

Version 2



# Table of Contents

Stain Summary	1
Thermo Scientific Fluorescent Dye-Based Stains	2
Krypton Fluorescent Protein Stain	
Krypton Infrared Fluorescent Protein Stain	
Krypton Fluorescent Glycoprotein Staining Kit	
GelCode 6xHis Protein Tag Stain	
Thermo Scientific Colorimetric Dye-Based Stains	8
GelCode Blue Safe Protein Stain	
The Original GelCode Blue Stain Reagent	
Imperial Protein Stain	
GelCode Glycoprotein Stain	
GelCode Phosphoprotein Staining Kit	
Thermo Scientific Metal-Based Stains	14
Pierce Silver Stain for Mass Spectrometry	
Pierce Silver Stain Kit II	
Pierce Silver Stain Rescue Reagent	
Pierce Color Silver Stain	
Pierce Zinc Reversible Stain	
Therman Colombific Duration Duration Colo	20

# Stain Summary

The staining of protein bands in gels after electrophoresis is one of the most routinely performed protocols in the life science laboratory. Because significant time is invested in preparing reagents, staining and destaining, even the simplest staining protocols can be tedious and take hours to complete.



Thermo Scientific Pierce Gel Stains provide speed, simplicity, convenience and economy over both homemade and other commercially available staining products. Life scientists around the world look to Pierce® Stains for speed, ease of use, sensitivity, superior results and economy.

This brochure summarizes the key features and benefits of several Pierce Staining Products that represent clear improvements in staining technology. Easy-to-follow protocols with schematics demonstrate just how easy staining can be with each of our staining kits. Although in-gel protein staining is a routine procedure, it does not have to be time-consuming. Choose Pierce Stain Products and reclaim the time you've been spending with homemade or less effective gel-staining products.

Table 1. Thermo Scientific Pierce Stain Products comparison.

Description	Number of Components	Number o Steps		Type of Detection⁵	Sensitivity	Mass Spec. Compatible
Krypton™ Fluorescent Protein Stain	1	3³	30-160 min	F	0.25 ng	Yes
Krypton Infrared Fluorescent Protein Stain	1	3³	30-160 min	F	0.25 ng	Yes
Krypton Fluorescent Glycoprotein Staining	Kit 3 <sup>2</sup>	6 <sup>3</sup>	3-4 hours	F	15 ng	Yes
GelCode® 6xHis Protein Tag Stain	<b>2</b> <sup>2</sup>	6	1 hour, 35 min		0.2 μg of a 35 kDa fusion prote	U <sup>6</sup>
GelCode Blue Safe Protein Stain	2	2	15-60 min	С	9 ng	Yes
GelCode Blue Stain Reagent	1	2	60 min	С	8 ng	Yes
Imperial™ Protein Stain	1	2	60 min	С	3 ng	Yes
GelCode Glycoprotein Stain	3 <sup>2</sup>	6 <sup>3</sup>	~ 2 hours	С	0.16 µg⁴	U <sup>6</sup>
GelCode Phosphoprotein Stain	<b>7</b> <sup>2</sup>	10	3 hours-overnig		0 ng phosvit 60 ng β-cas	
Silver Stain for Mass Spectrometry	6	<b>7</b> <sup>3</sup>	30 min <sup>3</sup>	С	0.25 ng	Yes
Silver Stain II	4	4	50 min	С	0.25 ng	Yes
Pierce Color Silver Stain	4	4	65 min <sup>1</sup>	С	0.1 ng	U <sup>6</sup>
Pierce Zinc Reversible Stain	3	2	15 min	С	0.25 ng	Yes

#### 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 (sensitivity will vary with extent of glycosylation of the protein under analysis)
- 5. C = colorimetric, F = fluorescent
- 6. U = Unknown

# Fluorescent Dye-Based Stains

# Thermo Scientific Krypton Fluorescent Protein Stain

#### A faster, affordable fluorescent stain that provides excellent performance

Thermo Scientific 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 1-2, Table 1). 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 3). 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 4).

#### 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

Table 1. 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	Krypton Protein Stain	Cost Savings
Stain cost per <b>20 mini-gels</b>	\$166	\$182	\$261	\$110	\$56-\$151
Stain cost per 100 mini-gels	\$779	\$767	\$946	\$399	\$368-\$547

Source: Online Catalogs (4/5/2009). All prices listed are USD.

