer	
	Binding/Wash Buffer, 6.5 ml of a 5X Stock Solution	
	Elution Buffer	5 ml
	Column Accessory Pack, 10 Spin Columns with Bottom Caps and 20 Collection Tubes	
89805	**Glycoprotein Isolation Kit, WGA** *Sufficient reagents for the isolation of glycoproteins with strong affinity for WGA from 10 samples of up to 640 µl (1-1.5 mg total protein) each.*	Kit
	Includes: WGA Lectin Resin, 1.1 ml settled resin supplied as a 50% slurry containing a hapten buffer	
	Binding/Wash Buffer, 6.5 ml of a 5X Stock Solution	
	Elution Buffer	5 ml
	Column Accessory Pack, 10 Spin Columns with Bottom Caps and 20 Collection Tubes	

References

23. Misenheimer, T.M., (2001). Disulfide connectivity of recombinant C-terminal region of human thrombospondin. *J. Biol. Chem.* **276,** 45882-45887.
24. Pio, R., *et al.* (2001). Complement factor H is a serum-binding protein for adrenomedullin, and the resulting complex modulates the bioactivities of both partners. *J. Biol. Chem.* **276,** 12292-12300.

Thermo Scientific Krypton Glycoprotein Staining Kit

Combine our glycoprotein stain with our fluorescent protein stain for multiplex proteomic analysis.

The Krypton Glycoprotein Staining Kit provides a fast and easy method for the fluorescent detection of glycoproteins in SDS-PAGE and 2-D gels (Figure 28). The stain exhibits sensitivity equivalent to, or greater than, other fluorescent glycoprotein stains and uses well-established periodate-oxidation chemistry that preferentially (15- to 20-fold more binding to glycoproteins) reacts with glycoproteins (Figure 29).

Highlights:

- **Excitation/emission maxima** – 654/673 nm
- **Multiplex-compatible** – after glycoprotein staining, gels can be stained with Krypton Protein Stain or colorimetric stains
- **Robust** – highly consistent, reproducible glycoprotein staining
- **Linear quantitative range** – 3 orders of magnitude
- **Sensitive** – detect down to 15 ng of glycoprotein
- **Fast** – total protocol time of 4 hours
- **Compatible** – well-suited to work with mass spec analysis and common imaging systems

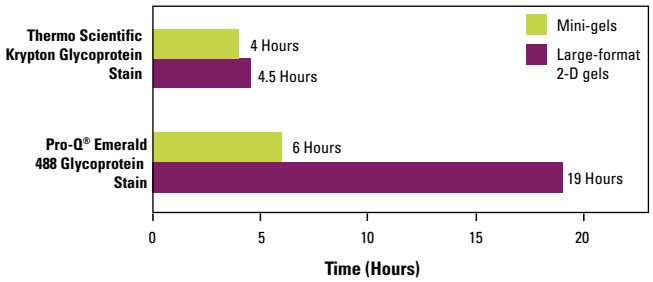


Figure 28. Thermo Scientific Krypton Glycoprotein Stain works up to four times faster than other fluorescent glycoprotein stain protocols.

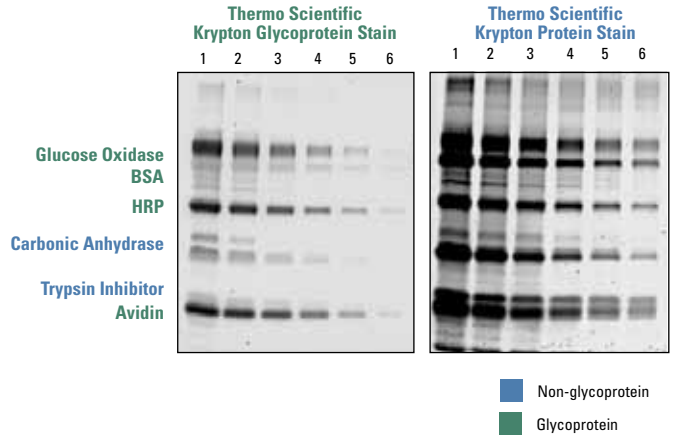


Figure 29. Thermo Scientific Krypton Glycoprotein Stain provides a fast and sensitive method for detecting glycoproteins in polyacrylamide gels. The gel was stained with Krypton Glycoprotein Stain followed by total protein staining with Krypton Protein Stain (Product # 46630). **Lanes 1-6** contain a mixture of the indicated proteins at the following concentrations: **Lane 1**. 500 ng, **Lane 2**. 250 ng, **Lane 3.** 125 ng, **Lane 4.** 63 ng, **Lane 5.** 31 ng and **Lane 6.** 15 ng.

