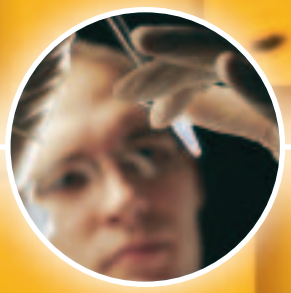
PIERCE

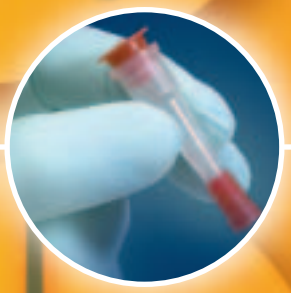
Grasp the Proteome®

Tools to Extract | Separate | Purify | Detect | Quantify Proteins

2-D MS







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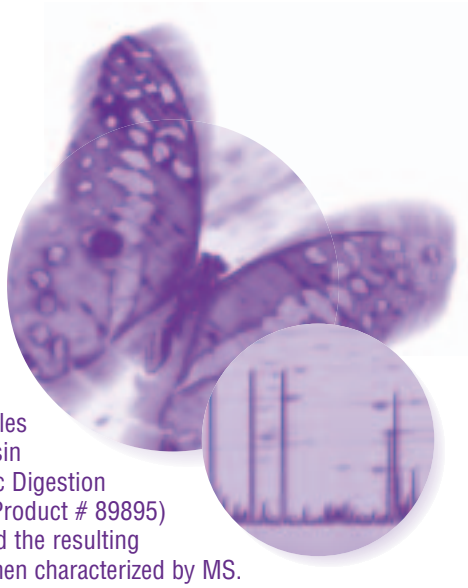


Pierce Novel 2-DE and Mass Spec Sample Preparation Kits and Other Tools

Proteomics is at the heart of modern drug discovery. In this rapidly growing area of research, the proteome (i.e., the mix of proteins in cells) of a diseased person is compared to that of a healthy person. This comparison creates a profile of a disease versus non-diseased state and, most importantly, helps identify potential drug targets.[1] The integrated technologies of two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) are the workhorses of proteomics, enabling scientists to separate, detect and quantify thousands of proteins in a single operation.[2]

Proteomic analysis begins with a protein extract (obtainable with any Poppers™ Cell Lysis Product) derived from a particular biological sample. At this stage, Poppers™ Products are used to prepare whole-cell extracts of a variety of cell types and tissues as well as fractionated extracts of nuclei, membrane proteins and intact mitochondria. These prepared protein extracts may include charged contaminants, dilute solutions, insoluble proteins and low-abundant proteins. Methods including dialysis, precipitation or desalting (Pierce offers four novel 2-D Sample Preparation Kits, Product #s 89863, 89864, 89865 and 89866) are often used to clean up these protein extracts. The prepared protein extracts can now be separated by 2-DE. A particular protein spot of interest is excised from the 2-DE gel and digested with an enzyme (often trypsin, as used in the In-Gel Tryptic Digestion Kit, Product # 89871) into peptide fragments that are 10 to 20 amino acids long. The sample is then cleaned (e.g., with PepClean™ C18 Columns, Product #s 89870 and 89873) and analyzed by mass spectrometry (e.g., MALDI-TOF mass spec). The end result is protein identification and analysis.[1]

In one common approach, protein samples are digested using trypsin (e.g., In-Solution Tryptic Digestion and Guanidination Kit, Product # 89895) or another protease, and the resulting peptide fragments are then characterized by MS. The parent proteins are identified by comparison of the m/z profile (where m is mass and z is charge) of the peptide fragments to predicted digest profiles of proteins with sequences that are known and available in public databases. One challenge with this approach, especially when the starting sample is a mixture of proteins, is that digestion results in a complex mixture of fragments that yield a large number of mass peaks and lead to data overload. Better purification of the starting protein sample is not always possible, but there are other strategies for decreasing the complexity of peptide fragments. One method is to select for only those peptide fragments that contain rare amino acids or amino acid modifications. Using Pierce phospho-selective (Product # 89853), methionine-selective (Product # 21300) and tryptophan-selective (Product # 21310) peptide isolation kits, a specific population of subproducts can be isolated and studied without the data overload associated with analysis of total peptide mixtures by LC/MALDI MS.

References

1. Masi, C.G. (2002). Analyzing the proteome with mass spectrometry. *Drug Discovery & Development,* **5(5)**, 67-70.
2. Aebersold, R. and Goodlett, D.R. (2001). Mass spectrometry in proteomics. *Chem. Rev.* **101**, 269-295.

An Introduction to Mass Spectrometry

Mass Spectrometry
MS is a powerful analytical technique used to identify unknown compounds, quantify known compounds and provide information on the structure and chemical properties of molecules. Detection is accomplished with very small samples. MS has a variety of analytical applications:

- Identifying structures of biomolecules such as carbohydrates, nucleic acids and steroids
- Identifying a known protein from the mass of its fragments
- Sequencing biopolymers such as proteins and oligosaccharides
- Determining structures of drug metabolites
- Performing forensic analyses such as confirmation of drugs of abuse

(See the American Society for Mass Spectrometry web site for a more detailed description.)

Mass spectrometers measure the masses of molecules by converting them in a vacuum into ions that are electrically charged. The results are determined as the ratio of the mass in daltons to the charge on the ions or m/z. A conceptually simplified mass spectrometer consists of a sample inlet leading to a source where gas-phase ions are created in a vacuum, analyzer (ion sorter), ion detector and data system. Data are usually reported in the form of a mass spectrum; i.e., a graph of intensity versus m/z.

Different types of MS and associated chromatographic instrumentation are used, depending on the sensitivity required and the amount of sample available. Properties of the analyte including its molecular weight (usable m/z range is approximately 1-300,000), polarity, volatility and state (solid, liquid or vapor) also influence the choice of analytical system. The amount of sample necessary ranges from low femtogram to several milligrams, depending on the complexity of the sample and the type of analysis used. Following is a brief description of some systems used for MS of peptide and protein samples.

- **Gas Chromatography/Mass Spectrometry (GC/MS)** is used for samples with nonpolar to intermediate polarity compounds or volatile derivatives up to ~800 daltons. For GC/MS, heat is usually used to vaporize the sample components for separation in the gas phase according to their approximate boiling points and absorption/desorption characteristics on a capillary support.
- **Electrospray Ionization (ESI)** is used for intermediate polarity to very polar samples of ~70-100,000 daltons. For electrospray ionization, the buffers actually provide the ion charges (e.g., whether positive or negative depends on the buffer pH). Volatile buffers such as ammonium acetate are preferred, and buffers containing nonvolatile solids such as sodium phosphate should be avoided. Different peptide ions are present at pH equilibrium in the buffer and result in a series of m/z ions that are measured by the detector. At least three ions with different m/z ratios are needed to accurately calculate the mass of a parent peptide or protein. The ion ratios can be varied by changing the buffer composition and pH.
- **Matrix-assisted Laser Desorption Ionization (MALDI)** is used from ~70-300,000 daltons. In MALDI the sample is co-crystallized with a cinnamic acid derivative (called the matrix), which is used to absorb nitrogen laser energy and leave the ionized protein in the gas phase.
- **Matrix-assisted Laser Desorption Ionization-time of Flight (MALDI-TOF)** combines a MALDI ion source with a TOF analyzer. This system consists of a linear instrument with a field-free path that ions pass through from the accelerator to the detector. It is sensitive, allowing for high resolution with few fragment ions while requiring little manipulation or optimization of the instrumentation.

Sample Requirements for MALDI-TOF
Typically, samples for protein and peptide analysis by MALDI-TOF should be at a 1 pmol/µl or greater concentration. Samples should be mixed together carefully to prevent the formation of any precipitates, then allowed to air-evaporate on the sample support. The crystal-growing portion of the matrix solution should be maintained at a pH below 4.0 to prevent ionization of the matrix. If a higher pH occurs, the addition of 0.1% TFA (see page 26) may improve results. Nonvolatile solvents should not be used.

Many common reagents such as urea, guanidinium hydrochloride, DTT, Tris buffer and ammonium carbonate are compatible with MALDI analysis at concentrations up to 0.5 M. Other compounds such as alkali metal salts should be kept below this concentration and phosphate buffer should not exceed 10 mM in the sample. Glycerol is compatible up to 1% in the sample. Most detergents are compatible up to 0.1%, with the exception of SDS, which should not exceed 0.01%.

STEP 1
Cell Lysis

Poppers™ Liquid Cell Lysis Reagents are pre-mixed and ready to use. They're the new wave of cell lysis! No hit-or-miss homemade recipes, no glass beads, no sonicators, no French presses, no freezing and no thawing! Just pour and explore!

Table 1. Poppers™ Cell Lysis Reagents.

Poppers™ Product (Product #)	Organisms/Samples	Dialyze[1]	Compatibility	Notes
Mitochondria Isolation Kit for Cultured Cells* 89874 **Mitochondria Isolation Kit for Tissue*** 89801	Mammalian Cells Mammalian Tissues	NA	Western blot, 2-D Western blots and electrophoresis. Applications include apoptosis, signal transduction and metabolic studies	Protease inhibitors may be added to prevent protein degradation (Halt™ Protease Inhibitor, EDTA-free, Product # 78415). Douncing will increase isolation efficiency vs. detergent alone; however, multiple samples can be processed simultaneously using the reagent-based method.
NE-PER® Nuclear and Cytoplasmic Extraction Reagents 78833	Tissue: calf liver. Cultured cells: epithelial (HeLa), fibroid (COS7), kidney (NIH3T3), liver (Hepa 1) and brain (C6)	No (CER) Yes (NER)	EMSA (if using <3 µl or 10%, otherwise dialyze first in SAL MINIs),[4] Western blot, reporter assays, IEF (after dialysis to reduce salt concentration)	Protease inhibitors[3] may be added to prevent protein degradation. Packed cell vol.: 2 x 10^6 HeLa cells =10 µl = 20 mg; Tissue Yield (calf liver): 3-4 mg cytoplasmic protein/100 mg tissue; 1-1.5 mg nuclear protein/100 mg tissue; Cell Yield (HeLa): 300-400 µg cytoplasmic protein/10^6 cells; 40-60 µg nuclear protein/10^6 cells. Positive controls tested: cytoplasmic (β-Gal, PKC, Hsp90) and nuclear (Oct-1, p53, DNA polymerase)
Mem-PER® Eukaryotic Membrane Protein Extraction Reagent 89826	Cultured cells: brain (C6), epithelial (HeLa), fibroblasts (NIH3T3) and yeast (*S. cerevisiae*)	Yes[5]	Western blot	Protease inhibitors[3] may be added to prevent protein degradation. Can dialyze against another detergent (e.g., CHAPS). Extraction efficiency is generally >50% with the cell lines tested (having proteins with up to two transmembrane segments).
B-PER® Bacterial Protein Extraction Reagent* 78248 500 ml 78243 165 ml **B-PER® PBS Reagent** 78266 500 ml	Gram(-) bacteria, *S. aureus H. pylori*, *E. coli* strains BL21(D3)> JM109> DH5α >M15, Archaebacteria, nematodes, *Acinetobacter* sp., Insect cells	Yes	Reporter assays, IPs,[2] Western blot, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents or reducing agents can be added for more efficient lysis. Do not exceed 0.5 M NaCl. Better lysis if cells are frozen in B-PER® Reagent.
B-PER® II Reagent 78260 250 ml (For smaller volume samples)	Gram(-) bacteria, *S. aureus H. pylori*, *E. coli* strains BL21(D3)> JM109> DH5α >M15, Archaebacteria, nematodes, *Acinetobacter* sp., Insect cells	Yes	Reporter assays, IPs,[2] Western blot, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents or reducing agents can be added for more efficient lysis. Better lysis if cells are frozen in B-PER® Reagent.
Y-PER® Yeast Protein Extraction Reagent 78990 500 ml 78991 200 ml	*S. cerevisiae, Schizo-saccharomyces pombe, C. albicans B. subtilis, E. coli, P. pastoris, Strep. avidinii, Acinetobacter sp.*	No	IPs,[2] Western blot, β-Gal enzyme assays, IEF after dialysis, GST- and His-tag purification	Protease inhibitors[3] may be added to prevent protein degradation. Use at room temperature. Double incubation time for use at 4°C. Use log-phase cells. For stationary-phase cells, add 0.1 M DTT or 20-50 mM TCEP. Will work with 1 mM EDTA. Does not lyse spores. Cannot use with ion exchange columns.
Y-PER® Plus Reagent 78999 500 ml 78998 25 ml	Yeast (*S. cerevisiae*) *Acinetobacter* sp.	Yes	GST- and His-tag purification and Western blot	Protease inhibitors[3] may be added to prevent protein degradation. The addition of up to 2 M NaCl may result in increased efficiency of lysis and protein yield.
M-PER® Mammalian Protein Extraction Reagent 78505 1 L 78501 250 ml	78503 25 ml Cultured mammalian cells. COS7, NIH3T3, Hepa 1-6, 293, CHO, MDA, MB231, FM2 and insect cells	Yes	Luciferase, β-Gal (low signal), CAT, kinase assays, ELISAs, immobilized glutathione and Western blot	Protease inhibitors[3] may be added to prevent protein degradation. Adding 150 mM NaCl results in increased efficiency of lysis and higher protein yield in some cells lines. A PBS rinse of cells prior to lysis removes contaminants such as phenol red and increases protein yield.
T-PER® Tissue Protein Extraction Reagent 78510 500 ml	Heart, liver, kidney and brain	Yes	Luciferase, β-Gal, CAT, kinase assays, Western blot, ELISAs and immobilized glutathione	Protease inhibitors[3] may be added to prevent protein degradation. Mechanical disruption of the tissue is still required. Can also be used for cultured cells.

1. The detergent can be removed by dialysis
2. Immunoprecipitation
3. Halt™ Protease Inhibitor Cocktail, Product # 78410 and 78415 (EDTA-free)
*4. Slide-A-Lyzer® MINI Dialysis Units.**
5. Samples prepared in Mem-PER® Reagent can be dialyzed if the buffer contains detergent (e.g., CHAPS).

**B-PER® Technology is protected by U.S. patent # 6,174,704. Slide-A-Lyzer® MINI Dialysis Unit Technology is protected by U.S. patent # 6,039,871. U.S. patent pending on Mitochondria Isolation Kit Technology.*

Mitochondria Isolation Kits

Rapid isolation of intact mitochondria with maximum yield.