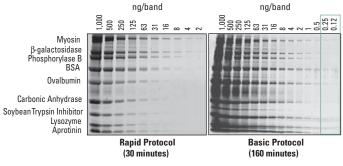
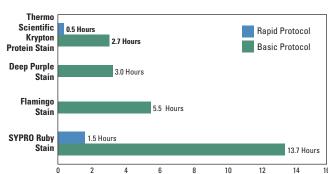


Figure 1. Thermo Scientific Krypton Protein Stain is fast and sensitive. Proteins were separated in 4-20% Tris-glycine gels and stained using the indicated protocols. The gels were imaged with the Typhoon® 9410 at 532 nm excitation and 580 BP30 emission.



Time (Hours)

Figure 2. Thermo Scientific Krypton Protein Stain works up to five times faster than other fluorescent stain protocols. There is no rapid protocol for the Deep Purple or Flamingo Stains.

#### **Minimal Protein-to-Protein Variation**

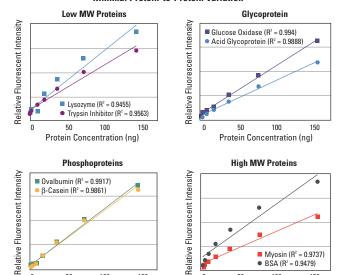
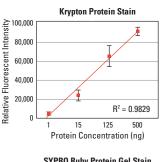


Figure 3. Thermo Scientific Krypton Protein Stain produces a linear response to staining with minimal protein-to-protein variation. Relative fluorescent intensity was plotted as a function of protein quantity for proteins of various sizes and containing post-translational modifications.

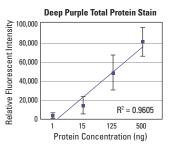
#### **Linear Quantitative Range**

150



100

Protein Concentration (ng)



50

Myosin ( $R^2 = 0.9737$ ) BSA (R<sup>2</sup> = 0.9479)

100

Protein Concentration (ng)

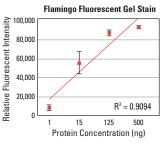


Figure 4. Thermo Scientific Krypton Protein Stain exhibits a more linear quantitative range than other fluorescent stains. Relative fluorescent intensity for each protein concentration is the average of nine different proteins separated in 4-20% Tris-glycine and Thermo Scientific Precise Gels. Error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

		SYPRO Ruby	Protein Gel Stain
.≧	100,000		-
Intens	100,000 80,000 60,000 40,000 20,000		
cent	60,000		
luores	40,000		/1
Je F	20.000		
elativ	0	I	$R^2 = 0.9455$
8	U	1 15	125 500
		Protein Cor	ncentration (ng)

Ordering	g Information		
Product #	Description	Pkg. Size	U.S. Price
46628	Krypton Protein Stain (10X)* Sufficient reagent to stain four mini gels (8 cm x 10 cm).	20 ml	\$ 39
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	\$110
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	\$399

<sup>†</sup> U.S. patent pending on Krypton Protein Stain Technology.

# Fluorescent Dye-Based Stains

# Thermo Scientific Krypton Infrared Fluorescent Protein Stain

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

Thermo Scientific Krypton Infrared Protein Stain is a fluorescent stain for detecting proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (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 1). The stain delivers substantial improvements in protein-staining performance compared to coomassie stains (Figure 2). Krypton Infrared Protein Stain exhibits minimal protein-to-protein variation and provides high signal intensity with a linear response to staining (Figures 3-4).

#### 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 1. Thermo Scientific Krypton Infrared Protein Stain protocol.

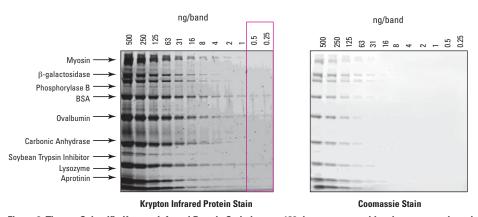


Figure 2. Thermo Scientific Krypton Infrared Protein Stain is up to 130 times more sensitive than coomassie stain. Proteins were separated in 4-20% Tris-glycine gels and stained with Krypton Infrared Protein Stain or coomassie stain (GelCode Blue Stain Reagent, Product # 24592). The gels were imaged with the Odyssey Infrared Imaging System at 680 nm excitation and 720 nm emission.