Ordering Information

Product #	Description		Pkg. Size
53074	**Krypton Glycoprotein Staining Kit**		Kit
	Sufficient reagents to stain 10 mini (8 cm x 10 cm) gels.		
	Includes:	Glycoprotein Stain Reagent	0.3 ml
		Staining Buffer	250 ml
		Oxidizing Reagent	2.5 mg
		Positive Control (Horseradish Peroxidase)	1 mg
		Negative Control (Soybean Trypsin Inhibitor)	1 mg

Step 6 — **Stain the gel**

Thermo Scientific GelCode 6xHis Protein Tag Staining Kit

Detect 6xHistidine-tagged protein directly on the gel!

You may never again need to perform a costly and time-consuming Western blotting step to verify 6xHis-tagged expressed protein.

Highlights:

- Works two- to three-times faster than Western blotting
- Detects directly on the gel[25]
- Ready-to-use, two-reagent formula (Figure 30)
- Fluorescent detection is designed to be specific for 6xHis-tagged proteins only
- Compatible with our GelCode Blue Stain Reagent Stain for 6xHis-tagged protein specifically and follow with GelCode Blue Stain Reagent for a total protein profile determination (Figure 31)
- Detects down to 5.7 picomoles histidine-tagged protein with CCD camera and 57 picomoles with a transilluminator

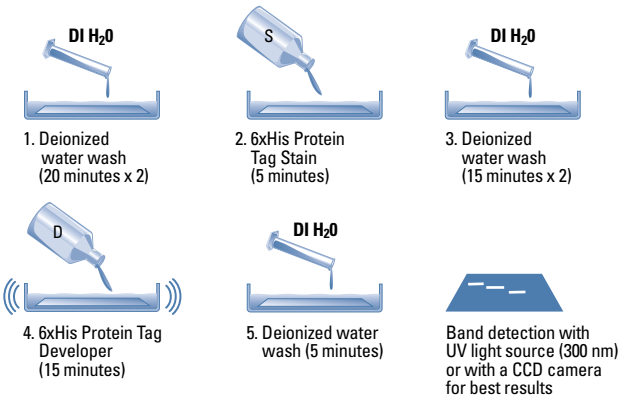
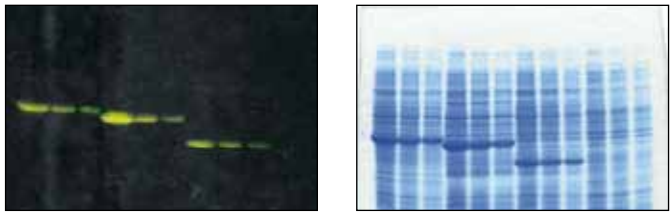


Figure 30. Thermo Scientific GelCode 6xHis Protein Tag Stain protocol.



A. **B.**

Figure 31. Total protein determination. 6xHis-tagged proteins stained with Thermo Scientific GelCode 6xHis Protein Tag Staining Kit. A. *Escherichia coli* lysates expressing 6xHis-tagged proteins, stained with the Pierce 6xHis Protein Tag Staining Kit. **B.** Identical lysates stained with GelCode Blue Stain Reagent.

Ordering Information

Product #	Description	Pkg. Size
24575	**GelCode 6xHis Protein Tag Staining Kit** *Sufficient reagent to stain 10 SDS-PAGE mini gels.* Includes: GelCode 6xHis Protein Tag Stain Reagent Set 6xHis Protein Control Set	Kit

Reference

25. Williams, N.K., *et al.* (2002). *In vivo* protein cyclization promoted by a circularly permuted *Synechocystis sp.* PCC6803 DnaB mini-intein. *J. Biol. Chem.* **277,** 7790-7798.