The Pierce Mitochondria Isolation Kits assist in the isolation of intact mitochondria from cultured mammalian cells or mammalian tissues. The kits offer two methods for the separation of mitochondria from cytosolic components, a reagent-based method and traditional Dounce homogenization. Both protocols rely on differential centrifugation to separate the mitochondrial and cytosolic fractions with a bench-top microcentrifuge and can be completed in approximately 40 minutes (post-cell harvest). Each procedure has been optimized for a maximum yield of mitochondrial protein(s) with minimal damage to the integrity of the mitochondria. Once isolated, the mitochondria may be used for a number of downstream applications, including apoptosis, signal transduction and metabolic studies as well as electrophoresis and Western blotting.

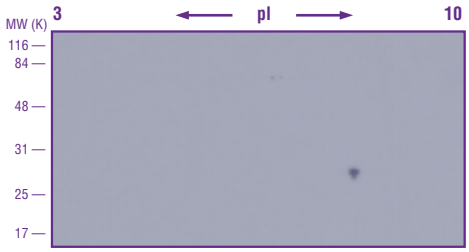


Figure 1. 2-D Western blot of superoxide dismutase (Mn-SOD) in isolated mitochondria. Mitochondria were isolated from NIH3T3 cells using the Dounce homogenization protocol and re-suspended in 2-D sample buffer (7 M urea, 2 M thiourea, 4% CHAPS, pH 3-10 carrier ampholytes, 50 mM DTT). Proteins were resolved on a pH 3-10 IPG strip followed by 8-16% SDS-PAGE and then analyzed by Western blot for superoxide dismutase.

Ordering Information

Product #	Description	Pkg. Size
89874	**Mitochondria Isolation Kit for Cultured Cells** *Sufficient reagents for 50 applications.*	Kit
	Mitochondria Isolation Kit Reagent A	50 ml
	Mitochondria Isolation Kit Reagent B	500 µl
	Mitochondria Isolation Kit Reagent C	70 ml
89801	**Mitochondria Isolation Kit for Tissue**	Kit
New!	Mitochondria Isolation Reagent A	50 ml
	Mitochondria Isolation Reagent B	500 µl
	Mitochondria Isolation Reagent C	65 ml
	BSA	235 mg
	BupH™ Phosphate Buffered Saline Each pack results in 0.1 M sodium phosphate, 0.15 M NaCl; pH 7.2 when reconstituted with 500 ml of ultrapure water	1 pack

Halt™ Protease Inhibitor Cocktails

Compatible protease inhibitors for all applications!

Now inhibit a variety of proteases using Halt™ Protease Inhibitor Cocktails. Both ready-to-use formulations inhibit serine, cysteine, calpain proteases and metalloproteases. The EDTA-free formulation is ideal for preparing samples that will be analyzed by 2-D gel electrophoresis.

Halt™ Kits are easy to use. Add the appropriate volume of inhibitor cocktail to the sample (10 µl per 1 ml of cell extract) and continue with your purification. The Halt™ Protease Inhibitor Cocktails are versatile enough to cease protease activity in animal tissues, bacteria, plants and yeast.

Figure 2. SDS-PAGE analysis of the inhibition of protease activity using Halt™ Protease Inhibitor Cocktails. Bovine serum albumin (BSA) was incubated overnight at 37°C in the presence of the protease papain. Lane 1 shows digestion of BSA in the absence of protease inhibitors. Lane 2 shows BSA digestion in the presence of Halt™ Protease Inhibitor Cocktail. Lane 3 shows BSA digestion in the presence of Halt™ Protease Inhibitor Cocktail, EDTA-Free.

Ordering Information

Product #	Description	Pkg. Size
78410	**Halt™ Protease Inhibitor Cocktail Kit** *Sufficient reagents for 200 ml of extract.*	Kit
78415	**Halt™ Protease Inhibitor Cocktail, EDTA-Free** *Sufficient reagents for 100 ml of extract.*	1 ml

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

New! Zeba™ Micro Desalt Spin Columns

Desalt sample volumes ranging from 2 to 12 µl with confidence!

Protein desalting is an established technique that researchers have used with little change for decades. Pierce has modernized protein-desalting applications by introducing Zeba™ Micro Desalt Spin Columns that allow you to process small sample volumes (2-12 µl), even at low-protein concentrations (25 µg/ml). These specially designed micro columns contain 50 µl of proprietary size-exclusion resin, exclusive to Pierce, to provide the ultimate protein recovery.

Highlights:

- Multiple sample processing in less than six minutes
- Exceptional protein recovery (Table 3)
- Designed for use with small sample volumes of 2-12 µl
- No cumbersome column preparation or equilibration
- No waiting for samples to emerge by gravity flow
- Minimal sample dilution

Table 2. Protein recovery after sample processing with commercially available desalting resins in a Zeba™ Micro Spin Column (Product # 89879). Samples of bovine serum albumin or ubiquitin at concentration of 250 ng/µl protein (2.5 µg total protein load) were desalted with Pierce high-performance desalting resin or the leading competitor's resin in a Zeba™ Micro Spin Column from Pierce. Sample volume was 10 µl plus a 3 µl buffer stacker placed over the sample. Recovery percentages analyzed by BCA™ Protein Assay (Product # 23225 and 23227).

Protein	Resin	% Recovery
BSA (66K M.W.)	Pierce	98
	Competitor B	47
	Competitor A	37
Ubiquitin (8.7K M.W.)	Pierce	76
	Competitor B	47
	Competitor A	50

Table 3. Effect of sample volume on protein recovery. Samples of bovine serum albumin at 1 mg/ml were desalted with a Zeba™ Micro Desalt Spin Column. Recovery percentages were analyzed by BCA™ Protein Assay (Product # 23225 and 23227). Sample volumes were 3 or 10 µl with a 3 µl buffer stacker placed over the sample.

Protein	Sample Volume	% Recovery
BSA (1 mg/ml)	10 µl	95
	3 µl	91

Ordering Information

Product #	Description	Pkg. Size
For 2-12 µl		
89877	Zeba™ Micro Desalt Spin Columns*	25/pack
89878	Zeba™ Micro Desalt Spin Columns*	50/pack
For 120 µl - 4 ml		
89882	Zeba™ Desalt Spin Columns, 0.5 ml	25/pack
89883	Zeba™ Desalt Spin Columns, 0.5 ml	50/pack
89889	Zeba™ Desalt Spin Columns, 2 ml	5/pack
89890	Zeba™ Desalt Spin Columns, 2 ml	25/pack
89891	Zeba™ Desalt Spin Columns, 5 ml	5/pack
89892	Zeba™ Desalt Spin Columns, 5 ml	25/pack
89893	Zeba™ Desalt Spin Columns, 10 ml	5/pack
89894	Zeba™ Desalt Spin Columns, 10 ml	25/pack
Empty Zeba™ Spin Columns		
89879	Zeba™ Micro Spin Empty Columns	50/pack
89896	Zeba™ Empty Spin Columns, 2 ml	25/pack
89897	Zeba™ Empty Spin Columns, 5 ml	25/pack
89898	Zeba™ Empty Spin Columns, 10 ml	25/pack

** Recommended for processing compounds > 7,000 MW.*

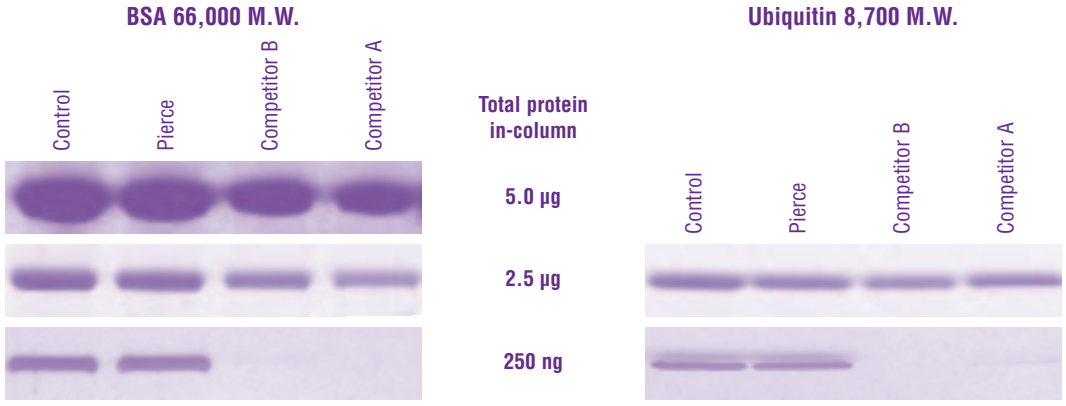


Figure 3. Protein recovery after sample processing with commercially available desalting resins in a micro-spin device. Samples of bovine serum albumin (BSA) or ubiquitin at a variety of concentrations were desalted with Pierce high-performance desalting resin or the leading competitor's resin in Zeba™ Micro Spin Columns (Product # 89879) from Pierce. In all cases sample volume was 10 µl plus a 3 µl buffer stacker placed over the sample. Significantly less protein was recovered using the competitor's resin.

STEP 2

2-D Sample Prep

Novel 2-DE Sample Preparation Kits and Other Tools

The mechanics of preparing gels have improved greatly over the years; however, sample preparation remains a bottleneck. Problems typically include charged contaminants, dilute solutions, insoluble proteins and low-abundant proteins. Furthermore, common methods to remove contaminants and concentrate proteins, such as dialysis and precipitation, are time-consuming and can be unreliable. Pierce has developed four 2-D sample preparation kits to address these challenges. The kits use protein desalting spin columns to remove small charged contaminants and concurrently buffer-exchange proteins into 2-D Sample Buffer, thereby increasing the amount of protein that can be loaded onto an immobilized pH gradient (IPG) strip. The 2-D Sample Prep for Membrane Proteins and the 2-D Sample Prep for Nuclear Proteins combine the isolation of membrane and nuclear proteins, respectively, with sample preparation. Alternatively, the 2-D Sample Preps for Soluble and Insoluble Proteins are for researchers who require clean up of samples isolated with their own methods. In addition to these products, Pierce also offers the SwellGel® Blue Albumin Removal and ProteoSeek™ Albumin and IgG Removal Kits. Albumin and IgG are abundant proteins in serum that can mask less abundant proteins of interest on 2-D gels. Cibacron™ Blue Dye is immobilized on a resin and used to effectively remove >90% of albumin contaminants from human serum. Finally, accurate determination of protein molecular weight and isoelectric point can be challenging. Pierce offers a 2-D Molecular Weight Marker Mix for estimating these values on broad-range 2-D gels.

2-D Sample Prep for Membrane Proteins

Cell fractionation and sample preparation are key tools for optimal 2-D gel analysis; however, both techniques can be challenging when working with membrane proteins. The 2-D Sample Prep for Membrane Proteins Kit combines membrane protein extraction with 2-D sample preparation in a fast, convenient and reliable protocol (Figure 4). Membrane proteins are first extracted from cultured cells using the Mem-PER® Eukaryotic Membrane Protein Extraction Kit. The Mem-PER® Kit also works on hard or soft tissue samples.[3] The prepared extract is subsequently cleaned up in a two-step process. Isolated membrane proteins are first treated with 2-D PAGEprep® Resin, a modified form of diatomaceous earth that selectively binds to proteins while contaminants are washed away. The resulting eluate is then desalted/buffer-exchanged, thus transferring the proteins into 2-D Sample Buffer so they can be electrophoresed in their entirety. Multiple chaotropes present in the 2-D Sample Buffer improve protein solubility[1,2] and result in high-resolution 2-D gels. The kit effectively isolates integral membrane proteins from both cultured mammalian and yeast cells (Figure 5) and concentrates proteins up to six-fold, improving detection of low-abundant proteins (Figure 6).

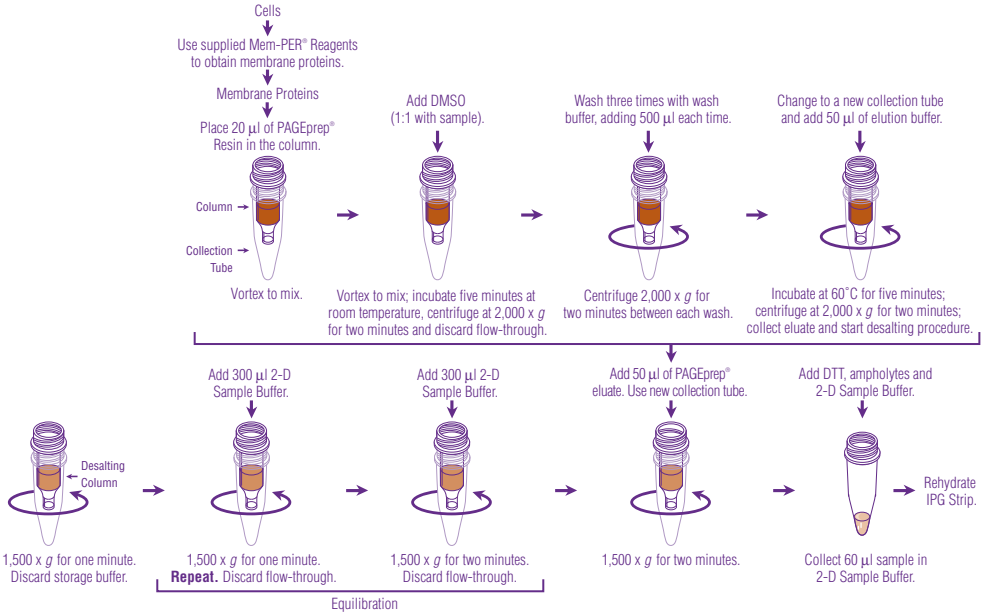


Figure 4. Protocol for the 2-D Sample Prep for Membrane Proteins Kit.

Approximately 30-100 µg of membrane proteins are ready for analysis in less than 90 minutes.