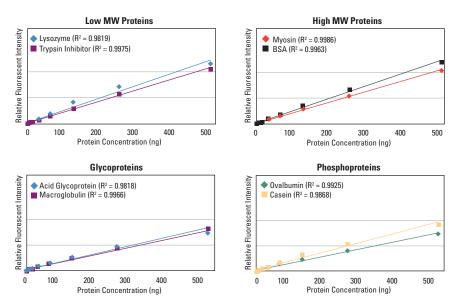


Figure 3. Thermo Scientific Krypton Infrared Protein Stain produces a linear response to staining with minimal protein-to-protein variation. Relative fluorescent intensity was plotted as a function of protein quantity for proteins of various sizes and containing post-translational modifications. The relative fluorescent intensity for each data point is the average value for triplicate gels.

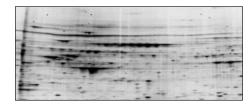


Figure 4. Thermo Scientific Krypton Infrared Protein Stain provides exceptional sensitivity and low background in 2-D analysis. Processed HeLa cell protein extract (28 μg) was focused on a pH 5-8 IPG strip followed by 4-20% SDS-PAGE. The gel was stained using the basic protocol (-2 hours) and imaged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

Orderin	g Information		
Product #	Description	Pkg. Size	U.S. Price
53070	Krypton Infrared Protein Stain (10X)† Sufficient reagent to stain 4 mini gels (8 cm x 10 cm).	20 ml	\$ 39
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	\$110
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	\$399

<sup>†</sup> US patent pending.

# Fluorescent Dye-Based Stains

# Thermo Scientific Krypton Glycoprotein Staining Kit

Combine Krypton Glycoprotein Stain with Krypton Protein Stain for multiplex proteomic analysis

The Thermo Scientific Krypton Glycoprotein Staining Kit provides a fast and easy method for the fluorescent detection of glycoproteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2-D gels (Figure 1). The stain exhibits sensitivity equivalent to, or greater than, other fluorescent glycoprotein stains and uses well-established periodate-oxidation chemistry that demonstrates 15- to 20-fold more binding to glycoproteins (Figure 2).

#### **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

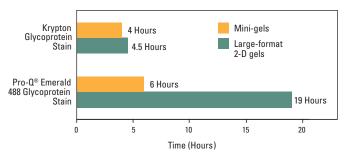


Figure 1. Thermo Scientific Krypton Glycoprotein Stain works up to four times faster than other fluorescent glycoprotein stain protocols.

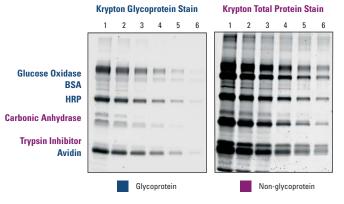


Figure 2. Thermo Scientific Krypton Glycoprotein Stain provides a fast and sensitive method for detecting glycoproteins in polyacrylamide gels. The gel was stained with Krypton Glycoprotein Stain followed by total protein staining with Krypton Protein Stain (Product # 46630). Lanes 1-6 contain a mixture 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	U.S. Price	
53074	Krypton Glycoprotein Staining Kit Sufficient reagents to stain 10 mini (8 cm x 10 cm) gels.	Kit	\$187	
	Includes: Glycoprotein Stain Reagent	0.3 ml		
	Staining Buffer	250 ml		
	Oxidizing Reagent	2.5 g		
	Positive Control (Horseradish Peroxidase)	1 mg		
	Negative Control (Soybean Trypsin Inhibitor)	1 mg		

# 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 gel1
- Ready-to-use, two-reagent formula (Figure 1)
- Fluorescent detection is designed to be specific for 6xHis-tagged proteins only
- After 6xHis-tagged protein staining, gels can be stained with GelCode Blue Stain Reagent for a total protein profile determination (Figure 2)
- Detects down to 5.7 picomoles histidine-tagged protein with CCD camera and 57 picomoles with a transilluminator





n 3.Deionized water wash

DI H<sub>2</sub>O





DI H<sub>2</sub>O



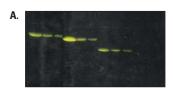
water wash (20 minutes x 2) Tag Stain (5 minutes)

Deionized 4. 6xHis Protein Tag water wash (15 minutes x 2) (15 minutes)

Deionized water wash (5 minutes)

Band detection with UV light source (300 nm) or with a CCD camera

Figure 1. Thermo Scientific GelCode 6xHis Protein Tag Stain protocol.



