



Run the gel

Step 5

Stain the gel

Step 6

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 various methods of in-gel detection. One method for detecting protein bands requires radio-labeling of the proteins before the electrophoretic separation and detecting the protein bands on X-ray film. As radiation is emitted, it produces metallic silver within the silver halide crystals in the film and can be seen once the film is developed. This is known as autoradiography. For example, proteins in a sample can be radioiodinated using ^{125}I and IODO-GEN[®] Iodination Tubes (Product # 28601). The labeled sample is run on an electrophoretic gel, which is then dried down and exposed to an X-ray film for 24-hours. When the film is developed, the iodine-labeled protein in the gel can be seen as gray bands in the sample lane. Autoradiography is a sensitive method for detecting small quantities of proteins in an electrophoretic gel. Other radioisotopes such as ^{14}C , ^{35}S and ^3H also can be used.

The most common method for visualizing proteins within the gel itself is staining with Coomassie Brilliant Blue Dye (Product # 20278). The mechanism of coomassie dye binding to proteins is not completely understood; however, it depends in part on basic and hydrophobic residues.

Therefore, binding varies widely among proteins. Coomassie staining turns the entire gel blue and, to see the protein bands, the gel must be destained with a methanol/acetic acid mixture to remove background stain. Destaining allows the gel to be reprobed with another dye or stain. Pierce has developed a unique coomassie-based stain, GelCode[®] Blue Stain Reagent, that does not require an organic solvent for destaining. GelCode[®] Blue Stain (Product # 24590 and 24592) is compatible with mass spectral analysis¹⁹⁻²² and N-terminal sequence analysis.²³ It simply requires a one-hour incubation with the stain and a one-hour water wash to see nanogram levels of protein (Figure 3).

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. Of the three methods commonly used for silver staining, the Pierce GelCode[®] Color Silver Stain is the most sensitive. This method produces colored protein bands when a strong base environment of sodium hydroxide and formalin are followed by incubation in a weak base of sodium carbonate. This silver staining system, developed by Sammons, *et al.*,²⁴ results

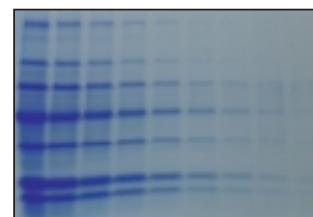
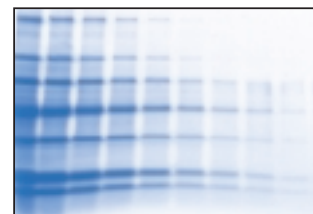
GelCode[®] Blue Stain ReagentGelCode[®] Blue Stain Reagent with Water Wash Enhancement[™] Step

Figure 3. GelCode[®] Blue Stain protocol and results.

Step 6 -- Stain the gel

Step 6

Gel Electrophoresis of Proteins

in protein staining in which the protein bands can be one of four colors (black, blue-brown, red or yellow), depending upon unique characteristics of the protein. The advantage of using a color vs. a monochromatic system is that overlapping protein bands can be seen easily. Color also helps to distinguish proteins that have been post-translationally modified. The intensity of color is also proportional to the amount of protein present in the bands. By scanning the gel with a densitometer, absolute amounts of proteins can be determined from a standard curve. This is less accurate 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.²⁵ GelCode® Color Silver Stain® (Product # 24597) can detect protein concentrations as low as 0.1 ng/mm. This is comparable to ³⁵S-methionine autoradiography. The GelCode® SilverSNAP® Staining System (Product # 24602) offers slightly less sensitivity but results can be obtained in less than 40 minutes.^{26,27}

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. GelCode® E-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.²⁸ 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 4 for a review of GelCode® Stain Products for detecting proteins.

Specific Functional Group Stains

It is often desirable to detect a subset of proteins on a gel rather than all proteins. Pierce has developed a unique line of specific gel stains that help to further characterize proteins separated on SDS-PAGE gels and 2-D gels according to specific modifications that are present. The GelCode® Glycoprotein Stain (Product # 24562) allows detection of proteins that have been post-translationally modified with carbohydrate.^{29,30} Glycoproteins stain a magenta color when developed with the GelCode® Glycoprotein Stain Kit. Both SDS-PAGE gels and proteins blotted onto nitrocellulose membranes can be stained for glycoproteins in approximately 2 hours. The process begins by fixing the gels in 50% methanol, followed by washing in acetic acid. Sugar residues on the glycoprotein are oxidized, reacted with the dye and then reduced. Because of the unique magenta color, it is easy to compare an identical gel stained with GelCode® Blue Stain to one stained with the Glycoprotein Stain and to identify the population of glycosylated proteins. The GelCode® Glycoprotein Stain Kit comes with a positive and negative control protein.

The GelCode® Phosphoprotein Staining Kit (Product # 24550) allows detection of abundant phosphorylated proteins. The stain produces a green to green-blue colored band if a protein is phosphorylated. The same gel can then be stained for total protein content with GelCode® Blue Stain for comparison.

For staining fusion proteins containing a polyhistidine tag, Pierce developed the GelCode® 6xHis Protein Tag Staining Kit (Product # 24575). This stain allows a histidine-tagged protein to be specifically stained in a gel. 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 appear as fluorescent bands in the gel that can be seen under UV light (300 nm) and photographed with a CCD camera.³¹ Expression levels in lysates as low as 40 µg/ml can be detected using the 6xHis Tag Stain since its sensitivity level for detection is as low as 0.2 µg. This staining method may also be followed by total protein staining with GelCode® Blue Stain.