Highlights:

- Requires no precipitation – eliminates difficult resolubilization steps
- Isolates efficiently – isolates 30-100 µg of membrane proteins
- Concentrates and cleans up – membrane proteins are concentrated up to six-fold and excess detergent is removed using 2-D PAGEprep® Resin
- Buffer-exchanges membrane proteins into 2-D Sample Buffer – maintains proteins in solution throughout the desalting process and allows the cleaned-up sample eluate obtained to be electrophoresed in its entirety
- Works quickly – membrane proteins are prepared in less than one hour and are cleaned up in 30-40 minutes
- Contains thiourea in the 2-D Sample Buffer – increases protein solubility and improves protein resolution on 2-D gels

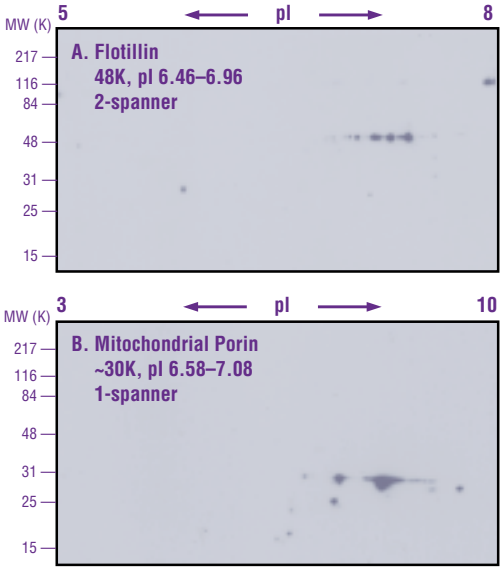


Figure 5. 2-D Western blots of membrane proteins prepared with the 2-D Sample Prep for Membrane Proteins Kit. Membrane protein extract was prepared from mammalian or yeast cells and then concentrated and cleaned. Processed membrane protein samples (approximately 30 µg each as determined by parallel BCA™ Assay of 2-D PAGEprep® Resin eluate) were resolved on 2-D gels and analyzed by Western blot for **A.** flotillin (NIH3T3 cells) and **B.** mitochondrial porin (*S. cerevisiae* EGY194 cells).

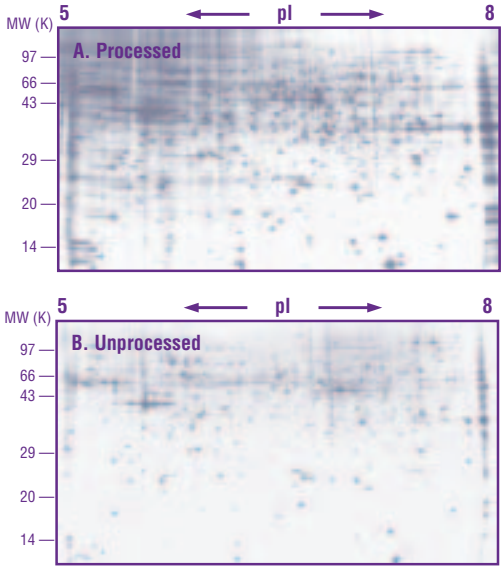


Figure 6. Concentration of membrane protein extract prepared with the 2-D Sample Prep for Membrane Proteins Kit. Membrane protein extract was prepared from C6 cells using the Mem-PER® Reagents supplied with the 2-D Sample Prep for Membrane Proteins Kit. **A.** Approximately 75 µl of extract (representing approximately four times the volume shown in B) was processed with 2-D PAGEprep® Resin and desalted/buffer-exchanged. The entire sample obtained (35 µg as determined by parallel BCA™ assay of 2-D PAGEprep® Resin eluate) was analyzed on a 2-D gel. **B.** Approximately 18.5 µl of unprocessed membrane protein extract, corresponding to the maximum volume recommended for IPG strip rehydration, was analyzed directly on a 2-D gel. Both samples were focused on 11 cm, pH 5-8 IPG strips followed by 8-16% SDS-PAGE and then silver stained.

References

1. Rabilloud, T., *et al.* (1997). *Electrophoresis* **18**, 307-316.
2. Lanne, B., *et al.* (2001). *Proteomics* **1**, 819-828.
3. Benton, B., *et al.* (2004). A better alternative to Dounce homogenization for the isolation of mitochondria. *Previews* **8(1)**, 1-3.

Ordering Information

Product #	Description	Pkg. Size
89864	**2-D Sample Prep for Membrane Proteins Kit**	Kit
	Sufficient reagents for 25 applications.	
	Includes: Mem-PER® Eukaryotic Membrane Protein Extraction Kit:	
	Mem-PER® Cell Lysis Reagent	5 ml
	Mem-PER® Buffer	12.5 ml
	Mem-PER® Membrane Protein Solubilization Reagent	20 ml
	2-D Sample Buffer for Membrane Proteins:	
	2-D Sample Buffer for Membrane Proteins Component A	18 ml
	2-D Sample Buffer for Membrane Proteins Component B	16.5 g
	2-D PAGEprep® Protein Clean-up and Enrichment Kit:	
	2-D PAGEprep® Protein Binding Resin	0.5 ml
	Binding/Wash Buffer Stock Solution	50 ml DMSO
	Protein Desalting Spin Columns	25 columns
	Elution Buffer	1.25 ml
	Spin Cups	25 cups
	Collection Tubes	50 tubes

2-D Sample Preparation for Nuclear Proteins

Preparation of nuclear proteins for 2-D gel electrophoresis (2-DE) can be time-consuming and frustrating as nuclear proteins are prone to aggregation both in solution and during isoelectric focusing.[1] Pierce researchers have developed the 2-D Sample Prep for Nuclear Proteins Kit to circumvent these issues. This kit combines nuclear protein extraction, using the popular NE-PER® Nuclear and Cytoplasmic Reagents, with a fast and reliable protocol for 2-D sample preparation.

Once isolated, nuclear proteins are buffer exchanged directly into a specially formulated 2-D Sample Buffer using equilibrated protein desalting columns (Figure 7). This procedure eliminates salts that interfere with isoelectric focusing (Figure 8A and Table 4), while multiple chaotropes[1,2] maintain the solubility of nuclear proteins, resulting in minimal sample loss and high-resolution 2-D gels (Figure 8B). Nuclear proteins from a variety of mammalian cell lines and tissues are recovered with ≥ 90% efficiency, while ≥ 98% of salt contaminants are removed. Moreover, as processed samples are recovered in 2-D Sample Buffer, they can be immediately applied to an IPG strip in their entirety, effectively increasing the allowable sample load volume.

The 2-D Sample Prep for Nuclear Proteins Kit offers a significant improvement over standard precipitation methods that require lengthy incubation and resolubilization steps (Table 5), often introducing contaminants that can partially obscure 2-D gels (Figure 8C).

Highlights:

- Removes small charged contaminants that interfere with 2-D electrophoresis – reduces the time for isoelectric focusing and prevents loss of data on 2-D gels due to salt fronts
- Buffer-exchanges nuclear proteins into 2-D Sample Buffer – "concentrates" protein by increasing amount of protein that can be applied to an IPG strip and maintains proteins in solution throughout the desalting process
- Uses NE-PER® Nuclear and Cytoplasmic Reagents – prepares a highly purified nuclear protein extract
- Streamlines nuclear protein extraction with 2-D sample preparation – contains a faster and more efficient protocol than the two procedures performed separately
- Contains thiourea in sample buffer – increases protein solubility and improves protein resolution on 2-D gels[1,2]
- Desalts faster than competing 2-D sample prep kits – allows multiple samples to be processed in less than 15 minutes instead of one-plus hours required for precipitation and dialysis

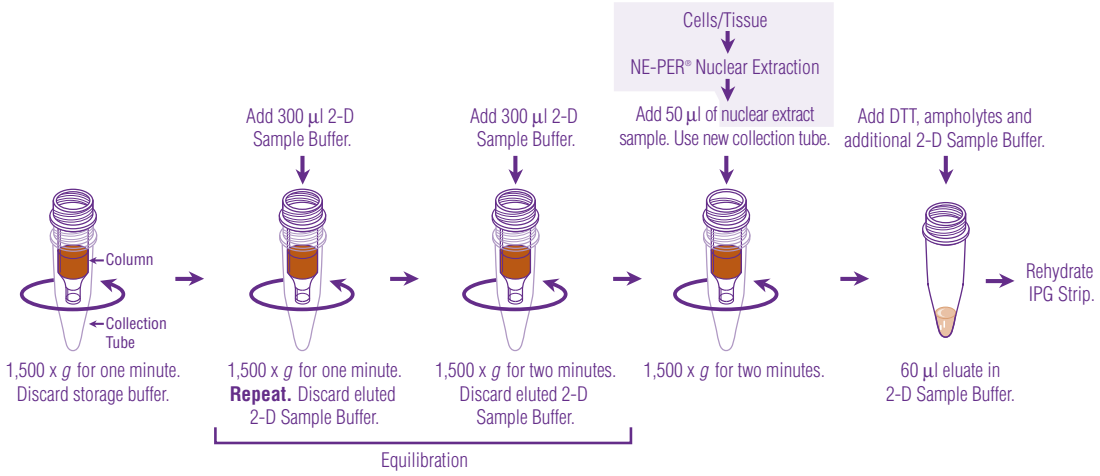


Figure 7. Protocol for 2-D Sample Prep for Nuclear Proteins Kit and 2-D Sample Prep for Soluble/Insoluble Proteins involving buffer exchange/desalting. The shaded area of the protocol applies to the Nuclear Proteins Kit only.

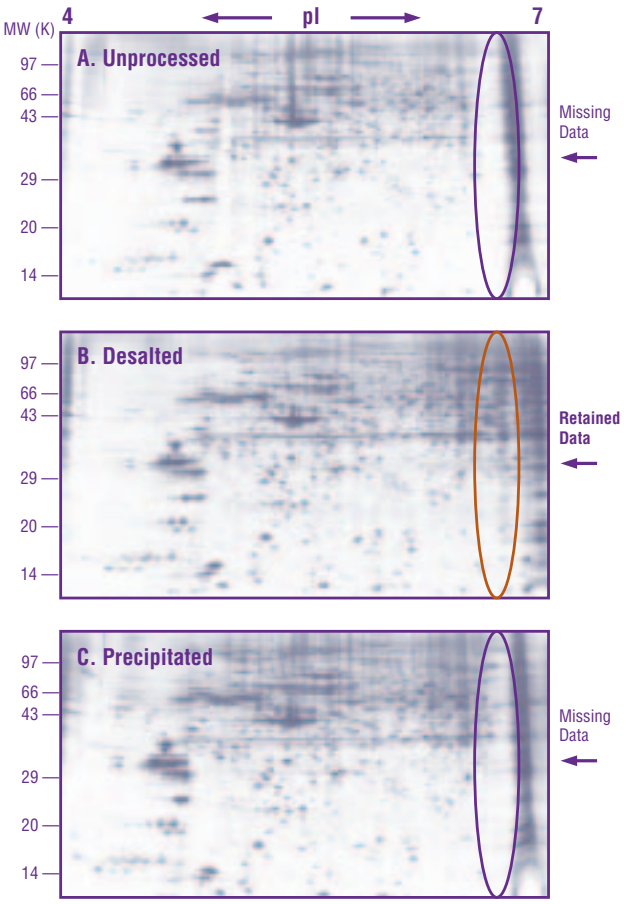


Figure 8. Comparison of unprocessed, desalted and precipitated NE-PER® Nuclear Extract. Nuclear extract (2 mg/ml) was prepared using the 2-D Sample Prep for Nuclear Proteins. Approximately 30 µg of protein was focused on a pH 4-7 IPG strip followed by 8-16% SDS-PAGE and the 2-D gels were silver-stained. 2-D gels shown are of extract that is **A.** unprocessed, **B.** desalted with the 2-D Sample Prep for Nuclear Proteins and **C.** precipitated with a commercial precipitation kit.

Table 4. Time required for isoelectric focusing of NE-PER® Nuclear Extract processed with the 2-D Sample Prep for Nuclear Proteins Kit compared with unprocessed extract.

Nuclear Extract	Time for IEF (hours)
Desalted	5.25
Unprocessed	6.5

Each time shown represents the average length of time to focus 30 µg of nuclear extract on 11 cm, pH 4-7 IPG strips.

Table 5. Time comparison.

Method	Time (minutes)
Desalted	15
Precipitation	90

Time required to process up to six desalted samples in the 2-D Sample Prep for Nuclear Proteins Kit compared to the same number of samples processed with precipitation.

References

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

Ordering Information

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

2-D Sample Preparation for Soluble/Insoluble Proteins

The 2-D Sample Prep Kits for Soluble and Insoluble Proteins are suitable for those who prefer to have a clean-up protocol without the membrane and/or nuclear protein prefractionation reagents. They were developed to rapidly remove salts, buffers and other small ionic contaminants from cultured cell extracts and tissues in preparation for 2-D gel electrophoresis (2-DE). Equilibrated protein desalting columns remove these contaminants and buffer-exchange proteins directly into 2-D Sample Buffer, thereby maintaining protein solubility and improving recovery (Figure 7, page 8). The 2-D Sample Prep for Soluble Proteins is for whole cell and tissue extracts as well as for fractionated proteins in which the proteins of interest are predominantly hydrophilic. The 2-D Sample Prep for Insoluble Proteins is for proteins that are more difficult to solubilize, including cell extracts containing larger and/or more hydrophobic proteins, proteins that tend to aggregate, and nuclear proteins.

Charged species prolong isoelectric focusing (IEF) and distort 2-D gels at pH extremes. The Pierce 2-D Sample Prep Kits remove ≥ 98% of these contaminants, significantly shortening the time required for IEF while maintaining high resolution on 2-D gels and retaining data normally obscured by salt fronts (Figure 8, page 9). Desalted proteins with a MW >7 kDa are recovered with approximately 80% efficiency from whole cell lysates and ≥ 90% efficiency from rat liver homogenates and nuclear extracts. Proteins are recovered in 2-D Sample Buffer so they can be immediately applied to an immobilized pH gradient (IPG) strip in their entirety, effectively increasing the allowable sample load volume (Figure 8, page 9).

Highlights:

- Removes small charged contaminants that interfere with 2-D electrophoresis – reduces time required for isoelectric focusing and prevents loss of data on 2-D gels due to salt fronts
- Buffer-exchanges proteins into 2-D Sample Buffer – "concentrates" protein by increasing amount of protein that can be applied to an IPG strip AND maintains proteins in solution throughout the desalting process
- Works faster than competing 2-D sample prep kits – processes multiple samples in less than 15 minutes instead of one-plus hours required for precipitation and dialysis
- Contains thiourea in the 2-D Sample Buffer for Insoluble Proteins – increases protein solubility and improves protein resolution on 2-D gels[1,2]

References

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

Ordering Information

Product #	Description	Pkg. Size
89865	**2-D Sample Prep for Soluble Proteins Kit** *Sufficient reagents for 25 applications.* Includes: 2-D Sample Buffer for Soluble Proteins: 2-D Sample Buffer for Soluble Proteins, Component A 2-D Sample Buffer for Soluble Proteins, Component B Protein Desalting Columns	Kit 19.5 ml 15 g 25 columns
89866	**2-D Sample Prep for Insoluble Proteins Kit** *Sufficient reagents for 25 applications.* Includes: 2-D Sample Buffer for Insoluble Proteins: 2-D Sample Buffer for Insoluble Proteins, Component A 2-D Sample Buffer for Insoluble Proteins, Component B Protein Desalting Columns	Kit 18 ml 16.5 g 25 columns

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

SwellGel® Blue Albumin Removal Kit

Offers ease-of-use, speed and performance not found in other kits.