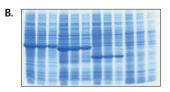


Figure 2. Total protein determination. 6xHis-tagged proteins stained with Thermo Scientific GelCode 6xHis Protein Tag Staining Kit. Figure 2A. Escherichia coli lysates expressing 6xHis-tagged proteins, stained with the Pierce 6xHis Protein Tag Staining Kit. Figure 2B. Identical lysates stained with GelCode Blue Stain Reagent.

# Ordering Information Product # Description Pkg. Size U.S. Price 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 \$275

#### Reference

 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.

# Colorimetric Dye-Based Stains

## Thermo Scientific GelCode Blue Safe Protein Stain

A safe, reliable and cost-effective stain for proteins

Thermo Scientific 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 1 and 2)
- 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 3), 2-D gel staining, nitrocellulose and PVDF membrane staining, and quantitative densitometry
- **Safe** non-corrosive 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



Figure 1. Thermo Scientific GelCode Blue Safe Protein Stain protocol.



Figure 2. Thermo Scientific GelCode Blue Safe Protein Stain is fast and sensitive. Panel 2A. A mixture of proteins (myosin, b-galactosidase, phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme and aprotinin) was electrophoresed on 4-20% 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 2B. 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.

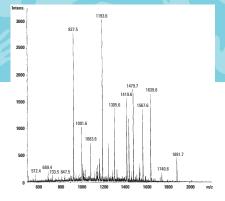


Figure 3. Mass spectrometry (MS)-compatible. BSA (2 μg ) was electrophoresed on a 4-20% Precise Protein Gel (Product # 25224) and stained with GelCode Blue Safe Stain prepared for MALDI-MS analysis using the In-Gel Tryptic

Reagent for one hour. BSA bands were excised from the gel and Digestion Kit (Product # 89871). Samples were purified using ZipTip® Pipette Tips before MS analysis on an LC/MSD Trap XCT (Agilent Technologies).

#### **Ordering Information**

Product #	Description	Pkg. Size	U.S. Price
24594	GelCode Blue Safe Protein Stain Sufficient reagent to stain 50 mini gels (8 cm x 10 cm).	1 L	\$ 77
24596	GelCode Blue Safe Protein Stain Sufficient reagent to stain 175 mini gels (8 cm x 10 cm).	3.5 L	\$170

# The Original Thermo Scientific GelCode Blue Stain Reagent

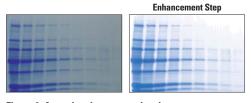
No need for methanol/acetic acid destaining ... ever!

#### Highlights:

- Ready-to-use stain reagent (Figure 1)
- Bands develop before your eves and can be viewed directly in the staining tray
- Some protein bands visible to 8 ng
- No sensitivity loss from over-destaining because the destaining step is eliminated
- Wide linear range for densitometric gel analysis1
- · Optional water wash enhancement step results in crystal-clear background and increased sensitivity (Figure 2)
- Compatible with MALDI-TOF analysis<sup>2,3,4</sup>
- Compatible with sequence analysis<sup>5</sup>



Figure 1. Thermo Scientific GelCode Blue Staining protocol.



Water Wash

Figure 2. 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	U.S. Price
24592	GelCode Blue Stain Reagent Sufficient reagent to stain 175 mini gels (8 cm x 10 cm).	3.5 L	\$234
72300	Pump (for 3.5 L package only)	1 pump	FREE*
24590	GelCode Blue Stain Reagent Sufficient reagent to stain 25 mini gels (8 cm x 10 cm)	500 ml	\$ 60

<sup>\*</sup>FREE upon request with purchase of Product # 24592

- 1. Mateer, S.C., et al. (2002). The mechanism for regulation of the F-actin binding activity of IQGAP1 by calcium/calmodulin. J. Biol. Chem. 227, 12324-12333.
- 2. Aulak, K.S., et al. (2001). Proteomic method identifies proteins nitrated in vivo during inflammatory challenge. P. Natl. Acad. Sci. USA. 98, 12056-12061.
- 3. Lim, J., et al. (2002). Metastable macromolecular complexes containing high mobility group nucleosome-binding chromosomal proteins in HeLa nuclei. J. Biol. Chem. 277, 20774-20782.
- 4. Hilton, J.M., et al. (2001). Phosphorylation of a synaptic vesicle-associated protein by an inositol hexakisphosphate-regulated protein kinase. J. Biol. Chem. 276, 16341-16347.
- 5. Tani, M., et al. (2000). Purification and characterization of a neutral ceramidase from mouse liver. J. Biol. Chem. 275, 3462-3468.