Table 4. GelCode® Stain Products Comparison

	Number of Components	Number of Steps	Staining Time (minutes)	Sensitivity	Applications
GelCode® Blue Stain Reagent	1	2	60	8 ng	<ul style="list-style-type: none"> • SDS-PAGE gels • Native PAGE gels • PhastGel® and IEF gels • Nitrocellulose and PVDF membranes
GelCode® SilverSNAP® Stain II	4	4	50	0.25 ng	<ul style="list-style-type: none"> • Two-dimensional (2-D) gels • Diluted protein samples • Purified protein samples • DNA and RNA
GelCode® Color Silver Stain	4	4	65 ¹	0.1 ng	<ul style="list-style-type: none"> • 1-D and 2-D SDS-PAGE gel staining • Color staining • Quantitative gel stain • Identification of post-translationally modified protein • Monitor proteins in biological fluids, sub-cellular fractions and disease states • Stains DNA, lipids and polysaccharides
GelCode® E-Zinc® Reversible Stain	3	2	15	0.25 ng	<ul style="list-style-type: none"> • Protein elution from PAGE gels • Staining before Western transfer • Diluted protein samples • Purified protein samples
GelCode® Glycoprotein Stain	3 ²	6 ³	> 2 hours	0.16 µg ⁴	<ul style="list-style-type: none"> • Detect sugar moieties of glycoproteins • Specific staining of glycoproteins on SDS-PAGE gels or on membrane after transfer
GelCode® 6xHis Protein Tag Stain	2 ²	6	1 hour 35 minutes	0.2 µg of a 35 kDa fusion protein	<ul style="list-style-type: none"> • Detect 6xHis-tagged proteins in a lysate • Monitor purification of fusion proteins
GelCode® Phosphoprotein	7 ²	10	3 hours – overnight	80 ng phosvitin 160 ng β-casein	<ul style="list-style-type: none"> • Detect phosphoproteins in crude samples • Locate high abundance phosphoproteins • Monitor purification of phosphoproteins

Notes

1. 0.75 mm gel thickness, does not include fixing and washing.
2. Also includes a positive and negative control protein.
3. After the initial gel fixation and wash protocol.
4. Horseradish peroxidase positive control (sensitivity will vary with extent of glycosylation of the protein under analysis).

References

19. Aulak, K.S., et al. (2001). *Proc. Nat. Acad. Sci.* **98**, 12056-12061.
20. Hughes, M.J.G., et al. (2002). *Infect. and Immun.* **70**, 1254-1259.
21. Hilton, J.M., et al. (2001). *J. Biol. Chem.* **276**, 16341-16347.
22. Lim, J., et al. (2002). *J. Biol. Chem.* **277**, 20774-20782.
23. Tani, M., et al. (2000). *J. Biol. Chem.* **275**, 3462-3468.
24. Sammons, D.W., et al. (1981). *Electrophoresis* **2**, 135-141.
25. Sliuz, M.L. and Van Frank, R.M. (1985). *Electrophoresis* **6**, 405-407.
26. Koszelak-Rosenblum, M.E. et al. (2002). *J. Biol. Chem.* **277**, 11664-11669.
27. Mattagajasingh, S.N., et al. (2000). *J. Biol. Chem.* **275**, 30573-30585.
28. Amano, S., et al. (2000). *J. Biol. Chem.* **275**, 22728-22735.
29. Pio, R., et al. (2001). *J. Biol. Chem.* **276**, 12292-12300.
30. Misenheimer, T.M. (2001). *J. Biol. Chem.* **276**, 45882-45887.
31. Williams, N.K., et al. (2002). *J. Biol. Chem.* **277**, 7790-7798.

Gel Electrophoresis of Proteins

GelCode® Blue Stain Reagent

Eliminates the pain of having to destain.



A remarkable new advancement in SDS-PAGE gel staining with features and benefits not available from homemade or commercial coomassie stain preparations ... and it's economically priced too!

Highlights:

No tedious, pungent methanol/acetic acid destaining step required

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

Fast, one-step, one-hour staining

- Ready-to-use stain reagent
- Bands develop before your eyes 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 analysis³²
- Allows gel staining after Western transfer
- Compatible with MALDI-TOF analysis³³⁻³⁵
- Compatible with sequence analysis³⁶

Optional Water Wash Enhancement™ Step further increases staining sensitivity

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

References

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

GelCode® Blue Staining Protocol

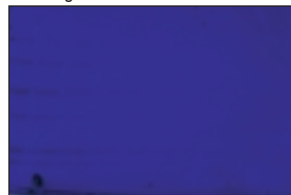


1. Wash gel three times with deionized water (15 minutes).
2. Add GelCode® Blue Stain Reagent (1 hour).
3. For increased clarity, use the optional Water Wash Enhancement™ Step (1 hour).

The results speak for themselves ...

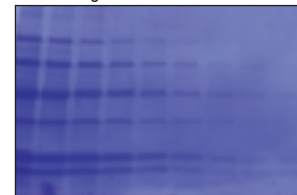
Classic Coomassie Stain Formulation

Staining Time: 1 hour



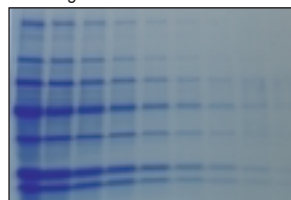
No bands observed prior to destaining.

Destaining Time: 1 hour



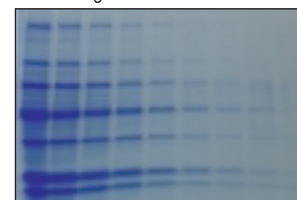
Pierce GelCode® Blue Stain Reagent

Staining Time: 1 hour



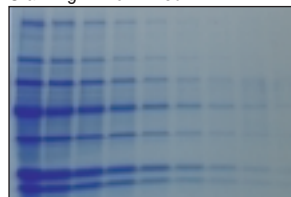
Bands clearly visible in the staining tray.

Destaining Time: 0



Pierce GelCode® Blue Stain Reagent

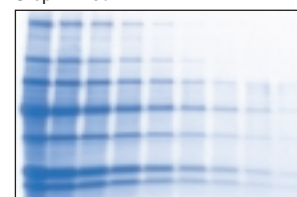
Staining Time: 1 hour



Crystal-clear background and excellent sensitivity to 8 ng.

Publication-quality stained gel ready for photo-documentation by computer or camera.

Water Wash Enhancement™
Step: 1 hour



Ordering Information

Product #	Description	Pkg. Size
24590	GelCode® Blue Stain Reagent Sufficient for staining up to 25 SDS-PAGE mini gels (8 cm x 10 cm). R: 20/21/22, 40/20/21/22; S: 26/37	500 ml
24592	GelCode® Blue Stain Reagent* Sufficient for staining up to 175 SDS-PAGE mini gels (8 cm x 10 cm). R: 20/21/22, 40/20/21/22; S: 26/37	3.5 liters
72300	Pump (for 3.5 liter package only)	1 pump

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

For more product information, or to download a product instruction booklet, visit www.piercenet.com.