SwellGel® Blue Albumin Removal Discs are designed for high-capacity albumin removal from small (10-100 µl) serum samples. Each disc can bind approximately 2 mg of human serum albumin.* Just add water and the discs hydrate in less than 20 seconds to form an equilibrated chromatographic resin bed of approximately 200 µl. The SwellGel® Blue Albumin Removal Kit is optimized for immediate downstream analysis by 2-D or MS applications. The simplicity of sample preparation and the versatility offered by the SwellGel® Technology make it ideal for standard chromatography, 2-D and micro-spin column applications.

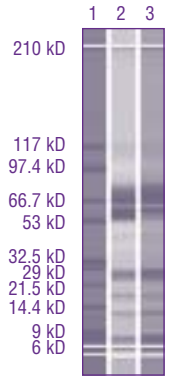


Figure 9. Gel-like images of serum samples obtained with Agilent 2100 Bioanalyzer using the Protein 200 Plus LabChip® Kit. Lane 1: molecular weight marker, **Lane 2:** normal human serum, and **Lane 3:** albumin-depleted human serum sample processed with the Pierce SwellGel® Blue Albumin Removal Kit. Note the significant reduction in the albumin interference in the 50-70 kD range.

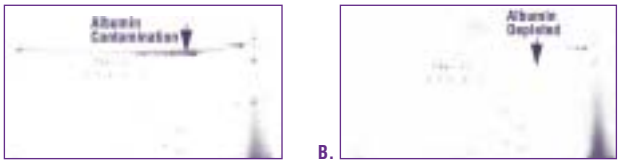


Figure 10. SwellGel® Albumin Removal Kit for 2-D analysis. Serum sample obtained by diluting 10 µl human serum with 40 µl TBS and loading 5 µl onto Gel A. Albumin-free sample obtained by diluting 50 µl human serum 1:1 with buffer, adding it to a SwellGel® Blue Disc, washing the resin three times with 50 µl of buffer, and combining the fractions; loading 5 µl onto Gel B. Both samples were focused using pH 4-7 IEF strips and run on 8-16% Tris-Glycine gels.

Highlights:

- **Excellent for 2-D applications** – allows the researcher to study less abundant proteins without interference from albumin (Figure 15) or processing buffers
- **SwellGel® Disc format** – offers customer stable product that outperforms existing kits and products (Figure 9)[1]
- **Convenient and versatile complete kit** – includes everything needed to remove albumin and works well with 96-well filter plates
- **Room temperature storage** – discs do not leak or spill, and the kit stores conveniently on bench top
- **Pre-measured, easy-to-handle discs with an easy-to-follow protocol (Figure 11)** – no waste – all resin can be used; no need to pipette messy slurries

SwellGel® Blue Albumin Removal Kits contain ready-to-hydrate Cibacron™ Blue Activated Discs, binding/wash buffer and Mini-Spin Columns. Separations are performed in microcentrifuge columns and can be processed by low-speed centrifugation or syringe-based methods.

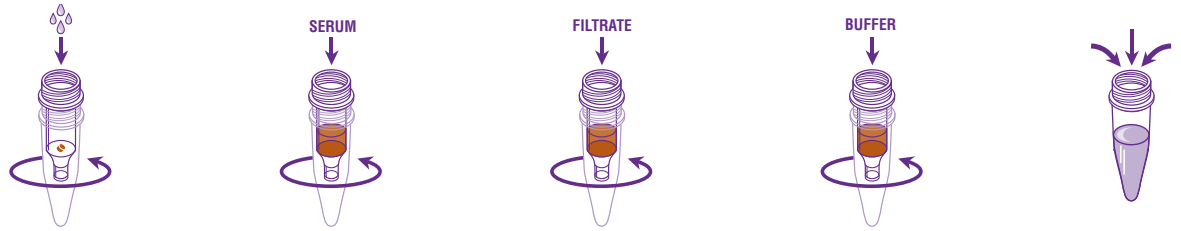
Reference

1. Steel, L.F., *et al.* (2003). Efficient and specific removal of albumin from human serum samples. *Mol Cell Proteomics* **2**, 262-270.

Ordering Information

Product #	Description	Pkg. Size
89845	**SwellGel® Blue Albumin Removal Kit***	12-25 Reactions
	Includes: SwellGel® Blue Albumin Removal Discs	25 discs
	Binding/Wash Buffer	6.25 ml
	Mini-Spin Columns	25 columns
89846	**SwellGel® Blue Albumin Removal Kit***	50-100 Reactions
	Includes: SwellGel® Blue Albumin Removal Discs	100 discs
	Binding/Wash Buffer	25 ml
	Mini-Spin Columns	50 columns

**SwellGel® Blue Discs have differing levels of affinity for species-specific albumins. SwellGel® Blue Discs bind human, swine and sheep albumin. They can also be used with a minor protocol change for bovine, calf and goat albumin. They do not bind mouse albumin.*



Step 1. Hydrate SwellGel® Disc in water. Spin.

Step 2. Add serum sample to the resin. Incubate 2 minutes and spin.

Step 3. Recover filtrate and add to the resin. Incubate 2 minutes and spin.

Step 4. Add Binding/Elution Buffer. Spin. Wash 1-4 times.

Step 5. Combine desired fractions. Concentrate if needed.

Time: 10-15 minutes

Figure 11. SwellGel® Blue Albumin Removal Kit Protocol.

New! ProteoSeek™ Albumin/IgG Removal Kits

Process multiple 2-D or 2D/LC samples in less than 40 minutes.

Low-abundant proteins in human serum and plasma can provide information about human diseases. However, human fluid analysis is often complicated by the presence of high concentrations of albumin and IgG, which can make up more than 70% of total serum protein (Figure 12). Pierce offers two kits for the removal of albumin and IgG from samples, allowing analysis of low-abundant proteins.

Highlights:

- **Efficient** – enable visualization of low-abundant proteins
- **Convenient** – choose the system appropriate for your application
- **Fast** – easy-to-optimize spin formats allow worry-free sample processing in less than 40 minutes

Figure 12. 2-D gel unprocessed human serum. Approximately 30 µg of unprocessed human serum was isoelectric-focused on a pH 5-8 strip and then separated in the second dimension on an 8-16% SDS polyacrylamide gel. SilverSNAP® Stain II (Product # 24612) was used to visualize bands. Large poorly resolved bands corresponding to human serum and IgG obscure much of the gel.

For Detailed Proteomic Analysis

The ProteoSeek™ Antibody-Based Albumin/IgG Removal Kit

This kit processes 12 samples of approximately 600 µg of human serum. It is suitable for detailed proteomic analysis because it uses highly specific immobilized antibodies for the removal of albumin and IgG, producing minimal nonspecific interactions (Figure 13).

Figure 13. 2-D gel of human serum processed with the ProteoSeek™ Antibody-Based Albumin/IgG Removal Kit. Approximately 30 µg of processed human serum was isoelectric-focused on a pH 5-8 strip and then separated in the second dimension on an 8-16% SDS polyacrylamide gel. SilverSNAP® Stain II (Product # 24612) was used to visualize bands. With albumin and IgG removed, low-abundant proteins are readily identified.

An Economical – Yet Effective – Method

The ProteoSeek™ Albumin/IgG Removal Kit

This Kit uses a classical mixture of Cibacron™ blue and Protein A resin for economical – yet effective – removal of albumin and IgG. The kit can process up to 25 samples of approximately 600 µg of serum using an optimized protocol to minimize nonspecific protein interactions and sample dilution (Figure 14).

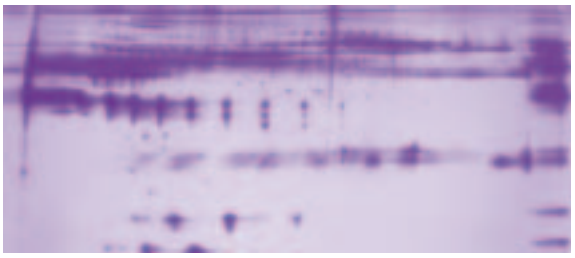


Figure 14. 2-D gel of human serum processed with ProteoSeek™ Albumin/IgG Removal Kit. Approximately 30 µg of processed human serum was isoelectric-focused on a pH 5-8 strip and then separated in the second dimension on an 8-16% SDS polyacrylamide gel. SilverSNAP® Stain II (Product # 24612) was used to visualize bands.

Each kit is optimized for downstream analysis by 2-D electrophoresis or 2D/LC mass spectrometric methods and allows for multiple samples to be processed simultaneously.

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

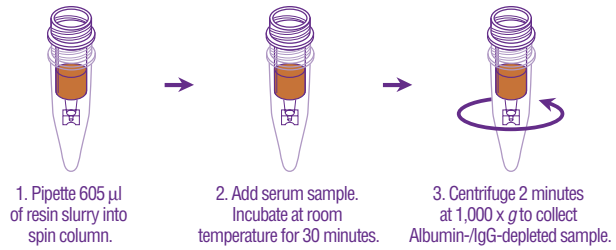


Figure 15. Protocol for the ProteoSeek™ Antibody-Based Albumin/IgG Removal Kit (Product # 89876).

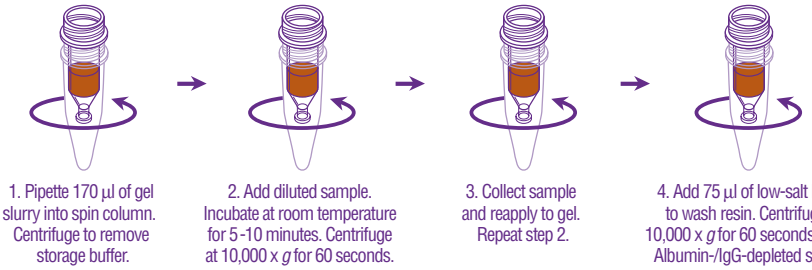


Figure 16. Protocol for the ProteoSeek™ Albumin/IgG Removal Kit (Product # 89875).

Ordering Information

Product #	Description	Pkg. Size
89876	**ProteoSeek™ Antibody-Based Albumin/IgG Removal Kit** *Sufficient material for up to 12 samples of 600 µg of serum.*	Kit
	Contents: ProteoSeek™ Antibody-Based Gel (*sold as 62% slurry [7.25 ml total volume]*)	4.5 ml of immobilized Anti-HSA/Anti-IgG Gel Bed
	Spin Columns and Caps	12

Product #	Description	Pkg. Size
89875	**ProteoSeek™ Albumin/IgG Removal Kit** *Sufficient material for up to 25 samples of 600 µg of serum.*	Kit
	Contents: ProteoSeek™ Gel (*sold as 50% slurry [4.25 total volume]*)	2.13 ml gel bed
	Handee™ Spin Columns and Accessories	27

STEP 3

Detection

2-D Protein Molecular Weight Marker Mix

A 2-D gel marker mix with all the proteins you want – with no document to sign.

Pierce ready-to-use 2-D gel marker mix was designed specifically to aid the proteome analyst. This 2-D gel marker mix contains a complement of seven reduced and denatured proteins. When performing protein 2-D separation and analysis, each protein in the mix provides important features for assessing system performance or estimating pI and molecular weight values. It's also convenient – this marker mix doesn't require you to sign a notarized document just to use it.

A unique complement of proteins

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

Table 6. Component proteins in the Pierce 2-D Protein Molecular Weight Marker Mix.

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

2-D Marker Shown Stained with Silver (left) and Coomassie Blue Dye (right)

Table 7. 500 µl of the Pierce 2-D Marker Mix is sufficient for the following number of applications, depending on gel size and staining method.

Gel Size	Stain Method	Marker Volume	# of Gels/ Vial
Mini-Gels	Coomassie Blue Dye	2.5 µl	200
Mini-Gels	Silver	0.5-1.0 µl	500-1,000
Large Format Gels	Coomassie Blue Dye	5-7.5 µl	66-100
Large Format Gels	Silver	1.0-2.5 µl	200-500

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

Ordering Information

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

Compatible Products for use in Proteome Analysis

Coomassie Blue Dye Stains		
24590	GelCode™ Blue Stain Reagent *Stains up to 25 mini-gels.*	500 ml
24592	GelCode™ Blue Stain Reagent *Stains up to 175 mini-gels.*	3.5 liters
Silver Stain		
24612	SilverSNAP® Silver Stain II *Stains up to 20 mini-gels.*	Kit
Other Pierce Protein Molecular Weight Markers		
26691	TriChromRanger™ Prestained Protein Molecular Weight Marker Mix	1 x 48 microtube plate
26681	BlueRanger® Prestained Protein Molecular Weight Marker Mix	1 x 48 microtube plate

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

SuperSignal® Chemiluminescent Substrates

A sensitive, user-friendly detection system for 2-D Western blotting.

Methods

Membrane protein extracts were prepared from cultured NIH3T3 or *S. cerevisiae* EGY194 cells using the 2-D-Sample Prep for Membrane Proteins Kit (Product # 89864). Nuclear protein extracts were prepared from TNFα-induced HeLa cells using the 2-D Sample Prep for Nuclear Proteins Kit (Product # 89863). Approximately 15-30 µg of purified protein was processed and focused on 11 cm pH 5-8 or pH 3-10 IPG strips. The second dimension was performed using 8-16% SDS-PAGE, and the resulting 2-D gels were transferred by electroblotting to nitrocellulose membranes for Western blot analysis. The nonspecific sites were blocked overnight using SuperBlock® (TBS) Blocking Buffer (Product # 37535) containing 0.05% Tween®-20 at 4°C with shaking. The blots were incubated with primary antibodies against the integral membrane proteins flotillin and mitochondrial porin, and the nuclear proteins NF-κB:p50 and p65 for one hour at room temperature (RT). The blots were then washed three times with TBS (Product # 28376) containing 0.05% Tween®-20. Following the wash, the blots were incubated in HRP-conjugated secondary antibodies (Product #s 31402, 31430 and 31460) for one hour at RT. After washing with TBS (Product # 28376) containing 0.1% Tween®-20, the blots were incubated for five minutes in SuperSignal® West Femto Maximum Sensitivity Chemiluminescent Substrate (Product # 34095). Excess substrate was drained and the blots were exposed to CL-XPosure™ Film (Product # 34090) for 1 second.