# Colorimetric Dye-Based Stains

#### A fast, sensitive and consistent coomassie stain

Thermo Scientific Imperial Protein Stain is a ready-to-use coomassie stain for the detection of protein bands in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (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.

Multiple staining protocols are provided to meet demanding time and sensitivity requirements (Figure 1). For fast results, a five-minute stain combined with a 15-minute water destain easily detects 6 ng protein bands (Figure 2).

Even greater levels of sensitivity and crystal-clear background can be achieved through increased staining time and destaining in water. Problems associated with coomassie G-250 stain preparations, such as inconsistent staining, are eliminated with Imperial Protein Stain. 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.

#### Highlights:

#### **Outstanding performance**

- Sensitive detect 3 ng protein/band with the enhanced protocol (3 hours); four to eight times more sensitive than the competition (Figure 3)
- Fast detect down to 6 ng protein/band in just 20 minutes
- Robust highly consistent, reproducible protein staining
- Excellent photo-documentation photographs/scans better than other coomassie stains

#### Convenience

- · Destain with water
- · No fixation step required
- · Ready-to-use reagent
- Store on your bench top for up to one year
- Multiple protocols to meet demanding time/sensitivity requirements
- No protein staining variability from lot-to-lot or gel-to-gel



Figure 1. Thermo Scientific Imperial Protein Stain protocol.

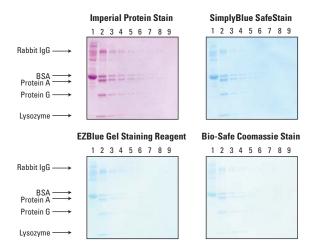


Figure 2. Thermo Scientific Imperial Protein Stain is fast and sensitive. Proteins were separated on Novex 4-20% Tris-glycine gels, stained for 5 minutes and destained 3 x 5 minutes in water. Lane 1: BSA only (6 μg), Lanes 2-9 contained the indicated proteins at the following concentrations: Lane 2: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

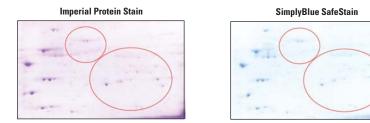


Figure 3. Thermo Scientific Imperial Protein Stain reveals spots that are faint or not detected with other coomassie stains. Mitochondrial protein extract was prepared from heart tissue of six-week-old Sprague-Dawley rat. Processed protein extract (72 µg) was focused on a pH 5-8 IPG strip followed by 8-16% SDS-PAGE. The gels were stained for 1 hour and destained overnight following manufacturer-recommended protocols.

Ordering	g Information		
Product #	Description	Pkg. Size	U.S. Price
24615	Imperial Protein Stain <sup>1</sup> Sufficient reagent to stain up to 50 mini gels (8 cm x 10 cm).	1 L	\$ 98
24617	Imperial Protein Stain Sufficient reagent to stain up to 150 mini gels (8 cm x 10 cm).	3 x 1 L	\$204

<sup>†</sup> U.S. patent pending on Imperial Protein Stain Technology.

# Colorimetric Dye-Based Stains

# Thermo Scientific GelCode 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 1)
- Three-reagent protocol yields results in less than two hours vs. four to five hours for other staining methods (Figure 2)
- 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

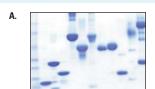




Figure 1. Sensitive staining of glycoproteins. Figure 1A. Glycoprotein-containing gel stained with GelCode Blue Stain Reagent. Figure 1B. Glycoprotein-containing gel stained with GelCode Glycoprotein Staining Kit.

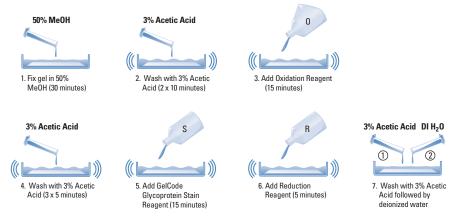


Figure 2. Thermo Scientific GelCode Glycoprotein Staining protocol.