InstaStain™ Blue Gel Stain Paper

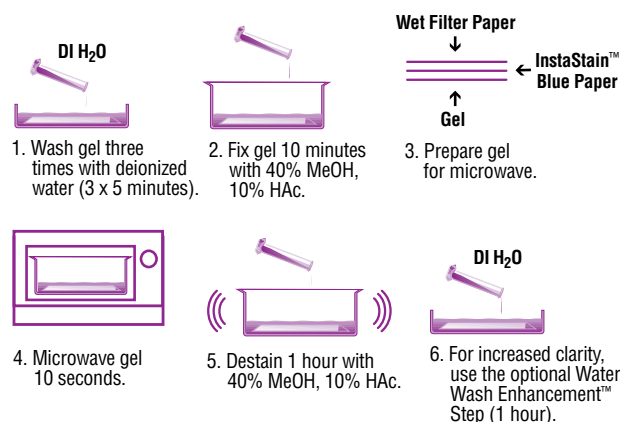
Stains in seconds and eliminates the need to prepare and handle messy coomassie dye stain solutions.

To use InstaStain™ Blue Gel Stain Paper, wash and fix the gel, put the paper in contact with the gel, microwave-activate the stain and destain in the traditional way. InstaStain™ Blue Gel Stain Paper is the shortest – and overall cleanest and neatest – approach for staining PAGE gels. Dispose of the paper after the staining. There is no messy dye solution to contend with. InstaStain™ Blue Gel Stain Paper dramatically shortens the staining process cutting, in most cases, hours out of the time required to obtain a fully destained gel suitable for photo-documentation and publication.

Highlights:

- Compatible with mass spectrometry³⁷
- Ends preparation of dye solution forever
- Fastest coomassie dye-based staining format known
- Solid-phase, microwave-activated staining
- Uses traditional methanol/acetic acid fixing and destain solutions
- Sensitivities comparable to classical coomassie gel stain formulations
- 10 cm x 10 cm sheets cover most popular mini gel sizes
- More environmentally friendly than classical staining and destaining methods

InstaStain™ Blue Gel Stain Paper Protocol



Reference

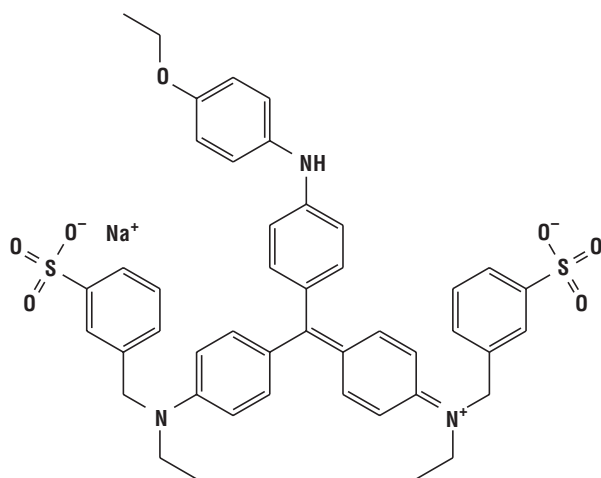
37. Simpson, R.J. (2003). Proteins and Proteomics. A Laboratory Manual. Cold Spring Harbor Laboratory Press. pp. 69-70.

Ordering Information

Product #	Description	Pkg. Size
24825	InstaStain™ Blue Gel Stain Paper	25 sheets/pkg.

Coomassie Brilliant Blue R-250 and G-250 Dyes³⁸

Ideal as a protein stain following electrophoresis.



Coomassie Brilliant Blue R-250 Dye
M.W. 825.97

Highlights:

- Develops intensely colored complexes with proteins
- Can determine as little as 0.5 µg/cm² 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

38. Syrový, I. and Hodný, Z. (1991). *J. Chromatogr.* **569**, 175-196.

Ordering Information

Product #	Description	Pkg. Size
20278	Coomassie Brilliant Blue R-250 Dye R: 36/37/38; S: 36/37/39	50 g
20279	Coomassie Brilliant Blue G-250 Dye (Colloidal)	50 g

Gel Electrophoresis of Proteins

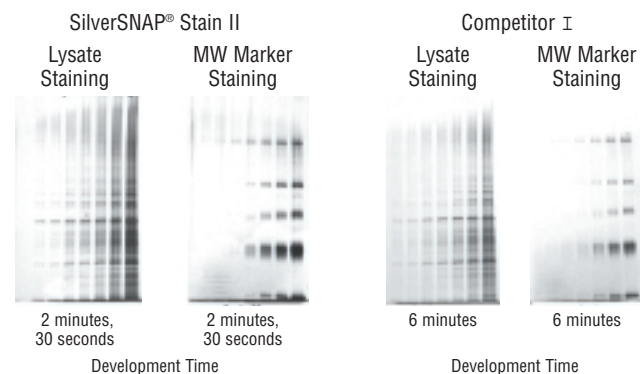
GelCode™ SilverSNAP® Stain II

A faster, more flexible silver stain

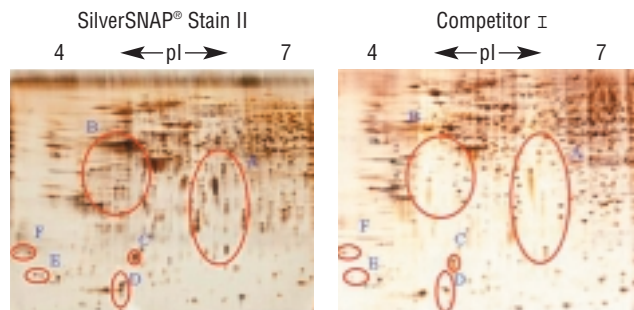
GelCode™ SilverSNAP® Stain II is a fast, more flexible and forgiving stain that yields results competitive with – and often better than – any homemade or commercially available silver stain. Most importantly, our improved silver stain delivers consistently reliable staining performance each and every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

Highlights:

- Remarkably low, uniform background
- Detect as little as 0.25 ng of protein
- Staining can be completed in less than 50 minutes
- Compatible with a wide assortment of homemade and precast gels
- Ideal for use with one- or two-dimensional PAGE and IEF gels
- Can also be used to stain DNA or RNA following electrophoresis
- Flexible protocol 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



Comparison of newly improved SilverSNAP® Stain II with another popular brand.