Results and discussion

SuperSignal® West Femto Chemiluminescent Substrate was highly sensitive and effective for detecting proteins in both low and high abundance in complex protein mixtures resolved on 2-D gels. The integral membrane proteins flotillin and mitochondrial porin were identified in mammalian and yeast membrane protein extracts, respectively (Figure 17). Similar sensitivity also occurred with transcription factors stimulated to translocate from the cytosol to the nucleus in response to TNFα. NF-κB p50 and its precursor, p105, were readily detected in nuclear extracts prepared from TNFα-stimulated cells (Figure 18A). Degradation of the p50 factor was also seen that is likely from inhibitor IkB loss. Another NF-κB transcription factor, p65, was also identified (Figure 18B).

Conclusion

Pierce SuperSignal® West Femto Chemiluminescent Substrate's enhanced sensitivity and signal duration allows for an improved signal-to-noise ratio that requires less sample, faster signal generation and greater light emission longevity.

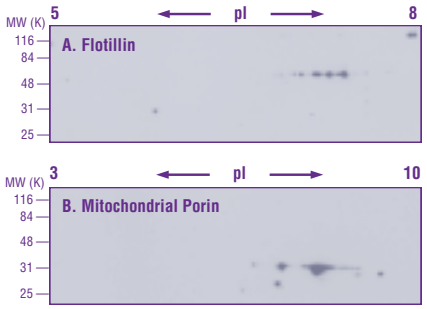


Figure 17. 2-D Western blots of membrane proteins prepared with the 2-D Sample Prep for Membrane Proteins Kit. Membrane protein extract was prepared from mammalian or yeast cells and then concentrated and cleaned up. Processed membrane protein samples (approximately 30 µg each as determined by parallel BCA™ Assay of 2-D PAGEprep® Resin eluate) were resolved on 2-D gels and analyzed by Western blot for **A.** flotillin (48 kDa, pI 6.46-6.96, NIH3T3 cells) and **B.** mitochondrial porin (30 kDa, pI 6.58-7.08, *S. cerevisiae* EGY194 cells).

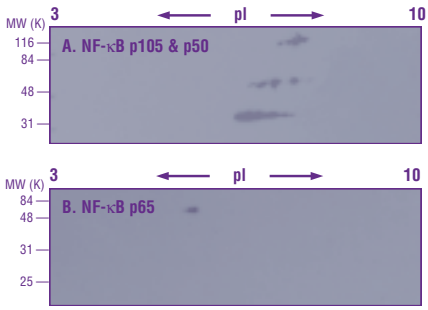


Figure 18. 2-D Western blots of nuclear proteins prepared with the 2-D Sample Prep for Nuclear Proteins Kit. Nuclear protein extract was prepared from TNFα-induced HeLa cells and then desalted/buffer-exchanged. Two-dimensional gels were prepared with 15 µg of protein each and analyzed by Western blot for **A.** NF-κB p105 and p50 and **B.** NF-κB p65.

Ordering Information

Product #	Description	Pkg. Size
34080	SuperSignal® West Pico Chemiluminescent Substrate	500 ml
34075	SuperSignal® West Dura Extended Duration Substrate	100 ml
34095	SuperSignal® West Femto Maximum Sensitivity Substrate	100 ml
89863	2-D Sample Prep for Nuclear Proteins	Kit
89864	2-D Sample Prep for Membrane Proteins	Kit

New! Imperial™ Protein Stain

Our most sensitive 2-DE Coomassie Stain that is mass spec-compatible.

Imperial™ Protein Stain from Pierce 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 19). For fast results, a 5-minute stain combined with a 15-minute water destain easily detects 6 ng protein bands. (Figure 20).

Even greater levels of sensitivity and crystal-clear background can be achieved through increased staining time and destaining in water (Figures 21-22). 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.

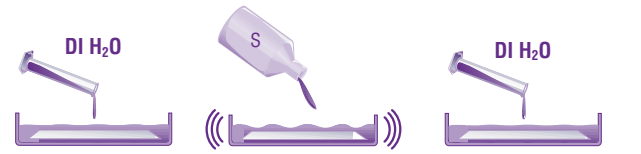
Highlights:

Outstanding Performance

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

Convenience

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



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

Figure 19. Imperial™ Protein Stain protocol.

Figure 20. 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.

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

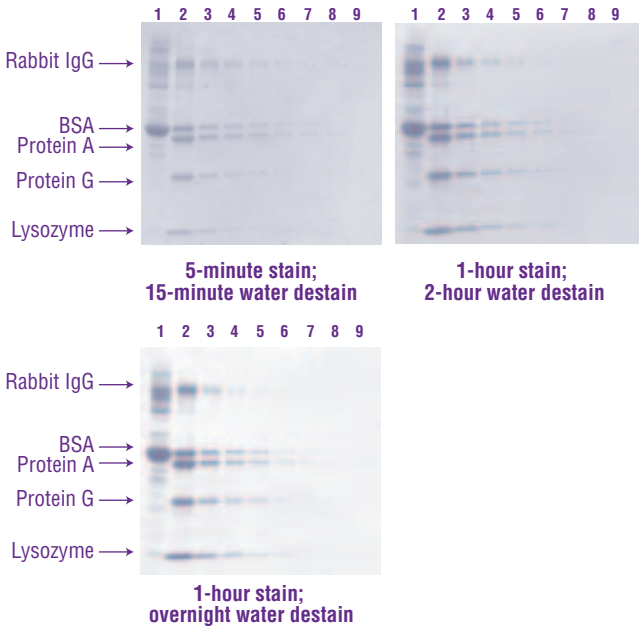


Figure 21. Enhanced sensitivity and crystal-clear background using Imperial™ Protein Stain. For even greater sensitivity and reduced background, gels can be stained with Imperial Protein Stain for 1 hour and destained in water from 1 hour to overnight. **Lane 1:** BSA only (6 µg), Lanes 2-9 contained 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.

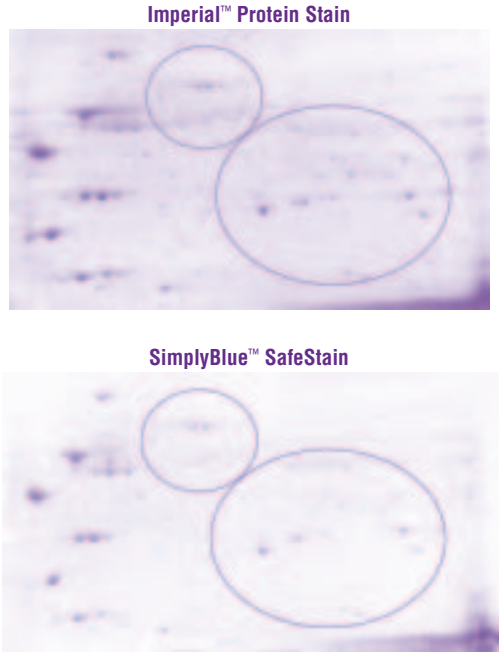


Figure 22. Imperial™ Protein Stain reveals spots that are faint or not detected with other Coomassie stains. Mitochondrial protein extract was prepared from heart tissue of six-week-old Sprague-Dawley rat. Processed protein extract (72 µg) was focused on a pH 5-8 IPG strip followed by 8-16% SDS-PAGE. The gels were stained for 1 hour and destained overnight following manufacturer-recommended protocols.

Ordering Information

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

** U.S. patent pending on Imperial™ Protein Stain.*

SilverSNAP® Stain II

SilverSNAP® Stain just got snappier!

Introducing a faster, more flexible silver stain

Improved SilverSNAP® Stain II is a fast, more flexible and forgiving stain. SilverSNAP® Stain II does all this with a remarkably clear and uniform gel background. Most importantly, this improved silver stain delivers consistently reliable staining performance.

Highlights:

- **Optimal flexibility built into two key steps – fixing and staining**
  This feature offers speed or gives you an "at-your-convenience" option. These "flextime" points provide added convenience compared with any other commercially available silver stain preparation that offers only one stopping point (i.e., the fixing step). The new improved SilverSNAP® Stain II from Pierce offers the following advantages:
  – The Fixing Step can be completed in 30 minutes or left overnight with no adverse affect on the staining result.
  – The Staining Step may be performed for just 5 minutes or you can leave your gel in stain reagent for more than **20 hours** with no noticeable negative affect on the results.
- **Uniform minimal backgrounds with a variety of gels offer contrast to aid band/spot visualization.**
  A short (one-minute) sensitization step performed after fixing yields results that are free of the characteristically dark or blotchy backgrounds often seen with homemade or other commercial stains. This feature offers you added benefits when you perform densitometric analysis of SilverSNAP® Stain II-stained gels.
- **Improved sensitivity often better than homemade or commercial preparations.**
  The SilverSNAP® Stain II allows you to see more of what you're looking for in-gel.

Ordering Information

Product #	Description	Pkg. Size
24612	**SilverSNAP® Silver Stain II**	Kit
	This product replaces Product # 24602.	
	Includes: SilverSNAP® Sensitizer	2 ml
	SilverSNAP® Stain	500 ml
	SilverSNAP® Enhancer	25 ml
	SilverSNAP® Developer	500 ml

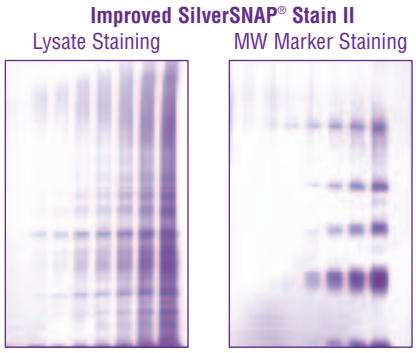


Figure 23. Comparison of newly improved SilverSNAP® Stain II with the silver stain of a popular competing brand. Dilutions of *Escherichia coli* lysate (1:25-1:1,000) and dilutions of pure protein were separated by electrophoresis using 10% Tris-glycine gels at 125 volts for 2 hours. The gels were then stained with SilverSNAP® Stain II and the competitor silver stain. The pure protein bands are myosin (14-0.2 ng), phosphorylase B (55-0.9 ng), BSA (14-0.2 ng), ovalbumin (55-0.9 ng) and carbonic anhydrase (55-0.9 ng). The development times indicated below each gel image refer to the amount of time the gel was in the developer solution before reaching the desired level of staining. The gel development was stopped after this time.

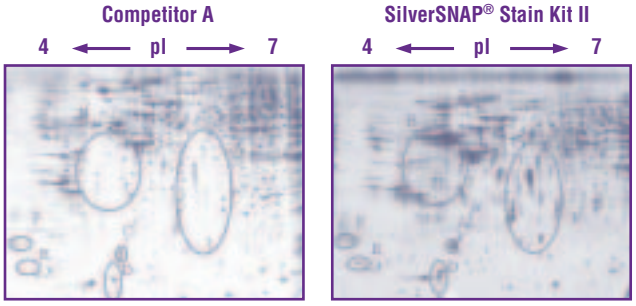


Figure 24. Comparison of identical 2-D gels stained with SilverSNAP® Stain II and a popular commercial silver stain formulation. Cytosolic protein extract (15 µg) was separated by 2-D gel electrophoresis. The gels were stained with the SilverSNAP® Stain Kit II and another popular commercial silver stain. Gels were stained according to the manufacturer's recommendations. Both gels were developed for 1 minute and 40 seconds to reveal protein spots. The circled regions A-E on the respective gels indicate comparable regions of each gel and illustrate the differences in spot intensity and overall detection sensitivity. A side-by-side comparison clearly shows that SilverSNAP® Stain Kit II was able to reveal spots that were faint or unobservable in the gel stained with the popular competing formulation.

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

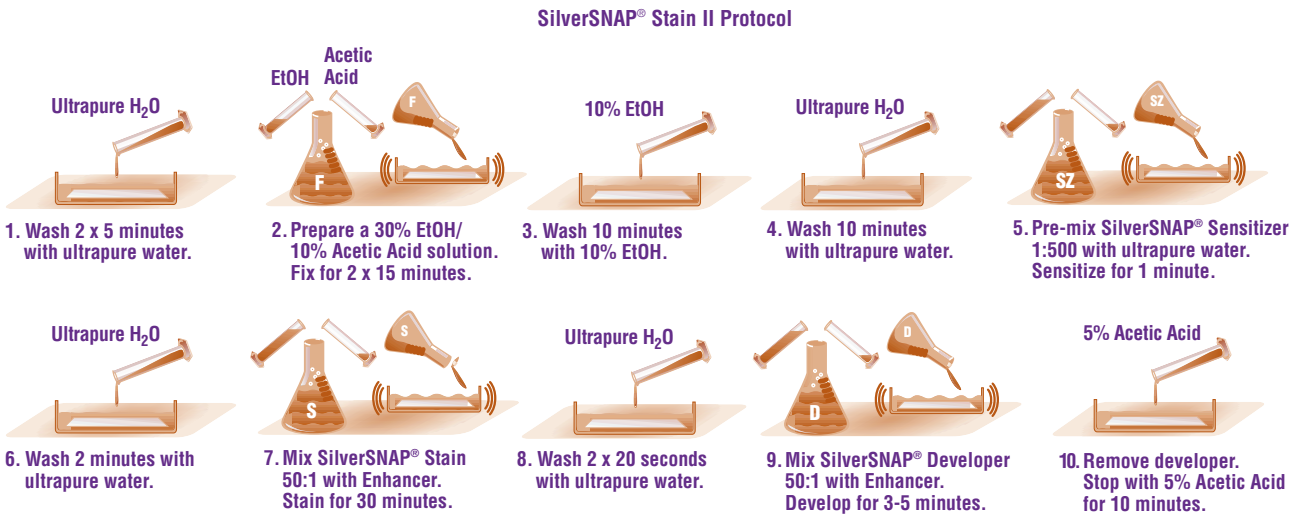


Figure 25. SilverSNAP® Stain II Protocol.

GelCode™ Blue Stain Reagent

A sensitive 2-DE stain that is mass spec-compatible.

Highlights:

- No destaining steps required for 2-DE – never needs pungent-smelling methanol/acetic acid solvents[1]
- Fast – works with a one-step, one-hour staining
- More sensitive than standard coomassie gel stain formulations
- Optional Water Wash Enhancement™ Step increases staining sensitivity
- Easily washed away prior to MS analysis[1,2]
- Compatible with MALDI-TOF analysis[3,4,5]
- Compatible with sequence analysis[6]

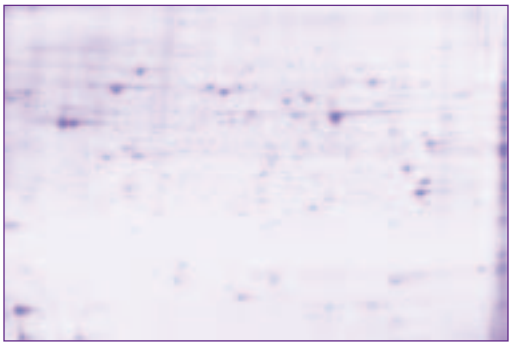


Figure 26. A GelCode™ Blue Stain Reagent-stained 2-D gel. The gel is an isolated hydrophilic fraction obtained using the Mem-PER® Kit (Product # 89826) from NIH3T3 cells run on a pH 5-8 IPG strip. Approximately 80 µg of protein was applied to the gel. The second dimension was performed on an 8-16% SDS-PAGE.

