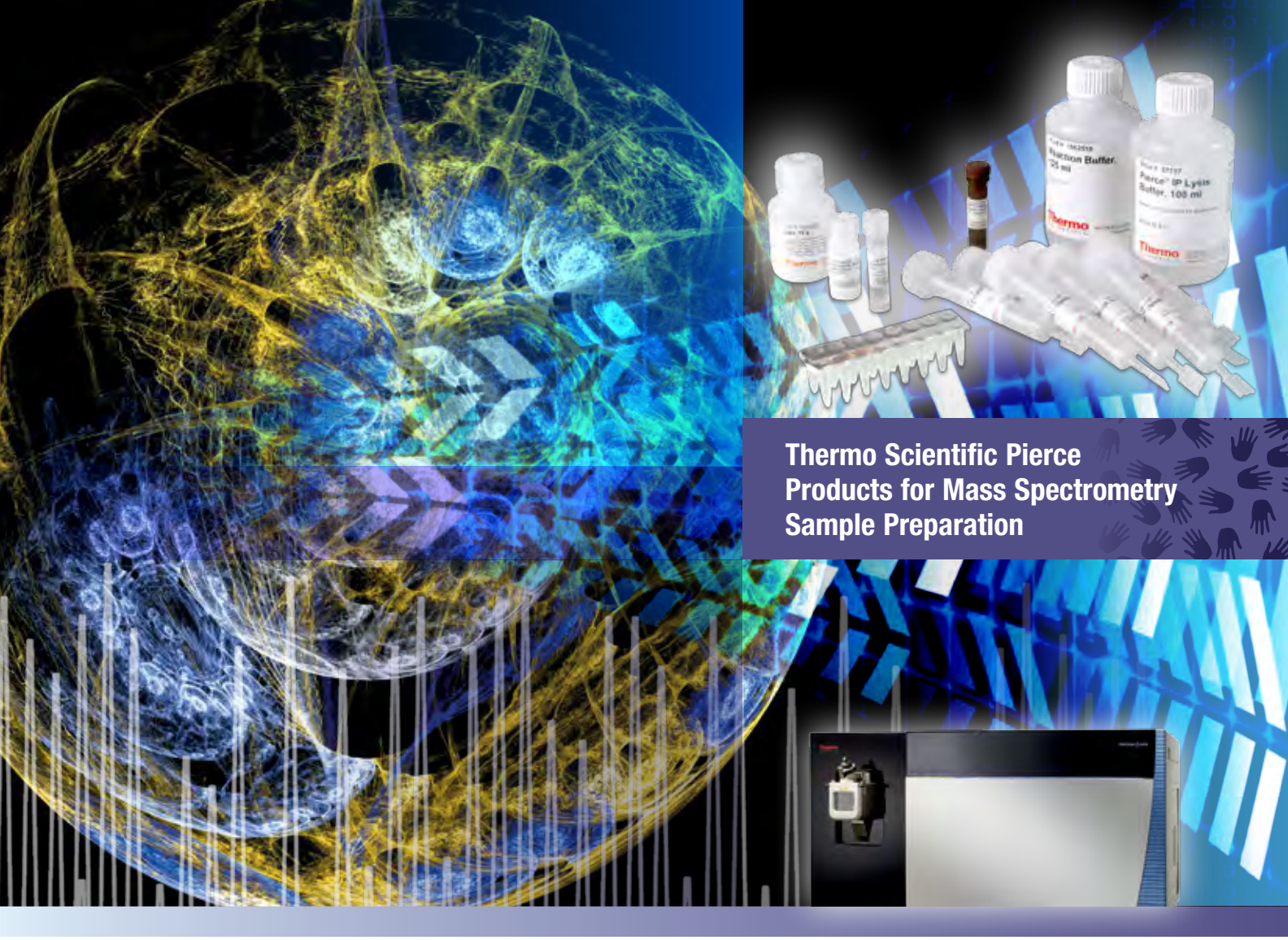

sample preparation
for mass spectrometry

Tools and reagents to extract, digest, enrich and clean up proteins and peptides

Thermo SCIENTIFIC

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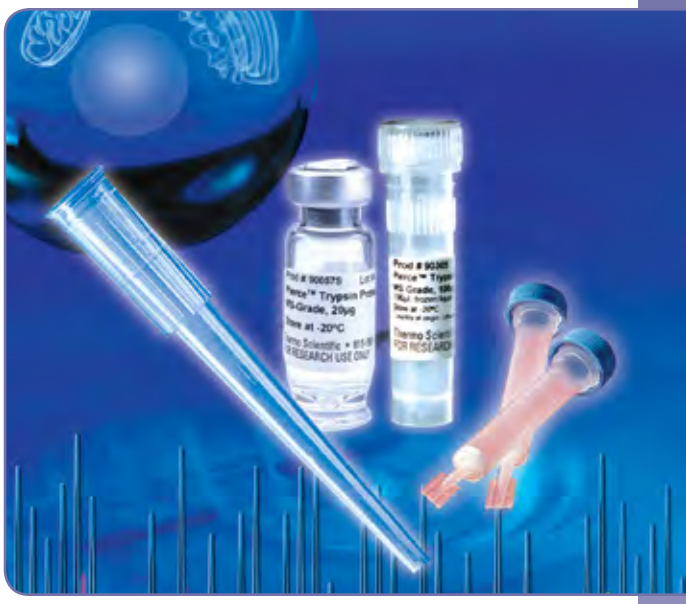
Thermo Scientific Pierce Products for Mass Spectrometry Sample Preparation

Overview of Mass Spectrometry Reagents, Instrumentation and Software



Mass spectrometry (MS) has become the method of choice for protein analysis. The accuracy, sensitivity and flexibility of MS instruments have enabled new applications in biological research, biopharmaceutical characterization and diagnostic detection. MS can identify and quantify known and unknown compounds by revealing their structural and chemical properties. With all of its many forms of ionization and measurement (e.g., ESI, MALDI, FT-MS, ion trap, time-of-flight, quadrupole, etc.), MS allows for the analysis of samples ranging in mass from 50-300,000 daltons, in attomole through nanomole quantities.

Proper sample preparation for MS-based analysis is a critical step in the proteomics workflow. This is because sample preparation is one of the most variable and time-consuming steps in the protein analysis, and the quality and reproducibility of sample extraction and preparation significantly impact the results from MS instruments. The most successful proteomics laboratories recognize that sample preparation, instrumentation and software are all critical to success in proteomics research, and that all three components must be properly integrated into robust workflows for consistent, high-quality results (Figure 1).

Because the proteome is so complex, there is no one standard method for preparing protein samples for MS analysis. Protocols differ depending on sample type, experimental goals and method of analysis. For example, preparing samples from a biological fluid involves a different set of procedures than those used for tissue or cultured cells, and different sample preparation techniques can be used to enrich for subcellular fractions or protein complexes. Many factors are considered when designing sample preparation strategies, including source, type, physical properties, abundance, complexity, matrix effects and cellular location of the proteins. Workflows that incorporate optimized cellular lysis, subcellular fractionation, enrichment via antibody or post-translational modification (e.g., phosphorylation, glycosylation, ubiquitination), and mass tagging tools enable accurate quantitation of global protein expression in complex samples under control versus experimental conditions.

Proper Sample Preparation Means Better Results

Proteins of interest to biological researchers are generally present in a complex mixture of other proteins. This presents two significant problems in MS analysis. First, the ionization techniques used for large molecules work well when the mixture contains roughly equal amounts of constituents. However, with biological samples, proteins tend to be present in widely differing amounts. If such a mixture is ionized using electrospray or MALDI, the more abundant species have a tendency to "drown" or suppress signals from less abundant ones. The second problem is that the mass spectrum from a complex mixture is very difficult to fully analyze because of the overwhelming number of components. This problem is exacerbated by enzymatic digestion of a protein sample into a large number of peptide products. The success of liquid chromatography tandem mass spectrometry (LC-MS/MS) depends on clean samples with limited sample complexity to minimize suppression of ionization by high-abundance species and to prevent MS undersampling of eluting peptides.

Preparation of proteins for MS analysis can be accomplished by many methods, so it is important to understand the steps leading to analysis. While intact proteins are typically studied by gel electrophoresis, the most common MS workflows for complex protein samples analyze peptides. Peptides are easier than proteins to fractionate by reversed-phase chromatography, they ionize and fragment more efficiently than proteins, and the resulting spectra are easier to interpret for protein identification.

introduction

An overview

Preparation of peptides involves reduction and alkylation of cysteines, digestion of the sample with a specific endoproteinase, desalting and concentration of the peptides, and then analysis of these peptides by MALDI-MS or LC-MS/MS. The LC-MS/MS system is configured so that peptides are ionized and sprayed into the mass spectrometer (e.g., electrospray ionization or ESI) as they elute from a C18 reversed-phase HPLC column. As peptides elute, the mass spectrometer cycles in real time, alternating on a millisecond time scale between measuring the mass of eluting peptides (MS stage), or isolating and fragmenting individual peptides to provide sequence information (MS/MS stage). This LC-MS/MS data contains the intact mass and fragmentation patterns of eluted peptides, which is compared to a protein sequence database to identify and assemble the information into protein identifications. Post-translational modifications are verified by including the corresponding mass shifts in the peptide and fragment ions during the database search. This requires knowledge of the source organism's genomic or proteomic sequences and likely post-translational protein modifications.

Thermo Fisher Scientific offers a complete workflow of sample preparation solutions designed for better MS analysis. These workflows are based upon the context of biology, which might include subcellular fractionation, enrichment based upon post-translational modifications (e.g., phosphorylation), or immunoprecipitation of low-abundant target. We recognize the need to provide complete solutions and technical support for protein research and analytical characterization using MS. These reagents and instructions are well tested and supported to assist the biologist and the mass spectrometrist to succeed in their research. Robust, integrated workflows provide consistent results between labs and eliminate wasted time spent troubleshooting experimental methods and results. This handbook highlights reagents and methods for preparing samples from a variety of starting materials for successful MS analysis.

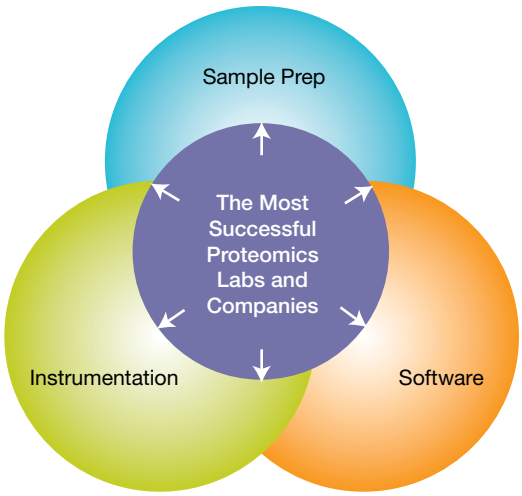


Figure 1. The key to proteomics success. Successful proteomics laboratories and companies recognize the importance of sophisticated sample preparation, instrumentation, and software technologies and skills. Workflows designed to maximize the overlap between these complementary technologies are an effective means of improving proteomics research.

Discovery vs. Targeted Proteomic Analysis

A successful proteomics experiment requires integration of the right sample preparation, instrumentation and software. In addition to these tools, a proteomics scientist also needs the right strategy to achieve the intended goals. Project managers are familiar with the conflicts of time, cost and scope; it is impossible to increase one of these without affecting the others.

For example, if the scope of a project is increased, it is understood that it will take more time or cost more money. Similarly, proteomics researchers must recognize the conflict of scalability, sensitivity and comprehensive analysis. It is impossible to achieve all three simultaneously. Strategies to improve sensitivity and comprehensiveness generally require large sample quantities and multi-dimensional fractionation, which sacrifices throughput. Alternatively, efforts to improve the sensitivity and throughput of protein quantification necessarily limit the number of features that can be monitored. For this reason, proteomics research is typically divided into two categories: discovery and targeted analysis. Discovery proteomics maximizes protein identification by spending more time and effort per sample and reducing the number of samples analyzed. In contrast, targeted proteomics strategies limit the number of features that will be monitored, and then optimize the chromatography, instrument tuning and acquisition methods to achieve the highest sensitivity and throughput for hundreds or thousands of samples.

Discovery Quantitation

Discovery proteomics experiments are intended to identify as many proteins as possible across a broad dynamic range. This often requires depletion of highly abundant proteins, enrichment of relevant proteins (e.g., protein immunoprecipitation), and fractionation steps to decrease sample complexity (e.g., SDS-PAGE or chromatography). These strategies reduce the dynamic range between components in an individual sample and reduce the competition between proteins or peptides for ionization and MS duty cycle time. Quantitative discovery proteomics experiments add a further challenge because they seek to identify *and* quantify protein levels across multiple samples. Quantitative discovery proteomics experiments utilize label-free or stable isotope labeling methods to quantify proteins. Label-free strategies require highly reproducible fractionation, increased instrumentation time and alignment of peptides across LC-MS/MS

experiments to compare spectral counts or ion intensities. Stable isotope protein labeling strategies (e.g., SILAC and Thermo Scientific™ Tandem Mass Tag™ methods) incorporate ^{2}H, ^{13}C, ^{15}N or ^{18}O isotopes into proteins and peptides, resulting in distinct mass shifts but otherwise identical chemical properties. This allows two to 10 samples to be labeled and combined prior to processing and LC-MS/MS analysis. Multiplexing reduces sample processing variability, improves specificity by quantifying the proteins from each condition simultaneously, and requires less LC-MS and data analysis time.

Quantitative proteomic studies are typically performed on high-resolution hybrid mass spectrometers, such as the Thermo Scientific™ Orbitrap™ Fusion™ Tribrid,™ Thermo Scientific™ Orbitrap Elite™ and Thermo Scientific™ Q Exactive™ Mass Spectrometers.

Targeted Quantitation

Targeted proteomics experiments are typically designed to quantify less than 100 proteins with very high precision, sensitivity, specificity and throughput. Targeted MS quantitation strategies use specialized workflows and instruments to improve the specificity and quantification of a limited number of features across hundreds or thousands of samples. Quantitative proteomic experiments are increasingly used in pharmaceutical and diagnostic applications to quantify proteins and metabolites in complex samples. These methods typically minimize the amount of sample preparation to improve precision and throughput.

Targeted quantitative proteomic workflows involve protein denaturation, reduction, alkylation, digestion and desalting prior to LC-MS/MS analysis. To improve assay sensitivity and selectivity, immunoprecipitation or abundant protein depletion may be used prior to sample processing. To further improve quantitative precision and accuracy, known amounts of synthetic peptides containing heavy stable isotopes, such as Thermo Scientific™ HeavyPeptide™ Reagents, are added to samples prior to MS analysis. These peptides serve as internal quantitative standards for absolute quantitation of the corresponding natural peptides in a biological sample. To better control for digestion efficiency, heavy proteins synthesized with the Thermo Scientific™ 1-Step Heavy Protein IVT Kit can be spiked into the sample prior to digestion, and then the resulting heavy peptides can be used for relative quantitation across all samples.

Targeted quantitative protein studies are typically performed on triple quadrupole mass spectrometers, such as the Thermo Scientific™ TSQ Quantiva™ Triple Stage Quadrupole Mass Spectrometer. A triple quadrupole mass spectrometer quantifies peptides by serially monitoring specific mass windows for peptides of interest, isolating the peptide(s), fragmenting, and then quantifying several fragment ions specific for each peptide of interest. This selective reaction monitoring (SRM) strategy for targeted quantitation, along with chromatographic retention time information, provides very high sensitivity and specificity. Alternatively, high-resolution and accurate MS instruments, such as the Q Exactive Mass Spectrometer, are being used to quantify proteins with even greater selectivity.

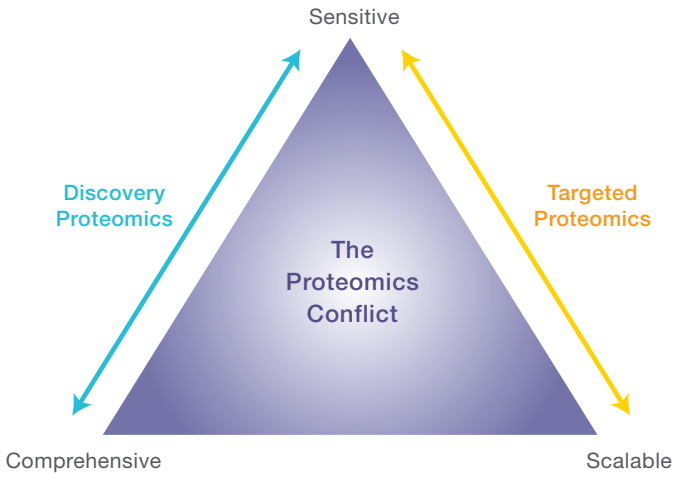
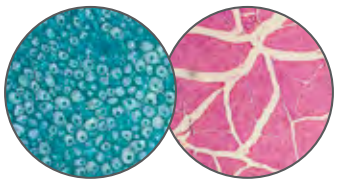


Figure 2. The proteomics conflict. It is impossible to optimize sensitivity, throughput and comprehensiveness simultaneously. Discovery proteomics strategies optimize sensitivity and comprehensiveness with few samples. Targeted proteomics strategies optimize sensitivity and scalability by limiting the number of monitored features.

Mass spectrometry workflow



- Cell lysis reagents
- Protease inhibitors

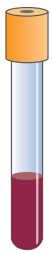
Cells and Tissue → **Lysis and Protein Extraction**

- SILAC stable isotope labeling

Protein Labeling and Enrichment

- Kinase
- GTPase
- Serine hydrolase
- Phosphoprotein
- Immunoprecipitation
- Cysteine-reactive mass tagging reagents

Serum, Plasma and Biofluids → **Abundant Protein Depletion**



- Albumin depletion kit
- Abundant protein depletion columns

- Coomassie stains
- Silver stain for MS

- In-gel tryptic digestion kit

Gel Separation

In-gel Digestion

Protein Clean Up and Quantitation

- Dialysis cassettes
- Desalting columns
- Protein assays

Complete Mass Spec Sample Prep Kit for Cultured Cells

Peptide Labeling and Enrichment

- Amine-reactive mass tagging reagents
- Glycan labeling mass tagging reagents
- Fe-NTA Phosphopeptide enrichment
- TiO_2 Phosphopeptide enrichment

In-solution Digestion

- MS-grade proteases
- In-solution tryptic digestion kit

Peptide Clean Up

- C18 columns, tips, spin tips
- Detergent removal columns
- Graphite spin columns

Mass Spectrometry Instrumentation

- Ion pairing reagents
- Chromatography solvents and blends
- MALDI matrices
- Calibration solutions

sample lysis and protein extraction

Introduction

Tissue or cell lysis is the first step in protein extraction, fractionation and purification. Numerous techniques have been developed to obtain the best possible yield and purity for different species of organisms, sample types (cells, biofluids or tissue), subcellular fractions or specific proteins. Both physical and reagent-based methods may be required to extract cellular proteins because of the diversity of tissue and cell types.

Physical lysis is commonly used to disrupt tissues or cells in order to extract cellular contents. However, it requires specialized equipment and protocols that can be difficult to repeat because of variability in the apparatus (e.g., different dounce pestles or sonication settings). Also, traditional physical disruption methods are typically not conducive for small samples volumes and high-throughput sample handling. Finally, physical lysis methods alone are unable to solubilize membrane-associated proteins. In contrast, reagent-based lysis methods using detergents not only lyse cells, but also solubilize proteins. By using different buffers, detergents, salts and reducing agents, tissue and cell lysis can be optimized to provide the best possible results for a particular cell type or protein fraction.

Separation of distinct subcellular fractions can be achieved through the careful optimization of physical disruption techniques, detergent-buffer solutions and density gradient methods. For example, with the phase-separating detergents, hydrophobic membrane proteins can be solubilized and extracted from hydrophilic proteins. Density gradient centrifugation is another technique that can be used to isolate intact nuclei, mitochondria and other organelles before protein solubilization.

All living organisms contain proteolytic enzymes (proteases and peptidases) for protein catabolism. Protease activities are tightly regulated by compartmentalization and inhibitors to prevent indiscriminate damage to cellular proteins. Cell lysis disrupts cell membranes and organelles resulting in unregulated proteolytic activity that can reduce protein yield and function. To prevent extracted protein degradation, it is often necessary to add protease inhibitors to cell lysis reagents. Protease inhibitors are biological or chemical compounds that function by reversibly or irreversibly binding to protease active sites. Most known proteases belong to one of four evolutionary distinct enzyme families classified by their active site functional groups. Due to the differences in the proteolytic mechanisms between these various classes of proteins (or proteases), no signal compound can effectively inhibit all proteases.

In addition to proteases, phosphatases are another class of enzymes that are liberated during cell lysis. Phosphatases play a key role in regulating signal transduction pathways by removing phosphoryl groups that are transferred to proteins by kinases. Phosphorylation is one of the most common post-translational modifications on proteins with approximately 80% occurring on serine, 20% on threonine and $< 1\%$ on tyrosine residues. Identification of protein phosphorylation sites and occupancy typically requires enrichment of phosphoproteins or phosphopeptides (see Protein Enrichment section) before MS analysis. These techniques require intact phospho group modifications for enrichment, which requires the use of phosphatase inhibitors to prevent dephosphorylation.

Most researchers use a mixture or “cocktail” of several different inhibitor compounds to ensure that protein extracts do not degrade before analysis of targets of interest. Protease inhibitors are nearly always needed, while phosphatase inhibitors are required only when investigating phosphorylation states (activation states). Particular research experiments may require the use of single inhibitors or customized mixtures, but most protein work is best served by using a broad-spectrum protease inhibitor cocktail.

When the goal of cell lysis is to purify or test the function of a particular protein(s), special attention must be given to the effects that the lysis reagents have on the stability and function of the target proteins. Certain detergents will inactivate the function of particular enzymes or disrupt protein complexes. Downstream analysis of extracted/purified proteins may also require salt and/or detergent removal in order to study proteins of interest or maintain long-term stability of the extracted protein.

Thermo Scientific Pierce Mass Spec Sample Prep Kit for Cultured Cells

Optimized isolation and digestion of protein samples for MS analysis.

The Thermo Scientific™ Pierce™ Mass Spec Sample Prep Kit for Cultured Cells is an easy-to-use, comprehensive kit for preparation of clean peptide mixtures from cultured cells for MS analysis.

The Pierce Mass Spec Sample Prep Kit for Cultured Cells contains all the necessary reagents and enzymes to prepare up to 20 samples (1 million cells each) for MS analysis. The simple and robust workflow uses a lysis protocol that generates approximately 100µg of protein per sample. The kit includes the Thermo Scientific™ Pierce™ Digestion Indicator as an internal protein/peptide control to monitor the efficiency of the two-step enzymatic digestion protocol. The procedure consistently and reproducibly produces clean peptide mixtures for protein identification.

Highlights:

- **Complete** – includes all reagents, a digestion indicator control, proteases and an optimized protocol needed to process up to 20 samples
- **Simple** – user-friendly kit can provide reproducible results even for non-expert MS analysts
- **Flexible** – can be adapted to handle sample sizes between 10-200µg
- **High yield** – total protein yield from one million cells is greater than 100µg
- **Optimized** – cysteine reduction and alkylation are 100% with less than 1% over-alkylation of non-cysteine residues
- **Efficient** – percentage of missed cleavages is less than 10%
- **Compatible** – final preparation is ready for direct MS analysis and other downstream applications, including mass-tag labeling

Sample prep for MS remains one of the largest bottlenecks associated with MS analysis, and consistent and reproducible sample preparation can make the difference between a successful analysis and a failed outcome. Current sample prep protocols are primarily homebrew and can be highly variable, making data analysis and interpretation difficult. The Pierce Mass Spec Sample Prep Kit for Cultured Cells provides researchers with all the necessary tools to generate consistent and reproducible protein digests that are directly compatible with LC-MS workflows. The digests do not require further processing, such as C18 clean up or detergent removal.

The Pierce Digestion Indicator, which can be purchased separately, is a non-mammalian recombinant protein (26kDa) with five signature peptides for use in determining the digestion efficiency and reproducibility across multiple samples. The protein sequence and recommended peptides to monitor across samples are supplied with the product instructions.

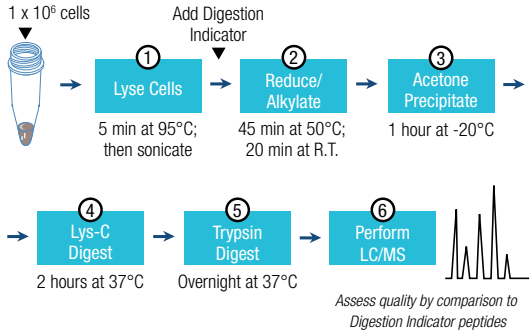


Figure 1. Protocol summary for the Thermo Scientific Pierce Mass Spec Sample Prep Kit for Cultured Cells. All reagent and enzymes are supplied in the kit, except trifluoroacetic acid (TFA), phosphate-buffered saline (PBS) and acetone. Thermo Scientific™ Pierce™ Universal Nuclease for Cell Lysis (Product # 88700) can be used as an alternative to sonication for cell lysis.

Table 1. Comparison of four MS sample prep methods. Summary of the optimized Pierce Mass Spec Sample Pre Kit for Cultured Cells sample preparation protocol compared to three other popular proteomic sample prep methods.

Pierce Kit	FASP	AmBic SDS	Urea
Extract with Lysis Buffer, heat	Extract with 4% SDS, DTT, heat	Extract in AmBic, 0.1% SDS, heat	Extract with 8M urea
Sonicate	Sonicate	Sonicate	Sonicate
Add Digestion Indicator, then reduce	Remove SDS by urea washes and spin concentrator	Reduce	Reduce
Alkylate	Alkylate	Alkylate	Alkylate
Acetone Precipitate	Remove urea and IAM by spin concentrator	–	–
Lys-C Digest	–	–	–
Trypsin Digest	Trypsin Digest	Trypsin Digest	Trypsin Digest
–	Recover peptides by NaCl washes and spin concentrator	–	–
–	C18 Desalt	C18 Desalt	C18 Desalt
LC-MS	LC-MS	LC-MS	LC-MS
Time: 4.5hr hands-on	**Time:** 7hr hands-on	**Time:** 5.5hr hands-on	**Time:** 5hr hands-on

sample lysis and protein extraction

Easily isolate and stabilize protein from samples

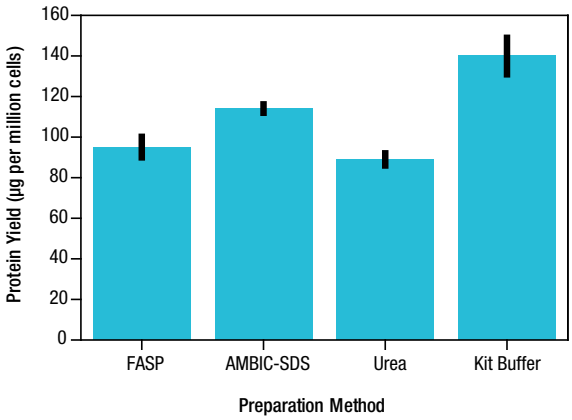


Figure 2. Comparison of protein yields by four MS sample prep lysis methods. From one culture of HeLa S3 cells, duplicate pellets containing 2 x 10^6 cells were resuspended and lysed using 0.2mL of the respective buffers and processed according to each protocol using sonication. Then, protein concentrations and yields were determined.

- FASP: 0.1M Tris-HCl, 4% SDS, 0.1M DTT, pH 7.6
- AmBic-SDS: 0.05M ammonium bicarbonate, 0.1% SDS, pH 8.0
- Urea: 0.1M Tris-HCl, 8M urea, pH 8.5
- Kit Buffer: Lysis Buffer from the Pierce Kit (Product # 84840)

Table 3. Comparison of peptide and protein identification results by four MS sample prep methods. From one source culture of HeLa cells, triplicate pellets (2 x 10^6 cells each) were lysed by each method. Subsequently, 100µg amounts of each replicate lysate were processed by the respective protocol. Finally, 500ng samples were analyzed by LC-FT MS/IT MS2 CID on an Orbitrap Elite Mass Spectrometer.

Feature	Pierce Kit	FASP	AmBic SDS	Urea
Number of Proteins	3964 ± 22	3894 ± 13	3716 ± 79	3756 ± 91
Number of Unique Peptides	19902 ± 190	18738 ± 128	17401 ± 587	19398 ± 689
Missed Cleavages (%)	7.3 ± 0.1	13.9 ± 1.2	17.5 ± 1.3	9.8 ± 1.0
Disulfide Bond Reduction (%)	100	100	100	100
Methionine Oxidation (%)	3.0 ± 0.1	11.3 ± 1.5	2.6 ± 0.1	5.3 ± 0.5
Cysteine Alkylation (%)	99.8 ± 0.4	99.8 ± 0.3	100.0 ± 0.0	100.0 ± 0.0
Over Alkylation (%)	0.7 ± 0.2	0.1 ± 0.1	0.8 ± 0.6	2.4 ± 0.4

Table 2. Consistent, clean and reproducible LC-MS/MS results of three biological replicates. HeLa cell lysate (200µg in 200µL of Lysis Buffer and spiked with 2µg Pierce Digestion Indicator) was processed by the Pierce Mass Spec Sample Prep Kit for Culture Cells and then analyzed by MS.

	Sample 1	Sample 2	Sample 3
Number of Proteins	3382	3228	3376
Number of Unique Peptides	16,333	15,939	17,048
Missed Cleavages (%)	< 10	< 10	< 10
Disulfide Bond Reduction (%)	100	100	100
Cysteine Alkylation (%)	300	100	100
Over Akylation (%)	0.1	0.3	0.9
Digestion Indicator Protein Sequence Coverage (%)	62.50	62.93	65.09

References

1. Wisniewski, JR., *et al.* (2009). Universal sample preparation method for proteome analysis. *Nat. Methods.* **6**:359-60.
2. Bereman, MS., Egertson, JD., MacCoss, MJ. (2011). Comparison between procedures using sodium dodecyl sulfate for shotgun proteomic analyses of complex samples. *Proteomics* **11**:2931-5.

Ordering Information

Product #	Description	Pkg. Size
84840	**Pierce Mass Spec Sample Prep Kit for Cultured Cells** *Sufficient for 20 protease digestions and preparations from 100µg of cell lysate.*	20-rxn kit
	Includes: Cell Lysis Buffer	5mL
	Digestion Buffer	5mL
	DTT, No-Weigh Format	24 tubes
	IAA, No-Weigh Format	24 tubes
	Trypsin Storage Solution	250µL
	Pierce Digestion Indicator	10µg
	Lys-C Protease, MS Grade	20µg
	Trypsin Protease, MS Grade	2 x 20µg
84841	**Pierce Digestion Indicator for Mass Spectrometry** *Sufficient for production of five signature peptides upon digestion for MS.*	10µg

Thermo Scientific Protease and Phosphatase Inhibitor Cocktails

Inhibit protease activity and/or protect against specific phosphatase activities during cell lysis and protein extraction with these ready-to-use inhibitor solutions or tablets.

Protease and phosphatase inhibitors are essential components of most cell lysis and protein extraction procedures. These inhibitors block or inactivate endogenous proteolytic and phospholytic enzymes that are released from subcellular compartments during cells lysis and that could degrade or modify proteins of interest.

Inhibitor Cocktails, Liquids and Tablets

In addition to individual protease inhibitors, we offer convenient, ready-to-use, broad-spectrum protease and phosphatase inhibitor cocktails and tablets. The inhibitor cocktails are available as both 100X cocktail solutions (i.e., Thermo Scientific™ Halt™ Inhibitor Cocktails) and quick-dissolving tablets (Thermo Scientific™ Pierce™ Inhibitor Tablets) to accommodate general and specific needs in cell lysis and protein extraction methods. These inhibitors are ideal for the protection of proteins during extraction or lysate preparation from cultured cells, animal tissues, plant tissues, yeast or bacteria.

These protease inhibitor cocktails and tablets target serine-, cysteine- and aspartic acid proteases, and aminopeptidases. Metalloproteases are inhibited by the optional addition of EDTA (available in a separate vial in the liquid format and included in the tablet format). The phosphatase inhibitor cocktails and tablets contain chemical compounds that target serine/threonine and tyrosine phosphatases.

For further savings and convenience, combined Thermo Scientific™ Protease and Phosphatase Inhibitor Cocktails and Tablets are offered. These prevent protein degradation and preserve phosphorylation simultaneously, providing complete protection in a single solution or tablet.

All Halt Inhibitor Cocktails and Pierce Inhibitor Tablets are compatible with Thermo Scientific™ Pierce™ Protein Extraction Reagents and most homemade and commercial cell lysis solutions.

Halt Inhibitor Cocktails are ready-to-use, 100X stock solutions. The cocktails are easy to use. Simply pipette the volume of concentrated cocktail your sample requires to ensure complete protection of the resulting protein extract. Halt Protease Inhibitor Cocktails and Combined Protease and Phosphatase Inhibitor Cocktails are available in both EDTA and EDTA-free formulations. Halt Inhibitor Cocktails are available in single-use format (24 x 100µL microtubes) and 1mL, 5mL and 10mL package sizes.

Pierce Protease, Phosphatase, and Combined Protease and Phosphatase Inhibitor Tablets are conveniently provided in vials and may be reconstituted before extract preparation for maximum protection. The formulations are available with or without EDTA. Each Pierce Inhibitor Tablet is sufficient for either 10mL or 50mL of solution.