Thermo Scientific GelCode Phosphoprotein Staining Kit

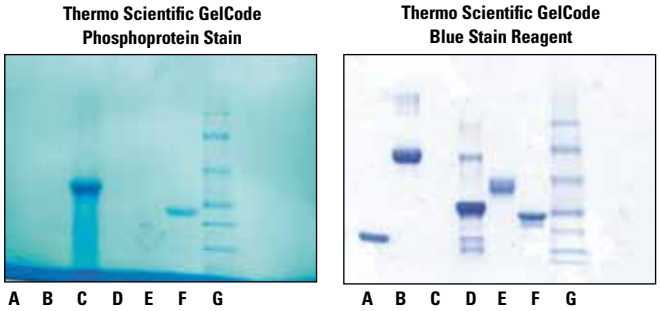
Green bands indicate specific, in-gel detection of phosphorylated proteins

Highlights:

- Specific stain for use with SDS-PAGE to detect abundant phosphoprotein components of the sample
- Phosphoproteins stain in-gel as green to green-blue bands (Figure 32)
- Easy-to-follow protocol yields results in three hours (Figure 33)
- Detects the phosphoproteins phosvitin and β-casein in the 40-80 ng/band and 80-160 ng/band range, respectively, in a 20% SDS-polyacrylamide gel
- Kit includes one positive control protein (Phosvitin) and one negative control protein (Soybean Trypsin Inhibitor)
- Phosphoprotein Stain Reagent Set is room temperature-stable, freeing up limited refrigerator space
- Phosphoprotein-stained gels can be stained with Thermo Scientific GelCode Blue Stain Reagent (Product #s 24590 and 24592) for total protein profiling

Applications:

- Use to evaluate the progress of a phospho-protein purification
- Excellent potential for use in dephosphorylation studies



A selection of commercially purified protein preparations with varying degrees of phosphorylation were stained with GelCode Phosphoprotein Stain. Approximately 10 µg of each protein was loaded per lane.

An identical gel was stained with GelCode Blue Stain Reagent.

Figure 32. Thermo Scientific GelCode Phosphoprotein Stain specifically detects phosphorylated proteins. An identical selection of proteins was run on two gels and stained with either GelCode Phosphoprotein Stain or GelCode Blue Stain for total protein detection. **A.** soybean trypsin inhibitor (negative control). **B.** bovine serum albumin, **C.** phosvitin, **D.** histone III-S, **E.** ovalbumin, **F.** β-casein and **G.** prestained protein molecular weight markers.

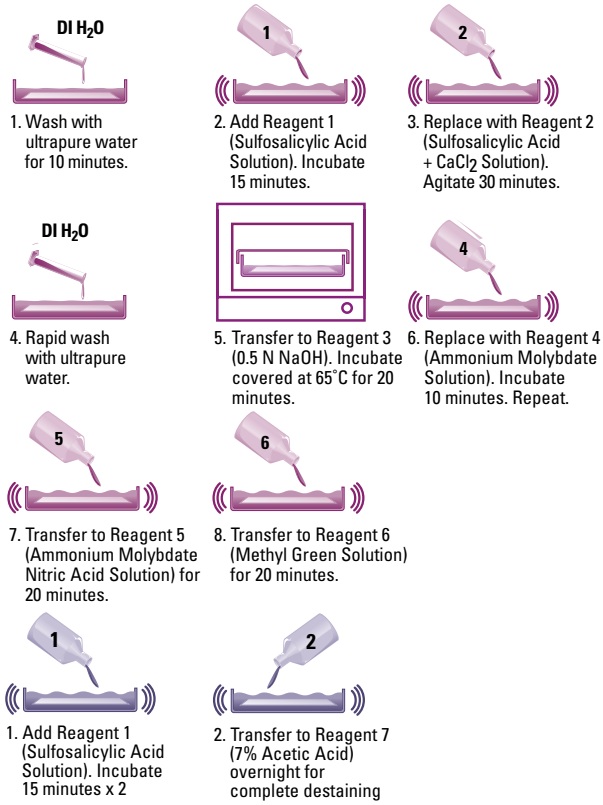


Figure 33. Thermo Scientific GelCode Phosphoprotein Stain protocol.