References

1. Aulak, K.S., *et al.* (2001). Proteomics method identified proteins nitrated *in vivo* during inflammatory challenge. *Proc. Natl. Acad. Sci. USA* **98(21)**, 12056-12061.
2. Hilton, J.M., *et al.* (2001). Phosphorylation of a synaptic vesicle-associated protein by an inositol hexakisphosphate-regulated protein kinase. *J. Biol. Chem.* **279(19)**, 16341-16347.
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6. Tani, M., *et al.* (2002). *J. Biol. Chem.* **275**, 3462-3468.

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).*	500 ml
24592	**GelCode™ Blue Stain Reagent*** *Sufficient for staining up to 175 SDS-PAGE mini gels (8 cm x 10 cm).*	3.5 liters
72300	**Pump (for 3.5 liter package only)**	1 pump

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

STEP 4

Mass Spec Sample Prep

New! In-Solution Tryptic Digestion and Guanidination Kit

Analyze proteins by mass spec with confidence

Highlights:

- **Optimized** – complete digestion is achieved for 0.025-10 µg protein samples with minimal to no side reactions
- **Quick** – protein can be reduced, alkylated, digested and guanidinated all in one day
- **Convenient** – kit includes reagents for reduction, alkylation, digestion and guanidination

Trypsin specifically cleaves peptide bonds at the carboxyl side of arginine and lysine residues, generating a peptide map unique for each protein. Analysis of tryptic peptides by mass spectrometry (MS) provides a powerful tool for identifying proteins or analyzing post-translational modifications. Reliable mass spectral analysis requires accurate and complete digestion of the target proteins as well as modification of peptides to optimize ionization and detection. The In-Solution Tryptic Digestion and Guanidination Kit from Pierce contains optimized procedures and reagents for reduction, alkylation, digestion and guanidination to provide reliable MS analysis of approximately 90 protein samples containing 0.025-10 µg of protein (Figure 27).

The In-Solution Tryptic Digestion and Guanidination Kit contains a proteomics-grade modified trypsin that produces clean, complete digests with minimal autolysis products present. A reduction and alkylation protocol eliminates disulfide bonds, improving peptide identification and simplifying data analysis. Guanidination eliminates ionization bias between peptides with C-terminal arginine residues over C-terminal lysine residues, improving detection and overall sequence coverage.

In the example below, a variety of proteins (200 ng per sample) were processed using the In-Solution Tryptic Digestion and Guanidination Kit. Following reduction, alkylation and digestion, each sample was divided in two. One-half was guanidinated, while the other half was saved as a no-guanidination control. Each sample was processed by PepClean™ C-18 Spin Columns and then analyzed on an Agilent LC/MSD Trap XCT equipped with an AP-MALDI ionization source using α-CHCA as the matrix.

Proteins processed with the In-Solution Tryptic Digestion and Guanidination Kit produced clean and reliable mass spectra with high sequence coverage (Table 8, Figure 28). Using the guanidination procedure to convert lysines to homoarginines enhanced the overall signal intensity of lysine-containing peptides by an average of 1.5- to four-times, eliminating the ionization bias for peptides with a terminal arginine and improving sequence coverage and the reliability of data analysis.

Table 8. Sequence coverage data for tryptic digestions with and without guanidination for three proteins.

	Sequence Coverage	
Protein	**No Guanidination**	**With Guanidination****
Lysozyme (14,000 MW)	6/8 peptides 66/86aa 77%	8/8 peptides 86/86aa 100%
Myoglobin (17,000 MW)	6/12 peptides 78/134aa 58%	8/12 peptides 90/134aa 67%
BSA (66,000 MW)	25/44 peptides 318/489aa 65%	28/44 peptides 344/489aa 70%

High levels of sequence coverage are seen for all test proteins processed with the In-Solution Tryptic Digestion and Guanidination Kit with significant increase in sequence coverage seen in samples undergoing the guanidination procedure. Sequence coverage based only on those peptides expected to be identified based on scanning from 600-2,000 m/z.

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

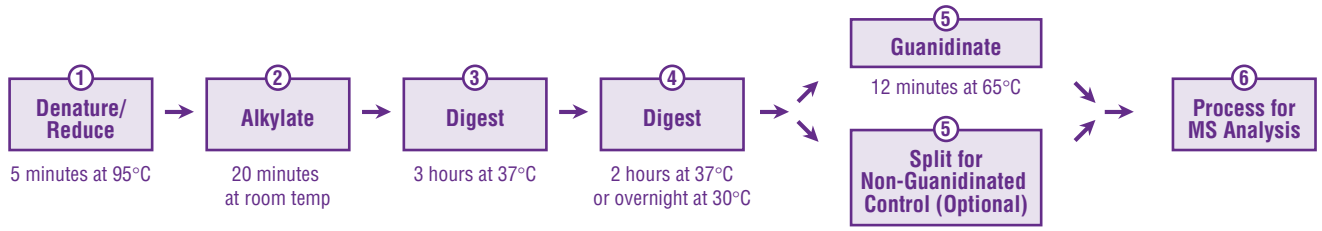

Figure 27. In-Solution Tryptic Digestion and Guanidination Kit protocol.

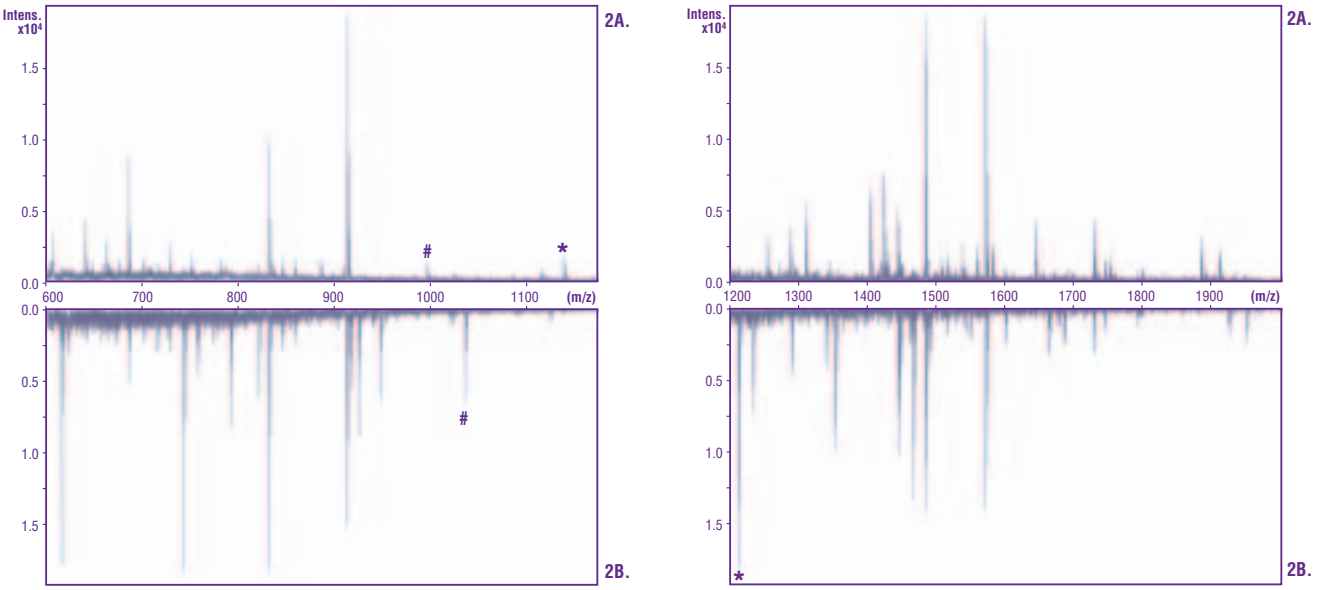

Figure 28. Comparison of a guanidinated digest with a non-guanidinated control demonstrates improved ionization and sequence coverage after guanidination. Figure 28A: MS spectra of digested BSA (100 ng). Figure 28B: MS spectra of digested BSA (100 ng) with guanidination. Guanidination results in a mass shift of +42 m/z for each lysine in a peptide. Symbols (#) and (*) indicated two lysine-containing peptides with and without guanidination for demonstration purposes.

Ordering Information

Product #	Description	Pkg. Size
89895	**In-Solution Tryptic Digestion and Guanidination Kit** *Sufficient reagents for approximately 90 digests of samples containing 0.025-10 µg of protein.*	Kit
	Includes: Modified Trypsin	20 µg
	Trypsin Storage Solution	50 µl
	Ammonium Bicarbonate	50 mg
	No-Weigh™ DTT	7.7 mg
	Iodoacetamide (IAA)	500 mg
	O-Methylisourea Hemisulfate Salt (OMI)	400 mg
	Ammonium Hydroxide	1 ml

Complementary Products

Product #	Description	Pkg. Size
28904	TFA, Sequanal Grade (10X)	10 x 1 ml
89870	PepClean™ C-18 Spin Columns	25/pkg.

In-Gel Tryptic Digestion Kit and PepClean™ C-18 Spin Columns

Highlights of the In-Gel Tryptic Digestion Kit:

- **Convenience** – includes all necessary reagents for the destaining of coomassie or fluorescent dye-stained proteins, reduction and alkylation of cystines, and tryptic digestion all in one kit
- **Robustness** – procedure and reagents are designed to produce reliable digestions and data generation across a wide range of conditions without requiring optimization
- **Accuracy** – contains highly purified and modified MS-grade trypsin that shows no chymotryptic activity and minimal autolytic activity

Highlights of the PepClean™ C-18 Spin Columns:

- **Efficient contaminant removal** – significantly reduces signal suppression, improves signal:noise ratios and sequence coverage and minimizes the need to repeat experiments due to failed or poor spectra
- **Robustness** – works with a wide variety of load volumes and concentrations; no need to reduce sample volume before application
- **Convenience** – easier handling and no special equipment required for processing multiple samples compared to tip-driven systems that require single-sample processing
- **Sensitivity** – special C-18 resin allows excellent recovery percentages, even at low sample loads (≤ 200 fmol).

Accurate identification and analysis of proteins is a central component of proteomic strategies for studying cellular functions and processes. Mass spectrometers are essential tools for these studies as they provide a high level of sensitivity and mass accuracy. However, this sensitivity and accuracy can be obtained only if proper sample preparation is performed. Two key sample preparation steps are the fragmentation of proteins into peptides and subsequent concentration and clean up of peptides. To help researchers perform these steps, Pierce offers the In-Gel Tryptic Digestion Kit and PepClean™ C-18 Spin Columns that are tailored to the needs of mass spectrometric (MS) analysis.

The In-Gel Tryptic Digestion Kit provides a complete set of reagents to perform approximately 150 digestions on colloidal coomassie or fluorescent dye-stained protein bands. The kit includes modified porcine trypsin, destaining buffers, reduction reagents, alkylation reagents and digestion buffers along with detailed and simple instructions. Each component and step has been optimized and balanced to produce complete, accurate and clean digests using a variety of conditions for dependable MS analysis (Figure 31).

PepClean™ C-18 Spin Columns concentrate peptide samples and remove contaminants common to biological systems, increasing the sensitivity, reliability and quality of MS-based analysis. Each spin column contains a porous C-18 reverse-phase resin with excellent binding and recovery characteristics for a wide range of peptide concentrations. The spin column format allows simultaneous processing of multiple samples (10-150 µl) in approximately 30 minutes without laborious, repetitive pipetting or specialized equipment (Figure 30). PepClean™ C-18 Spin Columns are used effectively for processing peptides derived from ≤ 10 ng or up to 30 µg of protein. Sensitivity and detection limits are dependent on the downstream application.

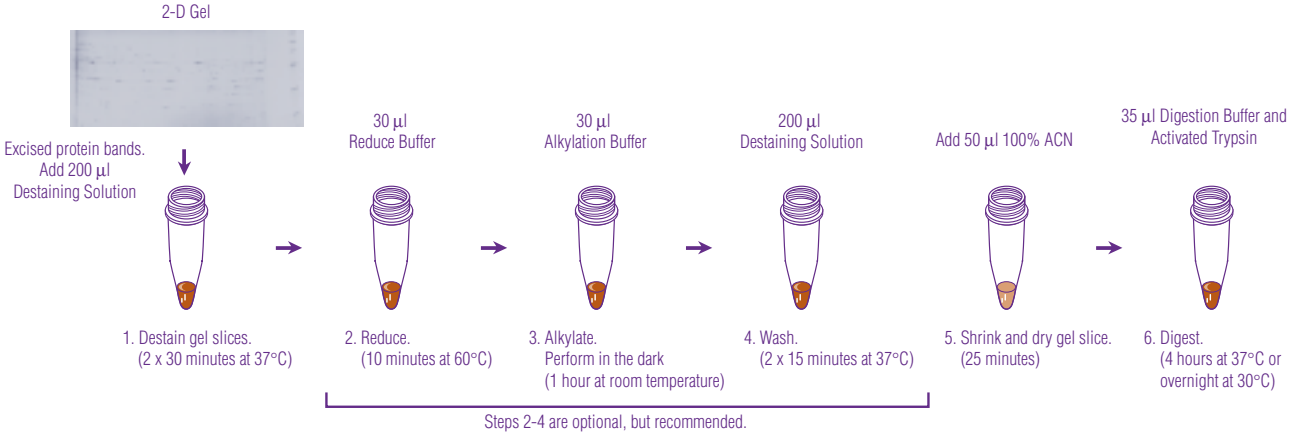

Figure 29. Schematic protocol for the In-Gel Tryptic Digestion Kit.