Ordering Information					
Product #	Description	Pkg. Size	U.S. Price		
24562	GelCode Glycoprotein Staining Kit Sufficient for staining up to 10 SDS-PAGE mini gels. (8 cm x 10 cm)	Kit	\$215		
	Includes: Glycoprotein Oxidation Reagent Glycoprotein Stain Reagent Reduction Reagent	Makes 250 ml 250 ml Makes 250 ml			
	Standards: Horseradish Peroxidase (positive control) Soybean Trypsin Inhibitor (negative control)	1 mg 1 mg			

#### References

Misenheimer, T.M., et al. (2001). Disulfide connectivity of recombinant C-terminal region of human thrombospondin. J. Biol. Chem. 276, 45882-45887.

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 GelCode Phosphoprotein Staining Kit

Specific in-gel detection of phosphorylated proteins

#### Highlights:

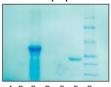
- Specific stain for use with SDS-PAGE to detect abundant phosphoprotein components of the sample
- Phosphoproteins stain in-gel as green to green-blue bands (Figure 1)
- Easy-to-follow protocol yields results in three hours (Figure 2)
- Detects the phosphoproteins phosvitin and β-casein in the 40-80 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 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

#### GelCode Phosphoprotein Stain



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.

#### GelCode Blue Stain Reagent



ABCDEFG

An identical gel was stained with GelCode Blue Stain Reagent.

Figure 1. 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:**  $\beta$ -casein and **G:** prestained protein molecular weight markers.



 Wash with deionized water for 10 minutes



 Add Reagent 1
 (Sulfosalicylic Acid Solution). Incubate



3. Replace with Reagent 2 (Sulfosalicylic Acid + CaCl<sub>2</sub> Solution). Agitate 30 minutes



4. Rapid wash with deionized water



5. Transfer to Reagent 3 (0.5 N NaOH). Incubate covered at 65°C for 20 minutes



6. [Replace with Reagent 4 (Ammonium Molybdate Solution). Incubate 10 minutes] x 2



7. Transfer to Reagent 5 (Ammonium Molybdate Nitric Acid Solution) for 20 minutes



8. Transfer to Reagent 6 (Methyl Green Solution) for 20 minutes



 Add Reagent 1
 (Sulfosalicylic Acid Solution). Incubate 15 minutes x 2



 Transfer to Reagent 7 (7% Acetic Acid) overnight for complete destaining

Figure 2. Thermo Scientific GelCode Phosphoprotein Stain protocol.

Orderin	g Information		
Product #	Description	Pkg. Size	U.S. Price
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	\$439

# Metal-Based Stains

# Thermo Scientific Pierce Silver Stain 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. The new Thermo Scientific Pierce Silver Stain for Mass Spectrometry Kit bundles a high-performance stain with an efficient geldestaining 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 1).

#### 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 pl proteins such as lysozyme (pl 10) and chymotrypsinogen A (pl 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 1. Sequence coverage comparison.

Fifty (50) ng each of BSA, ovalbumin, chymotrypsinogen A and myoglobin preparations were loaded onto separate sodium dodecyl sulfate-polyacrylamide gels. After electrophoresis, the respective gels were stained with Thermo Scientific Pierce Silver Stain for Mass Spectrometry; Supplier 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, the Pierce Silver Stain for Mass Spectrometry performed better than the alternative silver staining method.

Pierce Silver Stain for MS		Supplier I MS Stain		Original 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

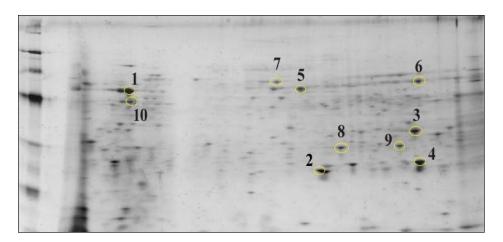


Table 1. Peptide mass fingerprinting bioinformatics data for 2-D rat mitochondrial protein analysis.