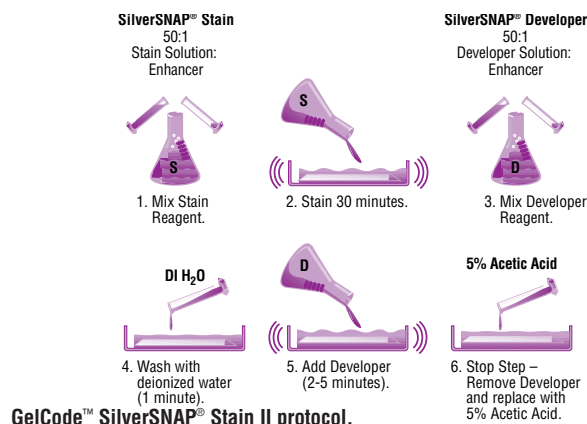
Comparison of identical 2-D gels stained with SilverSNAP® Stain II with another popular brand. (Circled regions indicate difference in staining intensity.)

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

Ordering Information

Product #	Description	Pkg. Size
24612	GelCode™ SilverSNAP® Silver Stain II <i>This product replaces Product # 24602.</i>	Kit
	Includes: SilverSNAP® Sensitizer	2 ml
	SilverSNAP® Stain	500 ml
	SilverSNAP® Enhancer	25 ml
	SilverSNAP® Developer	500 ml

*Criterion® is a trademark of Bio-Rad Laboratories.
SilverXpress® is a trademark of Invitrogen.*





GelCode® Color Silver Stain Kit³⁹⁻⁴⁷

The only silver staining system offering quantitative, reproducible color staining.



Highlights:

- Designed for one- and two-dimensional PAGE gel staining
- Total time, after fixing, varies from 11-90 minutes (depending on gel thickness)
- Sensitivity as low as 0.1 ng protein/mm² in the gel

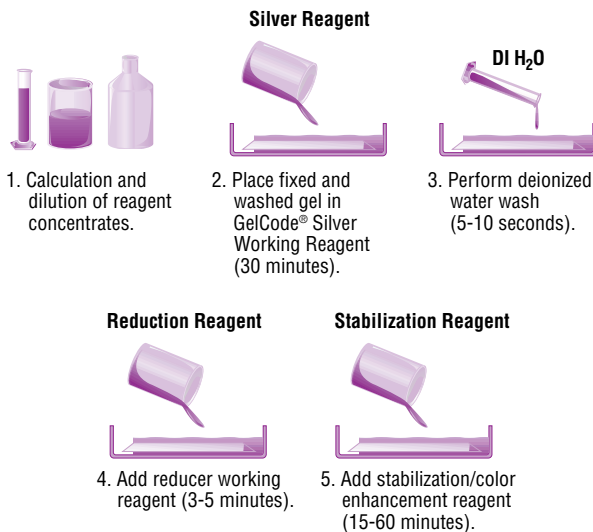
- Detects proteins that do not bind silver as yellow spots
- Proteins stain in five basic colors: black, blue, brown, red and yellow
- Four simple staining steps
- No stop baths or reversers needed
- Stains proteins, DNA, lipids and polysaccharides

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

GelCode® Color Silver Stain Protocol

Basic protocol, after gel fixing step, based on 0.75 mm gel.



References

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 40. Ochs, D.C., *et al.* (1981). *Electrophoresis* **2**, 304-307.
 41. Dunbar, B.D., *et al.* (1983). *Electrophoresis* **4**, 258-259.
 42. Schoenle, E.J., *et al.* (1984). *J. Biol. Chem.* **259**, 12112-12116.
 43. Sammons, D.W., *et al.* (1984). *Two-Dimensional Gel Electrophoresis-Proteins*. Eds. Celis, J.E. and Bravo, R. Academic Press. pp. 111-126.
 44. Slisz, M.L. and Van Frank, R.M. (1985). *Electrophoresis* **6**, 405-408.
 45. Dunbar, B.S., *et al.* (1990). *Methods Enzymol.* **182**, 441-459.
 46. Sammons, D.W., *et al.* (1981). *Electrophoresis* **2**, 141-147.
 47. Stoppler, H., *et al.* (1997). *J. Biol. Chem.* **272**, 13332-13337.

Ordering Information

Product #	Description	Pkg. Size
24597	GelCode® Color Silver Stain Kit 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. Includes: GelCode® Silver Concentrate GelCode® Reducer Base GelCode® Reducer Aldehyde GelCode® Stabilizer Base	Kit 500 ml 500 ml 500 ml 500 ml
R: 23/24/25, 36/37/38; S: 7/8, 26, 36/37/39		

Gel Electrophoresis of Proteins

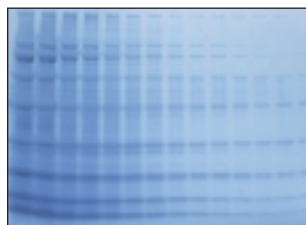
GelCode® E-Zinc® Reversible Stain Kit^{48, 49}

Reversible staining allows versatility other stains cannot offer.

When you need results quickly, GelCode® E-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 E-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.

Demonstrated superior staining sensitivity.

GelCode® E-Zinc® Reversible Stain



Vendor B's Zinc Reversible Stain



Highlights:

- Sensitive – see as little as 0.25 ng of protein
- Fast – results in 15 minutes
- All components are ready to use
- 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 before proceeding with purification or transfer for Western blotting

Reversibility of stain allows:

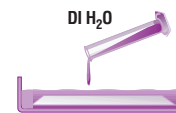
- Alternative staining of same gel
- Protein elution or transfer after gel staining and destaining

GelCode® E-Zinc® Staining Protocol



1. Add E-Zinc® Stain Solution for 10 minutes and remove stain.

2. Add E-Zinc® Developer Solution for 1-2 minutes and remove developer.



3. Stop with deionized water.

GelCode® E-Zinc® Destaining Protocol



1. Add E-Zinc® Eraser Solution.

2. Wash with deionized water.

Reference

48. Amano, S., *et al.* (2000). *J. Biol. Chem.* **275**, 22728-22735.

49. Steiglitz, B.M., *et al.* (2002). *J. Biol. Chem.* **277**, 49820-29830.

Ordering Information

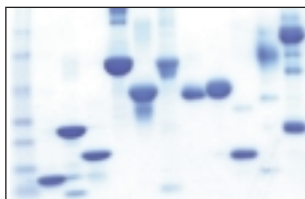
Product #	Description	Pkg. Size
24582	GelCode® E-Zinc® Reversible Stain Kit Sufficient reagent to stain up to 20 SDS-PAGE mini (8 cm x 10 cm) gels. Includes: E-Zinc® Stain E-Zinc® Developer E-Zinc® Eraser	Kit 500 ml 500 ml 500 ml
	R: 35; S: 23, 24/25, 36	



GelCode® Glycoprotein Staining Kit^{50, 51}

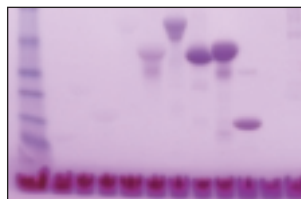
A fast and specific staining protocol for glycoprotein detection on gels or membranes.