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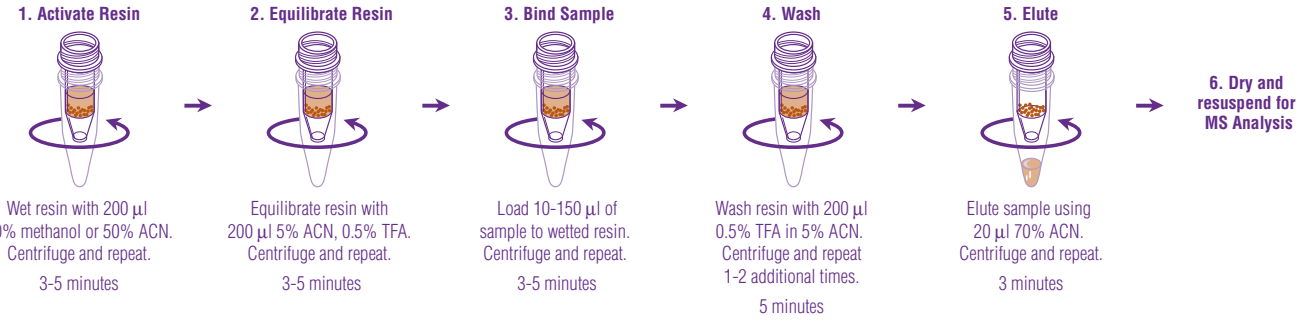


Figure 30. Schematic protocol for PepClean™ C-18 Spin Columns.

Methods

Cytosolic and mitochondrial protein extracts were prepared from NIH3T3 cells and separated by 2-D electrophoresis. Gels were stained with GelCode™ Blue Stain Reagent (Product # 24592) and excised using a manual spot picker. Protein bands were treated with the In-Gel Tryptic Digestion Kit according to instructions as outlined in Figure 29. Following digestion, each sample was prepared for MALDI analysis with PepClean™ C-18 Spin Columns according to instructions and as outlined in Figure 30. After processing the sample was dried in a vacuum evaporator for approximately 20 minutes, then resuspended in 1.1 µl of matrix (10 mg/ml α-cyano-4-hydroxycinnamic acid in 65% acetonitrile, 0.1% trifluroacetic acid). This mixture was spotted and analyzed on an Applied Biosystems Voyager DE™ PRO MALDI-TOF mass spectrometer.

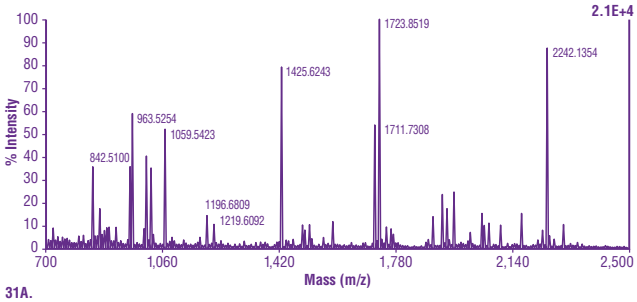
Results and discussion

The In-Gel Tryptic Digestion Kit and PepClean™ C-18 Spin Columns were used in the preparation of 2-D gel electrophoresis-separated proteins from mitochondrial isolates for MALDI-TOF analysis (see Figures 31A-C for the mass spectra). Database searches identified unknown proteins as A) glutamate dehydrogenase, B) ATP synthase alpha chain and C) voltage-dependent anion selective channel protein 1, with 55.8, 53.3, and 46.0% sequence coverage, respectively.

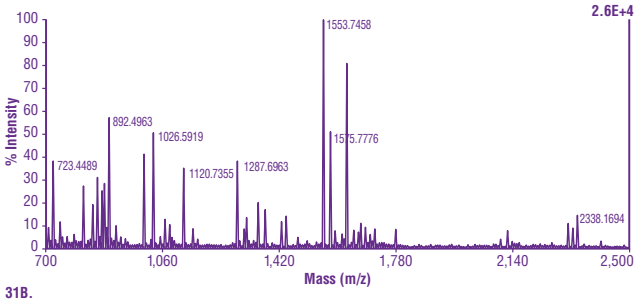
The In-Gel Tryptic Digestion Kit produced clean, high-sequence coverage digests containing minimal autolysis products. The PepClean™ C-18 Spin Columns efficiently purified and concentrated digests prior to MALDI-TOF analysis. MS analysis of digests that were not concentrated with C-18 yielded little to no signal (data not shown).

Ordering Information

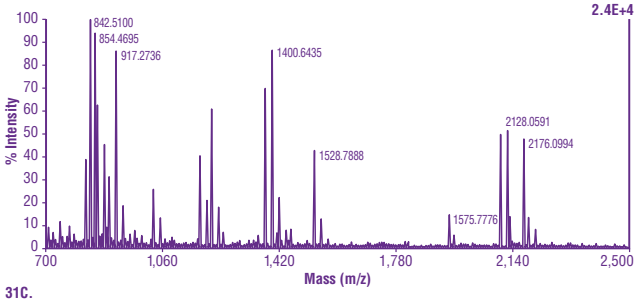
Product #	Description	Pkg. Size
89871	**In-Gel Tryptic Digestion Kit** *Sufficient for approximately 150 in-gel digestions.*	Kit
	Includes: Modified Trypsin	20 µg
	Trypsin Storage Solution	40 µl
	Acetonitrile	70 ml
	Ammonium Bicarbonate	300 mg
	Tris[2-carboxyethyl]phosphine (TCEP)	500 µl
	Iodoacetamide	500 mg
89870	**PepClean™ C-18 Spin Columns** *Each column contains 8 mg of C-18 resin.*	25 spin columns
89873	**PepClean™ C-18 Spin Columns** *Each column contains 8 mg of C-18 resin.*	50 spin columns



31A.



31B.



31C.

Figure 31. Mass spectra. MALDI-TOF MS of unknown proteins. Samples were processed with the In-Gel Tryptic Digestion Kit and PepClean™ C-18 Spin Columns.

Phosphopeptide Isolation Kit

A novel tool for isolating phosphorylated peptides from complex protein digests.

The central role of reversible protein phosphorylation/dephosphorylation in regulating cell function makes protein phosphorylation a major topic of study and one of the cornerstones of proteomic research. Identifying phosphoproteins and their phosphorylation sites is a difficult endeavor, requiring a variety of techniques. The new Pierce Phosphopeptide Isolation Kit (Product # 89853) is designed to simplify the isolation and identification of phosphopeptides from protein digests through the specific interaction of phosphate groups with immobilized gallium. This kit has a high binding capacity for phosphopeptides (~150 µg), making it compatible with classical methods for phosphopeptide analysis (e.g., ^{32}P radiolabeling, Edman degradation and TLC/HPLC), yet it has been optimized for more sensitive and more powerful LC-MS and MALDI-TOF mass spectrometric (MS) methods of phosphopeptide analysis because samples can be eluted directly in MS-compatible buffers.

Methods

Beta-Casein was digested with immobilized trypsin for 4 hours at 37°C and 3 µl (approximately 5 µg) of this digest was adjusted to pH 3.0 by adding 50 µl 0.1% acetic acid. The sample was applied to the column and washed with 75 µl of 0.1% acetic acid, followed by 75 µl of 0.1% acetic acid, 10% acetonitrile, and a final wash of 75 µl water. Sample was then eluted in three separate fractions using 20 µl of 100 mM ammonium bicarbonate. Elution # 1 (0.5 µl) was mixed with 0.5 µl of MALDI matrix (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 0.1% trifluoroacetic acid) and 0.5 µl ammonium citrate to improve detection of phosphopeptides. This mixture was spotted and analyzed in positive ion, linear, delayed-extraction mode on an Applied Biosystems Voyager DE™ PRO MALDI-TOF mass spectrometer (Figures 32 and 33).

Results

The Pierce Phosphopeptide Isolation Kit uses a gallium-chelated IDA-based resin that provides less nonspecific peptide binding and greater specificity for both single and multi-phosphorylated peptides than iron-chelated and NTA-based resins.[2,3,4] Additionally phosphopeptide isolation can be performed using MS-compatible elution buffers such as 0.1 N ammonium hydroxide or 100 mM ammonium bicarbonate, allowing direct analysis of sample with no desalting or additional sample handling required.[1,2,3]

The MALDI-TOF mass spectrum of peptides isolated from the tryptic-digested casein sample is shown in Figure 33. Peaks at masses 2,062 and 3,123 are the main signals detected in the eluted sample and represent peptides of 1,981 Da containing one phosphate group and of 2,802 Da containing four phosphate groups (+80 Da for each phosphate).

Highlights:

- **Fast** – total procedure time is just 15 minutes
- **Easy to use** – no metal chelating or resin equilibration required; each mini spin tube contains one ready-to-use SwellGel® Gallium Disc
- **High binding capacity** – each SwellGel® Gallium Disc is made of 25 µl gallium (III) resin with a binding capacity for phosphopeptides of approximately 150 µg
- **Ideal for MS analysis** – simplifies MS analysis by isolating phosphorylated peptides from complex mixtures; peptides can be eluted with MS-compatible buffers
- **Reliable** – isolates single and multiple phosphorylated peptides; low nonspecific binding
- **Room temperature stable** – one year shelf life from the date of purchase

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

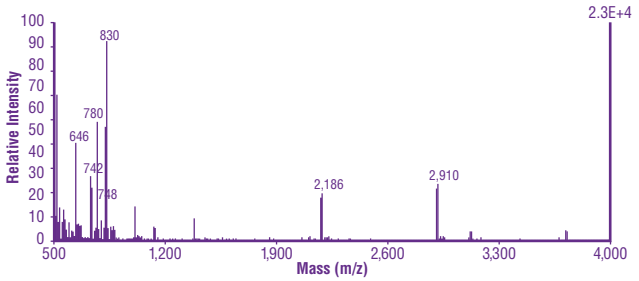


Figure 32. MALDI-TOF mass spectrum of tryptic digest of β-Casein. Peaks at masses 646, 742, 748, 780, 830 and 2,186 represent the expected peptides from a tryptic digest of β-casein. Peaks for the known phosphopeptides at 2,062 Da and 3,123 Da are not resolved in positive ion mode due to the additional negative charge. The peak at mass 2,910 represents a partial digest fragment.

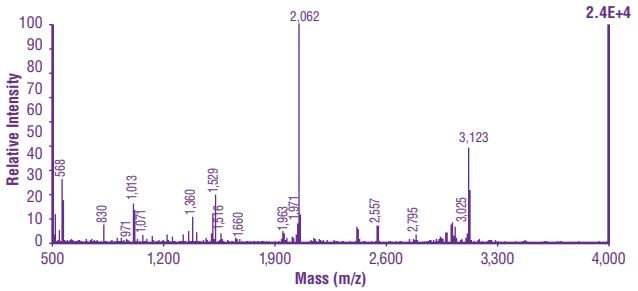


Figure 33. MALDI-TOF mass spectrum of tryptic digest of β-Casein after sample has been processed with the Phosphopeptide Isolation Kit. The 2,062 peak is the single phosphorylated peptide representing the amino acid sequence from residue 48 to 63. The 3,123 peak is the peptide from residue 16 to 40 containing four phosphate groups. None of the non-phosphorylated peptides of β-casein are detected in significant quantities.

Conclusion

MALDI-TOF MS spectra analyses show the effectiveness of the isolation columns in binding phosphorylated peptides from complex protein digests, making the Pierce Phosphopeptide Isolation Kit a useful sample preparation tool for MS-based proteomic analysis.

References

1. Giorgianni, F., *et al.* (2004). Identification and characterization of phosphorylated proteins in the human pituitary. *Proteomics* **4**, 587-598.
2. Poseqitz, M.C. and Tempst, P. (1999). Immobilized gallium (III) affinity chromatography of phosphopeptides. *Anal. Chem.* **71**, 2883-2892.
3. Zhou, W., *et al.* (2000). Detection and sequencing of phosphopeptides affinity-bound to immobilized metal ion beads by matrix-assisted laser desorption/ionization mass spectrometry. *J. Am. Soc. Mass. Spectrom.* **11**, 273-282.
4. Ficarro, S.B., *et al.* (2002). Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae. Nature Biotechnology* **20**, 301-305.

Acknowledgments: *Pierce would like to thank Dr. Bassam Wakim, Michael Pereckas and Dr. Joyce Thompson at the Protein and Nucleic Acid Core Facility at the Medical College of Wisconsin, Milwaukee, for their helpful contribution with mass spectrographic analysis of phosphopeptides.*

Ordering Information

Product #	Description	Pkg. Size
89853	**Phosphopeptide Isolation Kit** 30 mini-spin columns containing a SwellGel® Gallium (III) Disc. *Sufficient discs to analyze 30 protein digestions.*	Kit

Compatible Products

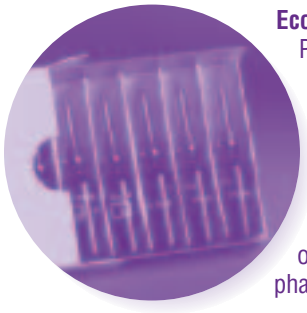
Product #	Description	Pkg. Size
20230	**Immobilized Trypsin, TPCK Treated**	2 ml gel
20151	**Immobilized V-8 Protease**	Kit
20199	**Submaxillaris Protease**	250 units (1 mg)
20212	**Carboxypeptidase Y**	30 units (0.5 mg)
20214	**Aminopeptidase M**	20 units
89871	**In-Gel Tryptic Digestion Kit***	Kit
89895	**In-Solution Tryptic Digestion and Guanidination Kit****	Kit

Product #	Description	Pkg. Size
89870	**PepClean™ C-18 Spin Columns**	25 spin
89873	**PepClean™ C-18 Spin Columns**	50 spin
21300	**PrepTide™ Methionine Peptide Selective Matrix Kit** *Contains sufficient material for two isolation reactions using a digestion of up to 5 nmol of protein.*	Kit
	Includes: Methionine Peptide Isolation Packs	2 each
	Accessory Pack	1 each
21310	**PrepTide™ Tryptophan Peptide Selective Matrix Kit** *Contains sufficient material for two isolation reactions using a digestion of up to 2 nmol of protein.*	Kit
	Includes: Tryptophan Peptide Isolation Packs	2 each
	Accessory Pack	1 each

** See page 23 for components.*
*** See page 21 for components.*

New! TFA (Trifluoroacetic Acid)

All trifluoroacetic acids are not equal – demand what is crucial for your application.



Economical Convenience

Pierce TFA is available in a variety of package formats and sizes, allowing researchers to save money by choosing the package that works best for their specific applications. For example, the 1 ml ampules provide a simple way to prepare liter quantities of 0.1% TFA for stationary and mobile phases in reverse-phase chromatography.