Highlights:

- **Multiple package sizes** – liquid cocktails are available in 100µL single-use format or 1, 5 and 10mL pack sizes; tablets come in two sizes – for 10 or 50mL volumes to accommodate different volume/pricing needs
- **Convenient** – the refrigerator-stable, 100X liquid or tablet format is more effective and easier to use than individual inhibitors; just pipette the amount you need, or add a tablet to a 10 or 50mL solution
- **No proprietary ingredients** – fully disclosed formulation
- **Two popular formulations** – available with or without EDTA; EDTA-free formulation ensures compatibility with isoelectric focusing or His-tag purification
- **Complete protection** – all-in-one formulations contain both protease and phosphatase inhibitors (combined cocktail only)
- **Compatible** – use with Thermo Scientific™ Pierce™ Cell Lysis Buffers or nearly any other commercial or homemade detergent-based lysis reagent; also works with standard protein assays, including BCA and coomassie (Bradford)

Table 1. Components present in the Thermo Scientific Halt Inhibitor Cocktails and Thermo Scientific Pierce Protease and Phosphatase Inhibitor Tablets.

Inhibitor Component	Target (mechanism)	Protease Liquid Cocktails and Tablets	Phosphatase Liquid Cocktails and Tablets	Combined Protease and Phosphatase Liquid Cocktails and Tablets
AEBSF•HCl	Serine Proteases (irreversible)	X		
Aprotinin	Serine Protease (reversible)	X		X
Bestatin	Aminopeptidase (reversible)	X		X
E-64	Cysteine (irreversible)	X		X
Leupeptin	Serine and Cysteine Proteases (reversible)	X		X
Pepstatin	Aspartic Acid Proteases (reversible)	X		
EDTA†	Metalloproteases (reversible)	X		X
Sodium Fluoride	Serine-Threonine and Acidic Phosphatases		X	X
Sodium Orthovanadate	Tyrosine and Alkaline Phosphatases		X	X
β-glycero-phosphate	Serine-Threonine Phosphatase		X	X
Sodium Pyrophosphate	Serine-Threonine Phosphatase		X	X

† EDTA not in EDTA-free formulations.

sample lysis and protein extraction

Easily isolate and stabilize protein from samples

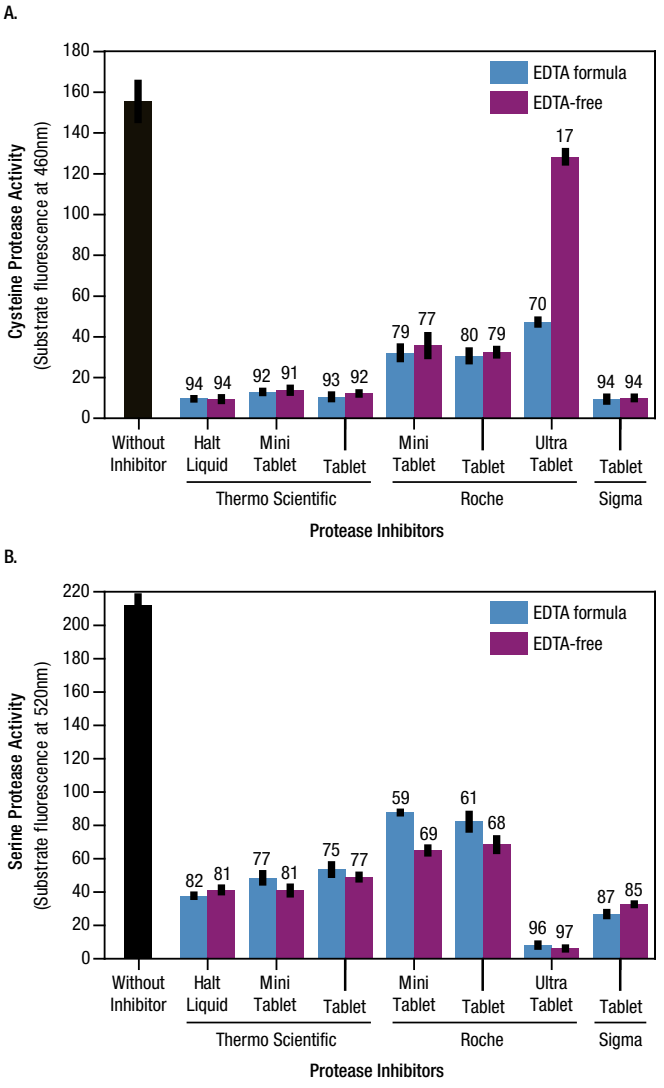


Figure 1. Comparison of commercially available protease inhibitor cocktails and tablets. Pancreatic extract (50µL; 1µg/µL protein) or trypsin (25µL, 0.1 units/µL) was incubated with a quenched-fluorescent, protease-cleavable substrate for cysteine (**A**) or serine proteases (**B**) in the presence or absence of commercially available protease inhibitors with EDTA-containing (blue) or EDTA-free (purple) formulations. Reactions were incubated for two hours at 37°C and the fluorescence determined at indicated detecting emissions. The percent protease inhibition is shown for each protease inhibitor formulation.

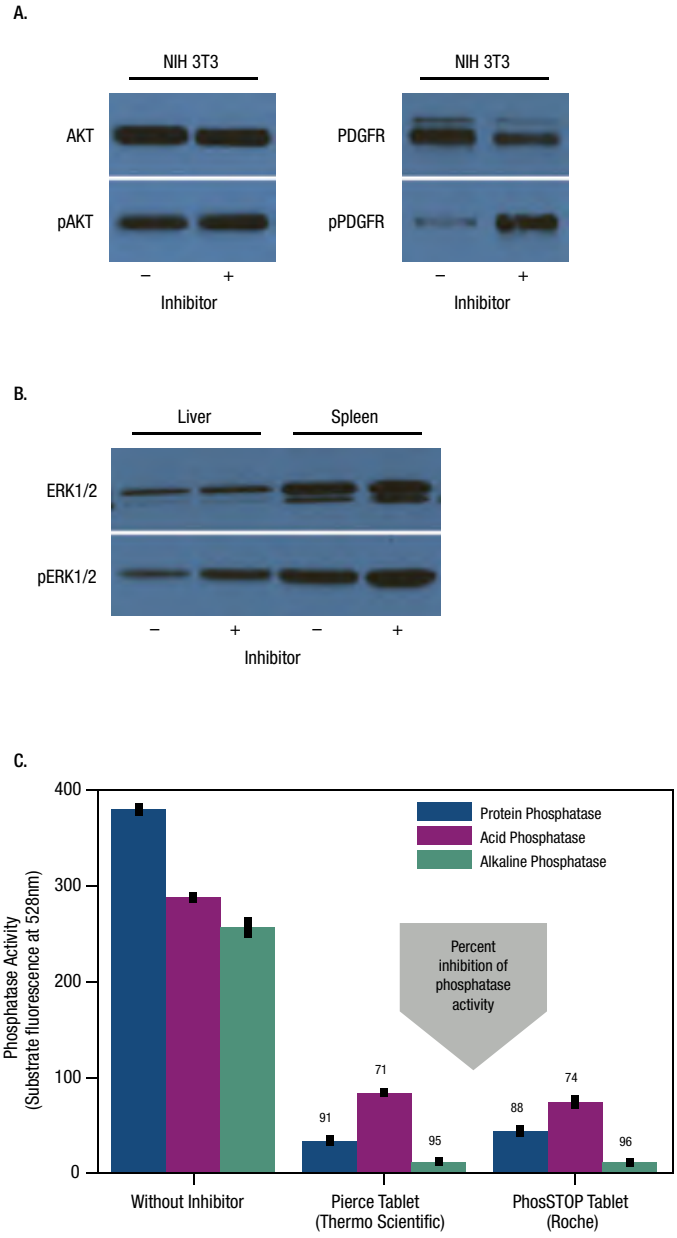


Figure 2. Protein phosphorylation is preserved in cell and tissue extracts. Relative levels of total and phosphorylated protein from extracts prepared in the absence or presence of phosphatase inhibitors were determined by Western blot analysis. (**A**): AKT and PDGFR in serum-starved, PDGF-stimulated (100ng/mL) NIH 3T3 cell extracts. (**B**): ERK1/2 in liver and spleen tissue extracts. (**C**): the degree of inhibition for protein, acid and alkaline phosphatase activity was determined in mouse brain extract after treatment with Pierce Phosphatase Inhibitor Tablets or another commercially available phosphatase inhibitor tablet. Percent inhibition is indicated.

Ordering Information

Product #	Description	Pkg. Size
Protease Inhibitor Cocktails		
78430	Halt Protease Inhibitor Single-Use Cocktail (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
87786	Halt Protease Inhibitor Cocktail (100X) *Sufficient for 100mL of sample.*	1mL
78429	Halt Protease Inhibitor Cocktail (100X) *Sufficient for 500mL of sample.*	5mL
78438	Halt Protease Inhibitor Cocktail (100X) *Sufficient for 1L of sample.*	10mL
78425	Halt Protease Inhibitor Single-Use Cocktail, EDTA-free (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
87785	Halt Protease Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 100mL of sample.*	1mL
78437	Halt Protease Inhibitor Cocktail, EDTA-Free (100X) *Sufficient for 500mL of sample.*	5mL
78439	Halt Protease Inhibitor Cocktail, EDTA-Free (100X) *Sufficient for 1L of sample.*	10mL
Phosphatase Inhibitor Cocktails		
78428	Halt Phosphatase Inhibitor Single-Use Cocktail *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
78420	Halt Phosphatase Inhibitor Cocktail *Sufficient for 100mL of sample.*	1mL
78426	Halt Phosphatase Inhibitor Cocktail *Sufficient for 500mL of sample.*	5 x 1mL
78427	Halt Phosphatase Inhibitor Cocktail *Sufficient for 1L of sample.*	10mL

Product #	Description	Pkg. Size
Combined Protease and Phosphatase Inhibitor Cocktails		
78442	Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
78440	Halt Protease and Phosphatase Inhibitor Cocktail (100X) *Sufficient for 100mL of sample.*	1mL
78444	Halt Protease and Phosphatase Inhibitor Cocktail (100X) *Sufficient for 500mL of sample.*	5 x 1mL
78446	Halt Protease and Phosphatase Inhibitor Cocktail (100X) *Sufficient for 1L of sample.*	10mL
78443	Halt Protease and Phosphatase Inhibitor Single-Use Cocktail, EDTA-Free (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 100mL of sample.*	1mL
78445	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 500mL of sample.*	5 x 1mL
78447	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 1L of sample.*	10mL

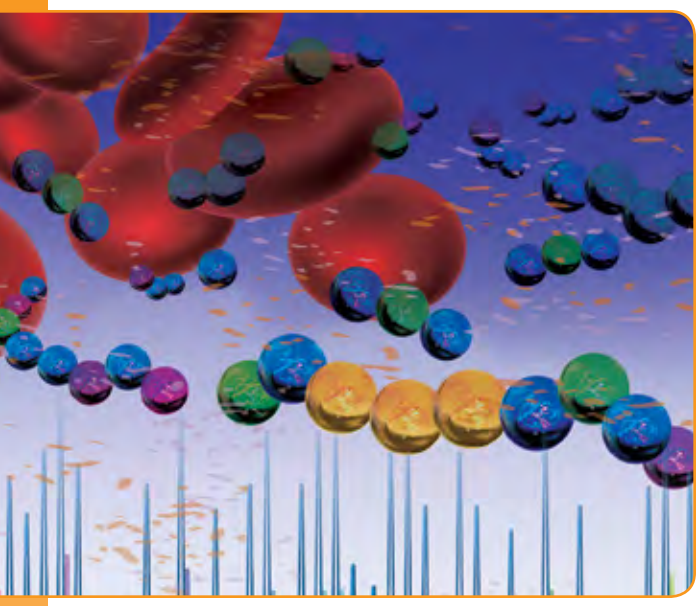
Ordering Information

Product #	Description	Pkg. Size
Protease Inhibitor Tablets		
88665	Pierce Protease Inhibitor Mini Tablets *Sufficient for 300mL of sample.*	30 tablets
88265	Pierce Protease Inhibitor Tablets *Sufficient for 1000mL of sample.*	20 tablets
88666	Pierce Protease Inhibitor Mini Tablets, EDTA-free *Sufficient for 300mL of sample.*	30 tablets
88266	Pierce Protease Inhibitor Tablets, EDTA-free *Sufficient for 1000mL of sample.*	20 tablets

Product #	Description	Pkg. Size
Phosphatase Inhibitor Tablets		
88667	Pierce Phosphatase Inhibitor Mini Tablets *Sufficient for 200mL of sample.*	20 tablets
Combined Protease and Phosphatase Inhibitor Tablets		
88668	Pierce Protease and Phosphatase Inhibitor Mini Tablets *Sufficient for 200mL of sample.*	20 tablets
88669	Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free *Sufficient for 200mL of sample.*	20 tablets

abundant protein depletion

Introduction



Sample complexity negatively affects the ability to detect, identify and quantify low-abundant proteins by MS because peptides from high-abundant proteins can mask detection of those from low-abundant proteins. Therefore, the more that a sample can be simplified and the dynamic range of protein concentrations reduced, the greater the ability to detect proteins at very low concentrations.

Depletion and enrichment strategies have been developed to remove high-abundant proteins or isolate target proteins in the sample, respectively (see Protein Enrichment section). Depletion is more often used to reduce the complexity of biological samples such as serum, plasma or biofluids, which contain high concentrations of albumin and immunoglobulins. Depletion strategies utilize immunoaffinity techniques using immobilized antibodies to remove the most abundant proteins, thus enhancing the detection of lower abundant proteins in both discovery and targeted proteomic analyses. As some high-abundant proteins interact with proteins of lower abundance, low-abundant proteins may also be depleted if they are in a complex with high-abundant proteins.

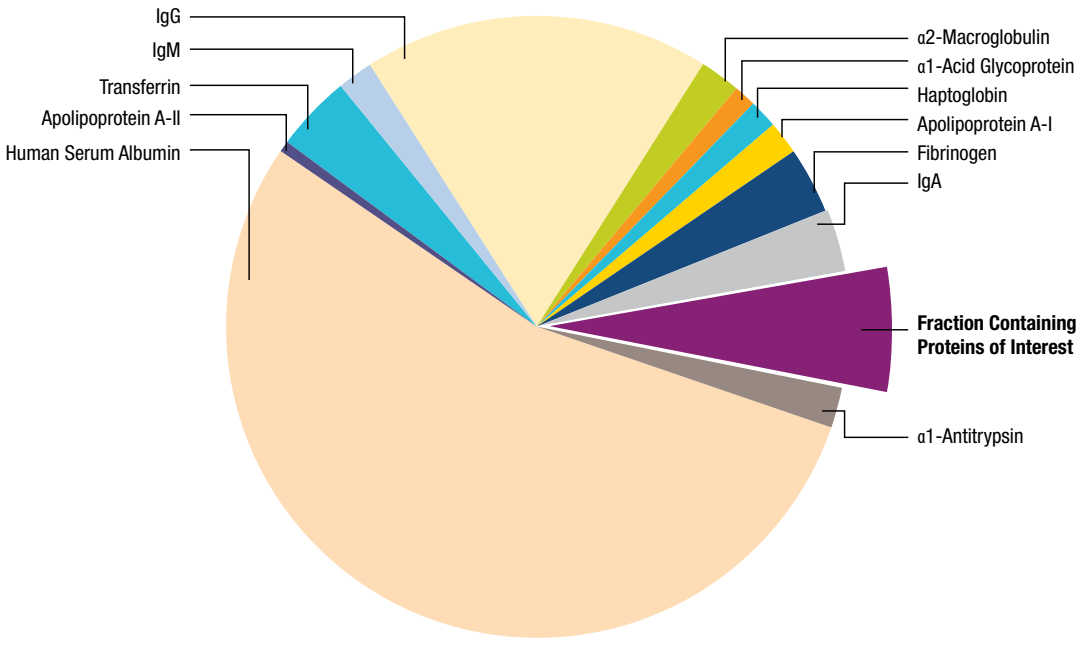


Figure 1. Ratio of protein abundance. Human Serum Albumin and IgG comprise almost 75% of all serum proteins. Other proteins with less (but still significant) abundance include α1-Acid Glycoprotein, α1-Antitrypsin, α2-Macroglobulin, Apolipoprotein A-I, Apolipoprotein A-II, Fibrinogen, Haptoglobin, IgA, IgM and Transferrin, which altogether comprise up to 95% of serum proteins.

Thermo Scientific Pierce Albumin Depletion Kit

Albumin-free serum samples in less than 15 minutes.

The Thermo Scientific™ Pierce™ Albumin Depletion Kit uses an agarose resin of the affinity ligand Cibacron™ Blue dye for high-capacity, selective extraction of human serum albumin from 10 to 50µL serum samples.

Pierce Albumin Depletion Resin is supplied as a 50% slurry. Dispense 200µL of slurry into the supplied microcentrifuge spin columns to obtain 100µL of settled beads for the standard protocol. Each aliquot of resin can be used to process 10 to 50µL of serum sample in a single reaction. Processed samples are ready for immediate downstream analysis by electrophoresis or MS applications. The ease and versatility offered by the slurry format make this kit ideal for single- or multi-sample processing with the microcentrifuge spin columns supplied in the kit.

Highlights:

- **Complete kit** – includes optimized buffer and microcentrifuge spin columns to remove albumin quickly and conveniently from 10 to 50µL samples
- **Easy-to-dispense slurry** – improves performance, eases processing of multiple samples, and can be adapted to larger or smaller columns or 96-well filter plates
- **Workflow compatible** – removing excess albumin facilitates MS or electrophoresis gel analysis of low-abundance serum proteins

Human serum albumin (HSA) often accounts for greater than 60% of the total protein present in serum samples and can have a concentration of approximately 40mg/mL. The high concentration of albumin hinders research, obscuring the detection of many proteins of biological interest. Traditionally, researchers have produced albumin-free samples using chromatography methods involving multiple purification steps. In addition to involving lengthy and tedious procedures, these purification steps also tend to give low protein yields. The Pierce Albumin Depletion Kit was developed to take advantage of the Cibacron Blue Dye albumin binding properties.

The kit is optimized to bind human, porcine and sheep albumin from serum samples. With a modification to the protocol, albumin from bovine, calf, goat and rat can be removed with this method. The kit is not effective for removal of mouse albumin.

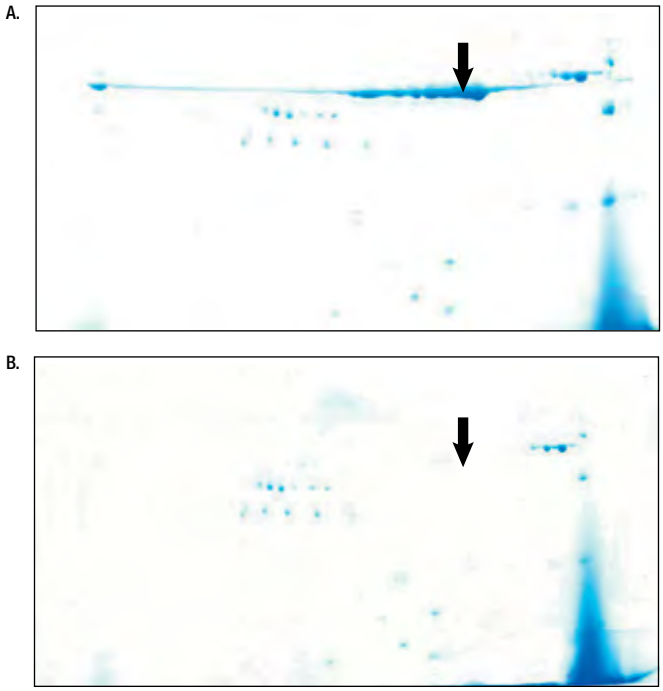


Figure 1. Effective albumin removal improves 2D gel analysis of serum. Panel A. Human serum was diluted five-fold in TBS (i.e., 10µL of serum added to 40µL of TBS), and 5µL of the diluted serum was separated by 2D-SDS PAGE. **Panel B.** Human serum was diluted two-fold in Binding/Wash Buffer (i.e., 50µL of serum added to 50µL of buffer) and processed using the Pierce Albumin Depletion Kit according to the instructions. Albumin-depleted samples and washes were combined (i.e., 100µL sample with 150µL wash buffer) and 5µL was separated by 2D SDS-PAGE. For 2D SDS-PAGE analysis, samples were diluted with 2D SDS-PAGE loading buffer, focused using pH 4-7 isoelectric focusing (IEF) strips and separated using 8-16% Tris-glycine gels. Gels were stained using Thermo Scientific™ GelCode™ Blue Stain (Product # 24590). The black arrow points to the gel region where albumin is located on the duplicate gels before (A) and after (B) processing.

Ordering Information

Product #	Description	Pkg. Size
85160	**Albumin Depletion Kit** *Sufficient for removing albumin from 24 human serum samples, 10 to 50µL each.*	24-rxn kit
	Includes: Pierce Albumin Depletion Resin	5mL
	Binding/Wash Buffer	11mL
	Pierce Spin Columns	24 columns

abundant protein depletion

Quickly reduce sample complexity for better target detection

Thermo Scientific Pierce Top 2 and Top 12 Abundant Protein Depletion Spin Columns

Deplete abundant plasma proteins quickly and economically.

Thermo Scientific™ Pierce™ Abundant Protein Depletion Spin Columns are optimized to decrease the abundant albumin and antibody components of human plasma samples in preparation for MS or 1D and 2D gel electrophoresis. Thermo Scientific™ Pierce™ Top 2 Abundant Protein Depletion Spin Columns use highly specific immobilized anti-HSA and anti-IgG antibodies to remove human serum albumin (HSA) and all major subclasses of gamma globulin (IgG) from serum, plasma or spinal fluids. Similarly, the Thermo Scientific™ Pierce™ Top 12 Abundant Protein Depletion Spin Columns are designed to remove HSA, IgG and 10 other high-abundance proteins from human serum or plasma. The resins are provided in an economical and convenient spin column specifically designed for one-step processing and for single use.

Highlights:

- **Optimized** – resin in spin columns is scaled and optimized for treating 10µL (600µg) of human plasma samples for MS and/or 1D and 2D electrophoresis
- **Efficient** – kits remove > 90% of IgG and > 95% of albumin, plus other abundant proteins (Top 12)
- **Fast** – spin columns process samples in 40 to 60 minutes (depending on resin)
- **Economical** – cost-effective spin columns are priced for single use
- **Consistent** – one-time use prevents abundant protein carryover and experimental variability
- **Flexible** – choose the system appropriate for your need: 2- or 12-protein depletion columns

Analysis of human serum is hindered by the presence of high concentrations of albumin and IgG that can account for more than 70% of the total protein present in the sample. Removal of these proteins is often essential for the study of low-abundant proteins of biological interest by MS or 1D and 2D gel electrophoresis. Traditionally, researchers have produced albumin-free samples using chromatography methods involving multiple purification steps. In addition to involving lengthy and tedious procedures, these purification steps also tend to give low protein yields and poor reproducibility.

The Pierce Top 2 Protein Depletion Columns and Pierce Top 12 Protein Depletion Columns facilitate the removal of highly abundant proteins from serum samples. The Pierce Top 2 Protein Depletion Columns can deplete both albumin (> 95%) and IgGs (> 90%) from human serum, while the Pierce Top 12 Protein Depletion Columns remove the 12 most abundant proteins (> 95%). Each pre-filled depletion column can process 10µL of human serum in 40 to 60 minutes using a convenient spin format compatible with low-speed centrifugation.

Table 1. Proteins removed by Thermo Scientific Pierce Abundant Protein Depletion Spin Columns. Binding and removal of proteins is achieved via specific antibodies, which are immobilized on the affinity support.

Top 2 Columns	Top 12 Columns	
• Albumin	• Albumin	• Apolipoprotein A-II
• IgG	• IgG	• Fibrinogen
	• α1-Acid Glycoprotein	• Haptoglobin
	• α1-Antitrypsin	• IgA
	• α2-Macroglobulin	• IgM
	• Apolipoprotein A-I	• Transferrin

Table 2. Protein removal achieved using Thermo Scientific Pierce Top 12 Abundant Protein Depletion Spin Columns. Values were determined by targeted MS. The albumin depletion percentage was cross-validated by ELISA and was in agreement with > 99% removal.

Protein	Fold Reduction	Percent Depletion
Albumin	3369	99.97
Transferrin	266	99.62
α1-Antitrypsin	37	97.30
Haptoglobin	127	99.21
α1-Acid Glycoprotein	402	99.75
α2-Macroglobulin	116	99.14

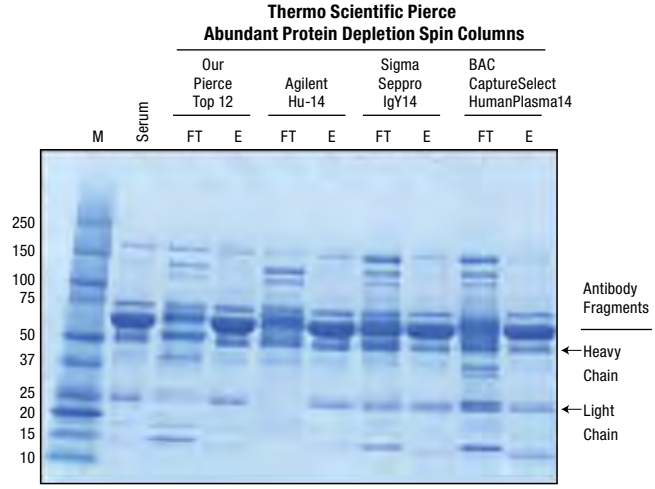


Figure 1. Performance of Thermo Scientific Pierce Top 12 Abundant Protein Depletion Spin Columns compared to equivalent products from other suppliers. Human serum (10-20µL, Product # 31876) was loaded onto each resin and processed according to the manufacturer's protocol (Agilent: Human 14 Multiple Affinity Removal Spin Cartridge, Product # 5188-6560; Sigma: SEPPRO™ IgY14 Spin Column, Product # SEP010; BAC: CaptureSelect™ HumanPlasma 14 Depletion Resin, Product # 291.9914) protocol. Total protein in the depleted fractions was estimated using Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Thermo Scientific, Product # 23225). Total amount of albumin in the depleted fractions was determined using AssayMax™ Human Albumin ELISA Kit (Assaypro, Product # EA2201-1).
FT = flow-through (i.e., depleted sample); **E** = eluate (i.e., proteins that were bound by the resin, plus stripped affinity antibodies of the column). Performance of all four products is comparable in this analysis. With the Top 12 proteins removed, low-abundance proteins are now visible in each depleted sample lane (FT).

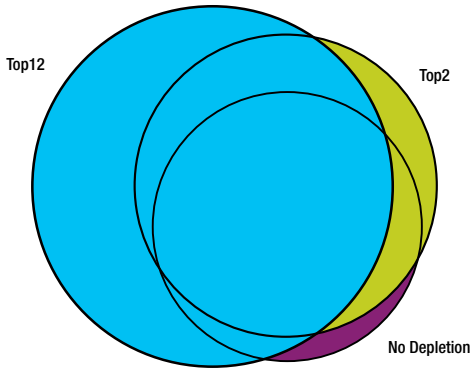


Figure 2. Greater numbers of peptides identified following abundant protein removal. This proportional Venn diagram displays the relative number of unique peptides identified by MS when human serum is depleted by the Pierce Top 2 or Top 12 Columns compared to non-depleted human serum. A simple, fast depletion using the Top 12 Columns doubled the number of unique peptides identified when compared to non-depleted human serum.

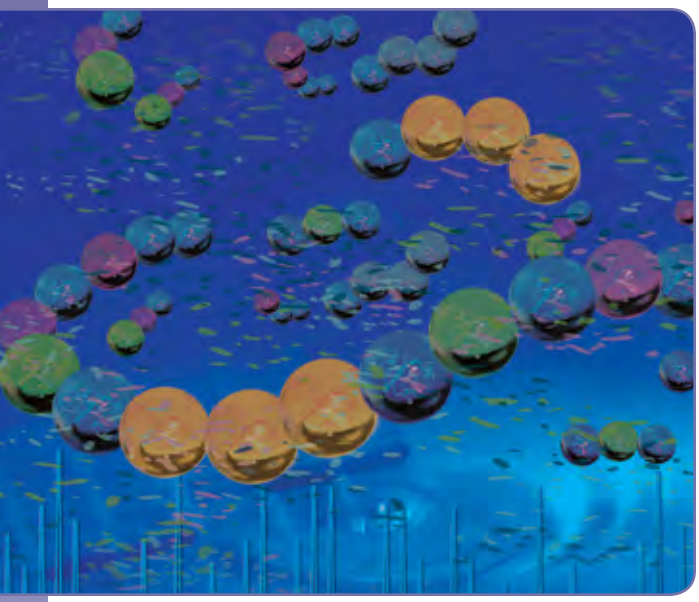
Ordering Information

Product #	Description	Pkg. Size
85161	**Top 2 Abundant Protein Depletion Spin Columns** *Sufficient for removing albumin and IgG from 6 human serum samples, 10µL each.*	6 columns
85162	**Top 2 Abundant Protein Depletion Spin Columns** *Sufficient for removing albumin and IgG from 24 human serum samples, 10µL each.*	24 columns

Product #	Description	Pkg. Size
85164	**Top 12 Abundant Protein Depletion Spin Columns** *Sufficient for removing α1-acid glycoprotein fibrinogen, α1-anti-trypsin, haptoglobin, α2-macroglobulin IgA, albumin, IgG, apolipoprotein A-I, IgM, apolipoprotein A-II and transferrin from 6 human serum samples, 10µL each.*	6 columns
85165	**Top 12 Abundant Protein Depletion Spin Columns** *Sufficient for removing α1-acid glycoprotein fibrinogen, α1-anti-trypsin, haptoglobin, α2-macroglobulin IgA, albumin, IgG, apolipoprotein A-I, IgM, apolipoprotein A-II and transferrin from 6 human serum samples, 10µL each.*	24 columns

protein enrichment

Introduction



Protein enrichment encompasses numerous techniques to isolate subclasses of cellular proteins based on their unique biochemical activity, post-translational modification (PTM) or spatial localization in a cell. Protein enrichment is essential for studying low-abundant proteins and for reducing the complexity of samples for proteomic analysis. Enrichment of specific proteins or protein complexes can most easily be accomplished using immunoaffinity techniques such as immunoprecipitation and co-immunoprecipitation. Although these antibody-based techniques are widely used, elution of immunoprecipitated proteins can sometimes result in low protein recovery or antibody contamination in samples.

Global protein enrichment strategies involve the selective isolation of distinct protein subclasses that share a common PTM or cellular localization. Post-translational modifications such as phosphorylation and glycosylation can be enriched using affinity ligands such as ion-metal affinity chromatography (IMAC) or immobilized lectins, respectively. In addition, PTM-specific antibodies can be used. Other techniques use metabolic or enzymatic incorporation of modified amino acids or PTMs to introduce unique protein chemistry that can be used for enrichment. Finally, proteins can also be enriched using various enzyme class-specific compounds or cell-impermeable labeling reagents that selectively label cell surface proteins.

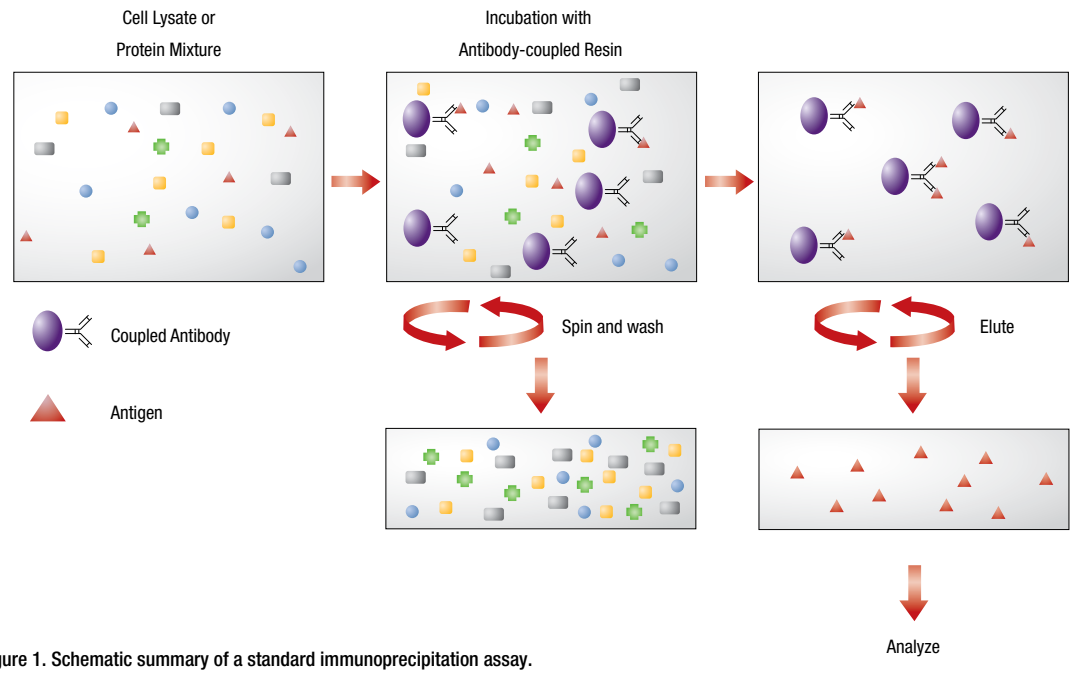


Figure 1. Schematic summary of a standard immunoprecipitation assay.