Stain total protein in a sample with GelCode® Blue Stain Reagent



Glycoprotein-containing gel stained with GelCode® Blue Stain Reagent.

Stain carbohydrate-containing protein with GelCode® Glycoprotein Stain



Identical gel stained with GelCode® Glycoprotein Staining Kit. Magenta bands indicate location of carbohydrate-containing protein.

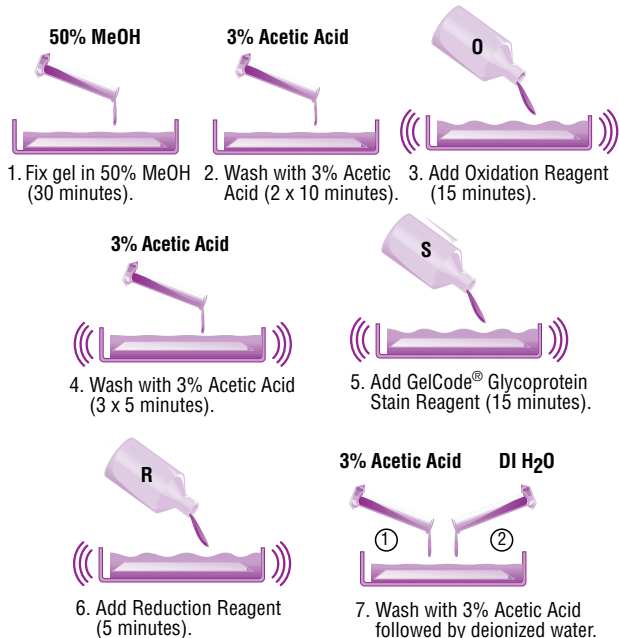
Highlights:

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

References

50. Misenheimer, T.M. (2001). *J. Biol. Chem.* **276**, 45882-45887.
 51. Pio, R., et al. (2001). *J. Biol. Chem.* **276**, 12292-12300.

GelCode® Glycoprotein Stain Protocol



Ordering Information

Product #	Description	Pkg. Size
24562	GelCode® Glycoprotein Staining Kit Sufficient for staining up to 10 SDS-PAGE mini (8 cm x 10 cm) gels. Includes: Glycoprotein Oxidation Reagent Glycoprotein Stain Reagent Reduction Reagent Standards: Horseradish peroxidase (positive control) Soybean trypsin inhibitor (negative control)	Kit Makes 250 ml 250 ml Makes 250 ml 1 mg 1 mg
	R: 36/37/38; S: 36/37/39	

Also available from Pierce:

Ordering Information

Product #	Description	Pkg. Size
23260	Glycoprotein Carbohydrate Estimation Kit	Kit

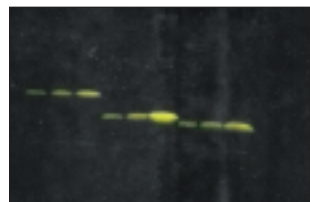
Gel Electrophoresis of Proteins

GelCode® 6xHis Protein Tag Staining Kit

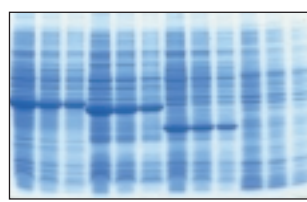
Detect 6xHistidine-tagged protein directly on the gel!

6xHis Protein-Tagged Preparations Stained with GelCode® 6xHis Protein Tag Staining Kit.

E. coli lysates expressing 6xHis-tagged proteins, stained with the Pierce 6xHis Protein Tag Staining Kit



Identical lysates stained with GelCode® Blue Stain Reagent

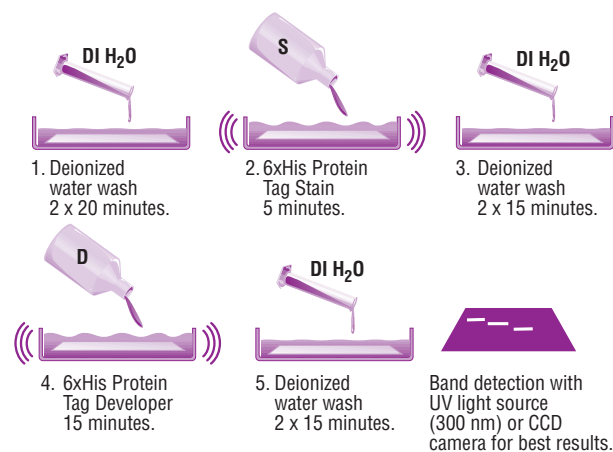


You may never again need to perform a costly and time-consuming Western blotting step to verify 6xHis-tagged expressed protein.

Highlights:

- Works two- to three-times faster than Western blotting – get results sooner and save hours of valuable research time
- Detects directly on the gel⁵² – can eliminate the need for a membrane transfer step and Western blot detection with as little as 0.2 µg of 6xHis-tagged protein
- Ready-to-use, two-reagent formula – no mixing, no diluting and no fuss; guarantees a simple-to-perform, mistake-free detection protocol
- Fluorescent detection is designed to be specific for 6xHis-tagged proteins only – see only what you want to see (CCD camera detection suggested for low-abundance proteins)
- Compatible with Pierce GelCode® Blue Stain Reagent – stain for 6xHis-tagged protein specifically and follow with GelCode® Blue Stain Reagent for a total protein profile determination

GelCode® 6xHis Protein Tag Staining Protocol



Reference

52. Williams, N.K., et al. (2002). *J. Biol. Chem.* **277**, 7790-7798.

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 (Product # 24570) 6xHis Protein Control Set (Product # 24572) R: 20/21/22, 40/20/21/22; S: 26/37	Kit
24572	6xHis Protein Control Set* For use with the GelCode® 6xHis Protein Tag Stain: Reagent Set. Sufficient material to control 50-100 SDS-PAGE runs.	Kit

*Also available for use with INDIA™ HisProbe™-HRP (Product # 15165).