Ordering Information

Product #	Description	Pkg. Size
24550	**GelCode Phosphoprotein Staining Kit** *Sufficient reagent to stain 10 mini gels (8 cm x 8 cm).* Includes: Protein Stain Reagent Set Phosphoprotein Control Set	Kit

Step 6 — **Stain the gel**

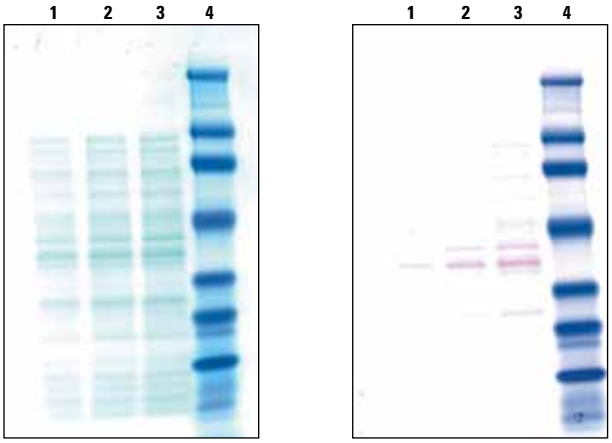
Thermo Scientific Pierce Reversible Protein Stains for Nitrocellulose Membranes

A great alternative to Ponceau S stain!

For years the red Ponceau S stain has been the best option for staining before Western blotting, despite its major shortcomings. Our Reversible Protein Stains decrease staining time, increase staining sensitivity and enhance the immunoreactivity of antigens in subsequent Western blotting. Try these new reversible protein stains for nitrocellulose and PVDF membranes and you will never use Ponceau S again.

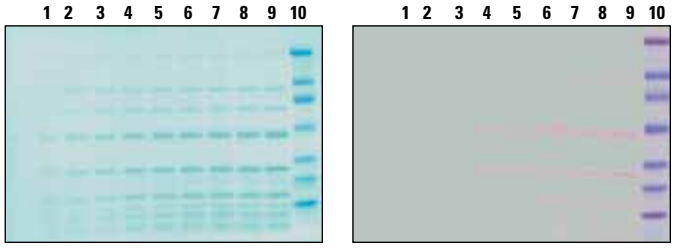
Highlights:

- Sensitive, high-avidity, general protein stain
- Stain is protein-specific, avoiding interference from other biomolecules
- From stain to destain to band erasure in minutes
- Turquoise bands are easily photographed
- Stained bands do not fade with time
- Enhances Western blot detection
- All components are room temperature-stable



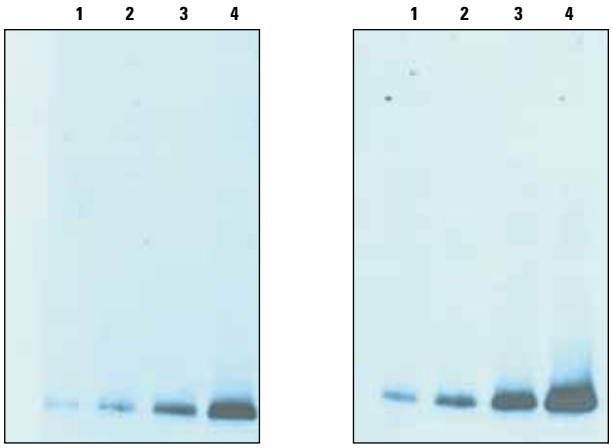
A. Thermo Scientific Pierce Reversible Stain **B. Ponceau S Stain**

Figure 34. Thermo Scientific Pierce Reversible Protein Stain and Ponceau S Stain: A comparison of GST lysate staining on nitrocellulose. Increasing amounts of GST Lysate protein were applied onto two 4-20% Tris-glycine SDS polyacrylamide gels. Both gels were electroblotted to nitrocellulose membrane. **Blot A** was treated with Pierce Reversible Stain for 30 seconds and destained according to the protocol. **Blot B** was stained with 0.1% Ponceau S stain for 5 minutes and destained. The blot stained with Pierce Reversible Stain demonstrates superior visual detection of bands. GST Lysate loading volumes (Lane 1-3). **Lane 1.** 5 µl, **Lane 2.** 10 µl, **Lane 3.** 15 µl and **Lane 4.** Thermo Scientific Pierce Prestained Marker Mix (Product # 26681), 10 µl.