Thermo Scientific Pierce Phosphoprotein Enrichment Kits

Process cell and tissue samples in less time and with greater purity.

Phosphorylation is one of the most frequently occurring PTMs in proteins. It is estimated that as many as 30% of all cellular proteins are transiently phosphorylated on serine, threonine and tyrosine residues.

Reversible protein phosphorylation regulates nearly all intracellular biological events, including signal transduction, protein-protein interactions, protein stability, protein localization, apoptosis and cell cycle control. Deregulation of protein phosphorylation is a hallmark of numerous human diseases, including cancer and metabolic and immune disorders.

Detecting changes in protein phosphorylation can be a difficult task because of the transient labile state of the phosphate group. Furthermore, low phosphoprotein abundance and poorly developed phospho-specific antibodies contribute to difficulties in phosphoprotein detection. Recent advances in MS technology, in combination with phosphoprotein enrichment using immobilized metal affinity chromatography (IMAC), have resulted in greater resolution of the phosphoproteome.

The Thermo Scientific™ Pierce™ Phosphoprotein Enrichment Kit efficiently enriches phosphorylated proteins derived from mammalian cells and tissues. The proprietary metal and buffer composition produces superior yields with negligible nonspecific binding.

Highlights:

- **Specific** – low contamination from nonspecific proteins
- **Fast** – easy-to-use spin format enriches phosphorylated proteins in less than 2 hours
- **Superior yield** – high yield from complex biological samples, cell culture lysate and mouse tissue extract
- **Convenient format** – complete kit includes pre-dispensed spin columns, buffers, reagents and Thermo Scientific™ Pierce™ Protein Concentrators
- **Compatible** – works with downstream applications, including MS, Western blotting and 2D-PAGE

Phospho-specific antibodies recognizing key regulatory proteins involved in growth factor signaling were used to monitor binding specificity of the Pierce Phosphoprotein Enrichment Kit (Figure 1). Specificity of the kit is further demonstrated by the absence of Cytochrome C (pI 9.6) and p15Ink4b (pI 5.5), two proteins not predicted to be phosphorylated, in the elution fraction and their emergence in the flow-through and wash fractions (Figure 1). Furthermore, dephosphorylation of HeLa cell extract *in vitro* resulted in diminished binding of PTEN, MAPK and GSK3β to the Thermo Scientific™ Pierce™ Phosphoprotein Enrichment Column as evidenced by their absence in the elution fraction. In contrast, all three proteins were present in the elution fraction from non-treated HeLa extract (Figure 2). The Pierce Phosphoprotein Enrichment Kit provided superior and efficient phosphoprotein enrichment yields when compared to other suppliers' products (Table 1). It also effectively enriched phosphoproteins from homogenized mouse liver tissue (Figure 3).

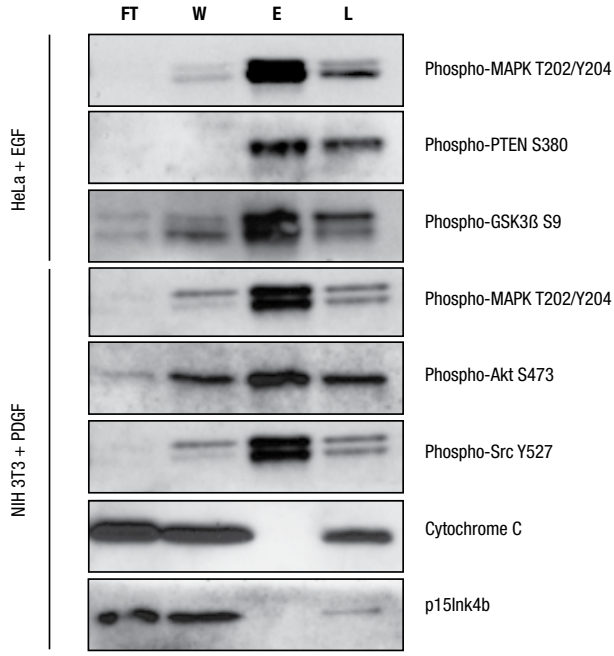


Figure 1. Highly pure phosphoprotein enrichment from complex biological samples. Serum-starved HeLa and NIH 3T3 cells were stimulated with EGF and PDGF, respectively. Cell lysate (2mg) was used for enrichment. Concentrated flow-through, wash and elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10µg/lane). Western blot analysis was performed using antibodies that detect site-specific phosphorylation events. Cytochrome C (pI 9.6) and p15Ink4b (pI 5.5) served as negative controls for nonspecific binding of non-phosphorylated proteins. FT = flow-through fraction, W = pooled wash fractions, E = pooled elution fractions and L = non-enriched total cell lysate.

protein enrichment

Improved detection by selective protein isolation

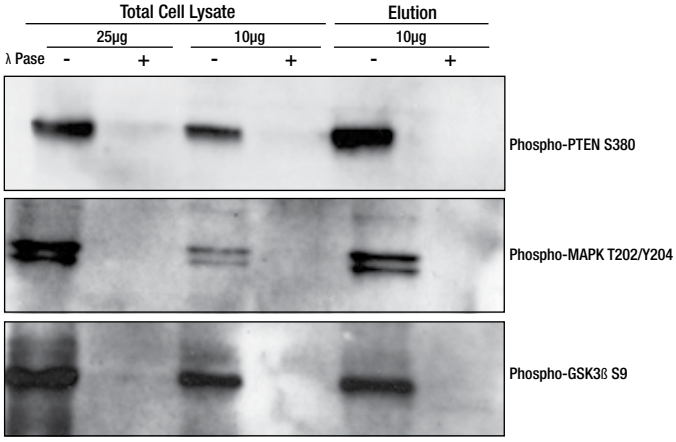


Figure 2. Highly specific phosphoprotein purification from lambda phosphatase-treated cells. Non-treated and lambda dephosphorylated HeLa cell extract (2mg) was loaded onto separate Pierce Phosphoprotein Enrichment Columns. Concentrated elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10µg/lane). To determine enrichment, 10µg and 25µg of non-treated or lambda phosphatase-treated total cell extract (non-enriched) was loaded onto each gel. Western blot analysis was performed using phospho-specific antibodies recognizing key proteins in the Ras-MAPK and PI3K-Akt signaling cascades.

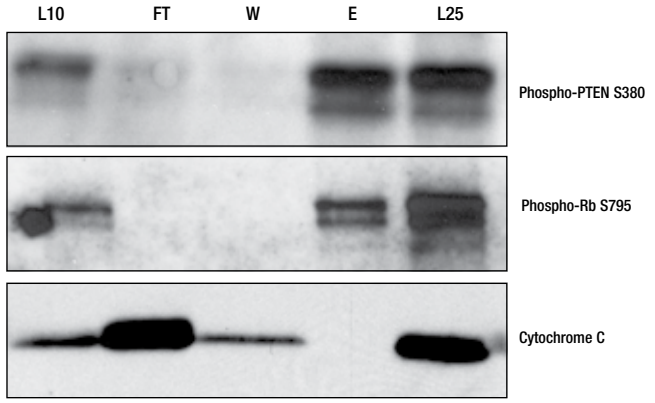


Figure 3. Efficient enrichment of phosphoproteins from mouse liver extract. Homogenized mouse liver extract (~2mg) was loaded onto a Pierce Phosphoprotein Enrichment Column. Concentrated flow-through, wash and elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10µg/lane). Western blot analysis was performed using antibodies that detect site-specific phosphorylation events. Cytochrome C (pI 9.6) served as a negative control for nonspecific binding. L10 = non-enriched total cell extract (10µg), FT = flow-through fraction, W = wash fraction, E = elution fraction and L25 = non-enriched total cell extract (25µg).

Table 1. The Thermo Scientific Pierce Phosphoprotein Enrichment Kit provides higher phosphoprotein yields in less time than other suppliers' kits.

Kit	Yield (%)	Enrichment Time (Hours)
Thermo Scientific Pierce Phosphoprotein Enrichment Kit	15	1.5
Supplier Q Kit	4.4	4.5
Supplier I Kit	2.6*	3.5
Supplier C Kit	8	3
Supplier E Kit	Too dilute to determine	5

** Based on maximum 1mg load per manufacturer's protocol.*

Ordering Information

Product #	Description	Pkg. Size
90003	**Pierce Phosphoprotein Enrichment Kit** *Sufficient for purifying 10 protein samples each containing up to 4mg total protein (400µg phosphoprotein).*	Kit
	Includes: Phosphoprotein Enrichment Column Resin Bed (1mL)	10 ea.
	Lysis/Binding/Wash Buffer	325mL
	Elution Buffer	60mL
	CHAPS	1g
	White Column Caps	10 caps
	Pierce Protein Concentrator 7mL/9K MWCO	10 Devices

Thermo Scientific Pierce Protein A/G Magnetic Beads

High-performance magnetic affinity particles optimized for immunoprecipitation applications.

The Thermo Scientific™ Pierce™ Protein A/G Magnetic Beads are high-performance affinity particles ideally suited for immunoprecipitation methods using manual or robotic magnetic separators.

These magnetic beads are coated with recombinant Pierce Protein A/G, a fusion protein that combines the IgG binding domains of both Protein A and Protein G. This enables capture of antibodies from a wider range of species and isotypes than either protein alone. Using our crosslinker chemistry, you can immobilize an antibody onto the magnetic particle and prevent IgG contamination in your immunoprecipitated sample. These beads can be used both manually with a magnetic stand, as well as with automated platforms such as the Thermo Scientific™ KingFisher™ Instruments.

Highlights:

- **High capacity** – nearly four times higher binding capacity than typical magnetic beads from other suppliers, allowing the use of smaller amounts per experiment
- **Low nonspecific binding** – stable, pre-blocked beads reduce nonspecific protein binding
- **Flexibility** – convenience of IgG binding domains of both Protein A and Protein G on one bead
- **Compatibility** – beads are compatible with manual and automated applications (e.g., KingFisher Instruments)
- **Assay consistency** – magnetic beads eliminate resin loss and provide for more efficient separation of solutions than traditional IP methods that use only microcentrifuge tubes

Applications:

- IP and Co-IP experiments
- Immunoprecipitation for analysis in non-reducing conditions
- Enrichment procedure amenable to both discovery and targeted (SRM) assay development

Product Details:

The recombinant Protein A/G that is immobilized onto the Pierce Magnetic Beads is a fusion of the IgG binding domains of both Protein A and Protein G. Protein A/G contains four Fc-binding domains from Protein A and two from Protein G, making it a convenient tool for investigating and purifying immunoglobulins. Thus, Pierce Magnetic Beads are not simply a mixed immobilization of separate Protein A and Protein G polypeptides, nor are they a mixture of Protein A magnetic beads and Protein G magnetic beads.

Table 1. Properties of Thermo Scientific Pierce Protein A/G Magnetic Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein A/G
Diameter	1µm
Concentration	10mg/mL
Binding Capacity	55 to 85µg rabbit IgG per mg magnetic particles

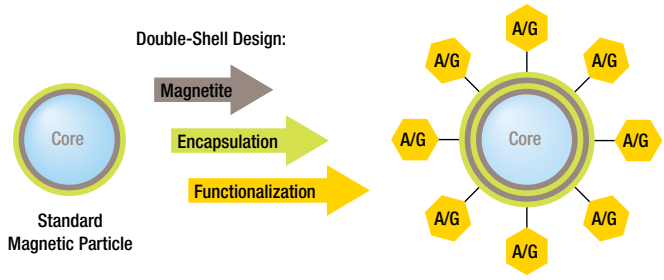
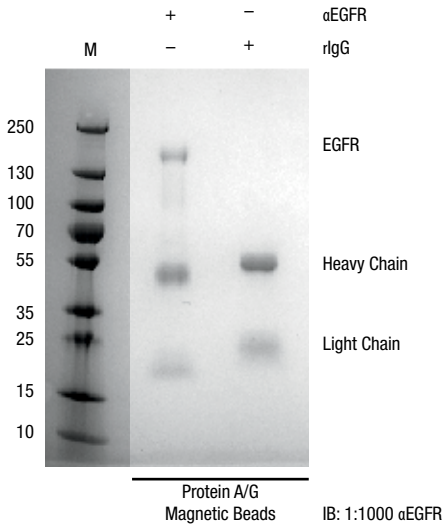


Figure 1. Diagram of Thermo Scientific Pierce Protein A/G Magnetic Beads. The magnetic particles are 1µm in diameter and are specially manufactured with two layers of magnetite and encapsulation. Recombinant Protein A/G is coupled to the bead surface.

A. Imperial Stain Gel



B. In-solution Digestion and LC-MS/MS Results

# Proteins Identified	EGFR % Sequence Coverage
31	61%

Figure 2. The Thermo Scientific Pierce Protein A/G Magnetic Beads effectively immunoprecipitate (IP) EGFR protein for MS application. (A) Thermo Scientific™ Imperial™ Stain SDS-PAGE Gel: A431 lysate (0.5mg) was incubated with 5µg anti-EGFR rabbit polyclonal IgG or 5µg normal rabbit IgG and 50µL Pierce Protein A/G Magnetic Beads for 1 hour at 4°C. The eluates were resolved by SDS-PAGE and stained with Thermo Scientific™ Imperial™ Protein Stain. **(B)** MS application: EGFR sequence coverage and background proteins were determined by LC-MS/MS after elution and trypsin digestion. IP using magnetic beads resulted low background and high EGFR sequence coverage.

Ordering Information

Product #	Description	Pkg. Size
88802	**Pierce Protein A/G Magnetic Beads** *Sufficient for binding 55 to 85µg rabbit IgG/mg beads.*	1mL
88803	**Pierce Protein A/G Magnetic Beads** *Sufficient for binding 55 to 85µg rabbit IgG/mg beads.*	5mL

Thermo Scientific Pierce Streptavidin Magnetic Beads

Superior biotin-binding particles for immunoprecipitation applications using magnetic separation.

Thermo Scientific™ Pierce™ Streptavidin Magnetic Beads accelerate throughput for magnetic purification of biotinylated molecules. These streptavidin magnetic particles are validated and optimized for use with high-throughput magnetic platforms, such as the Thermo Scientific™ KingFisher™ 96 and KingFisher™ Flex Instruments, but the beads also enable premium performance for simple benchtop applications using an appropriate magnetic stand. The iron oxide, super-paramagnetic particles offer superior performance (high capacity and low nonspecific binding) compared with other commercial magnetic beads, making them ideally suited for downstream MS analysis.

Highlights:

- **High-performance beads** – non-aggregating, pre-blocked, iron oxide, superparamagnetic microparticles provide exceptional uniformity for automated HTS and manual applications alike
- **Stable immobilization chemistry** – streptavidin is immobilized using leach-resistant chemistry
- **High capacity** – superior quality beads with high binding capacity provide rapid and efficient biomolecule enrichment from complex samples
- **Low nonspecific binding** – stable, pre-blocked beads reduce nonspecific protein binding
- **Superior performance** – nearly three times higher binding capacity than typical beads from other suppliers, allowing the use of smaller amounts per experiment

Applications:

- Immunoprecipitate antigens (using biotinylated antibodies) from a wide variety of sources for downstream MS analysis
- Enrichment procedure amenable to both discovery and targeted (SRM) assay development

Product Details:

Pierce Streptavidin Magnetic Beads use a recombinant form of streptavidin with a mass of 53kDa and a near-neutral isoelectric point (pI). The protein is a tetramer having four biotin-binding sites. Unlike avidin, streptavidin has no carbohydrate groups, resulting in low nonspecific binding. The high-affinity interaction between streptavidin and biotin cannot be dissociated efficiently except with very harsh conditions, such as boiling in sample loading buffer for SDS-PAGE or 8M guanidine•HCl, pH 1.5. Consequently, it is often possible to elute binding partners in an interaction complex without also eluting the biotinylated component.

Table 1. Characteristics of Thermo Scientific Pierce Streptavidin Magnetic Beads.

Composition	Iron oxide particles covalently coated with a monolayer of recombinant streptavidin protein
Magnetization	Superparamagnetic (no magnetic memory)
Mean Diameter	1µm
Density	2g/cm^3
Concentration	10mg/mL
Binding Capacity	~55µg biotinylated rabbit IgG per mg of beads; ~3500pmol biotinylated fluorescein per mg of beads

Note: These streptavidin magnetic bead solutions are NOT tested and certified to be RNase-free.

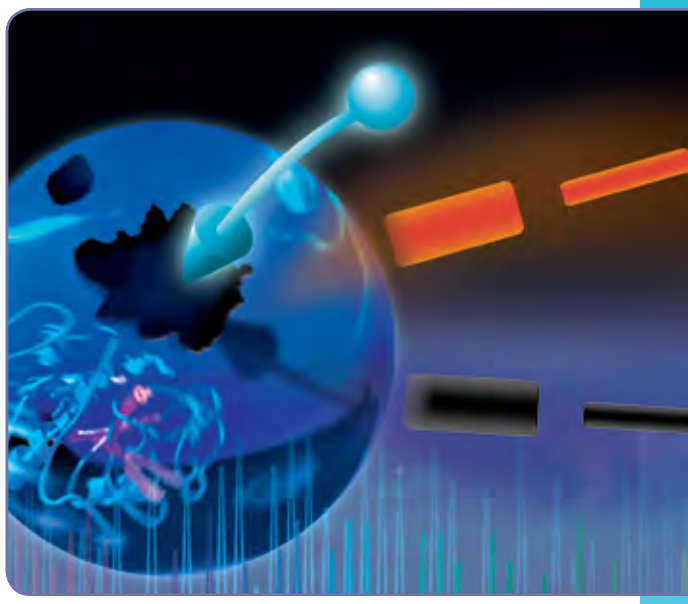
Table 2. Enrichment of medium- to low-abundant EGFR-AKT pathway targets using Thermo Scientific Pierce Streptavidin Magnetic Beads for MS analysis.
EGFR-AKT pathway targets were immunoprecipitated from two cell lines (A431 and HEK293) with biotinylated antibodies and captured with Pierce Streptavidin Magnetic Beads. IP eluates were digested in-solution, and analyzed by LC-MS/MS to assess sequence coverage and identify isoform-specific peptides.

		A431		HEK293	
	Target	**Anti-target Ab**	**Negative Control**	**Anti-target Ab**	**Negative Control**
% Sequence Coverage	**EGFR**	65%	0%	16%	0%
	AKT1	36%	2%	68%	6%
	AKT2	50%	0%	82%	0%
	AKT3	8%	0%	62%	0%
	PTEN	16%	0%	36%	0%

Ordering Information

Product #	Description	Pkg. Size
88816	**Pierce Streptavidin Magnetic Beads** *Sufficient for binding approx. 55µg biotinylated rabbit IgG per mg of beads (approx. 3500pmol biotinylated fluorescein per mg of beads).*	1mL
88817	**Pierce Streptavidin Magnetic Beads** *Sufficient for binding approx. 55µg biotinylated rabbit IgG per mg of beads (approx. 3500pmol biotinylated fluorescein per mg of beads).*	5mL

The new family of Thermo Scientific™ Pierce™ Enrichment Kits and Thermo Scientific™ ActivX™ Probes enables the specific targeting of several enzyme classes, including kinases, GTP-binding proteins and serine hydrolases, from tissues, cells and subcellular proteomes. The novel ActivX Probes covalently bind the active sites of the appropriate enzyme and offer a number of labeling and detection options (Figure 1). These reagents are ideal for selective subproteome enrichment, profiling inhibitor targets and determining inhibitor binding affinity.



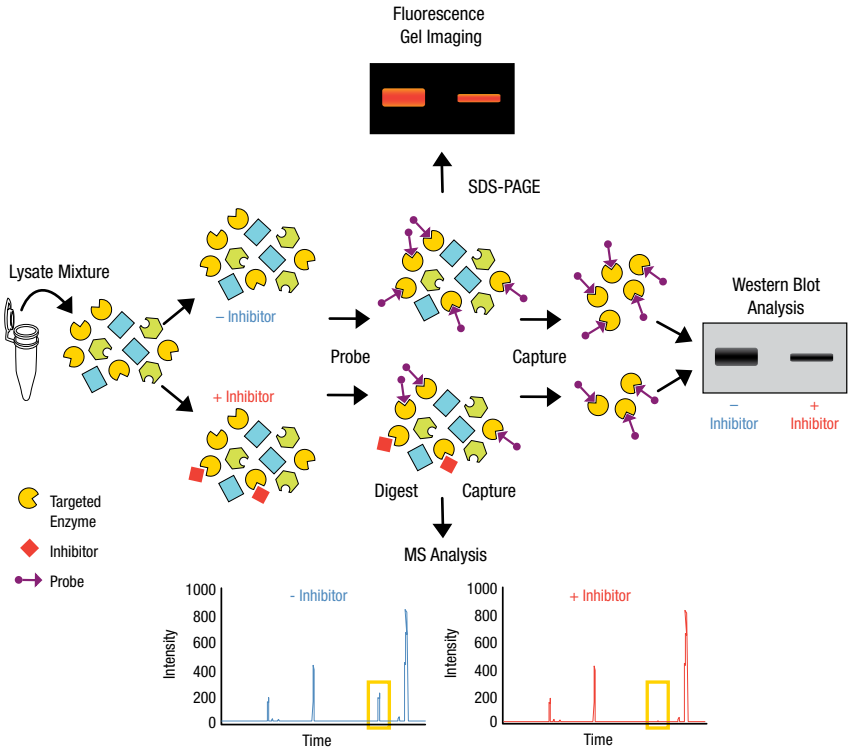


Figure 1. Western blot and MS workflows for targeted capture and analysis of enzymes. The schematic depicts two parallel workflows for the profiling, capture and detection of kinases, GTPases or serine hydrolases with ActivX Probes. Preincubation of enzymes with inhibitors allows for the determination of inhibitor specificity, binding affinity and potency by Western blot (or fluorescent SDS-PAGE for serine hydrolases only) of probe-labeled proteins or MS of probe-labeled peptides.

active site protein labeling and enrichment

Selectively capture and enrich proteins using active-site probes

Target: Nucleotide-binding Proteins

One class of ActivX Probes is based on derivatives of ATP, ADP or GTP nucleotides that select for nucleotide-binding proteins such as kinases, chaperones, metabolic enzymes and GTPases. These probes profile both active or inactive enzymes in a sample.

With these probes, the nucleotide is attached to desthiobiotin by a labile acyl-phosphate bond. The nucleotide binds to the active site of the nucleotide-binding protein, and the desthiobiotin tag allows for capture and enrichment of the bound protein. The desthiobiotin tag binds less tightly to biotin-binding proteins, making the capture bond easily reversible by biotin displacement, low pH or heat. Final analysis is achieved through Western blotting or MS.

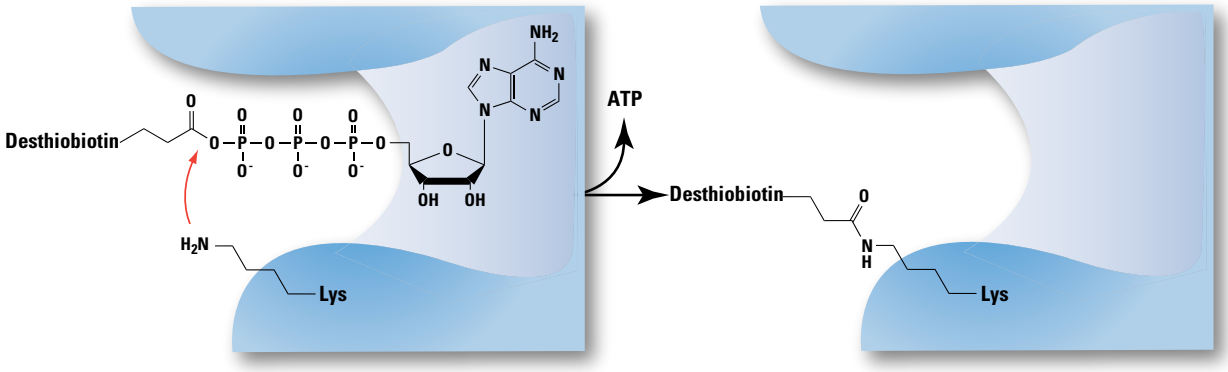


Figure 2. Labeling mechanism of the desthiobiotin-ATP. Desthiobiotin-ATP and -ADP bind to the active sites of ATPases and irreversibly transfer the desthiobiotin affinity tag to highly conserved lysine residues. Desthiobiotin derivatives bind streptavidin and are easily reversible using acidic elution conditions, allowing high recovery of labeled proteins and peptides.

Target: Active Serine Hydrolases

Another group of ActivX Probes uses fluorophosphonate (FP) to specifically target active serine hydrolases. Because these ActivX Probes only label active serine hydrolases, they are able to monitor enzymatic activity in addition to studying inhibitor binding affinities, unlike those probes that enrich nucleotide-binding proteins.

Three probes are available, differing by the enrichment/labeling tag. Probes are available with a desthiobiotin tag for selective enrichment and allow for Western blot and MS detection. Other serine hydrolase probes available use either a TAMRA fluorophore for fluorescent detection or a reactive-azido group (Staudinger reagents) that facilitates multiplex labeling when used with phosphine- or alkyne-derivatized tags.

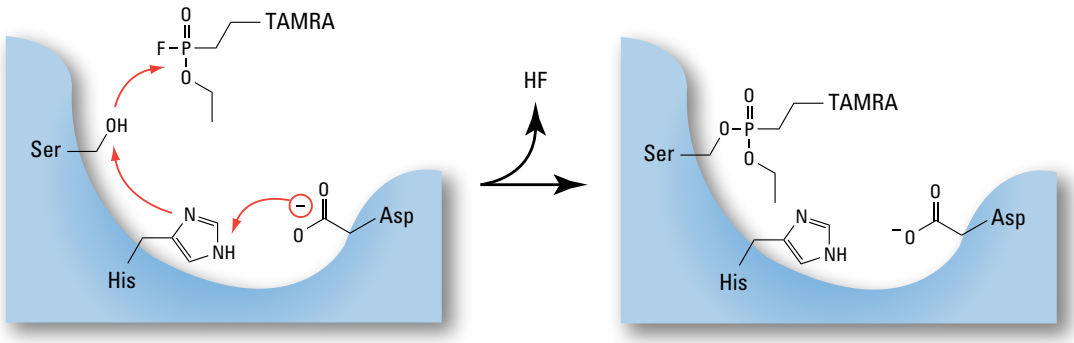


Figure 3. Labeling mechanism of Thermo Scientific ActivX Serine Hydrolase Probes. Fluorophosphonate probes covalently label the active-site serine of enzymatically active serine hydrolases.

Thermo Scientific Pierce Kinase Enrichment Kits with ActivX Desthiobiotin-ATP or Desthiobiotin-ADP Probes

Reagents for selective capture and enrichment of kinases using active-site probes.

Thermo Scientific™ Pierce™ Kinase Enrichment Kits with ActivX™ Desthiobiotin-ATP or -ADP Probes enable selective labeling and enrichment of ATPases including kinases, chaperones and metabolic enzyme.[1,2] The ActivX Desthiobiotin-ADP and -ATP Probes allow profiling of both inactive and active enzymes in a complex sample. Preincubation of samples with small-molecule inhibitors that compete for active sites can be used to determine inhibitor binding affinity. Active-site nucleotide probes also can be used to identify inhibitor off-targets. Analysis of enriched samples can be carried out with either Western blots or MS. Both analyses can be used to determine inhibitor target binding, but the MS workflow also can identify global inhibitor targets and off-targets and provide higher throughput for quantitative assays.[1,3]

The Pierce Kinase Enrichment Kits include all labeling and enrichment reagents for 16 reactions. The ActivX Desthiobiotin-ATP and -ADP Probes are supplied in the convenient Thermo Scientific™ No-Weigh™ Format and are also available separately.

Highlights:

- **Specific** – label only the conserved active-site lysines of nucleotide-binding proteins
- **Flexible** – use for *in vitro* labeling of ATPase enzymes derived from cells or tissues
- **Compatible** – use with Western blot or MS workflows

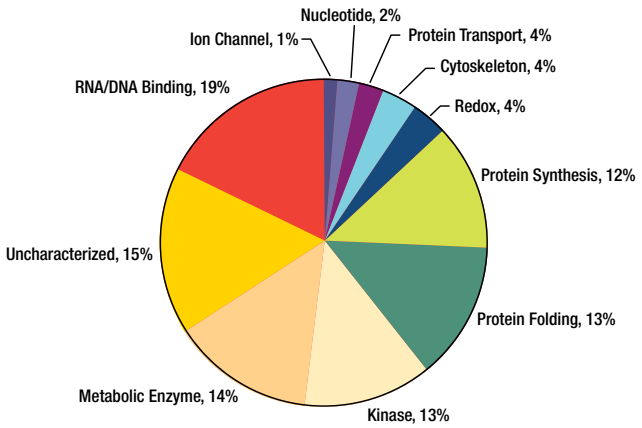


Figure 1. Mass spectrometry analysis of Thermo Scientific ActivX Desthiobiotin-ATP-labeled peptides. Active-site peptides (13% of total peptides) from more than 150 kinases were identified using desthiobiotin-ATP peptide pulldowns. K562 cell lysates from two independent biological replicates were used. A Thermo Scientific™ LTQ Orbitrap™ Mass Spectrometer was used for analysis.

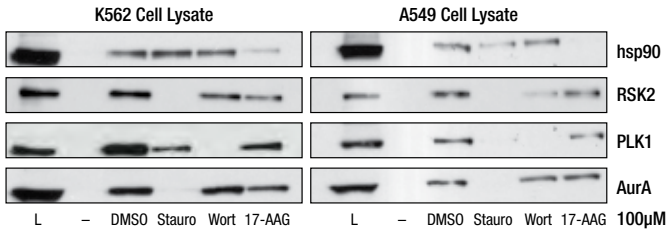


Figure 2. Screening of different inhibitors in K562 and A549 cell lines using Thermo Scientific ActivX Desthiobiotin-ATP Probes. Cell lysates (500µg) were pretreated with either DMSO or 100µM of staurosporine (Stauro), wortmannin (Wort) or 17-AAG before labeling with 5µM desthiobiotin-ATP probe and enriched using the Western blotting. Unlabeled lysate (-) was used as a control to show streptavidin pulldown specificity.

References

1. Patricelli, M.P. (2002). Activity-based probes for functional proteomics. *Brief Funct Genomic Proteomic* **1(2)**:151-8.
2. Patricelli, M.P., *et al.* (2007). Functional interrogation of the kinome using nucleotide acyl phosphates. *Biochemistry* **46**:350-8.
3. Okerberg, E.S., *et al.* (2005). High-resolution functional proteomics by active-site peptide profiling. *Proc Natl Acad Sci USA* **102(14)**:4996-5001.

Ordering Information

Product #	Description	Pkg. Size
88310	**Pierce Kinase Enrichment Kit with ATP Probe** *Sufficient for 16 pull-down assays.* Includes: ActivX Desthiobiotin-ATP Probe† Pierce IP Lysis Buffer Reaction Buffer Halt Protease/Phosphatase Inhibitor Cocktail (100X) Zeba Spin Desalting Columns, 7K MWCO High Capacity Streptavidin Agarose Resin (50% slurry) $MgCl_2$ Urea	16-rxn kit 16 x 12.6µg 100mL 125mL 1mL 8 x 5mL columns 1mL 500µL 12g
88311	**ActivX Desthiobiotin-ATP Probe**	16 x 12.6µg
88312	**Pierce Kinase Enrichment Kit with ADP Probe** *Sufficient for 16 pull-down assays.* Includes: ActivX Desthiobiotin-ADP Probe† Pierce IP Lysis Buffer Reaction Buffer Halt Protease/Phosphatase Inhibitor Cocktail (100X) Zeba Spin Desalting Columns, 7K MWCO High Capacity Streptavidin Agarose Resin (50% slurry) $MgCl_2$ Urea	16-rxn kit 16 x 9.9µg 100mL 125mL 1mL 8 x 5mL columns 1mL 500µL 12g
88313	**ActivX Desthiobiotin-ADP Probe**	16 x 9.9µg

† *ActivX Desthiobiotin-ATP, -ADP and -GTP Probes are exclusively licensed from ActivX Biosciences Inc. to Thermo Fisher Scientific for research use only.*

active site protein labeling and enrichment

Selectively capture and enrich proteins using active-site probes

Thermo Scientific Pierce GTPase Enrichment Kit with ActivX Desthiobiotin-GTP Probe

Selective capture and enrichment of GTPases using active-site probes.

The Thermo Scientific™ Pierce™ GTPase Enrichment Kits use the ActivX™ Desthiobiotin-GTP Probe to covalently modify conserved lysine residues in the binding site to selectively enrich and identify small GTPases and large G-protein subunits.[1,2,3] The ActivX Desthiobiotin-GTP Probe allows profiling of both inactive and active enzymes in a complex sample. Preincubation of samples with small-molecule inhibitors that compete for active sites can be used to determine inhibitor binding affinity. Active-site nucleotide probes also can be used to identify inhibitor off-targets. Analysis of enriched samples can be carried out with either Western blots or MS. Both analyses can be used to determine inhibitor target binding, but the MS workflow also can identify global inhibitor targets and off-targets and provide higher throughput for quantitative assays.