Ten spots that stained well with the Pierce Silver Stain for Mass Spectrometry, Supplier I MS-compatible Stain and 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 <sup>+</sup> transporting, mitochondrial F1 complex, beta subunit	All
2	AJ18 protein	Pierce Silver Stain for MS and GelCode Blue Stain Reagent
	ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, subunit d	Supplier I
3	Electron transfer flavo protein (ETF protein)	All
4	H <sup>+</sup> transporting two-sector ATPase (EC 3.6.3.14), alpha chain precursor	Pierce Silver Stain for MS and GelCode Blue Stain Reagent
	Unknown protein for MGC:93808	Supplier 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	Pierce Silver Stain for MS and GelCode Blue Stain Reagent
	Translocase of inner mitochondrial membrane homolog 44	Supplier I
9	Enoyl coenzyme A hydratase short chain mitochondrial	All
10	ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, beta subunit	All

Product #	Description	Pkg. Size	U.S. Price
24600	Pierce Silver Stain for Mass Spectrometry	Kit	\$165
	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.		
	Includes: Silver Sensitizer	2 ml	
	Silver Stain	500 ml	
	Silver Developer	500 ml	
	Silver Enhancer	25 ml	
	Silver Destain Reagent A	4 ml	
	Silver Destain Reagent B	14 ml	

# Metal-Based Stains

## Thermo Scientific Pierce Silver Stain Kit II

A faster, more flexible silver stain

#### 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 1 and 2)
- 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

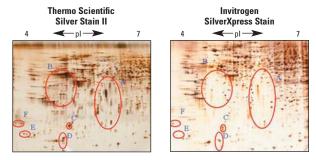


Figure 1. Comparison of identical 2-D gels stained with Thermo Scientific Pierce Silver Stain II and with Invitrogen SilverXpress® stain. Circled regions indicate difference in staining intensity.

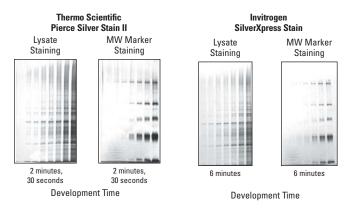


Figure 2. Thermo Scientific Pierce Silver Stain II provides more sensitive staining of a 1-D polyacrylamide gel than Invitrogen SilverXpress Stain.

Ordering Information				
Product #	Description	Pkg. Size	U.S. Price	
24612	Pierce Silver Stain II	Kit	\$109	
	This product replaces Product # 24602.			
	Sufficient reagent to stain 20 mini gels.			
	Includes: Silver Sensitizer	2 ml		
	Silver Stain	500 ml		
	Silver Enhancer	25 ml		
	Silver Developer	500 ml		

# Thermo Scientific Pierce Silver Stain Rescue Reagent

This 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 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 1)
- 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 \$0.55-US \$1.10 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 2)
- Compatible with any commercial or homemade silver stain – removes high background or non-uniform staining, regardless of the silver stain formulation



1. Prepare stop solution.



2. Wash gel 2 x 10 minutes with ultrapure water.



Destain gel in Rescue
 Reagent working solution.



4. Incubate gel in stop solution for 10 minutes. Repeat this step.

Figure 1. Thermo Scientific Silver Stain Rescue Reagent protocol.

Rescue Reagent B





Figure 2. High background removed by Thermo Scientific 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 Pierce Silver Stain Kit II (Product # 24612).

20 ml

# Product # Description 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. Includes: Rescue Reagent A 20 ml

# Metal-Based Stains

## 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 PAGE gel staining (Figure 1)
- Total time, after fixing, varies from 11-90 minutes (depending on gel thickness)
- Detect down to 0.1 ng/mm<sup>2</sup> protein in the gel
- · Proteins stain in five basic colors: black, blue, brown, red and yellow
- Five simple staining steps (Figure 2)
- Also stains DNA¹

#### Color aids in protein mapping by:

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



Figure 1. Pierce Color Silver Stain provides sensitive staining of 2-D gels.





1. Perform calculation and dilution of reagent concentrates



2. Place fixed and washed gel in Silver Working Reagent (30 minutes)



3. Perform deionized water wash (5-10 seconds)

#### **Reduction Reagent**



4. Add reducer working reagent (3-5 minutes)

#### Stabilization Reagent



5. Add stabilization/color enhancement reagent (15-60 minutes)

Figure 2. 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	U.S. Price		
24597	Pierce 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.	Kit	\$195		
	Includes: Silver Concentrate Reducer Base	500 ml 500 ml			
	Reducer Aldehyde Stabilizer Base	500 ml 500 ml			

#### References

<sup>1.</sup> 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.