A. **B.**

Figure 35. Comparison of Thermo Scientific Pierce Reversible Protein Stain with Ponceau S stain. Unstained Protein MW Markers (Product # 26671) were serially diluted and applied to two 4-20% Tris-glycine-SDS polyacrylamide gels **Lanes 1-9**. Both gels were electroblotted to PVDF membrane. **Blot A** was stained with Pierce Reversible Stain for 1 minute and destained according to the protocol. **Blot B** was stained with 0.1% Ponceau S in 5% acetic acid for 5 minutes and destained according to the published protocol. **Lane 10**. Pierce Prestained MW Marker Mix (Product # 26681).



A. Control **B. Thermo Scientific Pierce Reversible Stain**

Figure 36. Immunoblot analysis of GST by chemiluminescent detection after Thermo Scientific Pierce Reversible Staining, destaining and stain reversal. Different amounts of purified GST protein were applied to two 10% Tris-glycine SDS-polyacrylamide gels. Both gels were electroblotted to nitrocellulose membranes. The control membrane **(Panel A)** was not treated with Pierce Reversible Protein Stain. **Panel B** was subjected to the staining, destaining and stain erasing protocol of the Pierce Reversible Staining Kit. Both membranes were then probed with anti-GST incubated with goat anti-rabbit IgG-HRP conjugate and detected using Thermo Scientific Reversible SuperSignal West Dura Substrate (Product # 34075). **Lane 1.** 125 pg, **Lane 2.** 250 pg, **Lane 3.** 500 pg and **Lane 4.** 1 ng.

Table 6. Comparison of Thermos Scientific Pierce Reversible Protein Stain vs Ponceau S Stain.

Ponceau S Reversible Stain	Thermo Scientific Pierce Reversible Protein Stain
Weak-binding, low-sensitivity general protein stain	Tight-binding, higher sensitivity general protein stain
Detection limit: 250 ng	Detection limit: 25-50 ng
Red bands are difficult to photograph	Turquoise blue bands are easily photographed
Stained protein bands fade within hours	Turquoise bands do not fade over time, but they can be erased
Typical staining time: 5 minutes	Typical staining time: 60 seconds
	Background eliminated quickly with low pH wash

A. Nitrocellulose Membrane Staining Protocol 1. Wash membrane with ultrapure H_2O. 2. Add Pierce Reversible Stain. Shake 30 seconds. Protein bands appear turquoise in color.	**A. PVDF Membrane Staining Protocol** 1. Wash membrane with ultrapure H_2O. 2. Add Pierce Sensitizer. Shake for 2 minutes. 3. Add Pierce Stain. Shake for 1 minute. Protein bands appear turquoise in color.
B. Destaining Protocol 1. Rinse three times with Pierce Destain Solution. 2. Add Pierce Destain. Shake 5 minutes. 3. Rinse four times with ultrapure H_2O. 4. Wash on a shaker with ultrapure H_2O for 5 minutes.	**B. Destaining Protocol** 1. Rinse three times with Pierce Destain Solution. 2. Wash with Pierce Destain mixed 1:1 with MeOH on a shaker for 5 minutes. 3. Rinse five times with ultrapure H_2O.
C. Stain Erasing Protocol 1. Wash with Pierce Stain Eraser on a shaker for 2 minutes. 2. Rinse four times with ultrapure H_2O. 3. Wash with ultrapure H_2O on a shaker for 5 minutes.	**C. Stain Erasing Protocol** 1. Wash with Pierce Stain Eraser mixed 1:1 with MeOH on a shaker for 10-20 minutes. 2. Rinse five times with ultrapure H_2O.

Figure 37. Thermo Scientific Pierce Reversible Protein Stain protocols.