The Pierce GTPase Enrichment Kits include all labeling and enrichment reagents for 16 reactions. The ActivX Probes are supplied in the convenient Thermo Scientific No-Weigh Format and are also available separately.

Highlights:

- **Specific** – label only the conserved active-site lysines of nucleotide-binding proteins
- **Flexible** – use for *in vitro* labeling of ATPase enzymes derived from cells or tissues
- **Compatible** – use with Western blot or MS workflows

Table 1. GTPases identified by MS. Number for GTPase family members from human cell lysates identified by MS after labeling and enrichment using the desthiobiotin-GTP probe.

	Total of GTPases per Family
Rab family	38
Ras family	9
Arf family	8
Rho family	5
Gα family	4
Sar1 family	2

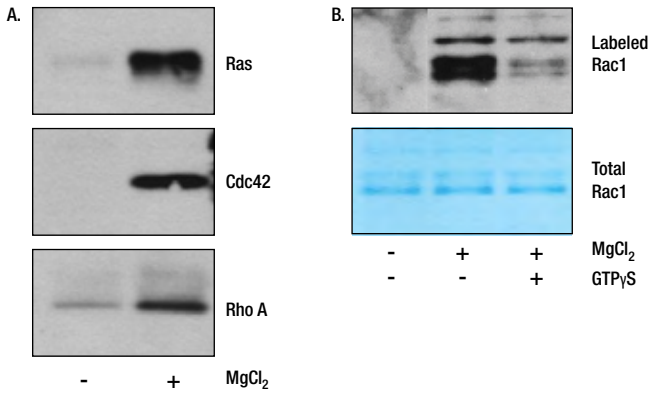


Figure 1. Desthiobiotin-GTP probe specifically labels small GTPases. Panel A: A549 cell lysates (500µg) were treated with (+) or without (-) 20mM of $MgCl_2$ after labeling with 20µM of desthiobiotin-GTP probe. Desthiobiotin-labeled proteins were denatured and enriched using streptavidin agarose before separation by SDS-PAGE and Western blotting with specific GTPase antibodies. **Panel B:** Recombinant Rac1 was treated with GTPγS before labeling with desthiobiotin-GTP probe. Labeling was performed in the presence (+) or absence (-) of 20mM $MgCl_2$. Samples were separated by SDS-PAGE and analyzed by Western blot (Labeled) to detect biotinylation of the active site. GelCode Blue Stain Reagent (Total) was used to stain a duplicate gel to show equal loading.

References

1. Patricelli, M.P. (2002). Activity-based probes for functional proteomics. *Brief Funct. Genomic Proteomic* **1(2)**:151-8.
2. Okerberg, E.S., *et al.* (2005). High-resolution functional proteomics by active-site peptide profiling. *Proc. Natl. Acad. Sci .USA* **102(14)**:4996-5001.
3. Cravatt, B.F., *et al.* (2008). Activity-based protein profiling: From enzyme chemistry to proteomic chemistry. *Ann. Rev. Biochem.* **77**:383-414.

Ordering Information

Product #	Description	Pkg. Size
88314	**Pierce GTPase Enrichment Kit with GTP Probe** *Sufficient reagents for 16 pull-down reactions.* Includes: ActivX Desthiobiotin-GTP Probe† Pierce IP Lysis Buffer Reaction Buffer Halt Protease/Phosphatase Inhibitor Cocktail (100X) Zeba Spin Desalting Columns, 7K MWCO High Capacity Streptavidin Agarose Resin (50% slurry) $MgCl_2$ Urea	16-rxn kit 16 x 12.9µg 100mL 125mL 1mL 8 x 5mL columns 1mL 500µL 12g
88315	**ActivX Desthiobiotin-GTP Probe**	16 x 12.9µg

† *ActivX Desthiobiotin-ATP, -ADP and -GTP Probes are exclusively licensed from ActivX Biosciences Inc. to Thermo Fisher Scientific for research use only.*

Thermo Scientific ActivX Serine Hydrolase Probes

Probes for specific detection and enrichment of active serine hydrolases.

The Thermo Scientific™ ActivX™ Serine Hydrolase Probes enable selective labeling and enrichment of active serine hydrolases. The serine hydrolase probe consists of a tag linked to a fluorophosphonate (FP) group that specifically and covalently labels serines of enzymatically active serine hydrolases.[1,2,3,4]

The ActivX Serine FP probes also can be used to screen small molecule inhibitors against enzymes derived from cell lysates, subcellular fractions, tissues and recombinant proteins.

Depending on the active-site probe tag group, FP probe-labeled enzymes can be detected and quantified by Western blot, fluorescent gel imaging or MS. TAMRA-FP probes can be used to label and detect serine hydrolase activity in samples using fluorescent gel imaging, capillary electrophoresis or MS.[1] Azido-FP probes are used in combination with phosphine- or alkyne-derivatized tags for either detection or enrichment. Desthiobiotin-FP probes can be used for both enrichment and detection of active-site-labeled proteins by Western blot and MS.

Highlights:

- **Specific** – label only the conserved active-site lysines of nucleotide-binding proteins
- **Flexible** – use for *in vitro* labeling of ATPase enzymes derived from cells or tissues
- **Compatible** – use with Western blot or MS workflows

Table 1. Serine hydrolases identified by MS with Thermo Scientific ActivX FP Probes. Number of serine hydrolase family members from mouse brain and liver tissue extracts identified by MS after labeling and enrichment using the desthiobiotin-FP probe.

Serine Hydrolase Family	Number Identified
Hydrolases	10
Sterases	6
Lipases	5
Peptidases	4
Other	4

References

1. Okerberg, E.S., *et al.* (2005). High-resolution functional proteomics by active-site peptide profiling. *Proc Natl Acad Sci USA* **102(14)**:4996-5001.
2. Liu,Y., *et al.* (1999). Activity-based protein profiling: The serine hydrolases. *Proc Natl Acad Sci USA* **96(26)**:14694-9.
3. Patricelli, M.P., *et al.* (2001). Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* **1**:1067-71.
4. Simon, G.M. and Cravatt, B.F. (2010). Activity-based proteomics of enzyme superfamilies: Serine hydrolases as a case study. *J. Biol. Chem.* **285(15)**:11051-5.

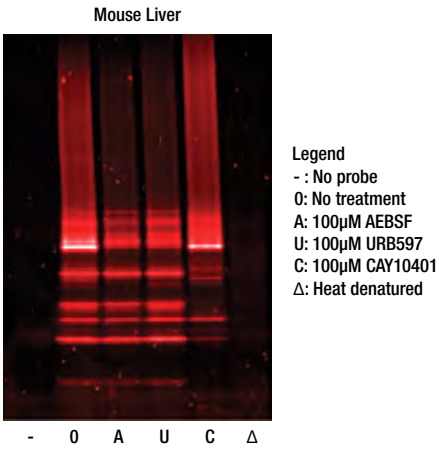


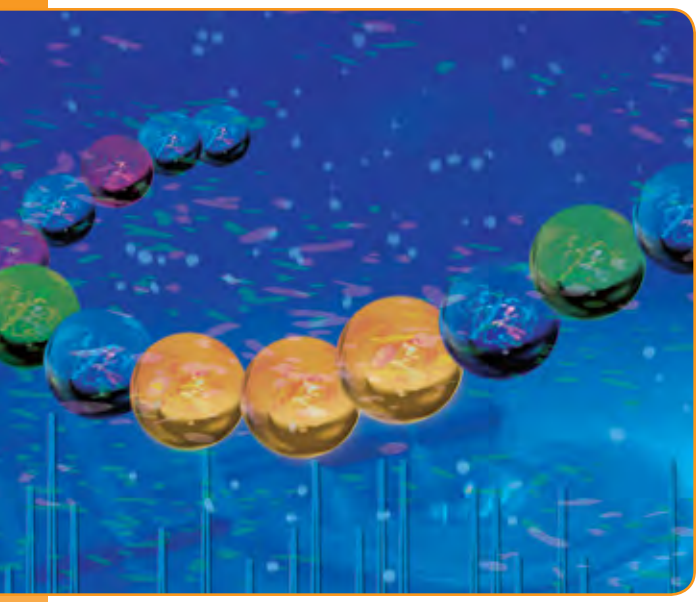
Figure 1. Screening of different inhibitors in mouse liver lysates using serine hydrolase probes. Mouse liver tissue lysates (50µg) were pretreated with either DMSO (0) or serine hydrolase inhibitors 100µM AEBSF (A), URB597 (U) or CAY10401 (C) for 1 hour before labeling with 2µM TAMRA-FP probe. Labeled proteins were separated by SDS-PAGE and analyzed by fluorescent gel scanning using a GE Healthcare Typhoon™ Imager. Unlabeled lysate (-) and heat denatured (Δ) lysate were used as a controls to show probe labeling specificity.

Ordering Information

Product #	Description	Pkg. Size
88316	**ActivX Azido-FP Serine Hydrolase Probe** *Sufficient for 100 labeling reactions or 10 pulldown reactions.*	3.5µg
88317	**ActivX Desthiobiotin-FP Serine Hydrolase Probe** *Sufficient for 100 labeling reactions or 10 pulldown reactions.*	4.6µg
88318	**ActivX TAMRA-FP Serine Hydrolase Probe** *Sufficient for 100 labeling reactions or 10 pulldown reactions.*	6.8µg

protein clean up

Introduction



Whether samples are simple or complex, they often need to be processed to ensure compatibility with downstream MS analysis. In addition, many of the techniques and products described in the Protein Enrichment section require upstream sample processing to ensure proper function.

Thermo Fisher Scientific offers a range of general protein clean up products for MS sample preparation. Dialysis, desalting and concentrating devices can be used for buffer exchange or to remove common small molecule contaminants such as salts or detergents. Concentration devices can also be used to concentrate dilute protein samples such as after abundant protein depletion. Detergent removal resins quickly remove a wide range of detergents that can interfere with peptide chromatography or MS analysis. Although most of these techniques are used for clean up of intact proteins, some can also be used for peptide clean up.

Thermo Scientific Zeba Desalting Columns, Plates and Cartridges

Quickly remove salts and small molecules from protein samples.

Our complete line of desalting columns, plates and cartridges, with either 7000 or 40,000 MWCO, contains the proprietary, high-performance Thermo Scientific™ Zeba™ Desalting Resin that offers rapid and exceptional protein desalting with high recovery of protein.

Available in five different spin-column sizes, 96-well spin plates and cartridges, Zeba Desalting Devices provide trouble-free desalting, buffer exchange or unincorporated tag removal for sample volumes ranging from 2µL to 4mL. The easy-to-use, spin-column format dramatically improves results over standard drip-column methodologies and the 96-well, spin-plate format enables high-throughput processing. The Zeba Desalting Cartridges, 7K MWCO are compatible with syringe processing or will attach to popular automated liquid-chromatography systems.

Highlights:

- **High recovery** – low-binding resin maximizes protein recovery
- **Fast** – no fraction screening or waiting for protein to emerge by gravity flow
- **Easy use** – no cumbersome column preparation or equilibration
- **Flexible** – available in spin columns, filter spin plates and cartridges for a range of needs

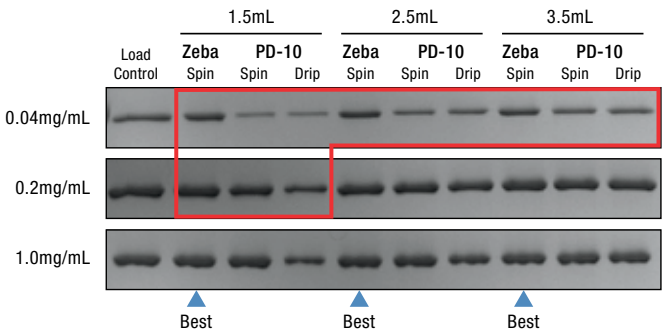


Figure 1. Better performance with Thermo Scientific Zeba Spin Desalting Columns. Zeba Columns provide higher protein recovery and less sample dilution over a wider range of sample concentrations and volumes compared to alternative products. Zeba Spin Desalting Columns (7K MWCO, 10mL) and Disposable PD-10 Desalting Columns (#17-0851-01, GE Healthcare) were used to desalt 1.5, 2.5 and 3.5mL samples of bovine serum albumin (BSA) at concentrations of 0.04, 0.2, and 1mg/mL. Desalting was performed according to each manufacturer's recommended protocols for either spin (centrifuge) or drip (gravity) procedures. Three sets of columns were equilibrated in final buffer and then loaded with 1.5, 2.5, and 3.5mL samples. For each electrophoresis gel, an aliquot of starting sample equal to 1µg of BSA was loaded in Lane 1 as the Load Control; all other desalted samples were loaded in the gel at the same volume as the Load Control. Differences in intensity between lanes are a combination of protein recovery and sample dilution caused by desalting.

Table 1. Comparison of protein recovery from the 7K MWCO and 40K MWCO, 0.5mL Thermo Scientific Zeba Spin Desalting Columns.

			Typical % Recovered From 100µL Sample	
Recovery of	**Concentration Loaded**	**Size of Molecule**	**7K Zeba Column**	**40K Zeba Column**
Ubiquitin	0.5mg/mL	8.7kDa	75%	60%
α-Lactalbumin	1.0mg/mL	14.1kDa	85%	75%
Soybean Trypsin Inhibitor	0.5mg/mL	20.1kDa	85%	65%
Carbonic Anhydrase	0.5mg/mL	29kDa	90%	75%
Ovalbumin	0.5mg/mL	44kDa	90%	85%
Bovine Serum Albumin	0.5mg/mL	66kDa	> 90%	> 90%
Human IgG	0.5mg/mL	150kDa	> 90%	> 90%

Table 2. Comparison of small molecule removal for the 7K MWCO and 40K MWCO, 0.5mL Thermo Scientific Zeba Spin Desalting Columns.

			Typical % Removed From 100µL Sample	
Removal of	**Concentration Loaded**	**Size of Molecule**	**7K Zeba Column**	**40K Zeba Column**
NaCl	1M	58.44Da	> 99%	> 99%
Dithiothreitol	0.5M	154Da	99%	99%
Sulfo-NHS-LC-Biotin	0.27mM	557Da	85%	85%
DyLight 549 Dye	0.2mM	1026Da	75%	90%
Bacitracin	0.5mg/mL	1200Da	75%	95%
Vitamin B12	0.5mg/mL	1386-Da	85%	95%

Table 3. Comparison of protein recoveries with different microcentrifuge desalting columns and conditions. Recoveries are expressed to the nearest 5% values based on several trials. Column products are Thermo Scientific Zeba Columns, SpinTrap G-25 (GE Healthcare) and Micro Bio-Spin 6 (Bio-Rad).

		Zeba Columns, 0.5mL	SpinTrap G-25 Columns	Micro Bio-Spin 6 Columns
Sample (concentration)	**Sample Volume**	**Recovery**	**Recovery**	**Recovery**
BSA 0.04mg/mL	40µL	70-80%	<10%	<10%
	80µL	80-95%	<10%	<10%
	120µL	>90%	<10%	<20%
BSA 0.2mg/mL	40µL	70-80%	<15%	25-35%
	80µL	80-95%	35-45%	45-60%
	120µL	>90%	55-70%	65-80%
BSA 1mg/mL	40µL	80-95%	45-60%	70-80%
	80µL	>90%	60-75%	80-90%
	120µL	>90%	75-90%	>90%
HeLa Lysate 0.2mg/mL	40µL	45-55%	<10%	<15%
	80µL	60-70%	15-25%	30-40%
	120µL	70-80%	45-55%	50-60%
HeLa Lysate 1mg/mL	40µL	65-75%	45-55%	45-55%
	80µL	75-85%	60-70%	70-80%
	120µL	80-90%	70-80%	80-90%

Ordering Information

Product #	Description	Pkg. Size
7K		
89877	**Zeba Micro Spin Desalting Columns, 7K MWCO, 75µL** *Sufficient for 25 samples, each 2 to 12µL.*	25 columns
89878	**Zeba Micro Spin Desalting Columns, 7K MWCO, 75µL** *Sufficient for 50 samples, each 2 to 12µL.*	50 columns
89882	**Zeba Spin Desalting Columns, 7K MWCO, 0.5mL** *Sufficient for 25 samples, each 30 to 130µL.*	25 columns
89883	**Zeba Spin Desalting Columns, 7K MWCO, 0.5mL** *Sufficient for 50 samples, each 30 to 130µL.*	50 columns
89889	**Zeba Spin Desalting Columns, 7K MWCO, 2mL** *Sufficient for 5 samples, each 200 to 700µL.*	5 columns
89890	**Zeba Spin Desalting Columns, 7K MWCO, 2mL** *Sufficient for 25 samples, each 200 to 700µL.*	25 columns
89891	**Zeba Spin Desalting Columns, 7K MWCO, 5mL** *Sufficient for 5 samples, each 500 to 2000µL.*	5 columns
89892	**Zeba Spin Desalting Columns, 7K MWCO, 5mL** *Sufficient for 25 samples, each 500 to 2000µL.*	25 columns
89893	**Zeba Spin Desalting Columns, 7K MWCO, 10mL** *Sufficient for 5 samples, each 700µL to 4mL.*	5 columns
89894	**Zeba Spin Desalting Columns, 7K MWCO, 10mL** *Sufficient for 25 samples, each 700µL to 4mL.*	25 columns
89807	**Zeba Spin Desalting Plates, 7K MWCO** *Sufficient for 2 × 96 samples, each 20 to 100µL.*	2 plates
89808	**Zeba Spin Desalting Plates, 7K MWCO** *Sufficient for 4 × 96 samples, each 20 to 100µL.*	4 plates
89934	**Zeba Desalting Chromatography Cartridges, 7K MWCO, 1mL** *Sufficient for samples requiring 1mL of resin for separation.*	5 cartridges
89935	**Zeba Desalting Chromatography Cartridges, 7K MWCO, 5mL** *Sufficient for samples requiring 5mL of resin for separation.*	5 cartridges

Product #	Description	Pkg. Size
40K		
87764	**Zeba Micro Spin Desalting Columns, 40K MWCO, 75µL** *Sufficient for 25 samples, each 5 to 14µL.*	25 columns
87765	**Zeba Micro Spin Desalting Columns, 40K MWCO, 75µL** *Sufficient for 50 samples, each 5 to 14µL.*	50 columns
87766	**Zeba Spin Desalting Columns, 40K MWCO, 0.5mL** *Sufficient for 25 samples, each 70 to 200µL.*	25 columns
87767	**Zeba Spin Desalting Columns, 40K MWCO, 0.5mL** *Sufficient for 50 samples, each 70 to 200µL.*	50 columns
87768	**Zeba Spin Desalting Columns, 40K MWCO, 2mL** *Sufficient for 5 samples, each 200 to 900µL.*	5 columns
87769	**Zeba Spin Desalting Columns, 40K MWCO, 2mL** *Sufficient for 25 samples, each 200 to 900µL.*	25 columns
87770	**Zeba Spin Desalting Columns, 40K MWCO, 5mL** *Sufficient for 5 samples, each 300 to 2000µL.*	5 columns
87771	**Zeba Spin Desalting Columns, 40K MWCO, 5mL** *Sufficient for 25 samples, each 300 to 2000µL.*	25 columns
87772	**Zeba Spin Desalting Columns, 40K MWCO, 10mL** *Sufficient for 5 samples, each 1mL to 4mL.*	5 columns
87773	**Zeba Spin Desalting Columns, 40K MWCO, 10mL** *Sufficient for 25 samples, each 1mL to 4mL.*	25 columns
87774	**Zeba 96-well Spin Desalting Plates, 40K MWCO** *Sufficient for 2 × 96 samples, each 20 to 100µL.*	2 plates
87775	**Zeba 96-well Spin Desalting Plates, 40K MWCO** *Sufficient for 4 × 96 samples, each 20 to 100µL.*	4 plates

Thermo Scientific Pierce 96-well Microdialysis Plate

Dialysis convenience in a 96-well plate.

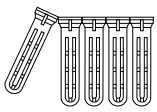
Thermo Scientific™ Pierce™ 96-well Microdialysis Plates are automation-compatible, microplate dialysis devices (3.5K or 10K MWCO) for rapid, simultaneous processing of 1 to 96 samples with volumes from 10 to 100µL.

Each microdialysis device has two regenerated cellulose membranes separated by < 2mm. This combination of short diffusion distance and large surface area allows for rapid dialysis. In addition, the small distance between the membranes allows highly efficient sample recovery using standard laboratory pipettes. The low-binding, regenerated cellulose membranes are rated to retain proteins and other macromolecules that are larger than the MWCO while allowing removal of buffer salts and small contaminants < 1000 daltons in size.

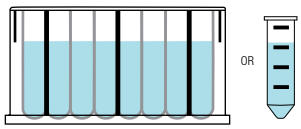
Highlights:

- **Efficient and rapid dialysis** – dialysis completed in 2-4 hours with > 99% salt removal
- **Excellent sample recovery** – > 90% protein recovery after dialysis
- **Ideal for small sample volume dialysis** – uses sample volumes from 10-100µL
- **Easy to use** – complete sample loading and retrieval with a standard pipette
- **Flexible** – detachable 8-unit strips; scalable from 1 to 96 samples
- **Automation-compatible** – plate format conforms to SBS Microplate Standard

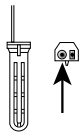
The dialysis chambers come in strips of eight units that can be easily separated allowing you to utilize just the number of units needed for your experiment. Dialysis can be efficiently performed in a standard 96 deep-well plate using a minimal amount of buffer. The assembled device is compatible with standard 96-well laboratory equipment and automated liquid-handling systems, making it an ideal option for high-throughput applications. Each device can also be used independently in a 2mL microcentrifuge tube. When used according to the method outlined in Figure 1, the Pierce 96-well Microdialysis Plate enables the removal of low-molecular weight contaminants, buffer exchange and desalting within two to four hours with typical protein recoveries of > 90%.



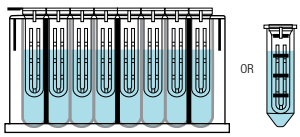
1. Remove one or more devices, as needed. If only one device is required, break it carefully from the 8-segmented cartridge.



2. Add dialysis buffer to a deep-well plate (≤ 1800µL) or a 2mL microcentrifuge tube (≤ 1400µL) and set aside.

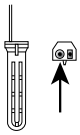


3. Insert an upright pipette tip filled with sample into the round opening (see arrow). Slowly add the sample (10-100µL).



4. Place device into the deep-well plate or 2mL microcentrifuge tube containing buffer.

5. Dialyze to remove low-molecular weight compounds (1 hour to overnight).



6. Remove device from plate or tube and recover sample by inserting upright pipette tip into round opening of device and slowly withdrawing the sample.

Figure 1. Protocol summary for the Thermo Scientific Pierce 96-well Microdialysis Plate.

Ordering Information

Product #	Description	Pkg. Size
88262	**Pierce 96-well Microdialysis Plate, 3.5K MWCO** *Sufficient for dialysis of 96 samples 10 to 100µL each.* Includes: 8-microdialysis device strip 96-deep well plate	1-plate set 12 strips 1 plate
88260	**Pierce 96-well Microdialysis Plate, 10K MWCO** *Sufficient for dialysis of 96 samples 10 to 100µL each.* Includes: 8-microdialysis device strip 96-well deep-well plate	1-plate set 12 strips 1 plate
88261	**96-well Deep-well Plate, 2.2mL** *Sufficient for 96 microdialysis devices.*	1 plate
88269	**Plate Seal for Pierce 96-well Microdialysis Plates** *Sufficient for 12 x 8 microdialysis plate wells.*	1 sheet

Thermo Scientific Slide-A-Lyzer MINI Dialysis Devices

Self-contained devices for sample volumes as small as 10µL.

Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Devices are disposable polypropylene cups with integrated, low-binding membranes for dialysis and high recovery of proteins and macromolecules in volumes from 10µL to 2mL.

Slide-A-Lyzer MINI Dialysis Devices allow easy sample addition and removal using a standard laboratory pipette and can be used for single or arrays of samples. The self-contained, single-use devices require no syringes, centrifuge, beakers or laborious steps. Using the Slide-A-Lyzer MINI Dialysis Devices, low-molecular weight contaminant removal, buffer exchange and desalting can be accomplished within 4-8 hours with high protein recovery (Table 1). Dialysis efficiencies, rates and recoveries are similar to conventional dialysis using a large volume of buffer (Figure 1).

Highlights:

- **Excellent sample recoveries** – low-binding plastic and small membrane surface area minimize sample loss compared to filtration and resin systems
- **One-step protocol** – pipette sample into the Slide-A-Lyzer MINI Device and place in tube containing the dialysis buffer; no laborious assembly, device preparation or expensive equipment is required
- **100% leak tested** – innovative design does not permit "wicking" that can occur in homemade devices
- **Minimal dialysis buffer required** – eliminates waste

The 0.1mL Slide-A-Lyzer MINI Dialysis Devices, for use with sample volumes of 10-100µL, are available in MWCOs of 2K, 3.5K, 7K, 10K or 20K and are used with standard 1.5mL microcentrifuge tubes (Figure 2).

The 0.5mL and 2mL Slide-A-Lyzer MINI Dialysis Devices, available in 3.5K, 10K or 20K MWCOs, are used for processing 50-500µL and 200-2000µL samples, respectively. The devices fit into and are capped by inserting them into the included 15mL or 50mL conical tubes (Figure 3). The tubes can be securely placed in standard laboratory racks or shakers, saving space, minimizing the risk of spills or contamination, and eliminating the need for floats or large beakers of buffer.

Slide-A-Lyzer MINI Dialysis Devices are manufactured in a HEPA-filtered clean-room facility, and 100% of units are vacuum-leak tested to ensure the highest quality and safety for your samples.

Table 1. High protein recovery is obtained using the 2mL Thermo Scientific Slide-A-Lyzer MINI Dialysis Device.†

Membrane MWCO (K)	Protein/Peptide	Recovery (%)
3.5	Insulin Chain B (3.5kDa)	90.13
10	Cytochrome C (12.4kDa)	94.44
20	Myoglobin (17kDa)	95

† Insulin chain B, cytochrome C and myoglobin (0.25mg/mL) in either 50mM sodium phosphate, 75mM NaCl at pH 7.2 or 0.2M carbonate-bicarbonate buffer at pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of protein in the retentate was determined using the Pierce BCA Protein Assay (Product # 23225).

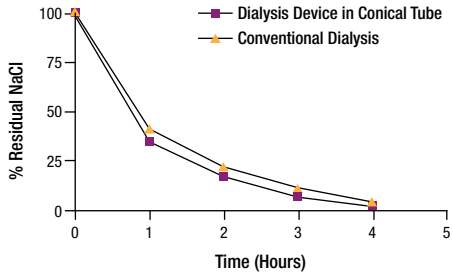


Figure 1. Rate of removal of NaCl using a 10K MWCO, 2mL Thermo Scientific Slide-A-Lyzer MINI Dialysis Device versus conventional dialysis. Bovine serum albumin (BSA) samples (2mL, 0.25mg/mL in 1M NaCl) were dialyzed against 45mL of water in 50mL disposable conical tubes on an orbital shaker (300 rpm) at room temperature. The water was changed once after 2 hours. Results are the average of two samples. Similar results were obtained with the 0.5mL device (data not shown). For conventional dialysis, the samples were dialyzed against 2L of water in a beaker with stirring. The rate of NaCl removal was determined by measuring the conductivity of the retentate at the indicated times. Greater than 95% of NaCl was removed within 4 hours.

1. Apply sample with a pipette.

2. Place the Slide-A-Lyzer MINI Dialysis Device into the float.

3. Insert the float into the beaker containing the dialysate.

4. Recover sample.

Figure 2. Sample dialysis using a 0.1mL Thermo Scientific Slide-A-Lyzer MINI Dialysis Device. The required float is sold separately.

1. Add sample into the device.

2. Place the device into the conical tube containing the buffer and cap the tube.

3. Shake gently on an orbital shaker.

4. Replace dialysis buffer after 2-3 hours and shake for an additional 2-4 hours or overnight.

5. Remove the device from the conical tube and recover the sample.

Figure 3. Sample dialysis with a 0.5mL or 2mL Thermo Scientific Slide-A-Lyzer MINI Dialysis Device.

Ordering Information

Product #	Description	Pkg. Size
2K		
69580	**Slide-A-Lyzer MINI Dialysis Device, 2K MWCO, 0.1mL** *Sufficient for 50 samples, each 10 to 100µL.*	50 devices
69553	**Slide-A-Lyzer MINI Dialysis Device, 2K MWCO, 0.1mL** *Sufficient for 250 samples, each 10 to 100µL.*	250 devices
3.5K		
69550	**Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 0.1mL** *Sufficient for 50 samples, each 10 to 100µL.*	50 devices
69552	**Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 0.1mL** *Sufficient for 250 samples, each 10 to 100µL.*	250 devices
69558	**Slide-A-Lyzer MINI Dialysis Device Kit, 3.5K MWCO, 0.1mL** *Sufficient for 10 samples, each 10 to 100µL.* Includes: Slide-A-Lyzer MINI Dialysis Device Slide-A-Lyzer MINI Dialysis Device Float Buoy	10-device kit 10 devices 1 float
88400	**Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 0.5mL** *Sufficient for 25 samples, each 50 to 500µL.*	25 devices
88403	**Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 2mL** *Sufficient for 25 samples, each 200 to 2000µL.*	25 devices
7K		
69560	**Slide-A-Lyzer MINI Dialysis Device, 7K MWCO, 0.1mL** *Sufficient for 50 samples, each 10 to 100µL.*	50 devices
69562	**Slide-A-Lyzer MINI Dialysis Device, 7K MWCO, 0.1mL** *Sufficient for 250 samples, each 10 to 100µL.*	250 devices

Product #	Description	Pkg. Size
10K		
69570	**Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 0.1mL** *Sufficient for 50 samples, each 10 to 100µL.*	50 devices
69572	**Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 0.1mL** *Sufficient for 250 samples, each 10 to 100µL.*	250 devices
69574	**Slide-A-Lyzer MINI Dialysis Device Kit, 10K MWCO, 0.1mL** *Sufficient for 10 samples, each 10 to 100µL.* Includes: Slide-A-Lyzer MINI Dialysis Units Microcentrifuge tubes	10-device kit 10 devices 10 tubes
69576	**Slide-A-Lyzer MINI Dialysis Device Kit, 10K MWCO, 0.1mL** *Sufficient for 10 samples, each 10 to 100µL.* Includes: Slide-A-Lyzer MINI Dialysis Units Slide-A-Lyzer MINI Dialysis Device Float Buoy	10-device kit 10 devices 1 float
88401	**Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 0.5mL** *Sufficient for 25 samples, each 50 to 500µL.*	25 devices
88404	**Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 2mL** *Sufficient for 25 samples, each 200 to 2000µL.*	25 devices
20K		
69590	**Slide-A-Lyzer MINI Dialysis Device, 20K MWCO, 0.1mL** *Sufficient for 50 samples, each 10 to 100µL.*	50 devices
69555	**Slide-A-Lyzer MINI Dialysis Device, 20K MWCO, 0.1mL** *Sufficient For: 250 samples, each 10 to 100µL.*	250 devices
88402	**Slide-A-Lyzer MINI Dialysis Device, 20K MWCO, 0.5mL** *Sufficient For: 25 samples, each 50 to 500µL.*	25 devices
88405	**Slide-A-Lyzer MINI Dialysis Device, 20K MWCO, 2mL** *Sufficient for 25 samples, each 200 to 2000µL.*	25 devices

Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassettes

Maximum convenience for high-performance dialysis.

Thermo Scientific™ Slide-A-Lyzer™ G2 Dialysis Cassettes provide maximum convenience, flexibility and performance for sample dialysis. These second-generation (G2) Slide-A-Lyzer Dialysis Cassettes are free-standing, self-floating and pipette-loadable. Sample loading and removal are easily accomplished by using a serological pipette or hypodermic needle (optional) attached to a syringe (Figure 1). The built-in air chamber provides sample buoyancy and vertical orientation of the cassette during dialysis.

Highlights:

- **Easy loading** – pipette-accessible for easy sample loading and retrieval
- **Self-floating** – integrated air chambers eliminate the need for float buoys
- **Sturdy construction** – ensures the highest possible sample integrity and protection
- **Superior design** – thoroughly researched and tested to provide fast and consistent dialysis with maximum sample recovery
- **Multiple sizes** – five cassette capacities to optimally match 0.25 to 70mL sample volumes
- **Versatile** – ideal for removing low-molecular weight contaminants, performing buffer exchange and desalting
- **Sterile option** – gamma-irradiated 10K MWCO cassettes are available for applications requiring sterile conditions

The single-use, disposable Slide-A-Lyzer G2 Dialysis Cassettes are available in five membrane MWCOs (2K, 3.5K, 7K, 10K and 20K) and in five different sizes for dialyzing sample volumes between 0.25 and 70mL. The membrane is composed of low-binding, regenerated cellulose, and the cassettes are manufactured using clean-room conditions. Select sizes of 10K MWCO Slide-A-Lyzer G2 Cassettes are also available in packages that have been gamma-irradiated to sterilize them. Gamma-irradiated Slide-A-Lyzer G2 Dialysis Cassettes are ideal for researchers culturing cells and microorganisms; purifying viruses, DNA and RNA; or performing sample preparation for other applications requiring sterile conditions to minimize the risk of sample contamination.