GelCode® Phosphoprotein Staining Kit⁵³

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

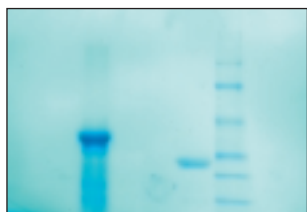
Highlights:

- Specific stain for use with SDS-PAGE to detect abundant phosphoprotein components of the sample
- Easy-to-follow protocol yields results in three hours
- Phosphoproteins stain in-gel as green to green-blue bands
- Detects the phosphoproteins phosphovitin and β -casein in the 40 ng-80 ng/band and 80 ng-160 ng/band range, respectively, in a 4-20% SDS-PAGE gel
- Kit includes one positive control protein (phosvitin) and one negative control protein (soybean trypsin inhibitor)
- Specific stain for use with SDS-PAGE to detect phosphoprotein (phosphoserine and phosphothreonine residues) components of the sample
- Phosphoprotein-stained gels can be stained with GelCode® Blue Stain Reagent (Product # 24590 and 24592) for total protein profiling

Applications:

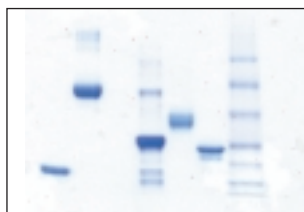
- Use to evaluate the progress of a phosphoprotein purification
- Excellent potential for use in dephosphorylation studies

GelCode® Phosphoprotein Stain



A B C D E F G

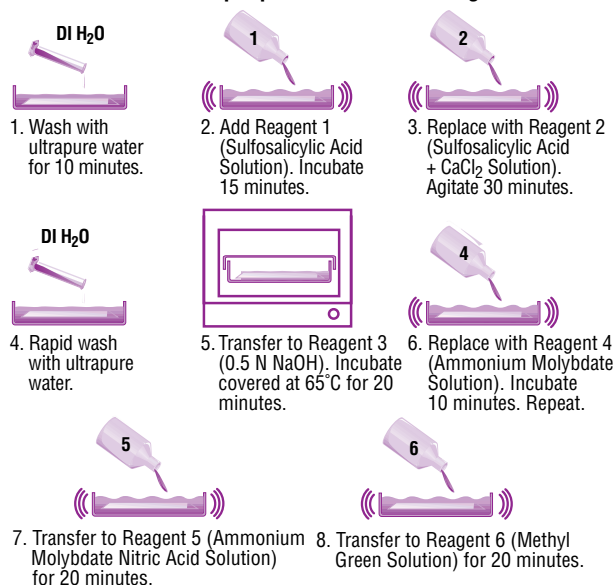
GelCode® Blue Stain



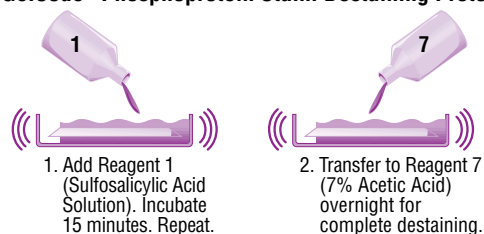
A B C D E F G

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.

GelCode® Phosphoprotein Stain: Staining Protocol



GelCode® Phosphoprotein Stain: Destaining 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: Phosphoprotein Stain Reagent Set (Product # 24551) Phosphoprotein Control Set (Product # 24552) R: 20/21/22; S: 36/37/39	Kit
23270	Phosphoprotein Phosphate Estimation Assay Kit	Kit

Also available from Pierce:

Reference

53. Simpson, R.J. (2003). *Proteins and Proteomics*. A Laboratory Manual. Cold Spring Harbor Laboratory Press. pp. 201-202.

Gel Electrophoresis of Proteins

MemCode™ Reversible Protein Stains for Nitrocellulose Membranes

A great NEW alternative to Ponceau S stain!

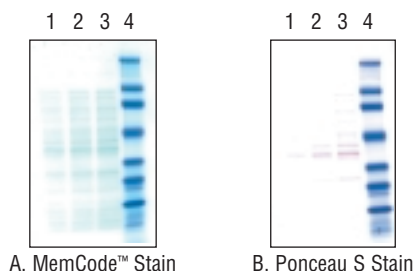


For years the red Ponceau S stain has been the best option for staining before Western blotting, despite its major shortcomings. MemCode™ Reversible Protein Stains decrease staining time, increase staining sensitivity and enhance the immunoreactivity of antigens

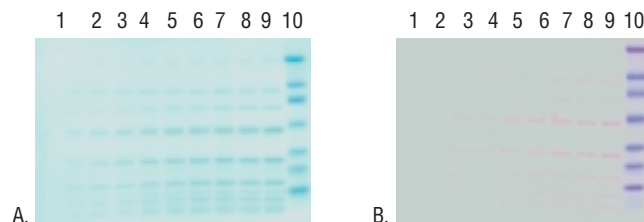
in subsequent Western blotting. Try these new reversible protein stains for nitrocellulose and PVDF membranes and you will never use Ponceau S again.

Highlights:

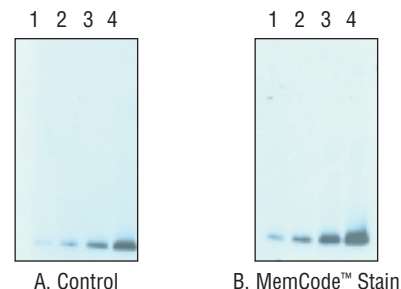
- Sensitive, high-avidity, general protein stain
- Stain is protein-specific, avoiding interference from other biomolecules
- From stain to destain to band erasure in minutes
- Turquoise bands are easily photographed
- Stained bands do not fade with time
- Enhances Western blot detection
- All components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



MemCode™ 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 MemCode™ 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 MemCode™ Stain demonstrates superior visual detection of bands. GST Lysate loading volumes (Lane 1-3). Lane 1: 5 μ l, Lane 2: 10 μ l, Lane 3: 15 μ l and Lane 4: BlueRanger® Marker Mix (Product # 26681), 10 μ l.



Comparison of MemCode™ Reversible Protein Stain with Ponceau S stain. ColorMeRanger™ Unstained Protein M.W. 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 MemCode™ 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**. BlueRanger® Prestained M.W. Marker Mix (Product # 26681).