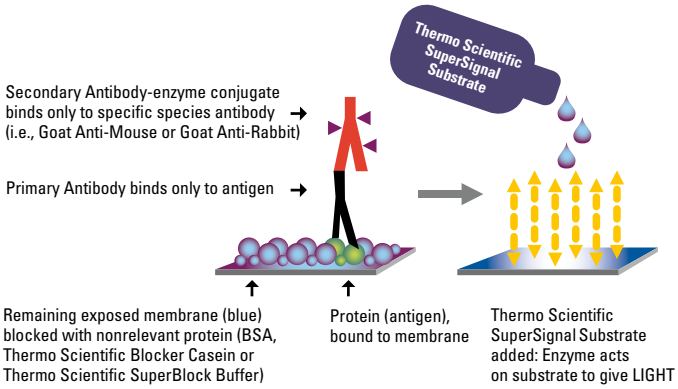
Ordering Information

Product #	Description	Pkg. Size	U.S. Price
24580	**Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes** *Sufficient material for 10 (8 cm x 8 cm) nitrocellulose membranes.*	Kit	$112
	Includes: Pierce Reversible Protein Stain (Component A)	250 ml	
	Pierce Destain (Component B)	2 x 500 ml	
	Pierce Stain Eraser (Component C)	250 ml	
24585	**Pierce Reversible Protein Stain Kit for PVDF Membranes** *Sufficient material to stain protein and reverse the stain from 10 (8 cm x 8 cm) PVDF membranes.*	Kit	$127
	Includes: Pierce Sensitizer *PVDF membrane pre-treatment agent*	250 ml	
	Pierce Reversible Stain *A broad-spectrum stain for proteins transferred to PVDF membrane*	250 ml	
	Pierce Destain *Enhances protein band detection by eliminating background staining*	1,000 ml	
	Pierce Stain Eraser *Reverses protein band staining on demand*	250 ml	

Step 7 — **Post-staining**

Western Blotting

Western blotting, also known as immunoblotting, is used to detect the presence and estimate the molecular weight of specific antigens in a crude mixture, to compare immunological cross-reactivity among proteins, and to study modifications of proteins during cellular processing. In Western blotting, a protein sample is separated by SDS-PAGE, electro-transferred to a membrane, and detected by a labeled antibody. The advantages of using membranes to immobilize proteins for protein detection are ease of membrane manipulation, reduced washing and reaction times, greater sensitivity, and possible reuse of the blot for more procedures after removal of probing reagents.

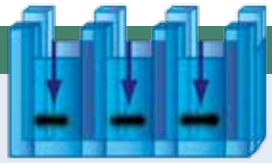


Western Blotting Handbook

Request this FREE handy guide for your laboratory or office! This booklet details our innovative products for Western blotting. Learn how you can obtain ultra-sensitive blotting detection using our SuperSignal Technology.

Product #: 1601775

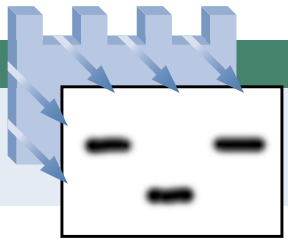
STEP 1



SDS-PAGE
Separate protein sample by electrophoresis.

- Pierce SDS-PAGE Sample Prep Kit (Product # 89888)
- Precise Protein Gels (many available, see page 7)
- Tris-Hepes-SDS Running Buffer (Product # 28398)
- Lane Marker Reducing Sample Buffer (5X) (Product # 39000)
- Lane Marker Non-Reducing Sample Buffer (5X) (Product # 39001)
- Pierce Blue Prestained Protein Molecular Weight Marker (Product #s 26681 and 26685)
- Pierce Chemiluminescent Prestained Peroxidase-labeled Protein Molecular Weight Marker (Product # 26651)
- Pierce Prestained 3-Color Protein Molecular Weight Marker (Product # 26691)
- DyLight Dual-Labeled Fluorescent Marker (Product # 22859 and 26665)

STEP 2

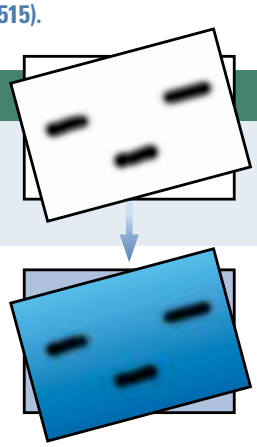


Electro-Transfer
Transfer proteins to membrane.