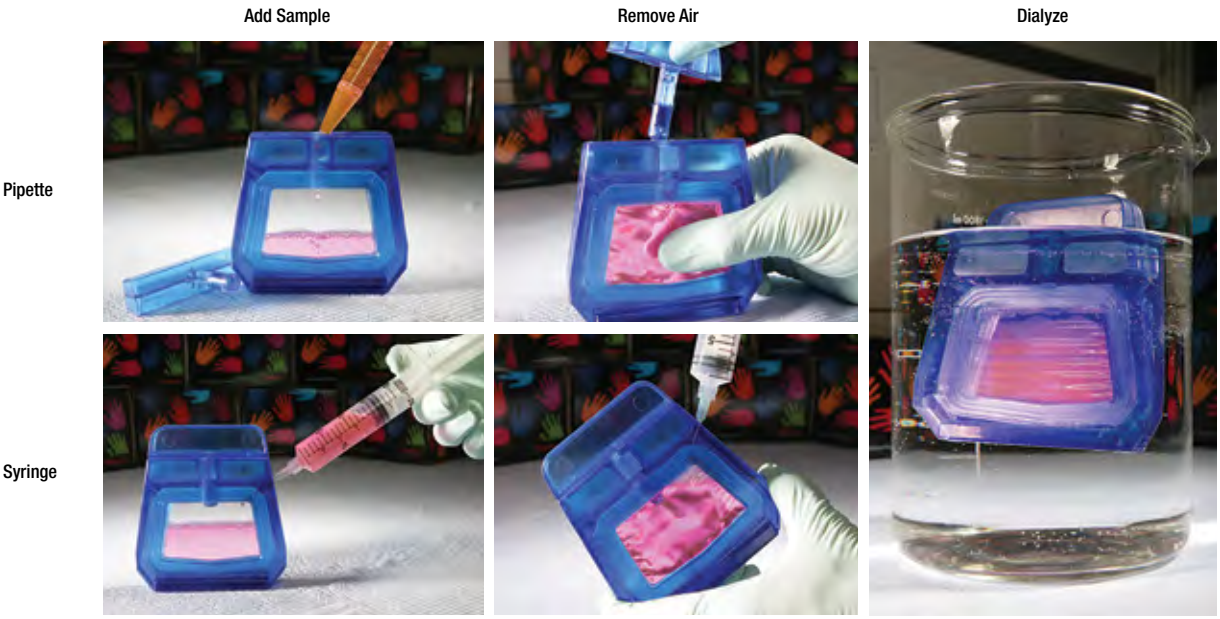


Figure 1. Easy sample loading and recovery. Samples can be loaded into a Slide-A-Lyzer G2 Dialysis Cassette with either a pipette or with a syringe through the unique sample port. The Slide-A-Lyzer Cassette design allows air to be removed from inside the cassette to maximize the surface area-to-volume ratio, providing better dialysis performance than dialysis tubing and tube-based systems. The sample is easily recovered through either the pipette or syringe sample ports. For a demonstration of the Slide-A-Lyzer G2 Cassettes, please go to thermoscientific.com/salG2

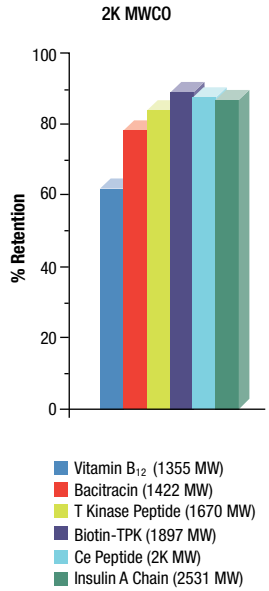


Figure 2. Sample retention by the 2K MWCO Thermo Scientific Slide-A-Lyzer Cassette membrane. Vitamin B_{12}, bacitracin, tyrosine kinase peptide 1, biotin-TPKs substrate, protein kinase Ce (PKCe) peptide substrate and insulin A chain model systems (0.5-1mg/mL) in either saline or 0.2M carbonate bicarbonate buffer, pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using either the Pierce BCA Protein Assay or absorption at 360nm (for vitamin B_{12}).

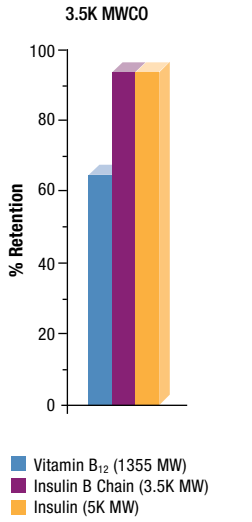


Figure 3. Sample retention by the 3.5K MWCO Thermo Scientific Slide-A-Lyzer Cassette membrane. Vitamin B_{12}, insulin B chain and insulin (1mg/mL) in either saline or 0.2M carbonate bicarbonate buffer, pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using either the Pierce BCA Protein Assay or absorption at 360nm (for vitamin B_{12}).

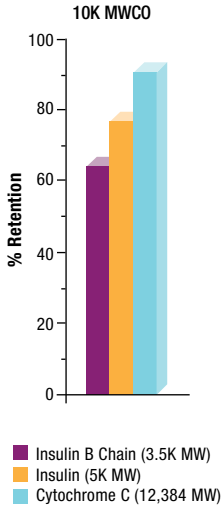


Figure 4. Sample retention by the 10K MWCO Thermo Scientific Slide-A-Lyzer Cassette membrane. Insulin B chain, insulin and cytochrome C (1mg/mL) in either 0.15M sodium chloride or 0.2M carbonate bicarbonate buffer, pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using the Pierce BCA Protein Assay.

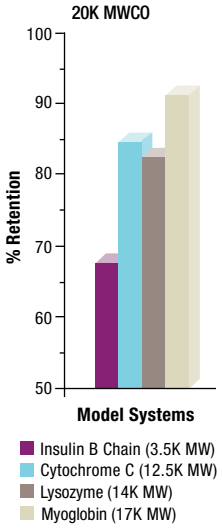


Figure 5. Sample retention by the 20K MWCO Thermo Scientific Slide-A-Lyzer Cassette membrane. Insulin B chain, cytochrome C, lysozyme and myoglobin were dialyzed overnight (17 hours) at 4°C in PBS, pH 7.4. The amount of retentate was estimated using the Pierce BCA Protein Assay.

Ordering Information

Product #	Description	Pkg. Size
2K		
87717	Slide-A-Lyzer G2 Dialysis Cassettes, 2K MWCO, 0.5mL *Sufficient for 10 samples, each 0.25 to 0.75mL.*	10 cassettes
87718	Slide-A-Lyzer G2 Dialysis Cassettes, 2K MWCO, 3mL *Sufficient for 10 samples, each 1 to 3mL.*	10 cassettes
87719	Slide-A-Lyzer G2 Dialysis Cassettes, 2K MWCO, 15mL *Sufficient for 8 samples, each 5 to 15mL.*	8 cassettes
87720	Slide-A-Lyzer G2 Dialysis Cassettes, 2K MWCO, 30mL *Sufficient for 6 samples, each 10 to 30mL.*	6 cassettes
87721	Slide-A-Lyzer G2 Dialysis Cassettes, 2K MWCO, 70mL *Sufficient for 6 samples, each 25 to 70mL.*	6 cassettes
3.5K		
87722	Slide-A-Lyzer G2 Dialysis Cassettes, 3.5K MWCO, 0.5mL *Sufficient for 10 samples, each 0.25 to 0.75mL.*	10 cassettes
87723	Slide-A-Lyzer G2 Dialysis Cassettes, 3.5K MWCO, 3mL *Sufficient for 10 samples, each 1 to 3mL.*	10 cassettes
87724	Slide-A-Lyzer G2 Dialysis Cassettes, 3.5K MWCO, 15mL *Sufficient for 8 samples, each 5 to 15mL.*	8 cassettes
87725	Slide-A-Lyzer G2 Dialysis Cassettes, 3.5K MWCO, 30mL *Sufficient for 6 samples, each 10 to 30mL.*	6 cassettes
87726	Slide-A-Lyzer G2 Dialysis Cassettes, 3.5K MWCO, 70mL *Sufficient for 6 samples, each 25 to 70mL.*	6 cassettes
7K		
87727	Slide-A-Lyzer G2 Dialysis Cassettes, 7K MWCO, 0.5mL *Sufficient for 10 samples, each 0.25 to 0.75mL.*	10 cassettes
87728	Slide-A-Lyzer G2 Dialysis Cassettes, 7K MWCO, 3mL *Sufficient for 10 samples, each 1 to 3mL.*	10 cassettes
10K		
87729	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 0.5mL *Sufficient for 10 samples, each 0.25 to 0.75mL.*	10 cassettes
87730	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 3mL *Sufficient for 10 samples, each 1 to 3mL.*	10 cassettes
87731	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 15mL *Sufficient for 8 samples, each 5 to 15mL.*	8 cassettes
87732	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 30mL *Sufficient for 6 samples, each 10 to 30mL.*	6 cassettes
87733	Slide-A-Lyzer G2 Dialysis Cassettes, 10K MWCO, 70mL *Sufficient for 6 samples, each 25 to 70mL.*	6 cassettes
20K		
87734	Slide-A-Lyzer G2 Dialysis Cassettes, 20K MWCO, 0.5mL *Sufficient for 10 samples, each 0.25 to 0.75mL.*	10 cassettes
87735	Slide-A-Lyzer G2 Dialysis Cassettes, 20K MWCO, 3mL *Sufficient for 10 samples, each 1 to 3mL.*	10 cassettes
87736	Slide-A-Lyzer G2 Dialysis Cassettes, 20K MWCO, 15mL *Sufficient for 8 samples, each 5 to 15mL.*	8 cassettes
87737	Slide-A-Lyzer G2 Dialysis Cassettes, 20K MWCO, 30mL *Sufficient for 6 samples, each 10 to 30mL.*	6 cassettes
87738	Slide-A-Lyzer G2 Dialysis Cassettes, 20K MWCO, 70mL *Sufficient for 6 samples, each 25 to 70mL.*	6 cassettes

Thermo Scientific Pierce Protein Concentrators, PES

Ultracentrifugation device for concentration of small protein samples.

The Thermo Scientific™ Pierce™ Protein Concentrators, PES are disposable ultrafiltration centrifugal devices with a vertical polyethersulfone (PES) membrane for concentrating, desalting and buffer exchanging biological samples with volumes of 0.5mL or less. The concentrators are available in four distinct molecular-weight cutoffs (MWCOs) of 3K, 10K, 30K and 100K. The 0.5mL Pierce Protein Concentrators are compatible with most bench-top microcentrifuges with fixed-angle rotors that accommodate 2.2mL tubes. The MWCOs are etched on the sides of the concentrators for easy identification, while a clear window with graduations marked on the side of each device allows for visual determination of the retentate volume.

Highlights:

- **Rapid concentration** – achieve 10- to 30-fold sample concentration in 10 minutes or less with greater than 90% protein recovery
- **Improved recovery** – unique design minimizes membrane fouling, even with particle laden solutions
- **Convenient** – spin format for sample volumes from 0.1 to 0.5mL
- **Versatile** – perform sample concentration, diafiltration or buffer-exchange on biological samples
- **Compatible** – use with most bench-top microcentrifuges with fixed-angle rotors

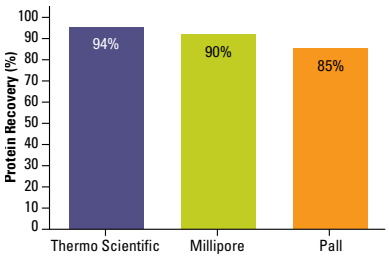


Figure 1. Thermo Scientific Pierce Protein Concentrators, PES, 10K MWCO provide exceptional recovery of low-concentration samples. Samples of Protein A (~46kDa) at approximately 0.05mg/mL starting concentrations were centrifuged in Pierce Protein Concentrators, PES, 10K MWCO and other suppliers' concentrators at 15,000 x *g* until a 15-fold decrease in sample volume was achieved. Protein recovery was determined by the Thermo Scientific™ Pierce™ Micro BCA Protein Assay.

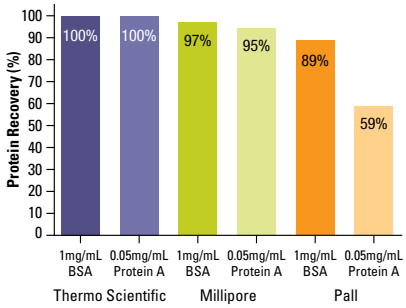


Figure 2. Thermo Scientific Pierce Protein Concentrators, PES, 30K MWCO provide exceptional recovery of low-concentration samples. Samples of Protein A (approximately 0.5mg/mL) and bovine serum albumin (BSA; 1.0mg/L) were centrifuged in Pierce Protein Concentrators, PES and other suppliers' concentrators at 15,000 x *g* until a 15-fold decrease in sample volume was achieved. Protein recovery was determined by the Pierce Micro BCA Protein Assay.

Table 1. Sample recovery and concentration using Thermo Scientific Protein Concentrators, PES, 100K. Samples of Fibrinogen and Apoferritin at 0.4mg/mL starting concentrations were centrifuged in Pierce Protein Concentrators and other suppliers' concentrators at 15,000 x *g* until a >15-fold decrease in sample volume was achieved. Protein recovery was determined by the Pierce Micro BCA Protein Assay.

Protein	Protein Concentrator	Time (15,000 x *g*)	Starting Concentration	Recovery (%)	Fold Concentration
Fibrinogen (334kDa)	0.5mL/100K	10 min	0.4mg/mL	89	18
Apoferritin (443kDa)			0.4mg/mL	95	28

Ordering Information

Product #	Description	Pkg. Size
88512	Pierce Protein Concentrators, PES, 3K MWCO, 0.5mL	25/pkg.
88513	Pierce Protein Concentrators, PES, 10K MWCO, 0.5mL	25/pkg.
88502	Pierce Protein Concentrators, PES, 30K MWCO, 0.5mL	25/pkg.
88503	Pierce Protein Concentrators, PES, 100K MWCO, 0.5mL	25/pkg.

Thermo Scientific Pierce Protein Concentrators

Disposable ultrafiltration centrifugal devices for protein concentration.

Thermo Scientific™ Pierce™ Protein Concentrators effectively combine simplicity, speed, capacity and recovery to yield enhanced concentration, purification and separation of proteins – even with dilute protein samples.

Their unique conical regenerated cellulose membrane provides a high degree of concentration in a single spin, while minimizing polarization and adsorption at the membrane surface. The concentrators provide efficient concentration of biological samples containing proteins, nucleic acids and viral particles. Pierce Concentrators are available with 9K, 20K and 150K MWCOs in 7mL and 20mL capacities and are compatible with swinging-bucket and fixed-angle rotors.

Highlights:

- **Superior protein concentration and recovery** – achieve greater than 110-fold protein concentration in less than 35 minutes with greater than 90% protein recovery
- **Convenient** – spin formats for concentrating 1-20mL of sample
- **Instrument-compatible** – universal rotor compatibility

Requires:

- Centrifuge equipped with a rotor for 15mL or 50mL conical tubes (for 7mL or 20mL Concentrators, respectively)

Applications:

- Protein concentration with tissue culture media, antiserum or monoclonal antibody preps
- Concentration of protein peaks following gel-permeation chromatography
- Removal of unincorporated protein label
- Concentration and desalt/buffer-exchange after eluting protein from ion-exchange, hydrophobic interaction (HIC), metal chelate or affinity chromatography columns

Table 2. Thermo Scientific Pierce Protein Concentrators provide exceptional recovery with low-concentration samples.

Pierce Concentrator	Protein†	Time (min)	Protein Concentration (mg/mL)	Recovery (%)	Fold Concentration‡
20mL/9K*	Cytochrome c	45	0.2	100	121
			0.01	96	117
20mL/20K**	BSA	45	0.2	98	137
			0.01	95	118
7mL/9K*	Cytochrome c	35	0.2	96	137
			0.01	88	129
7mL/20K**	BSA	35	0.2	97	81
			0.01	87	65
7mL/150K	IgG	15	0.33	95	325
20mL/150K			0.35	95	109

†For 9K and 20K samples, protein samples were centrifuged at 3,000 x g for 45 minutes at 22°C using a starting volume of 20mL with the 20mL concentrators and 5mL with the 7mL concentrators. For 150K samples, protein samples were centrifuged at 2,000 x g for 45 minutes.

‡Fold concentration was determined by dividing the starting volume by the recovered (retentate) volume.

**For the 9K concentrators, percent recovery was calculated by measuring the absorbance at 409nm of the retentate adjusted with buffer to the original volume.*

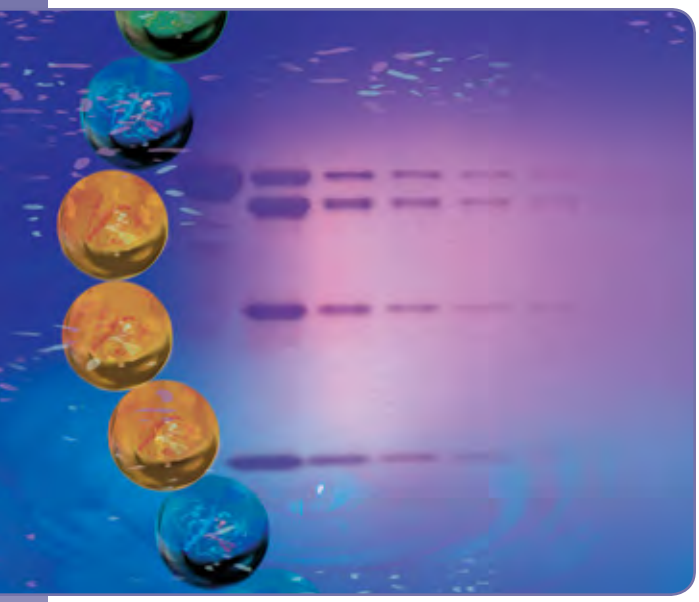
***For the 20K concentrators, percent recovery was determined using the Pierce Micro BCA Protein Assay (Product # 23235).*

Ordering Information

Product #	Description	Pkg. Size
87748	Pierce Protein Concentrators, 9K MWCO, 7mL Capacity	10/pkg.
89884A	Pierce Protein Concentrators, 9K MWCO, 7mL Capacity	25/pkg.
87749	Pierce Protein Concentrators, 9K MWCO, 20mL Capacity	10/pkg.
89885A	Pierce Protein Concentrators, 9K MWCO, 20mL Capacity	25/pkg.
87750	Pierce Protein Concentrators, 20K MWCO, 7mL Capacity	10/pkg.
89886A	Pierce Protein Concentrators, 20K MWCO, 7mL Capacity	25/pkg.
87751	Pierce Protein Concentrators, 20K MWCO, 20mL Capacity	10/pkg.
89887A	Pierce Protein Concentrators, 20K MWCO, 20mL Capacity	25/pkg.
89920	Pierce Protein Concentrators, 150K MWCO, 7mL Capacity	10/pkg.
89922	Pierce Protein Concentrators, 150K MWCO, 20mL Capacity	25/pkg.
89921	Pierce Protein Concentrators, 150K MWCO, 20mL Capacity	10/pkg.
89923	Pierce Protein Concentrators, 150K MWCO, 20mL Capacity	25/pkg.

protein quantitation and staining

Introduction



For workflows utilizing in-solution digestion protocols, it is critical to measure protein concentration following sample lysis using a standard protein assay in order to optimize the ratio of sample/protease (w/w) for digestion. It is also necessary to measure protein/peptide concentration upstream of isobaric labeling to ensure that equal amounts of sample are labeled before mixing. Although most protein assays are not sensitive enough to measure peptide concentration after digestion, the micro-BCA assay can sometimes be used for peptide quantitation.

For workflows utilizing in-gel digestion, proteins are separated by 1- or 2-dimensional (1D and 2D, respectively) SDS polyacrylamide gel electrophoresis (SDS-PAGE) to denature and separate proteins in a lysate. After electrophoresis, the protein bands are visualized using coomassie, fluorescence or silver stains; excised from the gel; and destained. The proteins in the excised gel band are reduced, alkylated and digested in-gel, and the resulting peptides are then extracted from the gel matrix for further processing.

Thermo Scientific Pierce BCA Protein Assay

Used in more labs than any other detergent-compatible protein assay.

The Thermo Scientific™ Pierce™ BCA Protein Assay Kit is a two-component, high-precision, detergent-compatible assay reagent set to measure (A562nm) total protein concentration compared to a protein standard.

Used in more labs than any other detergent-compatible protein assay, Pierce BCA Reagents provide accurate determination of protein concentration with most sample types encountered in protein research. The Pierce BCA Assay can be used to assess yields in whole cell lysates and affinity-column fractions, as well as to monitor protein contamination in industrial applications. Compared to most dye-binding methods, the BCA Assay is affected much less by protein compositional differences, providing greater protein-to-protein uniformity.

Highlights:

- **Colorimetric** – estimate visually or measure with a standard spectrophotometer or plate reader (562nm)
- **Excellent uniformity** – exhibits less protein-to-protein variation than dye-binding methods
- **Compatible** – unaffected by typical concentrations of most ionic and nonionic detergents
- **Moderately fast** – much easier and four times faster than the classical Lowry method
- **High linearity** – linear working range for BSA equals 20 to 2000µg/mL
- **Sensitive** – detect down to 5µg/mL with the enhanced protocol
- **Stability** – all reagents stable at room temperature for 2 years; working reagent stable for 24 hours

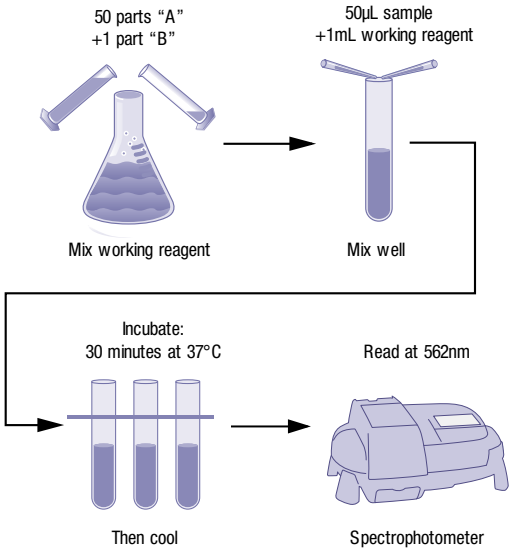


Figure 1. Thermo Scientific Pierce BCA Protein Assay protocol.

References

1. Smith, P.K., *et al.* (1985). *Anal. Biochem.* **150**:76-85.
2. Sorensen, K. (1992). *BioTechniques* **12**(2):235-236.
3. Ju, T., *et. al.* (2002). *J. Biol. Chem.* **277**:178-186.
4. Shibuya, T., *et. al.* (1989). *Tokyo Ika Daigaku Zasshi* **47**(4):677-682.
5. Hinson, D.L. and Webber, R.J. (1988). *BioTechniques* **6**(1):14-19.
6. Akins, R.E. and Tuan, R.S. (1992). *BioTechniques* **12**(4):496-499.
7. Tylianakis, P.E., *et. al.* (1994). *Anal. Biochem.* **219**(2):335-340.
8. Gates, R.E. (1991). *Anal. Biochem.* **196**(2):290-295.
9. Stich, T.M. (1990). *Anal. Biochem.* **191**:343-346.
10. Tuszynski, G.P. and Murphy, A. (1990). *Anal. Biochem.* **184**(1):189-191.

Ordering Information

Product #	Description	Pkg. Size
23225	**Pierce BCA Protein Assay Kit** *Sufficient for 500 tube assays or 5000 microplate assays.* Includes: BCA Reagent A BCA Reagent B, 25mL Albumin Standard Ampules, 2mg/mL	1L kit 2 x 500mL 25mL 10 x 1mL
23227	**Pierce BCA Protein Assay Kit** *Sufficient for 250 tube assays or 2500 microplate assays.* Includes: BCA Reagent A BCA Reagent B Albumin Standard Ampules, 2mg/mL	500mL kit 500mL 25mL 10 x 1mL
23221	**Pierce BCA Protein Assay Reagent A** *Sufficient for 1250 microplate assays when mixed with Reagent B.*	250mL
23228	**Pierce BCA Protein Assay Reagent A** *Sufficient for 2500 microplate assays when mixed with Reagent B.*	500mL
23223	**Pierce BCA Protein Assay Reagent A** *Sufficient for 5000 microplate assays when mixed with Reagent B.*	1L
23222	**Pierce BCA Protein Assay Reagent A** *Sufficient for 18,750 microplate assays when mixed with Reagent B.*	3.75L
23224	**Pierce BCA Protein Assay Reagent B** *Sufficient for 5000 microplate assays when mixed with Reagent A.*	25mL
23230	**Pierce BCA Solid** *Sufficient for preparing 2.5L of Reagent A.*	25g
23230B	**Pierce BCA Solid**	Custom

Thermo Scientific Pierce Micro BCA Protein Assay

Most sensitive BCA formulation measuring dilute protein solutions from 0.5 to 20µg/mL.

The Thermo Scientific™ Pierce™ Micro BCA Protein Assay is a special three-component version of our popular BCA Reagents, optimized to measure (A562nm) total protein concentration of dilute protein solutions (0.5 to 20µg/mL). Mixing together the three Micro BCA Reagents results in a working solution that is sufficiently concentrated to measure protein when mixed with an equal volume of sample. The result is an assay for accurately measuring 0.5 to 20µg/mL protein solutions. The assay is exceptionally linear and exhibits very low levels of protein-to-protein variability.

Highlights:

- **Sensitive** – accurately detect down to 0.5µg/mL (2µg/mL in microplate format)
- **High linearity** – linear working range for BSA equals 0.5 to 20µg/mL
- **Colorimetric** – measure with a standard spectrophotometer or plate reader (562nm)
- **Compatible** – unaffected by typical concentrations of most ionic and nonionic detergents
- **Convenient** – microplate and cuvette protocols provided with the instructions
- **Stable** – kit stable at room temperature; prepared working reagent stable for to 24 hours

References

1. Smith, P.K., *et al.* (1985). *Anal. Biochem.* **150**(1):76-85.
2. Kang, D.E., *et al.* (2002). *Cell* **110**:751-762.
3. Rawadi, G., *et al.* (1999). *J. Immunol.* **162**:2193-2203.
4. Blum, D., *et al.* (2002). *J. Neurosci.* **22**:9122-9133.
5. Paratcha, G., *et al.* (2003). *Cell* **113**:867-879.

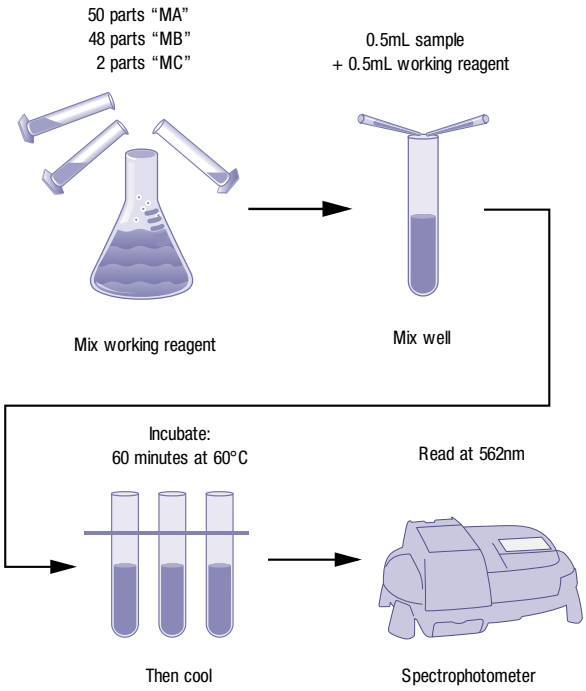


Figure 1. Thermo Scientific Pierce Micro BCA Protein Assay protocol.

Ordering Information

Product	Description	Pkg. Size
23235	**Pierce Micro BCA Protein Assay Kit** *Sufficient reagents to perform 480 standard tube assays or 3200 microplate assays.* Includes: Micro Reagent A (MA) (Sodium carbonate, sodium bicarbonate, and sodium tartrate in 0.2 N NaOH) Micro Reagent B (MB) (4% BCA in water) Micro Reagent C (MC) (4% cupric sulfate pentahydrate in water) Albumin Standard Ampules (2mg/mL)	Kit 240mL 240mL 12mL 10 x 1mL
23231	**Pierce Micro BCA Reagent A (MA)**	240mL
23232	**Pierce Micro BCA Reagent B (MB)**	240mL
23234	**Pierce Micro BCA Reagent C (MC)**	12mL
23209	**Albumin Standard Ampules, 2mg/mL** Contains: Bovine Albumin Fraction V in 0.9% NaCl solution containing sodium azide	Kit

Thermo Scientific Pierce Silver Stain for Mass Spectrometry

Fast and sensitive staining and destaining of protein gels for MS analysis.

Thermo Scientific™ Pierce™ Silver Stain for Mass Spectrometry is a complete kit for rapid and sensitive silver staining of proteins in polyacrylamide gels and efficient destaining of excised gel pieces for MS analysis. The kit bundles components of the high-performance Thermo Scientific™ Pierce™ Silver Stain Kit with optimized reagents to destain spots for subsequent in-gel tryptic digestion to recover peptide fragments for proteomics analysis. The resulting MS-compatible product and protocol deliver outstanding sensitivity and maintain favorable conditions for high-yield recovery and identification (sequence coverage) of proteins by MS. Silver staining of 2D gels is now an important intermediate step in a set of procedures that leads ultimately to identification of specific proteins in the proteome by mass fingerprinting methods.

Highlights:

- **Sensitive and fast staining** – the low-background, easy-to-use silver stain provides subnanogram sensitivity, detecting proteins at less than 0.25ng per spot in 30 minutes after fixing
- **Flexible staining protocol** – fix gel for 15 to 30 minutes or overnight without any affect on staining performance; stain for 1 to 30 minutes (typically 2 to 3 minutes)
- **Robust** – effective silver stain for even difficult-to-stain basic proteins, such as lysozyme (pI 10) and chymotrypsinogen A (pI 9.2), which are detectable at 0.2ng and 0.5ng, respectively
- **Simple, trouble-free spot preparation** – stained spots in excised gel pieces are easily destained and made ready for tryptic digestion in one hour
- **MS compatible** – reagents and protocol are optimized to provide excellent performance in MS following extraction of peptides from stained 1D or 2D gels (SDS-PAGE)
- **Complete kit** – contains all reagents necessary for staining and destaining before in-gel proteolysis and peptide recovery for analysis by MS
- **Convenient** – kit components are stable at room temperature; saves valuable refrigerator space and eliminates the need to equilibrate reagents before use

The Pierce Silver Stain protocol provides peak staining performance, flexibility, reliability and robustness for applications such as MALDI-MS. It enables both first-time and experienced users to achieve consistent and reliable staining using high-, low- and gradient-percentage polyacrylamide gels in single-dimension and 2D formats. The optimized staining method ensures extremely sensitive staining while minimizing covalent crosslinking of protein to the gel matrix, which can inhibit protein-peptide recovery following in-gel proteolysis. The destaining reagents facilitate complete removal of silver from stained protein bands and maximum protein recovery for subsequent MS analysis.

Silver staining methods generally use either glutaraldehyde or formaldehyde, which cause some covalent crosslinking of protein to each other and the gel matrix. To the extent that this crosslinking occurs, extraction or elution of protein from the gel will be inhibited. Pierce Silver Stain uses formaldehyde in the stain and developer working solutions. However, the procedure accompanying the Pierce Silver Stain Kit for Mass Spectrometry is optimized to maximize polypeptide recovery without greatly sacrificing sensitivity.

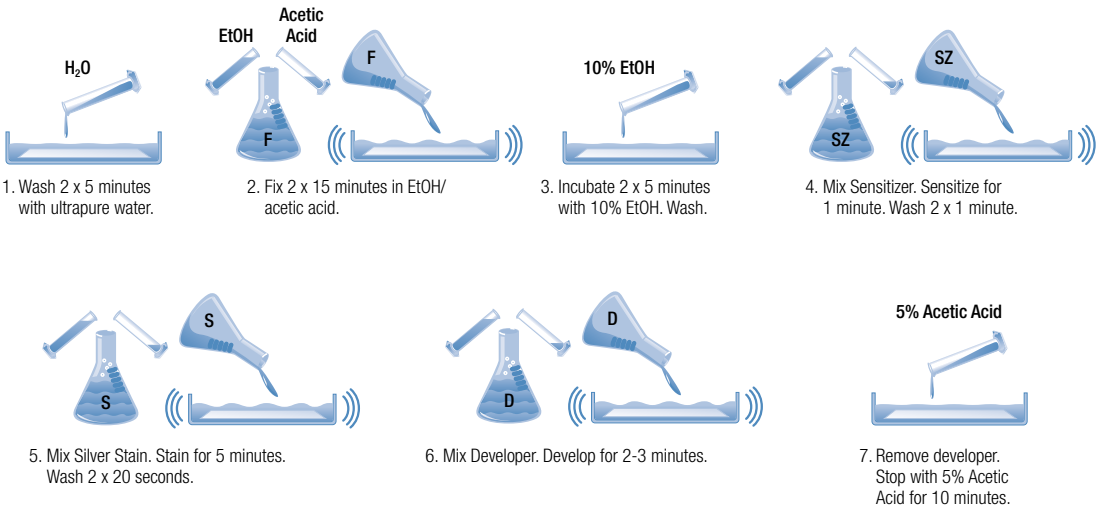


Figure 1. Staining protocol for the Thermo Scientific Pierce Silver Stain Kit for Mass Spectrometry.