Immunoblot analysis of GST by chemiluminescent detection after MemCode™ 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 MemCode™ Reversible Protein Stain. Panel **B** was subjected to the staining, destaining and stain erasing protocol of the MemCode™ Kit. Both membranes were then probed with anti-GST incubated with goat anti-rabbit IgG-HRP conjugate and detected using Pierce SuperSignal® West Dura Substrate (Product # 34075).* **Lane 1**: 125 μ g, **Lane 2**: 250 μ g, **Lane 3**: 500 μ g and **Lane 4**: 1 ng.



MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes, continued

Ponceau S Reversible Stain	MemCode™ 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

MemCode™ Reversible Protein Stain Protocols

A. Nitrocellulose Membrane Staining Protocol

1. Wash membrane with ultrapure H₂O.
2. Add MemCode™ Stain. Shake 30 seconds. Protein bands appear turquoise in color.

B. Destaining Protocol

1. Rinse three times with MemCode™ Destain Solution.
2. Add MemCode™ Destain. Shake 5 minutes.
3. Rinse four times with ultrapure H₂O.
4. Wash on a shaker with ultrapure H₂O for 5 minutes.

C. Stain Erasing Protocol

1. Wash with MemCode™ Stain Eraser on a shaker for 2 minutes.
2. Rinse four times with ultrapure H₂O.
3. Wash with ultrapure H₂O on a shaker for 5 minutes.

A. PVDF Membrane Staining Protocol

1. Wash membrane with ultrapure H₂O.
2. Add MemCode™ Sensitizer. Shake for 2 minutes.
3. Add MemCode™ Stain. Shake for 1 minute. Protein bands appear turquoise in color.

B. Destaining Protocol

1. Rinse three times with MemCode™ Destain Solution.
2. Wash with MemCode™ Destain mixed 1:1 with MeOH on a shaker for for 5 minutes.
3. Rinse five times with ultrapure H₂O.

C. Stain Erasing Protocol

1. Wash with MemCode™ Stain Eraser mixed 1:1 with MeOH on a shaker for 10-20 minutes.
2. Rinse five times with ultrapure H₂O.

Ordering Information

Product #	Description	Pkg. Size
24580	MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes	Kit
	<i>Sufficient material for 10 (8 cm x 8 cm) nitrocellulose membranes.</i>	
	Includes: MemCode™ Reversible Protein Stain (Component A)	250 ml
	MemCode™ Destain (Component B)	2 x 500 ml
	MemCode™ Stain Eraser (Component C)	250 ml
	<i>R: 34; S: 26, 36/37/39, 45</i>	
24585	MemCode™ Protein Reversible Protein Stain Kit for PVDF Membranes	Kit
	<i>Sufficient material to stain protein and reverse the stain from 10 (8 cm x 8 cm) PVDF membranes.</i>	
	Includes: MemCode™ Sensitizer	250 ml
	PVDF membrane pre-treatment agent	250 ml
	MemCode™ Reversible Stain	250 ml
	A broad-spectrum stain for proteins transferred to PVDF membrane	
	MemCode™ Destain*	1,000 ml
	Enhances protein band detection by eliminating background staining	
	MemCode™ Stain Eraser*	250 ml
	Reverses protein band staining on demand	
	<i>R: 34; S: 26, 36/37/39, 45</i>	

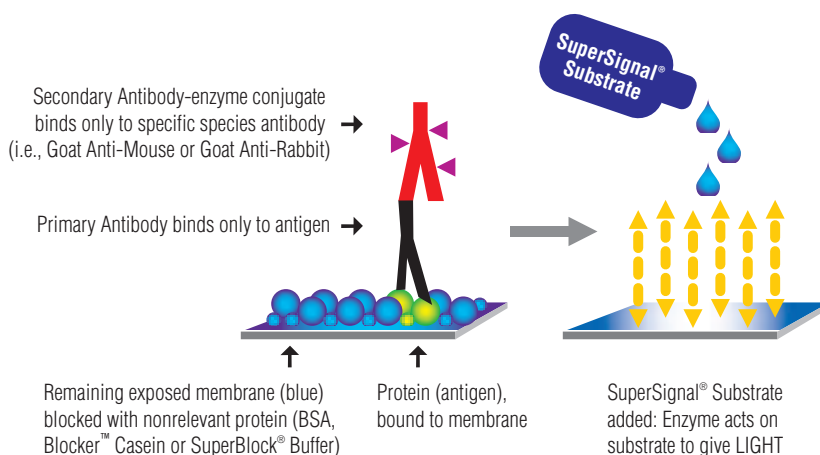
Step 7

Gel Electrophoresis of Proteins

Post staining Step 7

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.



FREE!

Western Blotting Handbook



Request this FREE handy guide for your laboratory or office! This booklet details Pierce's innovative products for Western blotting. Learn how you can obtain

ultra-sensitive blotting detection using SuperSignal® Technology. There's even information about UnBlot® Technology for in-gel probing and detection.

Product #: 1600991

In Western blotting, the protein sample is first separated by SDS-PAGE, then is electro-transferred onto a suitable membrane, and is ultimately detected by a labeled antibody.

STEP 1 SDS-PAGE

Separate protein sample by electrophoresis.

- PAGEprep® Protein Clean-up and Enrichment Kit (Product # 26800)
- Precise™ Protein Gels (many available, see www.piercenet.com)
- Tris-Hepes-SDS Running Buffer (Product # 28398)
- ImmunoPure® Lane Marker Reducing Sample Buffer (5X) (Product # 39000)
- ImmunoPure® Lane Marker Non-Reducing Sample Buffer (5X) (Product # 39001)
- BlueRanger® Prestained Protein Molecular Weight Marker Mix (Product #s 26681 and 26685)
- TriChromRanger™ Prestained Protein Molecular Weight Marker Mix (Product # 26691)
- ColorMeRanger™ Unstained Protein Molecular Weight Marker Mix (Product # 26671)



For detection of proteins that cannot be efficiently transferred to a membrane, Pierce developed UnBlot® Technology that allows positive identification of proteins directly in a gel. (Product #s 33500, 33505, 33510, 33515)

1. Prepare the gel

2. Prepare the sample

3. Prepare the Buffers

4. Choose MW markers

5. Run the gel

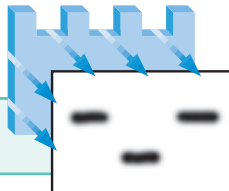
6. Stain the gel

7. Post staining

STEP 2 Electro-Transfer

Transfer proteins to membrane.