Sammons, D.W., et al., (1981). Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. Electrophoresis 2, 135-141

## Thermo Scientific Pierce Zinc Reversible Stain

Reversible staining feature allows versatility other stains cannot offer

#### Highlights:

- **Sensitive** detect down to 0.25 ng of protein (Figure 1)
- Fast results in 15 minutes
- **Convenient** all components are ready to use (Figure 2)
- 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

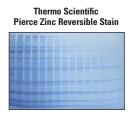




Figure 1. Thermo Scientific Pierce Zinc Reversible Stain is more sensitive than competing stains.

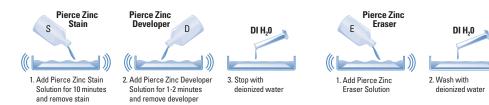


Figure 2. Thermo Scientific Pierce Zinc Reversible Staining protocol.

Ordering Information					
Product #	Description	Pkg. Size	U.S. Price		
24582	Pierce Zinc Reversible Stain Kit Sufficient reagent to stain up to 20 SDS-PAGE mini (8 cm x 10 cm) gels.	Kit	\$104		
	Includes: Zinc Stain Zinc Developer	500 ml 500 ml			
	Zinc Eraser	500 ml			

#### Reference

1. Amano, S., et al. (2000). Bone morphogenetic protein 1 is an extracellular processing enzyme of the laminin 5  $\gamma$ 2 chain. J. Biol. Chem. 275, 22728-22735.

# Precise Protein Gels

## Thermo Scientific Precise Protein Gels

Long shelf life ... short run time



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 (Figure 1).

#### 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

#### **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-HCI-SDS

#### **Compatible Gel Tanks**

Bio-Ñad® Mini-PROTEAN® II and 3 Novex®, XCell SureLock™ and XCell II™ IBI Universal Protein System Hoefer Mighty Small II (SE 260/ SE 250) Hoefer Tall Mighty Small II (SE 280) Owl Road Runner and Penguin Owl Single-Sided Vertical System

#### Migration Table by Gel Percentage



Figure 1. The migration table can help you choose the appropriate Thermo Scientific Precise Gel for your research.

#### Ordering Information

	Percent	# of Sample	Sample Well	Pkg.	U.S.
Product #	Acrylamide		Volume	Size	Price
25200	8%	10	50 μl	10 gels	\$99
25201	10%	10	50 μl	10 gels	\$99
25202	12%	10	50 μl	10 gels	\$99
25203	8-16%	10	50 μl	10 gels	\$99
25204	4-20%	10	50 μl	10 gels	\$99
25220	8%	12	30 μΙ	10 gels	\$99
25221	10%	12	30 μΙ	10 gels	\$99
25222	12%	12	30 μΙ	10 gels	\$99
25223	8-16%	12	30 μΙ	10 gels	\$99
25224	4-20%	12	30 μΙ	10 gels	\$99
25240	8%	15	25 µl	10 gels	\$99
25241	10%	15	25 μΙ	10 gels	\$99
25242	12%	15	25 μΙ	10 gels	\$99
25243	8-16%	15	25 μΙ	10 gels	\$99
25244	4-20%	15	25 ul	10 aels	\$99

Product #	Size	Price
Tris-HEPES-SDS Run	ning Buffer	Packs
28398	10 pack	\$29
Each pack yields 500 ml	of 1X buffer.	The 1 X
formulation is 100mM T	ris, 100 mM F	HEPES,
3 mM SDS, pH 8 ±0.25		

Pka.

U.S.



#### **Contact Information**

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France

Tel: 0 800 50 82 15

The Netherlands

Tel: 076 50 31 880

Germany

Tel: 0228 9125650

**United Kingdom** 

Tel: 0800 252 185

**Switzerland** Tel: 0800 56 31 40

Email: perbio.euromarketing@thermofisher.com www.thermo.com/perbio

**United States** 

Tel: 815-968-0747 or 800-874-3723 Customer Assistance E-mail: Pierce.CS@thermofisher.com www.thermo.com/pierce



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Areas of Interest (Please specify all that apply):    IP/Co-IP (A03)	☐ Fluorescent Dyes (C07) ☐ Affinity Supports (A01)		
☐ Desalting (A18) ☐ Crosslinkers (C11) ☐ Protein Assays (C12) ☐ Protein Detection (C19) ☐ Protein Interaction (C22) ☐ Chemiluminescent Substrates (C06) ☐ Mass Spectroscopy (A22)	□ Sample Prep (A01) □ Coated Plates (E04) □ Mass Spec Instrumentation □ Please have a proteomics applications specialist contact me to discuss		
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