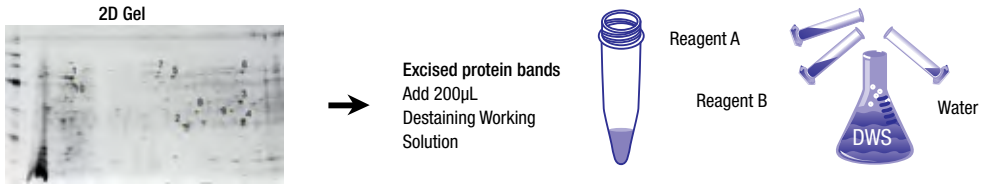


Figure 2. Spot excision and band destaining protocol summary. Place each gel piece in a 0.5mL microcentrifuge tube. Mix Destain Working Solution (DWS) at recommended volumes for Reagent A, B and water. Add 200µL of DWS to each gel piece, mix gently and incubate for 15 minutes. Repeat once.
Preparation for in-gel digestion. Wash three times with 25mM ammonium bicarbonate in 50% acetonitrile for 10 minutes each. Proceed with in-gel tryptic digestion (e.g., Thermo Scientific™ In-Gel Tryptic Digestion Kit, Product # 89871).

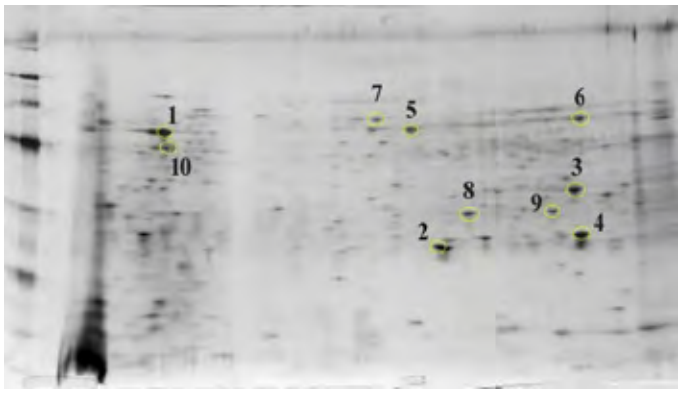


Figure 3. 2D gel stained with the Thermo Scientific Pierce Silver Stain Kit for Mass Spectrometry. Proteins of a rat mitochondrial extract were separated by IEF (pH 5-8 gradient) and SDS-PAGE, then stained. Ten spots were identified that stained well in three identical gels that were stained with three different stains (see Table 1). These spots were picked for in-gel digestion and MS analysis.

Table 1. Sequence coverage comparison for different gel stains. BSA, ovalbumin, chymotrypsinogen A and myoglobin (50ng each) were loaded onto mini gels and separated by SDS-PAGE. After electrophoresis, the respective gels were stained with Pierce Silver Stain for Mass Spectrometry, an MS-compatible stain silver stain from Supplier X and GelCode Blue Stain Reagent (Product # 24590). Bands were excised and destained, subjected to in-gel tryptic digestion (using Product # 89871) and prepared for analysis by MALDI/MS. Proteins were not reduced or alkylated before in-gel tryptic digestion. For all proteins, the Pierce Silver Stain for Mass Spectrometry performed better than or equal to the alternative silver staining method and GelCode Blue Stain.

	Thermo Scientific Silver Stain for MS			Supplier X Silver Stain for MS			Thermo Scientific GelCode Blue Stain		
Protein	**# Peptides**	**# Proteins**	**% Coverage**	**# Peptides**	**# Proteins**	**% Coverage**	**# Peptides**	**# Proteins**	**% Coverage**
BSA	63	13	21	53	6	11	40	7	18
Ovalbumin	40	5	13	44	1	2	42	1	2
Chymotrypsinogen A	47	4	9	41	2	5	41	1	2
Myoglobin	32	6	19	31	3	10	38	1	3

Ordering Information

Product #	Description	Pkg. Size
24600	**Pierce Silver Stain for Mass Spectrometry** *Sufficient for staining 20 mini gels (SDS-PAGE) and destaining 500 gel plugs.*	1L kit
	Includes: Silver Stain Sensitizer	2mL
	Silver Stain	500mL
	Silver Stain Enhancer	25mL
	Silver Stain Developer	500mL
	Silver Destain Reagent A	4mL
	Silver Destain Reagent B	14mL

Thermo Scientific Imperial Protein Stain

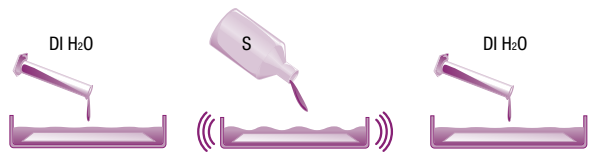
A fast and sensitive protein gel stain based on coomassie R-250 dye.

Thermo Scientific™ Imperial™ Protein Stain is a ready-to-use colorimetric stain formulated with coomassie dye R-250 that delivers consistent nanogram-level detection of proteins in polyacrylamide electrophoresis gels.

Imperial Protein Stain is a coomassie dye reagent for detection of protein bands in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2D gels. The stain is a unique formulation of coomassie brilliant blue R-250 that delivers substantial improvements in protein-staining performance compared to homemade or other commercial stains. Staining results in intensely colored protein bands that are easy to photograph and document with gel imagers. This reagent is one of the most sensitive colorimetric stains available, easily detecting 3 to 6 nanograms of protein per band. The Imperial Protein Stain protocol uses simple water-wash steps rather than methanol/acetic acid fixation and destaining, which saves valuable preparation time and minimizes reagent costs.

Highlights:

- **Sensitive** – detects less than 3ng protein per band with the enhanced protocol (3 hours)
- **Fast** – ready-to-use reagent detects less than 6ng protein per band in just 20 minutes
- **Robust** – highly consistent, reproducible protein staining technique
- **High contrast** – intense purple bands are easier to photograph or scan than typical coomassie blue stains
- **Versatile** – compatible with downstream MS analysis and protein sequencing
- **Convenient** – water washes only; no acid-fixative or methanol destaining solutions required
- **Stable** – one-year, room-temperature stability ensures consistent performance and saves refrigerator space
- **Flexible** – adjust staining and washing times to meet time and 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 1. Protocol summary for Thermo Scientific Imperial Protein Stain.

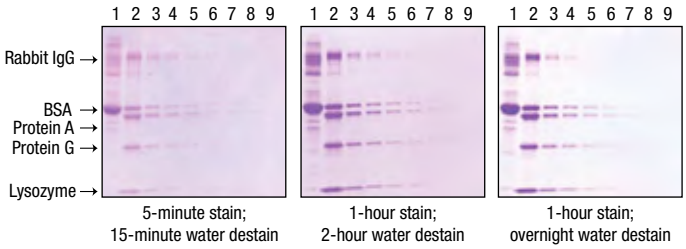


Figure 2. Enhanced sensitivity and crystal-clear background using Thermo Scientific Imperial Protein Stain. For even greater sensitivity and reduced background, gels can be stained with Imperial Protein Stain for 1 hour and washed with water from 1 hour to overnight. **Lane 1**: BSA only (6µg), **Lanes 2-9**: Loaded left to right at 1000ng, 200ng, 100ng, 50ng, 25ng, 12ng, 6ng and 3ng, respectively.

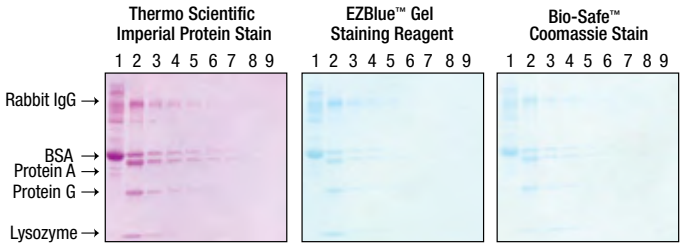


Figure 3. Thermo Scientific Imperial Protein Stain is fast and sensitive. Proteins were separated on 4-20% Tris-glycine gels, stained for 5 minutes and destained 3 x 5 minutes in water. **Lane 1**. BSA only (6µg), **Lanes 2-9**. Indicated proteins loaded left to right at 1000ng, 200ng, 100ng, 50ng, 25ng, 12ng, 6ng and 3ng, respectively.

Ordering Information

Product #	Description	Pkg. Size
24615	**Imperial Protein Stain** *Sufficient for 40 to 50 mini gels (SDS-PAGE).*	1L
24617	**Imperial Protein Stain** *Sufficient for 120 to 150 mini gels (SDS-PAGE).*	3 x 1L

Thermo Scientific GelCode Blue Safe Protein Stain

A coomassie gel stain that's safer and less costly to ship.

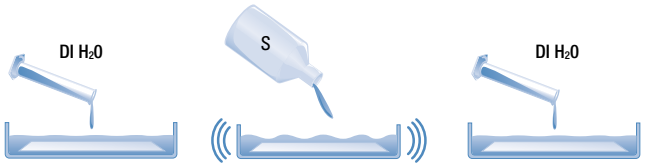
Thermo Scientific™ GelCode™ Blue Safe Protein Stain is a fast and sensitive coomassie G-250 dye-based reagent that provides one-step ultrasensitive detection of proteins in polyacrylamide gels and membranes and is formulated to avoid hazardous shipping costs.

GelCode Blue Safe Stain provides considerable sensitivity, allowing for the detection down to 9ng of protein. This protein-specific stain allows bands to be viewed directly on a gel or membrane during staining and is compatible with MS applications. The staining process is simple and flexible, usually requiring a 15-minute incubation of the gel or sample with the GelCode Blue Safe Protein Stain, followed by a simple water wash to yield blue protein bands and a clear background.

Because the supplied Activator Crystals are packaged and shipped dry in a separate bottle, GelCode Blue Safe Protein Stain is classified as nonhazardous under the U.S. Department of Transportation (DOT) shipping requirements.

Highlights:

- **Sensitive and fast** – detect down to 9ng of protein per band in 15 minutes
- **Safe** – non-corrosive to skin, non-flammable and safe to ship and store until activated
- **Convenient** – water washes only; no acid-fixative or methanol destain solutions required
- **Versatile** – suitable for staining 1D and 2D polyacrylamide gels and nitrocellulose and PVDF membranes (multiple protocols provided in the instructions)
- **Compatible** – useful for qualitative visualization, quantitative densitometry, and downstream excision and analysis by MS
- **Easy to use** – add and dissolve Activator Crystals before first use, then simply add reagent to stain gels
- **Stable** – store non-activated or activated stain at room temperature for up to one year



1. Wash the gel three times with deionized water (15 minutes).
2. Add activated GelCode Blue Safe Protein Stain (5 minutes-1 hour).
3. Destain in ultrapure water (1-2 hours or overnight).

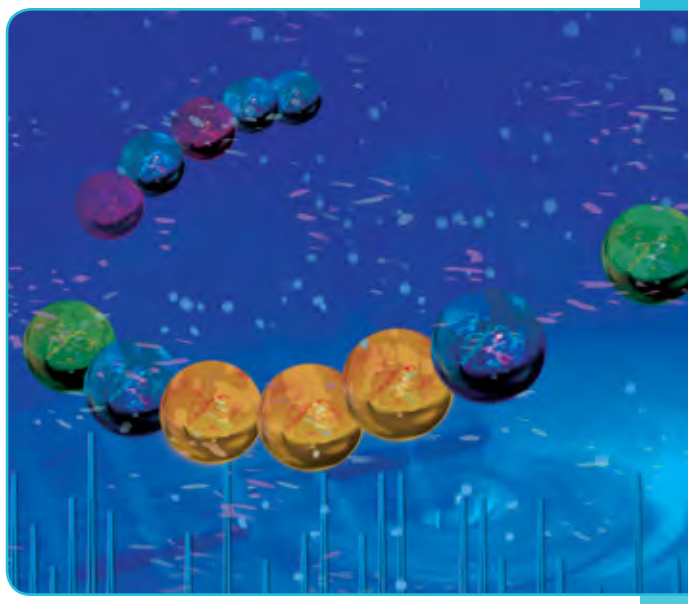
Figure 1. Protocol summary for the Thermo Scientific GelCode Blue Safe Stain. For proper protein staining, the Activator Crystals must be dissolved in the staining reagent before use. Add the entire contents of the Activator Crystals bottle into the GelCode Blue Safe Protein Stain bottle. Dissolve the crystals by mixing end-over-end for 1 minute, and then allow it to remain at room temperature for 5 minutes before use. Store the activated stain at room temperature.

GelCode Blue Safe Stain 5 minutes | GelCode Blue Safe Stain 15 minutes | GelCode Blue Safe Stain 1 hour

Figure 2. Thermo Scientific GelCode Blue Safe Protein Stain is fast and sensitive. A mixture of proteins (myosin, β-galactosidase, phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme and aprotinin) was electrophoresed on 4-20% Thermo Scientific™ Precise™ Protein Gels (Product # 25224). The gels were stained with GelCode Blue Safe Protein Stain for five minutes, 15 minutes and 1 hour. All gels were destained for 1 hour in ultrapure water. **Lanes 1-8**: Proteins at 1000ng, 500ng, 250ng, 125ng, 63ng, 31ng, 16ng and 8ng.

Ordering Information

Product #	Description	Pkg. Size
24594	**GelCode Blue Safe Protein Stain** *Sufficient for 40 to 50 mini gels (SDS-PAGE).*	1L
24596	**GelCode Blue Safe Protein Stain** *Sufficient for 140 to 175 mini gels (SDS-PAGE).*	3.5L



The decision to perform in-solution or in-gel digestion is generally determined by the sample amount and/or its complexity. In-solution digestion is recommended for small sample volumes because peptide extraction from the gel band after digestion can result in significant peptide loss. In-solution digestion is recommended for samples with low-to-moderate complexity. For highly complex samples, in-gel digestion has an inherent advantage because the SDS-PAGE workflow combines protein denaturation with separation, and provides a visual indication of the relative protein abundance in the sample. In addition, the peptide extraction protocol removes much of the salts and detergents that can be present in in-solutions workflows, although peptide recovery is reduced. In-solution digestion can be performed more rapidly than in-gel digestion because the extensive SDS-PAGE steps are eliminated. The in-solution protocol is also more amendable to high-throughput sample processing because the various steps can be automated, although there are automated processes for in-gel digestion and extraction as well.

Trypsin is the protease of choice for protein digestion. However, digestion with alternative proteases, such as Glu-C, LysN, Lys-C, Asp-N or chymotrypsin, can improve individual protein sequence coverage or generate unique peptide sequences for different MS applications. The Thermo Scientific™ Pierce™ Proteases are highly purified and modified for optimal protein digestion and validated for use in MS.

Cleavage sites of various proteases.

Protease	Cleavage Specificity
Trypsin	Carboxyl side of arginine and lysine residues
LysN	Amino side of lysine residues
Lys-C	Carboxyl side of lysine residues
Glu-C	Carboxyl side of glutamate or glutamate
Asp-N	Amino side of aspartate residues
Chymotrypsin	Carboxyl side of tyrosine, phenylalanine, tryptophan

Thermo Scientific Pierce Trypsin Protease, MS Grade

An economical alternative to Promega™ Trypsin Gold.

Thermo Scientific™ Pierce™ Trypsin Protease, MS Grade is a highly purified trypsin derived from porcine pancreatic extracts that has been chemically modified for maximum activity and stability in proteomics applications. The enzyme is TPCK-treated to eliminate chymotryptic activity and methylated to improve stability during protein digestion. This MS-grade, modified trypsin is then repurified and packaged in frozen liquid format (100µg at 1mg/mL), or lyophilized into glass vials and packaged in convenient 5 x 20µg, 5 x 100µg or 1mg fill sizes.

Highlights:

- **Exceptional selectivity** – cleaves at the carboxyl side of lysine and arginine residues with greater than 95% specificity
- **High purity** – no detectable chymotrypsin activity
- **Enhanced stability** – chemically modified for reduced autolytic activity
- **Economical** – available in multiple packaging formats including larger, more cost-effective sizes

Applications:

- In-gel digestion of proteins from 1- or 2-D gels
- In-solution tryptic digestion of proteins

Trypsin is a serine protease that specifically cleaves at the carboxyl side of lysine and arginine residues. The selectivity of this enzyme is critical for reproducible protein digestion and MS-based protein identification. Because chymotrypsin co-purifies with trypsin derived from natural sources, Pierce Trypsin Protease, MS Grade has been treated with TPCK to eliminate chymotrypsin activity, improving digestion specificity. Native trypsin is also subject to autolysis, which can reduce enzyme stability and efficiency. To reduce autolytic degradation, Pierce Trypsin Protease, MS Grade is chemically modified by methylation, yielding a highly active and more stable form of the enzyme.

In addition to possessing high specific activity and being resistant to autolytic digestion, Pierce Trypsin Protease, MS Grade can tolerate commonly used partially denaturing conditions such as 0.1% SDS, 1M urea and 10% acetonitrile. Pierce Trypsin Protease is most active in pH ranges of pH 7 to 9 and can be reversibly inactivated at pH < 4. The lyophilized enzyme is also stable for > 1 year when stored at –20°C.

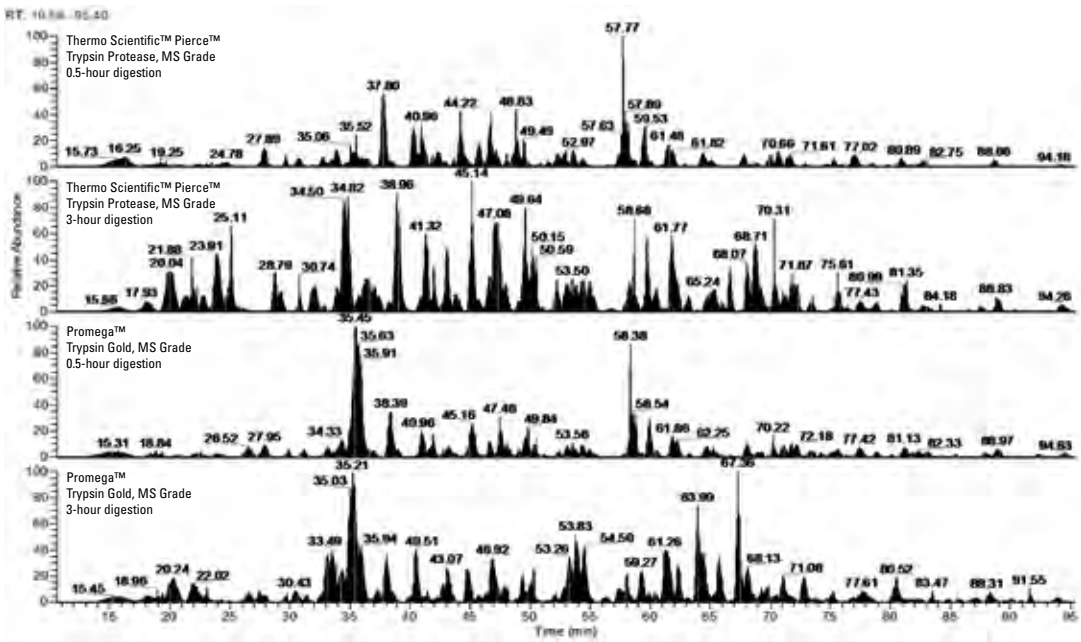


Figure 1. Excellent digestion performance with Thermo Scientific Pierce Trypsin Protease, MS Grade. Base peak chromatograms of a five-protein mixture sample digested with Pierce Trypsin Protease (top two) and Promega Trypsin Gold (bottom two). Samples (10µg each) were mixed with trypsin at a 1:50 ratio in a 50mM TEAB buffer (pH ~8) and incubated at 37°C for 30 minutes or 3 hours. Digested sample peptides (0.5µg each) were separated using nanoflow high-pressure liquid chromatography for analysis by a Thermo Scientific™ Velos™ Pro Mass Spectrometer.

Table 1. Comparison of Thermo Scientific Pierce Trypsin Protease, MS Grade to MS-grade trypsin from other suppliers. Enzyme purity, specific activity, chymotrypsin activity, activity retained after incubation and cost per microgram of Thermo Scientific Pierce, Sigma and Promega MS-Grade Trypsin Proteases.

Specifications	Thermo Scientific Pierce Trypsin Protease	Sigma Proteomics Grade Trypsin	Promega Trypsin Gold
Purity	> 95%	not specified	not specified
Specific activity (BAEE units)	> 15,000	> 10,000	> 15,000
Chymotrypsin activity (BTEE units)	< 0.1	not specified	not specified
Activity retained after 3-hour incubation at 37°C, pH 7.8	> 85%	not specified	> 85%
Cost/µg (based on 2014 US List Price)	$0.56 - $0.45	$1.85 - $0.50	$1.08

Table 2. Percent sequence coverage of selected proteins in a protein sample digest.

	Thermo Scientific Pierce Trypsin Protease, MS Grade		Promega Trypsin Gold, MS Grade	
Protein	30 min	3 hours	30 min	3 hours
Glutamate dehydrogenase	49	51	54	55
Myoglobin	68	82	68	78
Lactoperoxidase	41	45	46	45
Transferrin	63	64	67	64

Table 3. Percent sequence coverage for Erk1. Results were obtained by digestion with individual proteases, MS/MS analysis with CID or ETD fragmentation methods, and pair-wise combination of search results in Thermo Scientific™ Proteome Discoverer™ MultiConsensus Reports for Erk1.

	Sequence Coverage (%)	
	CID	ETD
Trypsin	87	51
Lys-C	45	52
Glu-C	47	43
Trypsin alone + Lys-C alone	93	74
Trypsin alone + Glu-C alone	93	71

Pairwise combinations of search results from two protease or fragmentation methods reveal complementary results. For example, trypsin digestion of Erk1 produces 87% coverage with CID but, when combined with Lys-C results, the total coverage increased to 93%. Peptide and protein sequence identifications are also improved for in-gel digestions of complex cell lysates. The combination of results from multiple individual protease digestions improves the number and confidence of protein identifications.

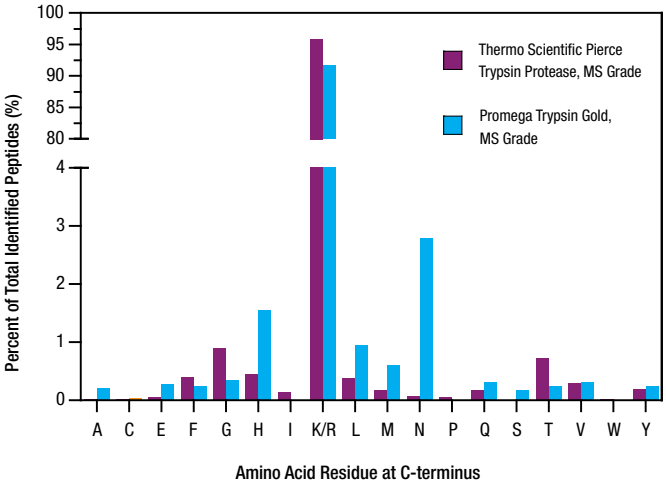


Figure 2. Comparison of the cleavage selectivity of MS-grade trypsin products. Five-protein mixture samples (10µg) were digested with Pierce Trypsin Protease or Promega Trypsin Gold for 3 hours and analyzed by LC/MS using a Thermo Scientific Velos Pro Mass Spectrometer. Data analysis was performed using a Mascot™ search engine with "no enzyme" digestion settings. Greater than 95% cleavage selectivity for lysine and arginine (K/R) was observed for Pierce Trypsin Protease.

Ordering Information

Product #	Description	Pkg. Size
90305	Pierce Trypsin Protease, MS Grade, Frozen	100µg
90057	Pierce Trypsin Protease, MS Grade	5 x 20µg
90058	Pierce Trypsin Protease, MS Grade	5 x 100µg
90059	Pierce Trypsin Protease, MS Grade	1mg

Thermo Scientific Pierce LysN Protease, MS Grade

Fast-digesting alternative to trypsin that yields complementary peptides.

Thermo Scientific™ Pierce™ LysN Protease, MS Grade, is highly purified native LysN protease that has been validated for maximum activity and stability in proteomic applications. Pierce LysN Protease is an MS-grade zinc metalloprotease derived from *Grifola frondosa*. The native Pierce LysN Protease has been highly purified to improve stability, specific activity and cleavage selectivity. Unlike trypsin, LysN protease cleaves at the amino-terminus of lysine residues. As a result, the peptides generated by LysN are longer than those generated by trypsin and have more prevalent charged amino terminal peptide fragments. Additionally, Pierce LysN Protease is more promising for epigenetic MS applications than Lys-C or trypsin because it is capable of cleaving methylated lysines.[1] The thermostability and chemical compatibility with denaturants make Pierce LysN Protease ideal for digestion of complex protein samples for "shotgun" proteomics.

Pierce LysN Protease is active over a wide range of temperatures and denaturing conditions. Efficient protein digestion can be completed in 2 hours at 50°C or 4 hours at 37°C; however, digestion is possible at both lower and higher temperatures. LysN also remains active under moderate denaturing conditions including 0.1% SDS, 6M urea or heating to 70°C. Maximal LysN activity occurs at pH 7-9. The lyophilized enzyme is stable for 2 years when stored at -20°C, and reconstituted stock solutions of LysN are stable at -80°C for 2 years or -20°C for one year without significant loss in activity.

Highlights:

- **Thermostable** – allows better digestion at higher temperatures (e.g., 50°C) in less time
- **Complementary to tryptic digests** – different cleavage site than trypsin
- **High purity** – no additional protease activity detected
- **N-terminal lysine cleavage specificity** – ≥ 90% for a complex protein sample
- **Versatile** – enzyme is effective over a wide temperature range and denaturing conditions
- **Stable** – provided in alyophilized format

Applications:

- Improved sequence coverage of protein digests
- *de novo* sequencing with Collision Induced Dissociation (CID)
- Epigenetic studies
- In-solution digestion of proteins

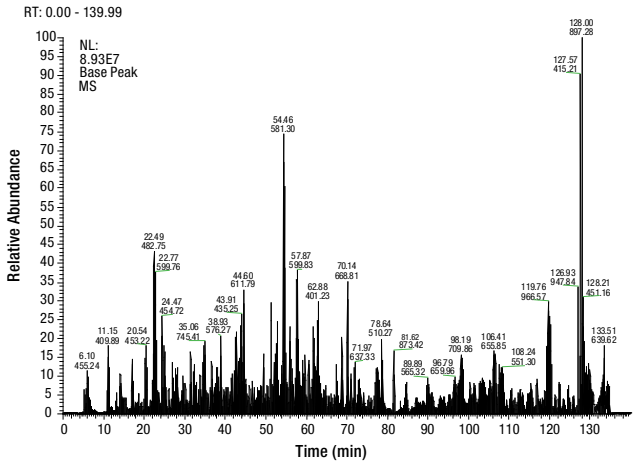


Figure 1. Exceptional digestion performance with Thermo Scientific Pierce LysN Protease, MS Grade. Base peak LC/MS chromatogram of an A549 cell lysate sample digested with Pierce LysN Protease. The sample (50µg) was prepared with the Pierce Mass Spec Sample Prep Kit for Cultured Cells and digested with LysN at a 1:75 protein:enzyme ratio and incubated at 37°C for 4 hours. Digested sample peptides (0.5µg) were separated using nanoflow high-pressure liquid chromatography and analyzed by a Thermo Scientific™ Orbitrap™ XL Mass Spectrometer.

Table 1. Percent sequence coverage of selected proteins in an A549 cell lysate upon in-solution digestion with LysN Protease or trypsin. The total (Merged) sequence coverage represents the combined coverage of separate LysN and trypsin digestions followed by LC-MS/MS analysis with CID.

Protein	LysN	Trypsin	Merged
Moesin	21%	24%	37%
Src substrate cortactin	25%	10%	30%
Glutamate dehydrogenase 1	16%	12%	28%
Epoxide hydrolase 1	13%	14%	23%

Ordering Information

Product #	Description	Pkg. Size
90300	Pierce LysN Protease, MS Grade	20µg
90301	Pierce LysN Protease, MS Grade	5 x 20µg

References

1. Taouatas, N., *et al.* (2010). Evaluation of metalloendopeptidase LysN protease performance under different sample handling conditions. *J Proteome Res.* **9(8)**:4282-8.
2. Taouatas, N., *et al.* (2008). Straightforward ladder sequencing of peptides using a LysN metalloendopeptidase. *Nat Methods* **5(5)**:405-7.
3. Nonaka, T., *et al.* (1998). Kinetic characterization of lysine-specific metalloendopeptidases from *Grifola frondosa and Pleurotus ostreatus* fruiting bodies. *Biochemistry* **124(1)**:157-62.

Thermo Scientific Lys-C Protease, MS Grade

Highly active alternative enzyme to trypsin that increases digestion efficiency.

Thermo Scientific™ Lys-C Protease, MS Grade, is purified native Lys-C protease that has been validated for maximum activity and stability in proteomic applications.

Lys-C Protease is a 30kDa serine protease isolated from *Lysobacter enzymogenes* that hydrolyzes proteins specifically at the carboxyl side of lysine. It can be used for in-solution or in-gel digestion workflows to produce peptides for LC-MS/MS protein identification. Lys-C has high activity and specificity for lysine residues, resulting in larger peptides and less sample complexity than trypsin (i.e., fewer peptides). Lys-C can also cleave lysines followed by prolines and remains active under highly denaturing conditions (i.e., 8M urea). For this reason, Lys-C is often used for sequential digestion of proteins followed by trypsin to decrease tryptic missed cleavages. These unique properties of Lys-C ensure high digestion efficiency alone or followed by tryptic digestion.

Lys-C prototypic peptides typically have higher charge states, making it the enzyme of choice for use with ETD fragmentation. Lys-C is also used commonly in phosphopeptide enrichment workflows and with isobaric-tagged peptide quantitation. Because Lys-C generates peptides with primary amines at both the N and C terminus of a peptide, each peptide can be double-labeled with amine-reactive isobaric tags. This results in enhanced peptide ionization and improved limits of quantitation since more fragment ions can be re-isolated during MS3 acquisition.

Highlights:

- **Enhanced digestion** – when used in tandem with trypsin, decreases tryptic missed cleavages
- **Increased sequence coverage** – better protein characterization results from overlapping peptides with complementary chromatographic, ionization and fragmentation properties
- **Carboxyl lysine cleavage specificity** – at least 90% for a complex protein sample
- **Efficient** – protein digestion can be completed in 2 hours at 37°C
- **Versatile** – effective even under highly denaturing conditions (e.g., 8M urea 2M guanidine HCl, 1% SDS, 2% CHAPS and 40% acetonitrile)
- **Stable** – store lyophilized protease for up to one year at -20°C

Applications:

- Improved sequence coverage of protein digests
- *De novo* sequencing
- Epigenetic studies
- In-gel digestion of proteins

Ordering Information

Product #	Description	Pkg. Size
90051	Lys-C Protease, MS Grade	20µg

Thermo Scientific Asp-N Protease, MS Grade

Highly complementary enzyme to trypsin that cleaves at the amino side of aspartate residues.

Thermo Scientific™ Asp-N Protease is a 27kDa metalloproteinase isolated from a mutant of *Pseudomonas fragi* that hydrolyzes proteins specifically at the amino side of aspartate and cysteic acid residues with high specificity. Asp-N can be used alone or in parallel with trypsin or other proteases to produce protein digests for peptide mapping and protein sequencing due to its highly specific cleavage of peptides, generating a limited number of peptide fragments. Asp-N Protease is suitable for either in-solution or in-gel digestion workflows. This MS-grade Asp-N Protease is packaged in a convenient, lyophilized format (2µg).

Cysteic acids result from the oxidization of cysteine residues. Cleavage can also occur at glutamic residues; however, the rate of cleavage at the glutamyl residues is significantly lower than the rate of cleavage at the aspartic acid residues.

Efficient protein digestion with Asp-N Protease can be completed in 2-20 hours at 37°C. Asp-N Protease remains active under denaturing conditions such as 1M urea, 2M guanidine HCl, 0.1% SDS, 2% CHAPS and 10% acetonitrile. Asp-N activity is optimal in the pH range of 6-8. The lyophilized enzyme is stable for one year when stored at -20°C.

Highlights:

- **Complementary to tryptic digests** – hydrolyzes proteins specifically at the amino side of aspartate and cysteic acid residues
- **Increased sequence coverage** – better protein characterization results from overlapping peptides with complementary chromatographic, ionization and fragmentation properties
- **High specific activity** – greater than 20,000units/mg protein
- **N-terminal arginine cleavage specificity** – at least 90% for a complex protein sample
- **Stable** – provided in a lyophilized format

Applications:

- Improved sequence coverage of protein digests
- In-solution digestion of proteins
- In-gel digestion of proteins

Ordering Information

Product #	Description	Pkg. Size
90053	Asp-N Protease, MS Grade	2µg

protein digestion

Ensure consistent performance using high-quality proteases

Thermo Scientific Glu-C Protease, MS Grade

Highly complementary enzyme to trypsin that cleaves at the carboxyl side of glutamate.