Purity

Clear, colorless Pierce Trifluoroacetic Acid (TFA) exhibits superior purity (99.8%) and exceptional clarity, allowing for sensitive, nondestructive peptide detection at low UV wavelengths in reverse-phase HPLC protein and peptide separation systems.[1]

Versatility

Pierce TFA also performs incomparably in protein sequencing applications[2,3] and solid-phase peptide synthesis[4] and as a protein/peptide solubilizing agent.[2,3]

High-performance Packaging

TFA is an extremely corrosive organic solvent that has vapors that corrode conventional bottle caps. Damaged packaging endangers TFA purity and it sabotages one's work, wasting valuable time. Because packaging is as important as product quality, Pierce packages its TFA in amber glass with protective Teflon® TFE-lined fluorocarbon caps or ampuled under nitrogen.

References

1. Chicz, R.M. and Regnier, F.E. (1990). High-performance liquid chromatography: effective protein purification by various chromatographic modes. *Methods Enzymol.* 392-421.
2. Smith, B.J. (1997). *Protein Sequencing Protocols.* Humana Press. (Product # 20016)
3. Allen, G. (1989). *Sequencing of Proteins and Peptides, Second Revised Edition.* Elsevier.
4. Stuart, J.M. and Young, J.D. (1984). *Solid Phase Peptide Synthesis, Second Edition.* Pierce Chemical Company.

Ordering Information

Product #	Description	Pkg. Size
28901	Trifluoroacetic Acid, Sequanal Grade	500 m
28902	Trifluoroacetic Acid, Sequanal Grade	10 x 1 g
28903	Trifluoroacetic Acid, Sequanal Grade	100 g
28904	Trifluoroacetic Acid, Sequanal Grade	10 x 1 ml

For more product information, or to download a product instruction booklet, visit *www.piercenet.com/2dms95d*

Downstream Applications

Cross-Linkers and Mass Spectrometry in Protein Structure and Protein Interaction Analysis

The ability to selectively conjugate two or more proteins together using cross-linking reagents permits the study of interacting proteins in complex mixtures. As the proteome become more defined, the interactions those protein molecules undergo will become increasingly important to understand. Pierce bioconjugation reagents are a critical factor in facilitating this knowledge.

In the drive to better understand protein structure and function, two powerful methods have been used in tandem to yield information for 3-D mapping of proteins and protein complexes.[1] The methods are mass spectrometry (MS) and protein cross-linking. Pierce Double-Agents™ Cross-linking Reagents have played an important role in the several studies published to date. Mass spectrometric analysis of the reaction products can yield low resolution three dimensional protein structure information giving insight into how a protein folds.[2] Analysis of interface sequences between interacting proteins yields insight into the composition and location of their respective molecular contact surfaces. Popular Pierce cross-linking reagents with application in MS analysis:

- Sulfo-DST (Product # 20591): Disulfosuccinimidyl tartarate
- BS^3 (Product # 21580): *Bis*(sulfosuccinimidyl) suberate
- Sulfo-EGS (Product # 21566): Ethylene glycol *bis*(sulfosuccinimidyl succinate
- DTSSP (Product # 21578): 3,3′-Dithiobis(sulfosuccinimidyl propionate)

These reagents are all homobifunctional, amine-reactive and water-soluble. Sulfo-DST, Sulfo-EGS and DTSSP are cleavable by periodate, hydroxylamine and thiols, respectively. BS^3 is not cleavable.

Deuterated (Heavy) Cross-linking Agents

To simplify the resulting mass spectra from an inter- or intramolecular cross-linking experiment, cross-linking reagents with defined isotope tags have been employed.[3] Application of a 1:1 ratio of two identical cross-linking agents differing only in the number of deuterium atoms vs. hydrogen atoms in their chemical composition (e.g., d_4 vs. d_0) can easily distinguish low-abundant cross-linked peptides. Characteristic isotopic MS patterns differing by four mass units, for example, after enzymatic digestion of the cross-linked complex identifies the cross-linked sequences.

References

1. Sinz, A. (2003). *J. Mass Spectrom.* **38**, 1225-1237.
2. Dihazi, G.H. and Sinz, A. (2003). *Rapid Commun. Mass Spectrom.* **17**, 2005-2014.
3. Muller, D.R., *et al.* (2001). *Anal. Chem.* **73**, 1927-1934.

New! Deuterated Cross-Linking Reagents

For the analysis of protein tertiary structure and protein interactions by MS.

Highlights:

- Well-characterized, high-purity deuterium-labeled cross-linkers and their hydrogen-containing analogs
- Instructions targeted to the application of structure and interaction analysis
- Suberate- and glutarate-based reagent pairs offer a "molecular ruler" option to study of inter- and intra-molecular interactions
- Requires only microgram amounts of protein to perform the analysis
- Excellent alternative to NMR or X-ray crystallography-based methods that require large amounts of protein, special solvents and crystal formation

Ordering Information

Product #	Description	Pkg. Size
21595	**BS^3-d_4** *Bis*(Sulfosuccinimidyl) 2,2,7,7 suberate-d_4 M.W. 576.45, Spacer Arm: 11.4 Å	10 mg
21590	**BS^3-d_0** *Bis*(Sulfosuccinimidyl)suberate-d_0 M.W. 572.43, Spacer Arm: 11.4 Å	10 mg
21615	**BS^2G-d_4** *Bis*(Sulfosuccinimidyl) 2,2,4,4 glutarate-d_4 M.W. 534.38, Spacer Arm: 7.7 Å	10 mg
21610	**BS^2G-d_0** *Bis*(Sulfosuccinimidyl)glutarate-d_0 M.W. 530.35, Spacer Arm: 7.7 Å	10 mg

d_0 designates that the analog contains only hydrogen-substituted methylene carbon atoms in its spacer arm. d_4 designates that the analog contains two methylene carbon atoms in the spacer fully substituted with deuterium.

Cross-linking Reagents for the Analysis of Protein Interactions by Mass Spectrometry Analysis

These reagents have been successfully applied to the inter- and intra-molecular analysis of proteins by MS methods.[1] Watch the Pierce web site (*www.piercenet.com*) and *Previews*, our new products newsletter, for exciting new developments to advance the use of mass spectrometry for three-dimensional and protein interaction analysis of proteins.

Homobifunctional Cross-linking Reagents for MS Analysis

Ordering Information

Product #	Description		Key Features	Ref.	Pkg. Size
21580	**BS³** *Bis*(Sulfosuccinimidyl) suberate	M.W. 572.43 Spacer Arm 11.4 Å	• Water soluble • Amine reactive • Non-cleavable	1,2,3,10	50 mg
21525	**DFDNB** 1,5 Difluoro-2,4-dinitrobenzene)	M.W. 204.09 Spacer Arm 3.0 Å	• Amine reactive • Short distance cross-links	4	1 g
20663	**DMA** Dimethyl adipimidate•2HCl	M.W. 245.15 Spacer Arm 8.6 Å	• Amine reactive • Retains charge character	2.9	50 mg
20700	**DMS** Dimethyl suberimidate•2HCl	M.W. 273.20 Spacer Arm 11.0 Å	• Amine reactive • Retains charge character	2	1 g
20593	**DSG** Disuccinimidyl glutarate	M.W. 326.26 Spacer Arm 7.7 Å	• Amine reactive • Non-cleavable	1,3	50 mg
21578	**DTSSP** 3,3′Dithio*bis*(sulfosuccinimidyl propionate)	M.W. 608.51 Spacer Arm 12 Å	• Amine reactive • Reducing agents cleavable • Water soluble	5,6	50 mg
20591	**Sulfo-DST** Disulfosuccinimidyl tartrate	M.W. 548.32 Spacer Arm 6.4 Å	• Water soluble • Periodate cleavable • Protein S-S bonds remain intact	1,2	50 mg
21566	**Sulfo-EGS** Ethylene glycol *bis*(sulfosuccinimidyl succinate)	M.W. 660.45 Spacer Arm 16.1 Å	• Water soluble • Hydroxyl-amine cleavable	1,2	50 mg
33030	**SAED** Sulfosuccinimimidyl 2-(7-azido-4-methyl-coumarin-3-acetamido)ethyl-1,3′dithio-propionate	M.W. 621.60 Spacer Arm 23.6 Å	• Amine reactive • Photo-reactive • Fluorescent	7	5 mg
21857	**SPDP** *N*-Succinimidyl 3-(2-pyridyldithio)propionate	M.W. 312.37 Spacer Arm 6.8 Å	• Amine reactive • Thiol reactive • Cleavable by reducing agents	8	50 mg
22980	**EDC** 1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide HCl	M.W. 191.70	• Cross-links –COOH with $–NH_2$ • Amide linkage • Zero-length linkage	1	5 g

References

1. Sinz, A. (2003). *J. Mass Spectrom.* **38**, 1225-1237.
2. Dihazi, G.H. and Sinz, A. (2003). *Rapid Commun. Mass Spectrom.* **17**, 2005-2014.
3. Muller, D.R., *et al.* (2001). *Anal. Chem.* **73**, 1927-1934.
4. Chen, X., *et al.* (1999). *Anal. Biochem.* **273**, 192-203.
5. Bennett, K.L., *et al.* (2000). *Protein Sci.* **9**, 1503-1518.
6. Back, J. W., *et al.* (2002). *Protein Sci.* **11**, 2471-2478.
7. Wine, R.N., *et al.* (2002). *Anal. Chem.* **74**, 1939-1945.
8. Itoh, Y., *et al.* (2001). *Proc. Natl. Acad. Sci. USA* **98**, 4883-4887.
9. Pearson, K.M., *et al.* (2002). *Rapid Commun. Mass Spectrom.* **16**, 149-159.
10. Schutz, D.M., *et al.* (2004). *Biochemistry* **43**, 4703-4715.

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Authoritative descriptions of the newest and most effective spectrometric techniques.

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Ordering Information

Product #	Description
20011	**Protein and Peptide Analysis by Mass Spectrometry** *Chapman, J.R., Ed., Published by Humana Press, Inc., 1996, 352 pages, Hardcover*

Complementary Products

Ordering Information

Product #	Description	Pkg. Size
Protein Assay Products		
23236	Coomassie Plus™ Protein Assay Reagent Kit	950 ml
23225	BCA™ Protein Assay Kit	Kit
23235	Micro BCA™ Protein Assay Reagent Kit	480 assays
23240	Modified Lowry Protein Assay Reagent	Kit
Molecular Weight Marker Mix		
26681	BlueRanger® Prestained Protein Molecular Weight Marker Mix	1 x 48 plate
Reducing Agent		
20291	No-Weigh™ DTT	48 Tubes
20290	Dithiothreitol (DTT)	5 g
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69705	Handee™ Mini-Spin Columns	25 units
69715	Handee™ Microcentrifuge Tubes, 1.5 ml	72/pkg.
69710	Handee™ Resin/Serum Separators	25 units

Product #	Description	MWCO	Pkg. Size
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20439	D-Salt™ Excellulose™ Desalting Columns	5K	5 x 2 ml
20449	D-Salt™ Excellulose™ Desalting Columns	5K	5 x 5 ml
D-Salt™ Dextran Desalting Columns			
43230	D-Salt™ Dextran Desalting Columns	5K	5 x 5 ml
43233	D-Salt™ Dextran Desalting Columns	5K	5 x 10 ml
D-Salt™ Polyacrylamide Desalting Columns			
43426	D-Salt™ Polyacrylamide Desalting Columns	1.8K	5 x 5 ml
43240	D-Salt™ Polyacrylamide Desalting Columns	6K	5 x 5 ml
43243	D-Salt™ Polyacrylamide Desalting Columns	6K	5 x 10 ml
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Visit www.piercenet.com or see the Pierce Applications Handbook & Catalog for the complete line of Slide-A-Lyzer® Dialysis Cassettes (U.S. Patent # 5,503,741) and Slide-A-Lyzer® MINI Dialysis Units (U.S. Patent # 6,039,871).

Protein Concentration

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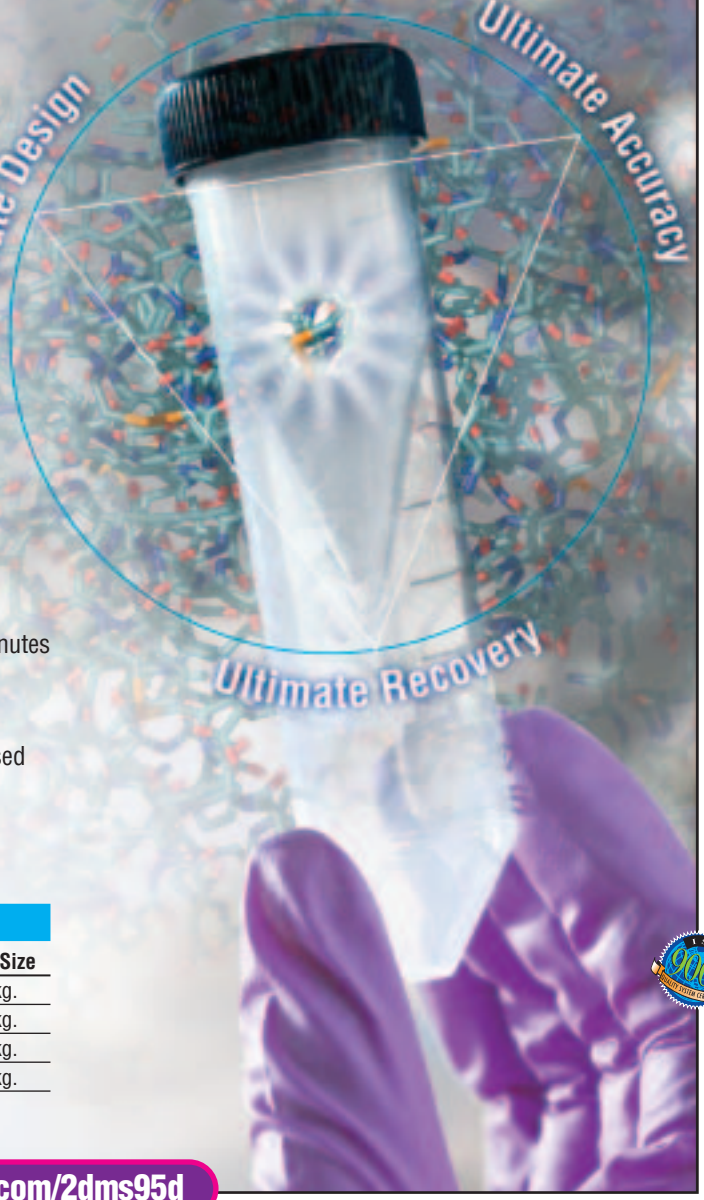

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- Excellent recovery of dilute proteins

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89886	iCON™ Concentrators, 7 ml, 20K MWCO	25/pkg.
89887	iCON™ Concentrators, 20 ml, 20K MWCO	25/pkg.

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