- Fast Semi-Dry Blotter (Product # 88217)
- Methanol-Free Transfer Buffer (Product # 35040)
- Fast Semi-Dry Transfer Buffer (Product # 35035)
- Tris-Glycine Transfer Buffer (Product # 28380)
- Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes (Product # 24580) and for PVDF Membranes (Product # 24585)
- Pierce Western Blot Signal Enhancer (Product # 21050)
- Pierce Antibody Extender NC (Product # 32110 and 32105)
- Nitrocellulose Membrane, 0.2 µm (Product #s 77012, 88013 and 88024)
- Nitrocellulose Membrane, 0.45 µm (Product #s 77010, 88014 and 88025)
- PVDF Membrane, 0.45 µm (Product #s 88585 and 88518)
- Low-fluorescence PVDF Membrane, 0.2 µm (Product # 22860)
- Western Blotting Filter Paper (Product # 88600)
- Extra Thick Blotting Filter Paper (Product # 88605, 88610, 88615, 88620)

For detection of proteins that cannot be efficiently transferred to a membrane, Thermo Scientific Pierce In-Gel Detection Technology† allows positive identification of proteins directly in a gel (Product #s 33500, 33505, 33510 and 33515).

STEP 3



Blocking
Block nonspecific sites.

- Protein-free Blocking Buffer (Product #s 37570, 37571, 37572 and 37573)
- Thermo Scientific StartingBlock Blocking Buffer in PBS (Product # 37538) and in TBS (Product # 37542)
- StartingBlock™ T20 Blocking Buffer (Contains 0.05% Tween®-20) in PBS (Product # 37539) or TBS (Product # 37543)
- Thermo Scientific SuperBlock Buffer in PBS (Product # 37515 and 37518) and in TBS (Product # 37535)
- SuperBlock® T20 Blocking Buffer (Contains 0.05% Tween-20) in PBS (Product # 37516) or TBS (Product # 37536)
- SuperBlock Blocking Buffer – Blotting in PBS (Product # 37517) and in TBS (Product # 37537)
- Casein in PBS (Product # 37528) and in TBS (Product # 37532)
- BSA in PBS (Product # 37525) and in TBS (Product # 37520)
- SEA BLOCK Buffer (Product # 37527)
- BLOTTO in TBS (Product # 37530)

STEP 4A

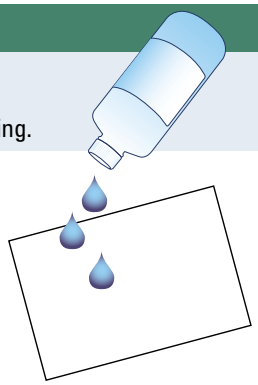
Formulate Wash Buffers
Choose a buffer.

- Phosphate Buffered Saline (PBS, Product #s 28372 and 28348)
- Tris Buffered Saline (TBS, Product #s 28376, 28379 and 28358)
- Modified Dulbecco's PBS (Product #s 28374 and 28344)
- Carbonate-Bicarbonate Buffer Packs (Product # 28382)
- MES Buffered Saline (Product # 28390)
- Thermo Scientific BupH Borate Buffer Packs (Product #s 28384 and 28341)
- BupH™ Citrate-Carbonate Buffer Pack (Product # 28388)

Step 7 — **Post-staining**

STEP 4B

Formulate Wash Buffers
Add detergent to blocking/wash buffers to reduce nonspecific binding.



Skip this step if you use StartingBlock T20 Blocking Buffer in PBS (Product # 37539) or TBS (Product # 37543) or SuperBlock T20 Blocking Buffer in PBS (Product # 37516) or TBS (Product # 37536). These buffers already contain Tween-20 Detergent at optimized concentrations.

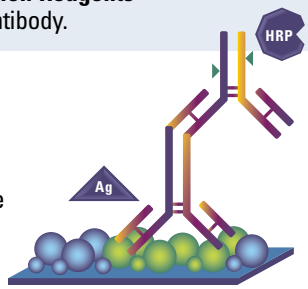
Thermo Scientific Surfact-Amps Detergents containing:

- Tween-20 (Product # 28320) and Tween-80 (Product # 28328)
- Triton® X-100 (Product # 28314) and Triton X-114 (Product # 28332)
- Nonidet P-40 (Product # 28324)
- Brij®-35 (Product # 28316) and Brij-58 (Product # 28336)

For convenience and economy, we also offer complete Western blotting kits that include chemiluminescent substrates, enzyme-conjugated antibodies, blocking buffers and standard buffers.