Thermo Scientific™ Glu-C Protease, also referred to as V-8 Protease, is a 27kDa serine protease isolated from *Staphylococcus aureus* that hydrolyzes proteins specifically at the carboxyl side of glutamic acids with high specificity. Glu-C Protease can be used alone or in combination with trypsin or other proteases to produce protein digests for peptide mapping and protein sequencing due to its highly specific cleavage of peptides, generating a limited number of peptide fragments. Only the glutamic residues are cleaved in ammonium bicarbonate and ammonium acetate buffers, while glutamic and aspartic residues are cleaved in phosphate buffers. Glu-C Protease is suitable for either in-solution or in-gel digestion workflows. This MS-grade Glu-C is packaged in a convenient, lyophilized format (5 x 10µg).

Efficient protein digestion with Glu-C Protease can be completed in 5-18 hours at 37°C. Glu-C Protease remains active under denaturing conditions such as 2M urea, 1M guanidine HCl, 0.1% SDS, 2% CHAPS and 20% acetonitrile. Glu-C activity is optimal at pH 8. The lyophilized enzyme is stable for one year when stored at -20°C.

Highlights:

- **Complementary to tryptic digests** – hydrolyzes proteins specifically at the carboxyl side of glutamic acids
- **Increased sequence coverage** – better protein characterization results from overlapping peptides with complementary chromatographic, ionization and fragmentation properties
- **High specific activity** – greater than 500units/mg protein
- **C-terminal glutamine cleavage specificity** – at least 90% for a complex protein sample
- **Stable** – provided in a lyophilized format

Applications:

- Improved sequence coverage of protein digests
- In-solution digestion of proteins
- In-gel digestion of proteins

Ordering Information

Product #	Description	Pkg. Size
90054	Glu-C Protease, MS Grade	5 x 10µg

Thermo Scientific Chymotrypsin Protease, MS Grade

Highly complementary enzyme to trypsin that cleaves at the carboxyl side of tyrosine, phenylalanine, tryptophan and leucine.

Thermo Scientific™ Chymotrypsin Protease, MS Grade, is purified bovine chymotrypsin that has been treated with TLCK for maximum activity and selectivity in proteomic applications.

Chymotrypsin Protease, MS Grade, is a 25kDa serine protease derived from bovine pancreatic extracts. The enzyme is TLCK-treated to eliminate tryptic activity. In general, chymotrypsin generates a larger number of shorter peptides than trypsin. This MS-grade Chymotrypsin is packaged in a lyophilized format (convenient 4 x 25µg).

Chymotrypsin specifically cleaves at the carboxyl side of tyrosine, phenylalanine, tryptophan and leucine. Two predominant forms of chymotrypsin, A and B, are found in equal amounts in bovine pancreas. They are similar proteins (80% homology), but have different proteolytic characteristics. Both forms of chymotrypsin are present in Thermo Scientific Chymotrypsin Protease, MS Grade.

The selectivity of this enzyme is important for reproducible protein digestion and MS-based protein identification. Because Chymotrypsin co-purifies with trypsin derived from natural sources, it has been treated with TLCK to eliminate potential tryptic activity, improving digestion specificity.

In addition to possessing high specific activity, Chymotrypsin Protease, MS Grade can tolerate commonly used partially denaturing conditions, such as 0.1% SDS, 2M urea, 2M Guanidine HCl, 1% CHAPS and 30% acetonitrile. The enzyme is most active in pH ranges from 7.5 to 8.5. The lyophilized protease is stable for >1 year when stored at -20°C.

Highlights:

- **High selectivity** – cleaves at the carboxyl side of tyrosine, phenylalanine, tryptophan and leucine
- **Increased sequence coverage** – better protein characterization results from overlapping peptides with complementary chromatographic, ionization and fragmentation properties
- **High purity** – treated with TLCK to eliminate trypsin activity
- **High activity** – greater than 45units/mg protein

Applications:

- In-gel digestion of proteins from 1-D or 2-D gels
- In-solution tryptic digestion of proteins

Ordering Information

Product #	Description	Pkg. Size
90056	Chymotrypsin Protease, MS Grade	4 x 25µg

Thermo Scientific In-Gel Tryptic Digestion Kit

Excellent for mass spectrometric analysis sample preparation.

The Thermo Scientific™ In-Gel Tryptic Digestion Kit provides a complete set of reagents for performing 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, easy-to-follow instructions. Each component and step has been optimized and balanced to produce accurate and clean digests for dependable MS analysis with high sequence coverage.

Highlights:

- **Convenient** – includes all necessary reagents for destaining coomassie- or fluorescent dye-stained proteins, reduction and alkylation of cystines, and tryptic digestion
- **Robust** – the procedure and reagents produce reliable digestions and data generation using a wide range of conditions without optimization
- **Accurate** – contains highly purified and modified MS-grade trypsin that shows no chymotryptic activity and minimal autolytic activity

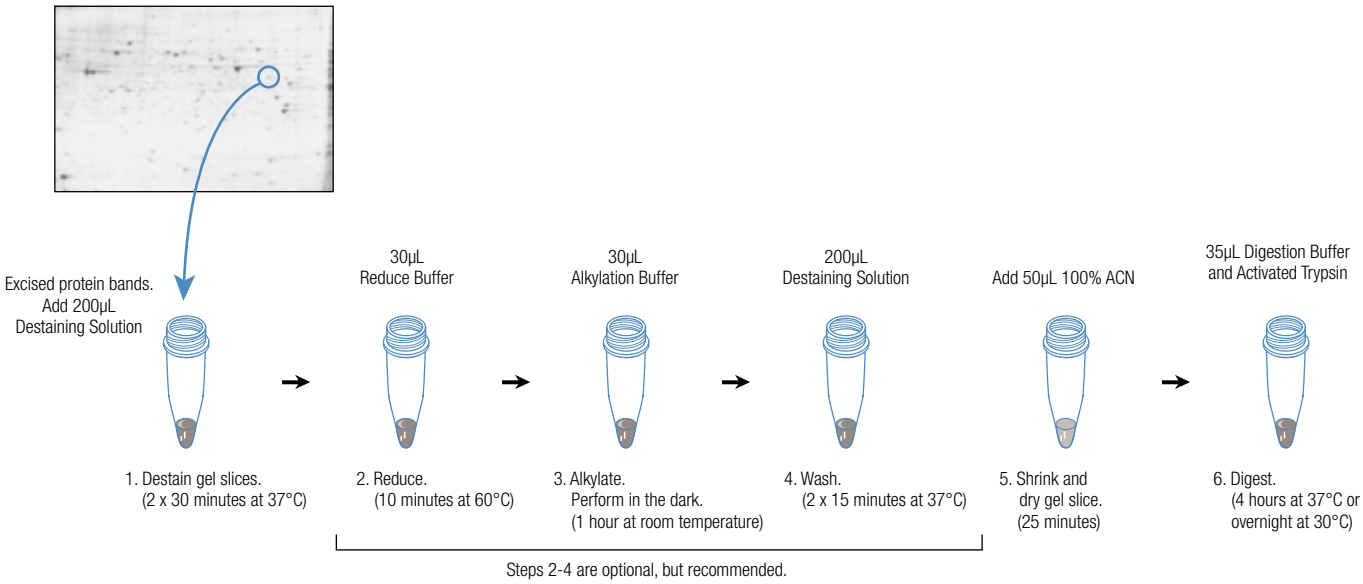


Figure 1. Schematic protocol for the Thermo Scientific In-Gel Tryptic Digestion Kit.

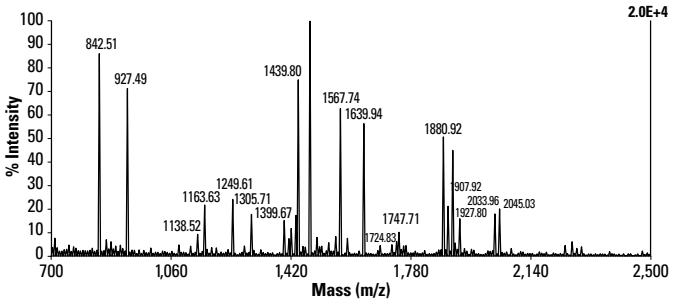


Figure 2. MALDI-TOF MS analysis of bovine serum albumin (BSA) digest. Ten nanograms (150fmol) of BSA was separated by SDS-PAGE and stained with GelCode Blue Stain Reagent and then processed with the In-Gel Tryptic Digestion Kit. The resulting digest was treated with Thermo Scientific™ Pierce™ C18 Spin Columns (Product # 89870) and then subjected to analysis on an Applied Biosystems Voyager DE™-PRO MALDI-MS in positive ion, linear, delayed-extraction mode. Database searches identified BSA with 47.0% sequence coverage.

Ordering Information

Product #	Description	Pkg. Size
89871	**In-Gel Tryptic Digestion Kit**	Kit
	Sufficient for approximately 150 in-gel digestions.	
	Includes: Trypsin, Modified	20µg
	Trypsin Storage Solution	40µL
	Acetonitrile	70mL
	Ammonium Bicarbonate	300mg
	Tris[2-carboxyethyl]phosphine (TCEP)	500µL
	Iodoacetamide	500mg

Thermo Scientific In-Solution Tryptic Digestion and Guanidination Kit

Analyze proteins by MS with confidence.

Accurate identification of proteins and analysis of PTMs by MS require accurate and complete protein digestion and peptide modification. The Thermo Scientific™ In-Solution Tryptic Digestion and Guanidination Kit provides an optimized procedure and reagents for approximately 90 protein digests.

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 MS provides a powerful tool for identifying proteins or analyzing PTMs. 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 contains optimized procedures and reagents for reduction, alkylation, digestion and guanidination to provide reliable MS analysis of approximately 90 protein samples containing 0.025-10µg of protein.

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.

Proteins processed with the In-Solution Tryptic Digestion and Guanidination Kit produce clean and reliable mass spectra with high sequence coverage (Table 1). Using the guanidination procedure to convert lysines to homoarginines enhances the overall signal intensity of lysine-containing peptides by an average of 1.5- to 4.0-times, eliminating the ionization bias for peptides with a terminal arginine and improving sequence coverage and the reliability of data analysis.

Highlights:

- **Optimized** – complete digestion is achieved for 0.025-10µg 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

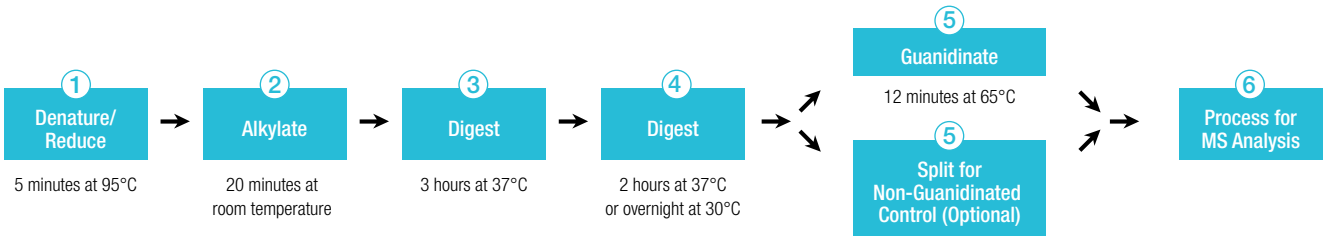


Figure 1. Thermo Scientific In-Solution Tryptic Digestion and Guanidination Kit protocol.

Table 1. Sequence coverage data for tryptic digestions with and without guanidination for three proteins.

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

† *High levels of sequence coverage were obtained for all test proteins processed with the In-Solution Tryptic Digestion and Guanidination Kit, especially when the guanidination procedure was used. Sequence coverage based only on those peptides expected to be identified based on scanning from 600-2,000m/z.*

Ordering Information

Product #	Description	Pkg. Size
89895	**In-Solution Tryptic Digestion and Guanidination Kit** *Sufficient reagents for preparing 90 digests.*	Kit
	Includes: Trypsin, Modified	20µg
	Trypsin Storage Solution	50µL
	Ammonium Bicarbonate	50mg
	No-Weigh DTT	7.7mg
	Iodoacetamide (IAA)	500mg
	O-Methylisourea Hemisulfate Salt (OMI)	400mg
	Ammonium Hydroxide	1mL

Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH

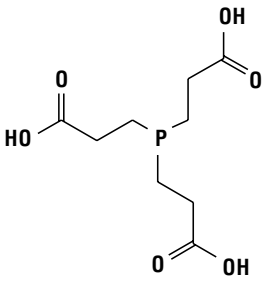
Efficient, odor-free alternative to sample reduction prior to SDS-PAGE analysis.

Thermo Scientific™ Bond-Breaker™ TCEP Solution, Neutral pH is a stable, 0.5M solution of the thiol-free, phosphine-based TCEP compound, useful as a 10X stock for addition to SDS-PAGE sample loading buffers to reduce protein disulfide bonds.

Bond-Breaker TCEP Solution is a potent, odorless, thiol-free reducing agent with broad application to protein and other research involving reduction of disulfide bonds. This product is an effective and convenient replacement for β-mercaptoethanol or DTT in SDS-PAGE sample buffers. The neutral pH of this reagent provides sharp bands and avoids exposing proteins to the strong acid of TCEP•HCl, which can result in hydrolysis and carbohydrate modification.

Highlights:

- **Odorless** – unlike DTT and β-ME, TCEP is odor-free, contributing to a healthier lab environment
- **Efficient** – 5 to 50mM TCEP thoroughly reduces most peptide or protein disulfide bonds within a few minutes (i.e., just as effective as DTT)
- **Specific** – selective and complete reduction of even the most stable water-soluble alkyl disulfides
- **Fast** – reduces protein disulfides at room temperature and pH 5 in less than five minutes
- **Stable** – resistant to air oxidation; nonvolatile and nonreactive toward other functional groups found in proteins
- **Versatile** – reduces peptides and proteins over a broad range of pH, salt, detergent and temperature conditions
- **Compatible** – removal of the reducing agent is not necessary before most applications, (e.g., histidine-tagged protein purification, maleimide conjugations), because TCEP does not contain sulfhydryl groups



TCEP
M.W. 250.15

Considerations for use of Bond-Breaker TCEP Solution:

- Reduction occurs over a wide range of pH (pH 4.0-9.0) and temperature (5-95°C).
- Most proteins are reduced efficiently without a denaturant. However, adding a denaturant such as guanidine•HCl may aid in exposing internal disulfides to the Immobilized TCEP.
- Urea is not recommended as a denaturant as it forms cyanates that react with sulfhydryl groups.
- Do not allow metals to contact the TCEP solution as this will decrease TCEP activity.
- Including 5-20mM EDTA in the sample buffer during reduction helps prevent reoxidation of the sulfhydryl groups by divalent metals such as Zn^{2+}, Cu^{2+} and Mg^{2+}.
- The reduced sample should be used immediately after reduction because disulfides will reform over time.

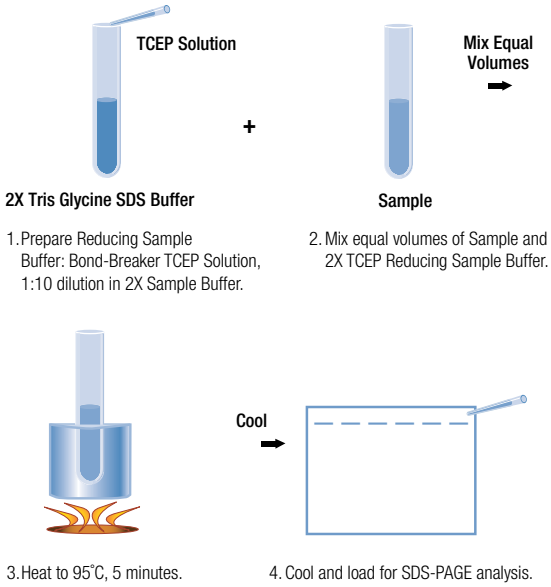


Figure 1. Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH protocol.

Reference

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

Ordering Information

Product #	Description	Pkg. Size
77720	**Bond-Breaker™ TCEP Solution, Neutral pH** Stable, 0.5 M solution	5mL

protein digestion

Streamline protein digestion with high-quality reagents

Thermo Scientific DTT and No-Weigh DTT

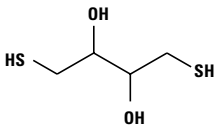
Popular disulfide reducing agent for protein electrophoresis (SDS-PAGE) and other methods.

Thermo Scientific™ Pierce™ DTT (Dithiothreitol) is offered as 7.7mg x 48-tube microplates for convenient preparation of 100µL of 0.5M stocks for addition to protein samples for effective reduction of disulfide bonds.

Dithiothreitol is a popular reducing agent for many protein research applications, including reducing polyacrylamide electrophoresis (SDS-PAGE). The No-Weigh Format consists of a 48-microtube tray containing dry, stable, single-use aliquots of the DTT. Just puncture the seal of one microtube and add 100µL of water or buffer. In just a few seconds you will have a solution of 500mM DTT, ready for a variety of laboratory methods. Dilute as necessary or add the appropriate volume of the 500mM stock solution to achieve the amount of reducing agent required for your specific application.

Highlights (of the No-Weigh Format):

- **Saves time** – just add 100µL water to make 500mM DTT solution in seconds
- **Eliminates waste** – makes 100µL at a time, just the right amount for most applications
- **Always fresh** – ensures full reducing strength for every use; no need to store stock solutions



DTT
Dithiothreitol
M.W. 154.25

Product Details:

With the convenient No-Weigh Format, a 500mM DTT solution can be made in less than 30 seconds. The unique packaging ensures that the reducing agent is at full strength and able to protect proteins from oxidative damage or reduce any disulfides prior to electrophoresis.

Reference

1. Zahler, W.L. and Cleland, W.W. (1964). *Biochemistry* **3**:480-482.

Thermo Scientific Iodoacetic Acid and Iodoacetamide

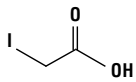
Rapid carboxymethylation of reduced cysteine residues.

Thermo Scientific™ Pierce™ Iodoacetic Acid (IAA) can react with several protein functional groups but is typically used for specific S-carboxymethylation of sulfhydryls (reduced cysteines).

Iodoacetic acid reacts with sulfhydryls on cysteines, imidazolyl side chain nitrogens of histidines, the thioether of methionine and the primary amine group of lysines. The rate of reaction and specificity is dependent on the ionization level, which can be manipulated by the pH of the reaction condition.

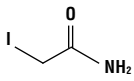
Highlights:

- React at slightly alkaline pH for specific S-carboxymethylation of free sulfhydryls
- React at low pH for specific carboxymethylation of methionines
- React at high pH to favor carboxymethylation of histidines and lysines
- Methylate reduced cysteine peptide fragments in protease digests for MS



IAA
Iodoacetic Acid
M.W. 185.95

- Chemical name: iodoacetic acid
- Reactivity: sulfhydryl groups
- Chemical formula: $C_2H_3IO_2$
- CAS number: 64-69-7
- Molecular weight: 185.95
- Form: solid, powder



IAM
2-iodoacetamide
M.W. 184.96

- Chemical name: iodoacetamide
- Reactivity: sulfhydryl groups
- Chemical formula: C_2H_4INO
- CAS number: 144-48-9
- Molecular weight: 184.96
- Form: solid, powder

References

1. Hall, J., *et al.* (1989). Role of specific lysine residues in the reaction of Rhodobacter sphaeroides cytochrome c2 with the cytochrome bc1 complex. *Biochem.* **28**:2568.
2. Hermason, G.T. (2008). *Bioconjugate Techniques*, Second Edition. Academic Press. pp. 109-111.

Ordering Information

Product #	Description	Pkg. Size
20290	**Pierce DTT (Dithiothreitol)** *Sufficient for preparing 64.8mL of 0.5M solution.*	5g
20291	**Pierce DTT (Dithiothreitol), No-Weigh Format** *Sufficient for preparing 100µL of 0.5M DTT per microtube.*	48 x 7.7mg

Ordering Information

Product #	Description	Pkg. Size
35603	**Pierce Iodoacetic Acid** *Sufficient for 27mL of 100mM solution.*	500mg
90034	**Pierce Iodoacetamide, Single-Use** *Sufficient for 24 separate uses for alkylation before MS, each with up to 75 protein samples.*	24 x 9.3mg

Thermo Scientific Pierce MMTS (Methyl methanethiosulfonate)

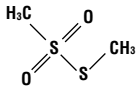
Reversibly blocks cysteines and other sulfhydryl groups.

Thermo Scientific™ Pierce™ MMTS (methyl methanethiosulfonate) is a small compound that reversibly blocks cysteines and other sulfhydryl groups, enabling the study of enzyme activation and other protein functions.

MMTS is a sulfhydryl-reactive compound that can reversibly sulfenylate thiol-containing molecules. Reacting MMTS with reduced sulfhydryls (-SH) results in their modification to dithiomethane (-S-S-CH_3). Treatment with reducing agents, such as dithiothreitol (DTT), 2-mercaptoethanol (2-ME) or Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), will cleave the disulfide groups to restore the original sulfhydryl. MMTS is commonly used to study biochemical pathways involving thiol-dependent enzymes.

Highlights:

- Converts sulfhydryl groups on cysteine side chains into -S-S-CH_3
- Reaction is reversible with DTT or TCEP, restoring the free sulfhydryl
- Used to modify thiol groups in creatine kinase



MMTS
S-methyl methanesulfonothioate
M.W. 126.20

- Chemical name: methyl methanethiosulfonate
- Reactivity: sulfhydryl groups (adds methyl group via disulfide bond)
- Chemical formula: $C_2H_6O_2S_2$
- CAS number: 2949-92-0
- Molecular weight: 126.20
- Form: liquid
- Boiling point: 85-87°C/0.8mm

References

1. Kirley, T.L. (1989). Reduction and fluorescent labeling of cyst(e)ine-containing proteins for subsequent structural analyses. *Anal. Biochem.* **180**:231.
2. Smith, D.J., *et al.* (1975). Simple alkanethiol groups for temporary blocking of enzymes. *Biochemistry* **14**:766.

Ordering Information

Product #	Description	Pkg. Size
23011	**Pierce MMTS** *Sufficient for 1585mL of typical 1X (1mM) reaction solution, or approx. 100 uses at 2mg reagent per use.*	200mg

Thermo Scientific Pierce N-Ethylmaleimide (NEM)

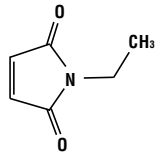
Permanently blocks sulfhydryl groups.

Thermo Scientific™ Pierce™ N-Ethylmaleimide (NEM) is a small compound that forms stable, covalent thioether bonds with sulfhydryls (e.g., reduced cysteines), enabling them to be permanently blocked to prevent disulfide bond formation.

NEM is an alkylating reagent that reacts with sulfhydryls. At pH 6.5-7.5, the maleimide reaction is specific for sulfhdryls; however, at pH values greater than 7.5, reactivity with amino groups occurs. Maleimide groups react with sulfhydryls by nucleophilic attack of the thiolate anion on one of the carbons of the double bond. When sufficient sulfhydryls have been blocked, the reaction can be monitored by measuring the decrease in absorbance at 300nm as the double bond becomes a single bond. The resulting thioether group is non-reversible and terminates in an ethyl group, blocking or capping the sulfhydryl.

Highlights:

- Permanently block sulfhydryls to prevent disulfide bond formation.
- Monitor the reaction by measuring the decrease in absorbance at 300nm
- Block sulfhydryl-containing reagents that interfere in enzyme assays



NEM
N-Ethylmaleimide
M.W. 125.13

- Functional groups: maleimide (sulfhydryl-reactive)
- Chemical name: N-ethylmaleimide
- Chemical formula: $C_6H_7NO_2$
- CAS number: 128-53-0
- Molecular weight: 125.13
- Form: white to off-white crystals or powder

Reference

1. Partis, M.D., *et al.* (1983). Cross-linking of protein by ω-maleimido alkanoyl N-hydroxysuccinimido esters. *J. Prot. Chem.* **2(3)**:263-77.

Ordering Information

Product #	Description	Pkg. Size
23030	**Pierce N-Ethylmalemide (NEM)** *Sufficient for 200mL of typical 1X (1mM) reaction solution, or approx. 12 uses at 2mg reagent per use.*	25g

peptide enrichment

Introduction

Successful analysis of low-abundant proteins and identification of post-translationally modified peptides requires at least two steps: enrichment and clean up. Phosphorylation is arguably the most intensively studied PTM in biology and identification of sites of phosphorylation often requires several enrichment steps. Enrichment of phosphoproteins and phosphopeptides is facilitated using kits specifically designed for this purpose: The Thermo Scientific™ Phosphoprotein Kit (see Protein Enrichment section) for proteins or the Thermo Scientific™ Pierce™ Fe-NTA Phosphopeptide and Thermo Scientific™ Pierce™ Magnetic TiO_2 Phosphopeptide Enrichment Kits for phosphopeptides.

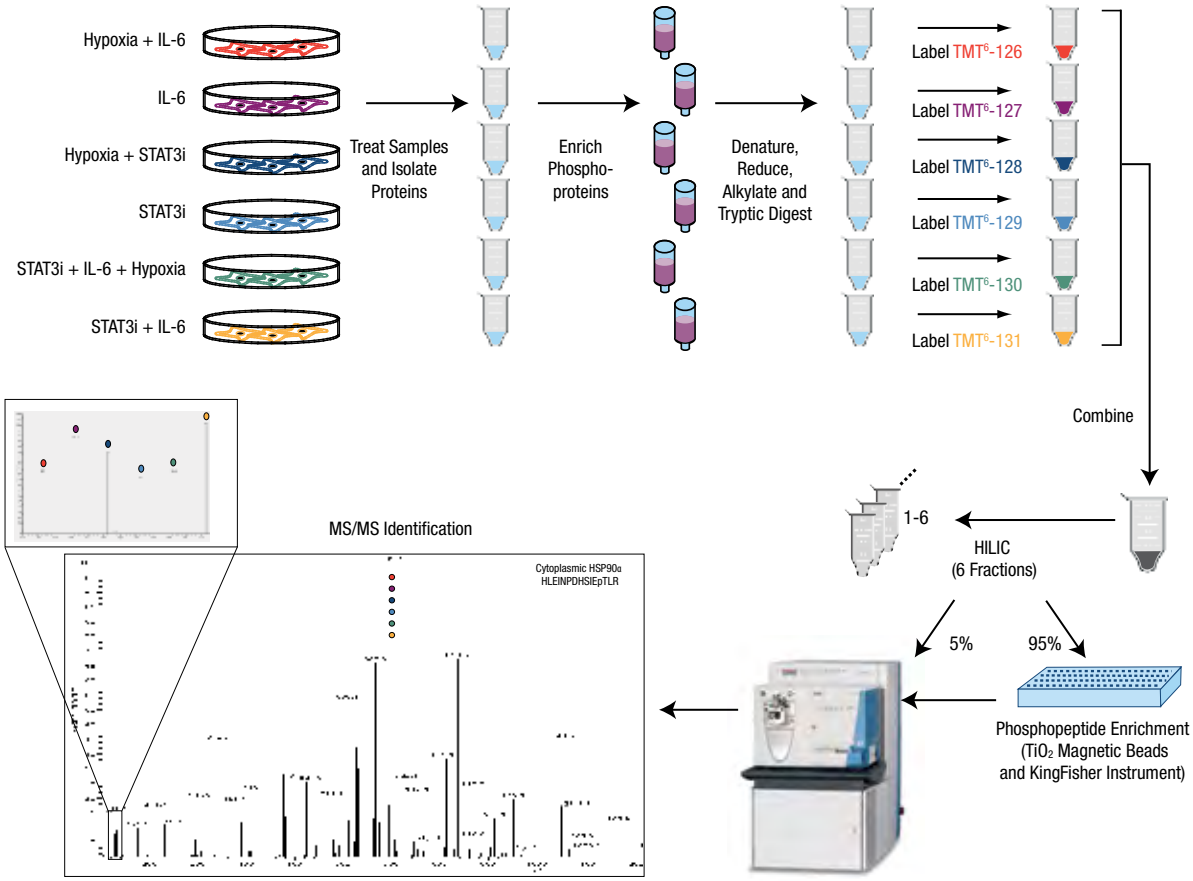


Figure 1. Protein phosphoprotein profiling with Thermo Scientific TMT tags. Protein samples from up to six treated cell samples are: 1) denatured; 2) digested with trypsin; 3) labeled with Thermo Scientific TMT6 tags; 4) combined; 5) cleaned or fractionated by hydrophilic interaction chromatography (HILIC); 6) chromatographically separated, isolated, and fragmented as peptides by in-line, reversed-phase LC-MS/MS; and 7) identified and quantified with Thermo Bioworks™, Discoverer or Matrix Science Mascot software.

Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit

Fe-NTA format optimized for capture and recovery of phosphopeptides.

The Thermo Scientific™ Pierce™ Fe-NTA Phosphopeptide Enrichment Kit enables fast and efficient enrichment of phosphorylated peptides. The Thermo Scientific™ Pierce™ Fe-NTA Spin Columns included in the kit are easy to use and require less than one hour to process protein digests or strong cation-exchange peptide fractions for analysis by MS.

Highlights:

- **Convenient** – spin-column format enables parallel processing of multiple samples
- **High-binding capacity** – each column enriches up to 150µg of phosphopeptides
- **Excellent recovery** – enriches more total and unique phosphopeptides than other commercially available resins

Protein phosphorylation is essential to biological functions, including cell signaling, growth, differentiation, division and programmed cell death. Over 500 protein kinases catalyze phosphorylation of specific targets, primarily on serine, threonine, and tyrosine residues.

MS is increasingly being used to identify and quantify phosphorylation changes; however, phosphoprotein and phosphopeptide analysis by MS is limited by many factors, including digestion efficiency, low stoiochiometry, low abundance, hydrophilicity, poor ionization and poor fragmentation. As a result, phosphopeptide enrichment is essential to successful MS analysis. The Pierce Fe-NTA Phosphopeptide Enrichment Kit is compatible with our lysis, reduction, alkylation, and digestion reagents and with Thermo Scientific™ Pierce™ Graphite Spin Columns to provide a complete workflow for phosphopeptide enrichment.

Product Details:

To assess phosphopeptide enrichment from lysates, cultured U2-OS cells arrested with nocodazole (100ng/mL, 25 hours) were lysed with 6M urea in 50mM Tris, pH 8.0 containing Halt Protease and Phosphatase Inhibitor Cocktail (Product # 78440). Protein concentration was determined with the Thermo Scientific™ Pierce™ 660nm Protein Assay (Product # 22660). Proteins were reduced with Bond-Breaker TCEP Solution, Neutral pH (Product # 77720), alkylated with single-use iodoacetamide (Product # 90034), digested overnight with Pierce Trypsin Protease, MS-grade (Product # 90057), and desalted with Thermo Scientific™ HyperSep™ C18 Cartridges (Product # 60108-305). An equivalent of 200µg of peptides were dried and dissolved in 5% acetic acid or Sigma PHOS-Select™ Buffer. Phosphopeptides were enriched with Pierce Fe-NTA Phosphopeptide Enrichment Kit or Sigma PHOS-Select Reagents and then desalted and concentrated with Pierce Graphite Spin Columns (Product # 88302) according to instructions.

Enriched phosphopeptide samples were analyzed by LC-MS/MS. A NanoLC™-2D HPLC with a ProteoPrep™ II C18 Column (75µm ID x 20cm) was used to separate peptides using a 4-40% gradient of solvents (A: water, 0.1% formic acid; B: acetonitrile, 0.1% formic acid) at 250nL per minute for 60 minutes. Peptides were identified with a Thermo Scientific™ LTQ Orbitrap™ XL ETD Mass Spectrometer using a top four experiment consisting of high-resolution MS followed by acquisition of four MS/MS spectra using the CID mode of fragmentation. LC-MS/MS data were interpreted with Mascot™ 2.2 and Scaffold™ 2.6.

To achieve robust MS results, enrichment of phosphopeptide samples is essential because of low stoichiometry and abundance and poor ionization relative to nonphosphorylated peptides. We have developed an efficient means to enrich phosphopeptides from complex samples. The Pierce Fe-NTA Spin Columns effectively capture, enrich and recover phosphopeptides. These columns enrich a higher percentage of phosphopeptides than other resins and with an overall higher number of total and unique phosphopeptides (Figure 1 and Table 1).

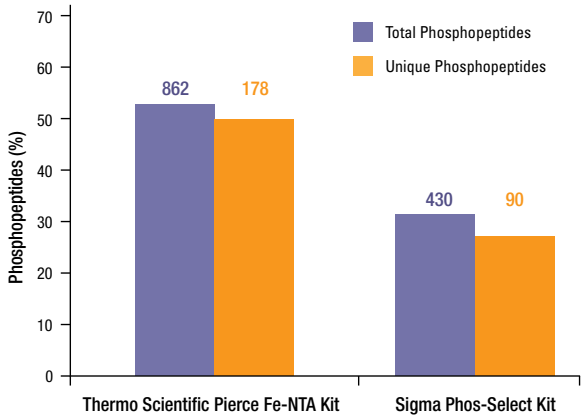


Figure 1. The Thermo Scientific Pierce Fe-NTA Kit enriched a greater percentage of total and unique phosphopeptides from U2-OS cell lysate. Numbers refer to the total and unique number of phosphopeptides identified in each condition. A summary of results is listed in Table 1.

peptide enrichment

Easily isolate phosphopeptides using convenient kits

Multiple phosphorylated amino acids within a peptide contribute to the complexity of phosphopeptide analysis. Pierce Fe-NTA Spin Columns enrich peptides with three or more phosphorylated sites and significantly outperform other commercially available columns (Figure 2). Phosphopeptide enrichment greatly reduces sample complexity and enables effective identification and characterization of phosphorylated peptides by MS (Figure 3).