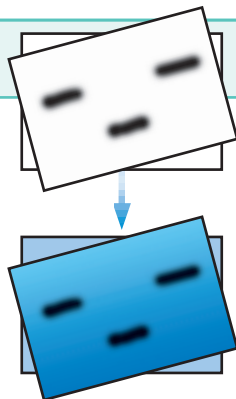
- MemCode™ Reversible Protein Stain Kit – for Nitrocellulose Membranes (Product # 24580) – for PVDF Membranes (Product # 24585)
- Tris-Glycine Transfer Buffer (Product # 28380)
- Qentix™ Western Blot Enhancer (Product # 21050)
- Nitrocellulose Membrane, 0.2 µm (Product #s 77012, 88013 and 88024)
- Nitrocellulose Membrane, 0.45 µm (Product #s 77010, 77011, 88014 and 88025)
- HiBind™ PVDF Membrane, 0.45 µm (Product #s 88585 and 88518)



STEP 3 Blocking

Block nonspecific sites.

- Starting Block™ Blocking Buffer (Product #s 37538 and 37542)
- SuperBlock® Buffer in TBS (Product # 37535) and in PBS (Product # 37515)
- SuperBlock® Blocking Buffer – Blotting in PBS (Product # 37517) and in TBS (Product # 37537)
- Casein in TBS (Product # 37532) and in PBS (Product # 37528)
- BSA in PBS (Product # 37525) and in TBS (Product # 37520)
- SEA BLOCK Buffer (Product # 37527)
- BLOTTO in TBS (Product # 37530)



STEP 4 Formulate Wash Buffers

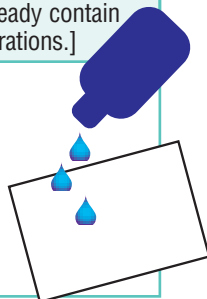
Wash the membrane.

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

Add detergent to blocking/wash buffers to reduce nonspecific binding. [Skip this step if you use StartingBlock™ T20 Blocking Buffer in PBS (Product # 35739) or TBS (Product # 37543) or SuperBlock® T20 Blocking Buffer in PBS (Product # 37536). These buffers already contain Tween®-20 Detergent at optimized concentrations.]

Surfact-Amps® Brand 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)



STEP 5 Primary and Secondary Detection Reagents

Incubate the membrane with antibody.

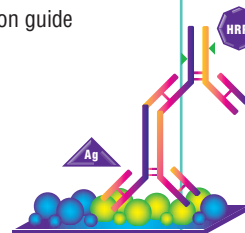
For a complete list, visit the antibody selection guide on our web site (see below).

For direct detection methods we offer:

- Monoclonal Antibodies
- Fluorescent 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 NeutrAvidin™ Biotin-Binding Protein labeled with fluorescein, rhodamine, HRP or AP
- Secondary antibodies labeled with fluorescein, rhodamine, HRP, AP or biotin



STEP 6 Enzyme Substrates

Add the detection reagent.

Chemiluminescent Substrates:

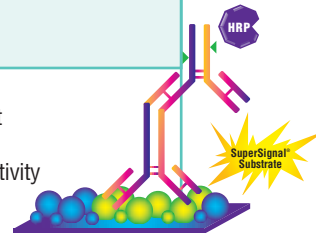
- SuperSignal® West Pico Chemiluminescent Substrate (Product #s 34077 and 34080)
- SuperSignal® West Femto Maximum Sensitivity Substrate (Product #s 34095 and 34096)
- SuperSignal® West Dura Extended Duration Substrate (Product #s 34075 and 34076)
- Lumi-Phos™ WB Substrate (Product # 34150)

Chemifluorescent Substrates:

- FluoroBlot™ Peroxidase Substrate (Product #s 33050 and 33250)

Colorimetric Substrates:

- 1-Step™ 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, or 8" x 10" sheets, Product #s 34091 and 34093)
- Erase-It® Background Eliminator Kit (Product # 21065)



STEP 8 Stripping Buffer

Reprobe the blot if necessary.

- Restore™ Western Blot Stripping Buffer (Product # 21059)
- IgG Elution Buffer (Product #s 21004 and 21009)



For convenience and economy, Pierce also offers complete Western Blotting Kits. These kits include chemiluminescent substrates, enzyme-conjugated antibodies, blocking buffers and standard buffers.

PIERCENET

Grasp the Proteome™



• Sample Prep



• Electrophoresis



• Markers



• Staining

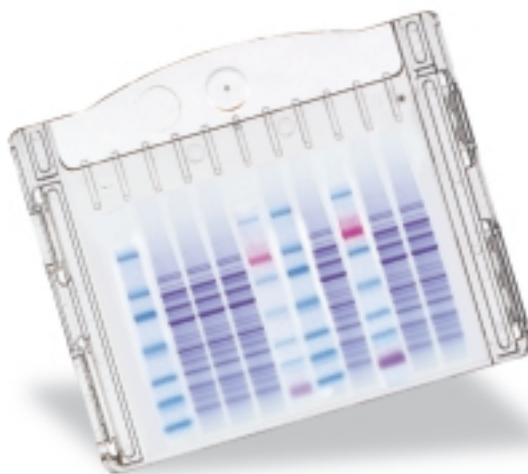


• Blotting

Precisely our points

Introducing Precise™ Protein Gels.

- **Easy-to-load lanes** with durable plastic dividers
- **Long shelf life** – 12-month guarantee ensures consistent performance
- **Unique running buffer** for excellent separation and high-resolution protein bands
- **Compatible** with Laemmli sample buffer and standard mini-gel tanks



- **Easy-to-open**, no need for special tools
- **Short run time** – 45-minute run time provides results quickly
- **1 mm thick gels** stain quickly and with high sensitivity using coomassie and silver stains
- **Transfers quickly and efficiently** to nitrocellulose and PVDF membranes for Western blotting

Precise™ Protein Gels are cast in a durable plastic cassette using a neutral pH buffer that prevents polyacrylamide breakdown. The gels are individually packaged in an easy-to-open plastic pouch and are ready to run with no comb or tape to remove. They are available in both gradient and fixed-concentrations and in 10-, 12- and 15-well formats. Visit our web site for the complete selection.

www.piercenet.com



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Technical Assistance E-mail: TA@piercenet.com • Customer Assistance E-mail: CS@piercenet.com

Outside the United States, visit our web site or call 815-968-0747 to locate your local Perbio Science branch office (below) or distributor

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