STEP 5

Primary and Secondary Detection Reagents
Incubate the membrane with antibody.



For a complete list, visit the secondary antibody selection guide at www.thermo.com/pierce. For a complete listing of primary antibodies, request a copy of the Thermo Scientific Pierce Antibody Handbook featuring over 35,000 antibodies in 42 research areas or visit www.thermo.com/abr.

For direct detection methods we offer:

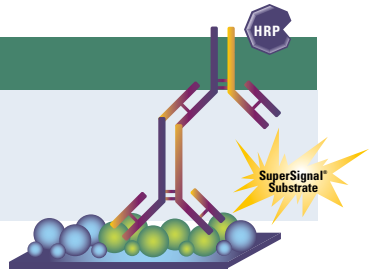
- Monoclonal Antibodies
- Fluorescent Probes and Labeling Kits
- Enzyme Labeling Kits

For indirect detection methods we offer:

- Biotinylation Kits
- Protein A, Protein G and Protein L labeled with fluorescein, rhodamine, HRP, AP or biotin
- Avidin, Streptavidin and Thermo Scientific NeutrAvidin Biotin-Binding Protein labeled with fluorescein, rhodamine, HRP or AP
- Secondary antibodies labeled with fluorescein, rhodamine, HRP, AP or biotin
- Clean-Blot IP Detection Reagents (HRP/AP)
- DyLight Secondary Antibody and Streptavidin Conjugates [Photostable and inexpensive alternatives to CyDye™ Fluors (GE) and Alexa Fluor Dye (Invitrogen)].

STEP 6

Enzyme Substrates
Add the detection reagent.



Chemiluminescent Substrates:

- Pierce ECL Substrate (Product #s 32106, 32209 and 32109)
- Pierce Fast Western Blot Kits: SuperSignal West Pico Substrate (Product # 35065, 35060, 35066 and 35061); SuperSignal West Dura Substrate (Product # 35075, 35070, 35076 and 35071); SuperSignal West Femto Substrate (Product # 35080 and 35081); ECL Substrate (Product #s 35050 and 35055)
- SuperSignal® West Pico Chemiluminescent Substrate (Product #s 34077 and 34080); also available in an economical 1-L package (Product # 34078)
- SuperSignal West Femto Maximum Sensitivity Substrate (Product #s 34096 and 34095)
- SuperSignal West Dura Extended Duration Substrate (Product #s 34076 and 34075)
- Lumi-Phos WB Substrate (Product # 34150)

Colorimetric Substrates:

- Pierce Chloronaphthol (Product # 34012)
- TMB-Blotting (Product # 34018)
- NBT/BCIP (Product # 34042)
- Metal Enhanced DAB (Product # 34065)

STEP 7

Film
Expose the membrane to X-ray film.

- CL-XPosure™ Film 5 x 7" sheets (Product #s 34090 and 34092); 8 x 10" sheets (Product #s 34091 and 34093); 18 x 24 cm sheets (Product # 34089)
- Pierce Background Eliminator Kit (Product # 21065)

STEP 8

Stripping Buffer
Reprobe the blot if necessary.

- Thermo Scientific Restore Western Blot Stripping Buffer (Product # 21059 and 21063)
- Restore™ PLUS Western Blotting Stripping Buffer (Product #s 46428, 46430 and 46431) for High-Affinity Antibodies
- IgG Elution Buffer (Product #s 21004 and 21009)

Thermo Scientific SuperSignal® Technology is protected by U.S. patent 6,432,662.
Thermo Scientific Pierce Direct Detection of Biomolecules Technology is protected by U.S. patent 7,112,411.
U.S. patent pending on Thermo Scientific Dual-Labeled Fluorescent Molecular Weight Marker Technology, Imperial Protein Stain Technology and Krypton Protein Stain Technology.

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