The Pierce Fe-NTA Phosphopeptide Enrichment Kit contains detailed instructions and all necessary components to load, wash and elute phosphopeptides within an hour. This kit is compatible with samples digested in solution or after in-gel digestion using the Thermo Scientific In-Gel Tryptic Digestion Kit (Product # 89871).

Table 1. Average phosphopeptide enrichment results from duplicate experiments.[§]

	Thermo Scientific Pierce Fe-NTA	Sigma PHOS-Select
Total Phosphopeptides	862	430
Total Peptides	1753	1665
Total Unique Peptides	393	395
Total Unique Phosphopeptides	178	90
Total Phosphopeptides (%)	53	31
Unique Phosphopeptides (%)	50	27.5

[§] *Peptide summary results were exported from Scaffold and analyzed and summarized with Microsoft™ Excel™ and Access™ software.*

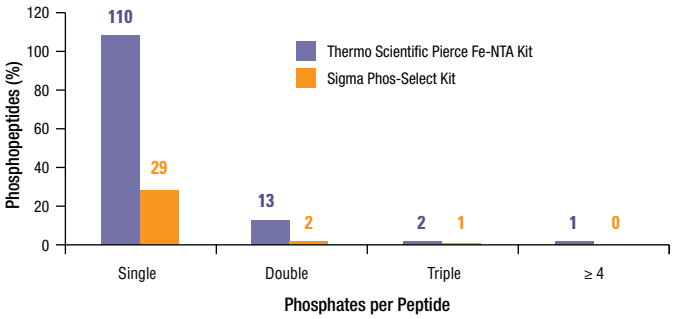


Figure 2. The Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit effectively captures phosphopeptides with multiple phosphates.

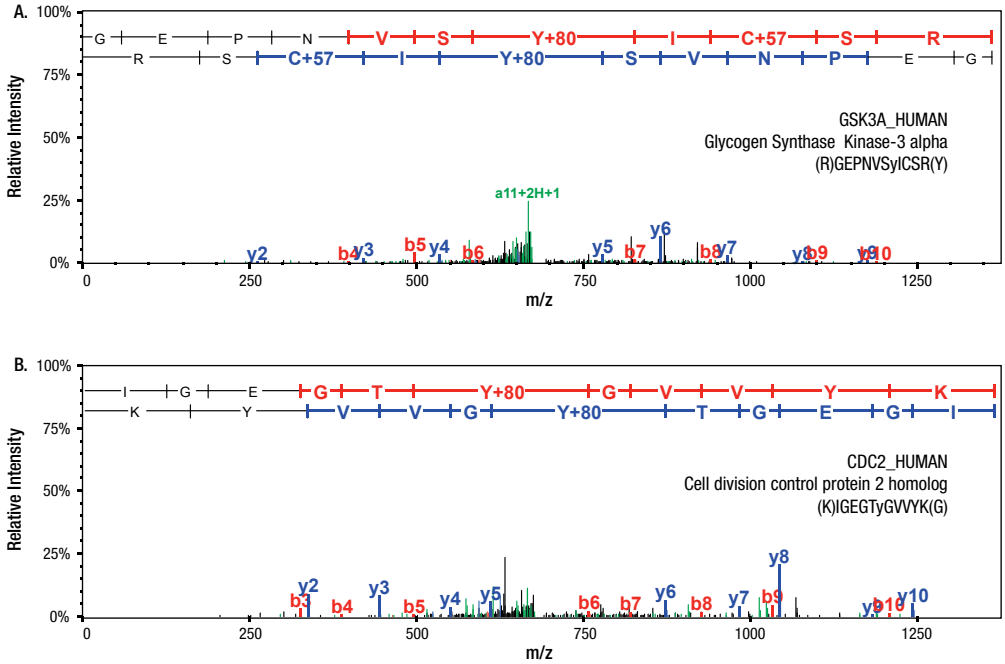


Figure 3. Example MS/MS spectra from enriched phosphopeptides. Lowercase letters indicate the position of tyrosine phosphorylation in enriched peptides. **Panel A**: Glycogen synthase-3 alpha. **Panel B**: cdc2/cyclin dependent kinase 1.

Ordering Information

Product #	Description	Pkg. Size
88300	Pierce Fe-NTA Phosphopeptide Enrichment Kit *Sufficient for 30 samples.*	Kit

Thermo Scientific Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit

New TiO_2 spin tips and graphite columns are selective for phosphopeptides.

Phosphopeptides have high hydrophilicity and are low in abundance, resulting in poor chromatography, ionization, detection and fragmentation. Phosphopeptide enrichment is therefore essential to successful MS analysis. The Thermo Scientific™ Pierce™ TiO_2 Phosphopeptide Enrichment and Clean-up Kit enables efficient isolation of phosphorylated peptides from complex and fractionated protein digests for analysis by MS.

Highlights:

- **Convenient** – spin-column format enables parallel processing of multiple samples
- **High capacity** – enrich up to 100µg of phosphopeptides from 300-1000µg of protein digest
- **Complementary** – TiO_2 enriches a unique set of phosphopeptides that complements our Fe-NTA IMAC kit
- **High selectivity** – recover phosphopeptides with > 90% selectivity

This phosphopeptide enrichment and clean-up kit contains spherical porous titanium dioxide (TiO_2) spin tips, graphite spin columns, optimized buffers and an easy-to-use protocol that produces high yield of clean phosphopeptide samples ready for MS analysis. The kit is compatible with our lysis, reduction, alkylation and digestion reagents to provide a complete workflow for studying phosphopeptides.

Comprehensive phosphopeptide identification using TiO_2 and Fe-NTA IMAC

TiO_2 primarily enriches monophosphorylated peptides[1,2] and typically enriches more phosphopeptides than immobilized metal affinity chromatography (IMAC). The preferential enrichment of singly phosphorylated peptides by TiO_2 is likely because of its high binding affinity, making it difficult to elute multiply phosphorylated peptides.[1,2] Iron (Fe)-NTA IMAC enriches more peptides with multiple phosphorylation sites (Table 1). Because of unique binding characteristics of each method, the Pierce TiO_2 Phosphopeptide Enrichment and Clean-up and Fe-NTA IMAC Phosphopeptide Enrichment Kits bind a complementary set of phosphopeptides from complex samples (Figure 1).[3]

The IMAC and TiO_2 methods are ideal combined in series; the flow-through and washes from IMAC can be acidified and applied to TiO_2 to enrich a more comprehensive set of phosphopeptides.[2] To significantly increase the number of identified phosphopeptides, fractionation with strong-cation exchange chromatography can be used for reducing sample complexity before phosphopeptide enrichment with TiO_2 or IMAC.[4]

Clean phosphopeptide samples ready for MS analysis

The stringent washing conditions used for the TiO_2 method increases the selectivity for phosphopeptides to > 90% (Figure 2). These wash conditions reduce nonspecific binding of acidic peptides while allowing the TiO_2 to enrich a higher total number of phosphopeptides than IMAC. Because some of the salts in these stringent washes might be present in the eluted samples, the kit includes graphite spin columns to desalt and concentrate enriched phosphopeptides. These convenient columns enrich hydrophilic phosphopeptides with high recovery, resulting in more successful MS results (Figure 3).[5]

The Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit contains detailed instructions and all necessary components to load, enrich and clean phosphopeptides within two hours. This kit is compatible with samples digested in solution or after in-gel digestion with trypsin or other MS-grade proteases.

Table 1. Selective enrichment of singly and multiply phosphorylated phosphopeptides with TiO_2 and Fe-NTA IMAC.†

	Number of Phosphates per Peptide						
	1	**2**	**3**	**4**	**5**	**6**	**Total**
TiO_2	492	103	8	4	0	1	608
Fe-NTA IMAC	234	34	216	3	1	0	488
Overlap	155	0	1	0	0	0	156

†Average phosphopeptide enrichment results from duplicate experiments showing the number of phosphopeptides containing one or more phosphate per peptide enriched using either method. Peptide spectrum summary results were exported from Proteome Software Scaffold 3.0 and analyzed with Microsoft Excel Software.

Figure 1. TiO_2 and Fe-NTA IMAC Resins enrich a complementary set of phosphopeptides. The Venn diagram details the number of unique phosphopeptides identified from 250µg of peptides prepared from nocodazole-treated U2OS cells. Phosphopeptides were enriched with the Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit and the Pierce Fe-NTA IMAC Phosphopeptide Enrichment Kit. Eluted peptides were desalted and concentrated with graphite spin columns before LC-MS/MS analysis on an Orbitrap XL Mass Spectrometer. Data was analyzed with Proteome Discoverer 2.1 and Proteome Software Scaffold 3.0.

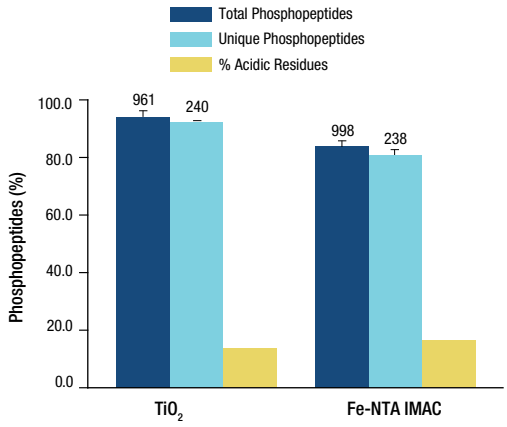


Figure 2. TiO_2 selectively enriches more phosphopeptides than Fe-NTA IMAC. The numbers refer to the total and unique number of phosphopeptides enriched by each method. The Y-axis is the percentage of phosphopeptides in the number of total and unique peptides, or the percentage of acidic residues in enriched unique phosphopeptides.

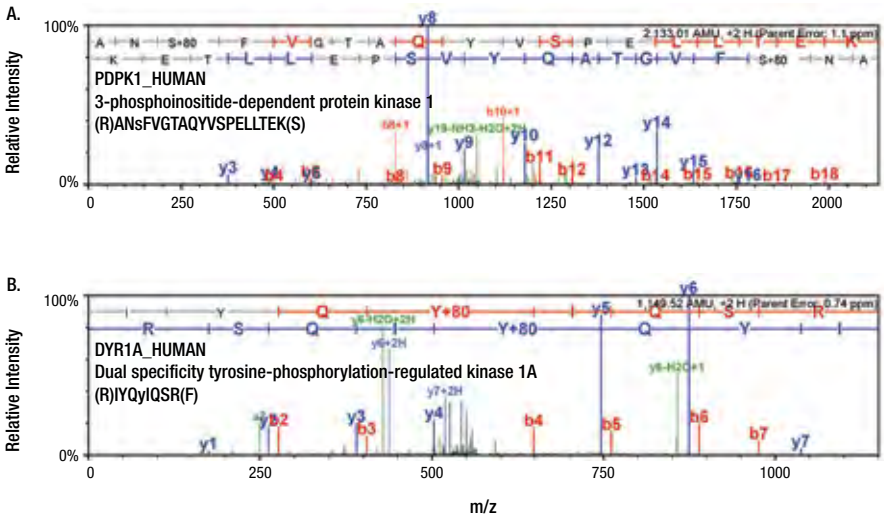


Figure 3. Representative MS/MS spectra from enriched phosphopeptides. Panel A: Phosphorylated Ser-241 in regulatory region of 3-phosphoinositide-dependent protein kinase. **Panel B:** Phosphorylated Tyr-321 in Y-X-Y regulatory region of dual-specificity tyrosine phosphorylation-regulated kinase 1A. Lowercase letters indicate the position of phosphorylation in enriched peptides, and letters in parentheses indicate amino acids adjacent to the identified peptide in the protein sequence.

References

1. Larsen, M.R., *et al.* (2005). Highly selective enrichment of phosphorylated peptides from peptide mixtures using titanium dioxide microcolumns. *Mol Cell Proteomics* **4(7)**:873-86.
2. Sugiyama, N., *et al.* (2007). Phosphopeptide enrichment by aliphatic hydroxy acid-modified metal oxide chromatography for nano-LC-MS/MS in proteomics applications. *Mol Cell Proteomics* **6**:1103-9.
3. Carrascal, M. *et al.* (2008). Phosphorylation analysis of primary human T lymphocytes using sequential IMAC and titanium oxide enrichment. *J Proteome Res.* **7(12)**:5167-76.
4. Wilson-Grady, J.T., *et al.* (2008). Phosphoproteome analysis of fission yeast. *J Proteome Res* **7(3)**:1088-97.
5. Larsen, M.R., *et al.* (2004). Improved detection of hydrophilic phosphopeptides using graphite powder microcolumns and mass spectrometry: evidence for *in vivo* doubly phosphorylated dynamin I and dynamin III. *Mol Cell Proteomics* **3**:456-65.

Ordering Information

Product #	Description	Pkg. Size
88301	**Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit** *Sufficient reagents for phosphopeptide enrichment and clean-up of 24 samples.*	Kit
88303	**Pierce TiO_2 Phosphopeptide Enrichment Spin Tips**	96 tips

Thermo Scientific Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit

TiO_2 magnetic particles for high-throughput phosphopeptide isolation.

The Thermo Scientific™ Pierce™ Magnetic TiO_2 Phosphopeptide Enrichment Kit is used for isolating phosphopeptides from complex biological samples using titanium dioxide-coated magnetic beads. The TiO_2 ligand selectively binds peptides containing phosphorylated serine (Ser), tyrosine (Tyr) or threonine (Thr), enabling phosphopeptide enrichment from protease-digested samples. The isolated phosphopeptides are compatible for analysis downstream by MS (Table 1).

The high-performance, iron oxide, superparamagnetic particles are validated and optimized for use with high-throughput magnetic platforms, such as the KingFisher 96 and KingFisher Flex Instruments. The beads also enable premium performance for simple benchtop applications using an appropriate magnetic stand.

Highlights:

- **Complete MS-compatible kits** – include ready-to-use binding, wash and elution buffers that are optimized for phosphopeptide enrichment and downstream analysis by MALDI and ESI MS
- **Optimized for high-throughput screening** – procedure validated for processing 1 to 96 samples at a time; complete entire assay in about 15 minutes using a KingFisher Flex Instrument
- **Stable affinity ligand** – titanium dioxide is specially coated as a film on the magnetic particles
- **Selective** – affinity system is selective for phosphorylated Ser, Tyr and Thr; exhibits minimal nonspecific binding to acidic residues
- **Sensitive** – affinity provides more than 1000 times greater sensitivity than traditional IMAC technologies; enables enrichment and MS measurement of less than 100fmol of phosphoprotein

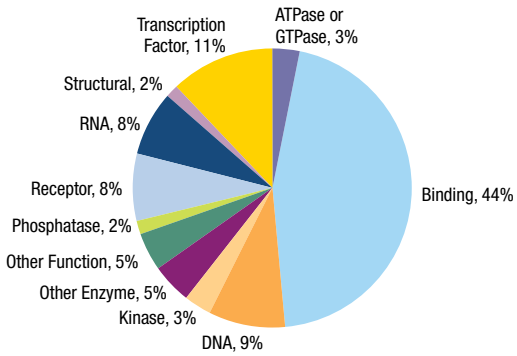


Figure 1. Major protein functions identified in a phosphoprotein- and phosphopeptide-enriched MS data set using the Thermo Scientific Magnetic TiO_2 Phosphopeptide Enrichment Kit.

Table 1. Phosphopeptide enrichment improves MS identification of phosphoproteins. Two milligrams of a tryptic digest prepared from peripheral blood mononuclear cells (lymphocytes) with and without phosphopeptide enrichment were analyzed by MS. Enrichment was performed with the Pierce TiO_2 Phosphopeptide Enrichment Kit using the KingFisher 96 Instrument. Samples were analyzed on an LTQ Orbitrap Mass Spectrometer.

	Enriched	Non-Enriched
Total Number of Proteins Identified	185	247
Total Number of Phosphoproteins Identified	160	1
Total Number of Peptides Identified	2347	2457
Total Number of Phosphopeptides Identified	2009	7
Total Number of Unique Phosphopeptides Identified	177	1
Relative Enrichment for Phosphopeptides (%)	86	0.3

Ordering Information

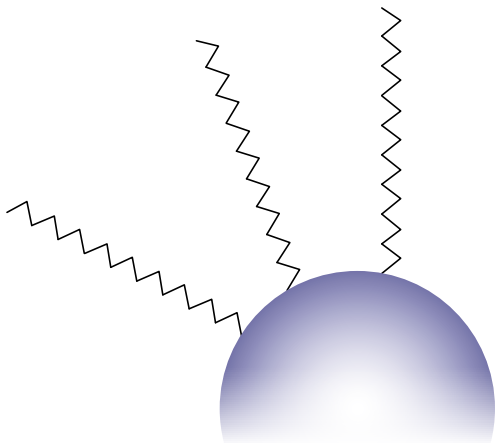
Product #	Description	Pkg. Size
88811	**Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit** *Sufficient for purifying 96 x 100µg peptide samples.* Includes: TiO_2 Magnetic Beads (20X) Binding Buffer Washing Buffer Elution Buffer Thermo-Fast 96 Robotic PCR Plate (0.2mL wells)	96-rxn kit 1mL 100mL 25mL 3mL 2 plates
88812	**Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit, Trial Size** *Sufficient for purifying 24 x 100µg peptide samples.* Includes: TiO_2 Magnetic Beads (20X) Binding Buffer Washing Buffer Elution Buffer Thermo-Fast 96 Robotic PCR Plate (0.2mL wells)	24-rxn kit 0.25mL 100mL 25mL 3mL 2 plates

peptide clean up

Introduction

After isolation of peptides, salts and buffers can be removed using either Thermo Scientific™ Pierce™ C18 Spin Columns or Tips. Pierce C18 Columns utilize reversed phase (RP) resin. The resin consists of a film of non-polar matrix that has been designed to be chemically anchored to an inert material (silica gel particles). Pierce C18 Columns have packing materials coated with C18 hydrophobic units, which are more hydrophobic than other types of RP columns (e.g., C4 and C8). C4 is typically used for proteins and C18 for the capture of peptides or small molecules. The larger protein molecules tend to have more hydrophobic moieties to interact with the column, thus a shorter chain length is more appropriate. The peptides bind to reverse-phase columns in high-aqueous mobile phase, salts and buffers are then washed off, and the peptides are eluted using a high-organic mobile phase. In RP, HPLC compounds are separated based on their hydrophobic character. As very hydophilic peptides, including phosphopeptides, may bind to C18 resins poorly, graphite spin columns may provide better peptide recovery.

However, C18 and graphite resins do not efficiently remove contaminants such as detergents. Detergent removal from peptide samples is a challenge, especially for MS analysis in which even low detergent concentrations contaminate instruments and interfere with column binding, elution and ionization. Traditional techniques for removing detergents include SCX and acetone precipitation. However, SCX can only remove anionic detergents such as SDS. Acetone precipitation is primarily used for removing detergents from proteins, and is not as effective for peptides. Acetone precipitation can also lead to low protein recovery. The Thermo Scientific™ Detergent Removal Resins and Kits efficiently bind to and remove high concentrations of a wide variety of detergents and surfactants that are commonly used in protein extraction and preparation, from samples ranging in volume from 10µL to 1mL, with minimal sample loss.



Thermo Scientific Pierce C18 Spin Tips

Easy-to-use C18 spin tips for fast and efficient clean up of peptides for MS analysis.

Thermo Scientific™ Pierce™ C18 Spin Tips enable fast and efficient capture, concentration, desalting and elution of up to 10µg peptides per 20µL sample for MS analysis.

Pierce C18 Spin Tips are 20µL-capacity pipette tips with accompanying adaptors for microcentrifuge sample processing. The tips contain a C18 reversed-phase sorbent that minimizes flow resistance and provides excellent binding and recovery characteristics at a wide range of peptide concentrations upstream of matrix-assisted laser desorption ionization (MALDI) or nanoelectrospray ionization techniques. Sample clean up with C18 resin significantly improves protein analysis results by removing urea, salts and other contaminants before MS. Each Pierce C18 Spin Tip has a 20µL volume capacity with a 10µg peptide-binding capacity.

Highlights:

- **Rapid** – C18 fast-flow tips have low resistance and improved flow characteristics compared to other commercially available tips
- **High capacity** – up to 10µg peptide per 20µL solution
- **Convenient** – spin tips come with tip adaptors for easy centrifugation
- **Cleaner sample** – device design filters out particulates that can cause autosampler and column clogging

Product Details:

Pierce C18 Spin Tips offer excellent flow properties with a high-efficiency C18 sorbent for fast wetting, loading, washing and eluting. Sample is simply loaded in prepared tip and washed and eluted using multiple centrifugation steps. The procedure is simple and requires less than five minutes to process protein digests, strong cation exchange fractions and other protein and peptide samples for mass spectrometric analyses. The unique tip design also removes any particulates in the sample, eliminating clogging of the auto sampler or column upstream of the MS.

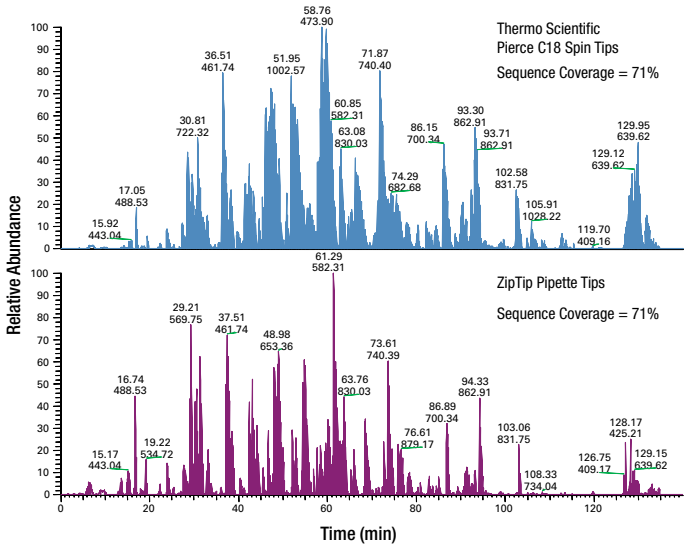


Figure 1. Thermo Scientific Pierce C18 Spin Tips outperform other popular C18 tips. BSA tryptic digests were analyzed on an LTQ™ Orbitrap™ XL ETD Mass Spectrometer after processing 10µg aliquots of the same digest with Pierce C18 Spin Tips or ZipTip™ Pipette Tips (10µL tips with 0.6µL C18 resin; EMD Millipore). Base peak chromatograms of the peptide elution were extracted from the data sets to evaluate sample complexity and chromatographic resolution. MS results were analyzed using the SEQUEST™ search engine and the SwissProt database to determine protein sequence coverage.

Ordering Information

Product #	Description	Pkg. Size
84850	**Pierce C18 Spin Tips** *Sufficient for processing up to 10µg peptide per tip.*	96 tips

peptide clean up

Purify peptides using optimized formats

Thermo Scientific Pierce C18 Tips

Monolithic C18 sorbent in a pipette tip for fast sample desalting and concentrating.

Thermo Scientific™ Pierce™ C18 Tips enable efficient purification of peptides and small proteins before MS, HPLC, capillary electrophoresis and other analytical techniques. They provide a reproducible method for capturing, concentrating, desalting and eluting femtomole to nanomole quantities of peptides for improved data generation and analysis. The Pierce C18 Tips have unique monolithic C18 sorbent technology and offer superior flow and exceptional binding capacity, delivering uniform flow and strong analyte-to-surface interactions. They consistently achieve better sequence coverage, higher peak intensities and improved peptide capture for accurate protein identification. During the quick and easy-to-use protocol, peptides and small proteins bind to C18 resin while contaminants are washed away. The target peptides are then recovered in their concentrated and purified form with an aqueous, organic solvent.

Highlights:

- **Better sequence coverage** – obtain high sequence coverage for more reliable protein identification
- **Higher peak intensities** – assure correct protein identification with significant signal improvements
- **Increased recoveries** – isolate more peptides using Pierce C18 Tips' superior binding capacity
- **Flexible tip formats** – available in 10 and 100µL bed volumes processing up to 8 or 80µg of samples, respectively
- **Expandable** – our design conveniently adapts to a variety of automated liquid handling systems with pipetting stations for maximum performance, speed and hands-off convenience

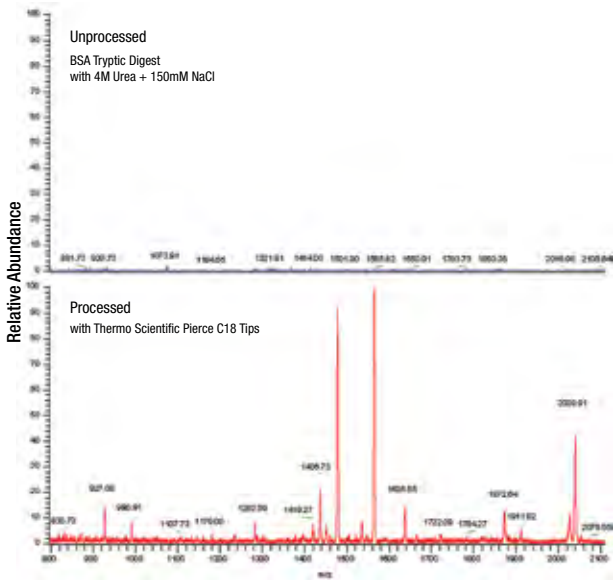


Figure 1. Removing urea and NaCl eliminates interference in MS chromatograms. A bovine serum albumin (BSA) tryptic digest was analyzed on a Thermo Scientific™ MALDI-Orbitrap™ XL Hybrid Mass Spectrometer. Digests and samples containing 150mM NaCl and 4M urea were analyzed with or without processing with Pierce C18 Tips (10µL).

Improve protein analysis results with Pierce C18 Tips by removing urea, salts and other contaminants before MS analysis (Figures 1 and 2). The tips are ideal for matrix-assisted laser desorption ionization (MALDI) or nanoelectrospray ionization techniques. They are available in convenient 10 and 100µL tips with binding capacities of 8 and 80µg, respectively.

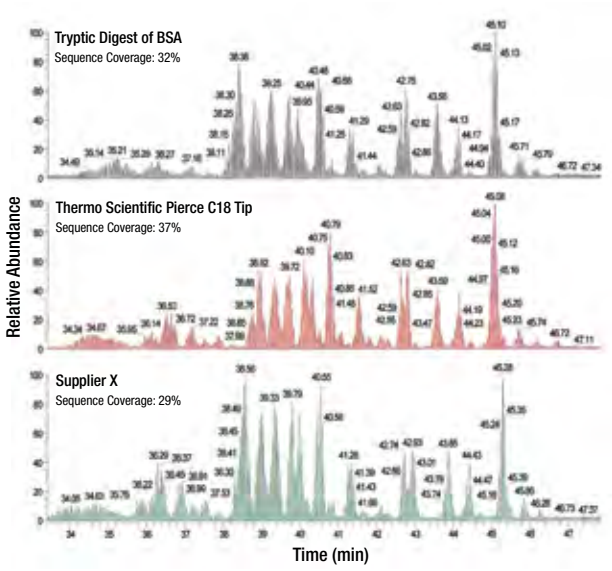


Figure 2. The Thermo Scientific Pierce C18 Tips outperform other suppliers' tips. BSA tryptic digests were analyzed either directly on a Thermo Scientific™ LTQ-XL™ Ion Trap Mass Spectrometer or after processing with Pierce C18 Tips (100µL) or supplier X tips. Base peak chromatograms of the peptide elution were extracted from the data sets to evaluate sample complexity and chromatographic resolution. MS results were analyzed with Matrix Science Mascot and the SwissProt™ Release 52 database to determine protein sequence coverage.

Ordering Information

Product #	Description	Pkg. Size
87781	Pierce C18 Tips, 10µL bed	8 tips
87782	Pierce C18 Tips, 10µL bed	96 tips
87783	Pierce C18 Tips, 100µL bed	8 tips
87784	Pierce C18 Tips, 100µL bed	96 tips

Thermo Scientific Pierce C18 Spin Columns

Purify and/or concentrate multiple peptide samples in less than 30 minutes.

Peptide samples can be purified and concentrated for a variety of applications using Pierce C18 Spin Columns. Each spin column contains a porous C18 reversed-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 (10-150µL) in approximately 30 minutes without laborious repeat pipetting or specialized equipment. Pierce C18 Spin Columns can be used effectively for processing peptides derived from 10ng to 30µg of protein. Sensitivity and detection limits are dependent on the downstream application.

Highlights:

- **Removes MS-interfering contaminants** – significantly reduces signal suppression and improves signal-to-noise ratios and sequence coverage; works on a variety of reverse-phase-compatible contaminants
- **Robust** – works with a wide variety of load volumes (10-150µL) and concentrations; no need to reduce sample volume before application
- **Convenient** – easy to handle and requires no special equipment for processing multiple samples simultaneously (unlike tip-driven systems that require one sample be processed at a time)
- **Sensitive** – special C18 resin allows excellent recovery percentages, even at low (sub-picomole) sample loads

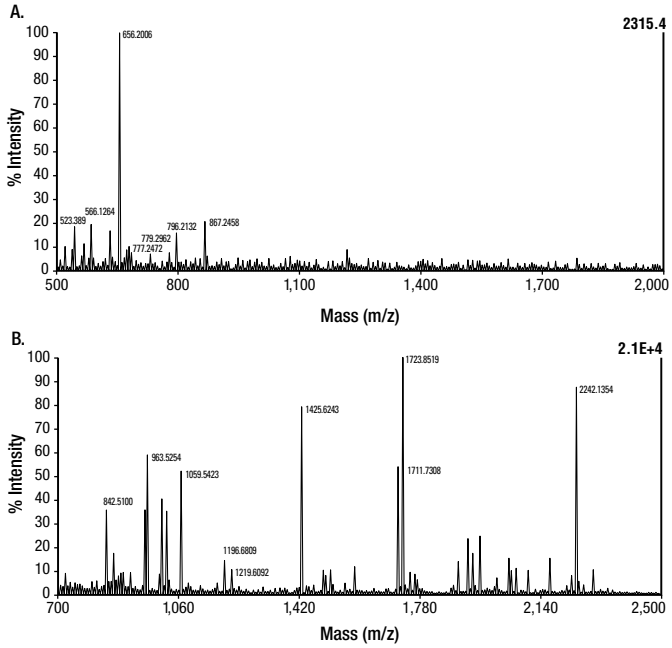


Figure 2. Effective clean up of MS sample with Thermo Scientific Pierce C18 Spin Columns. Panel A. MALDI-TOF MS analysis of an unknown protein isolated from a mitochondrial extract separated by 2D electrophoresis and subjected to in-gel tryptic digestion followed by processing with Pierce C18 Spin Columns. **Panel B.** MALDI-TOF MS analysis of an identical digest that has not been C18 processed.

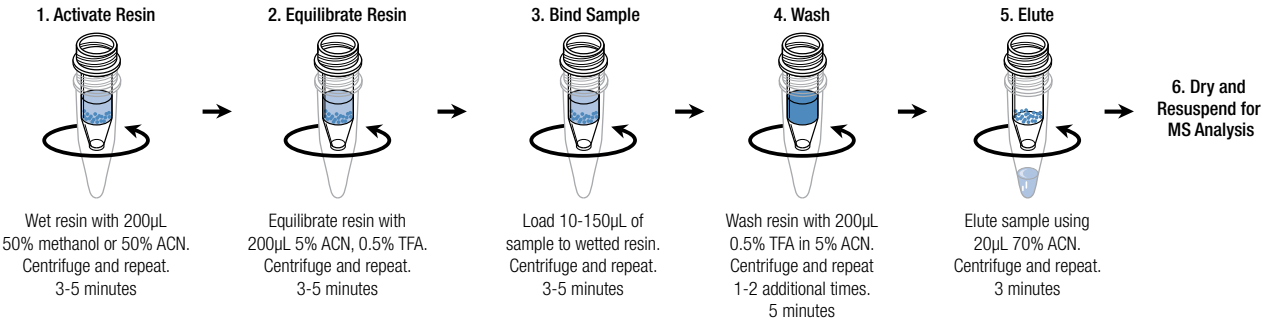


Figure 1. Thermo Scientific Pierce C18 Spin Column protocol summary.

Ordering Information

Product #	Description	Pkg. Size
89870	**Pierce C18 Spin Columns** Each column contains 8 mg of a porous C18 reversed-phase resin.	Kit 25 columns
89873	**Pierce C18 Spin Columns** Each column contains 8 mg of a porous C18 reversed-phase resin.	Kit 50 columns

Thermo Scientific Pierce Graphite Spin Columns

Graphite spin columns efficiently purify and concentrate hydrophilic phosphopeptides.

The Thermo Scientific™ Pierce™ Graphite Spin Columns enable fast and efficient capture, concentration, desalting and elution of hydrophilic peptides. The five-step procedure is simple and requires less than 10 minutes to process. These columns are ideal for improving mass spectrometric analyses of samples from protein digests (Figure 1), strong-cation exchange fractions, and enriched phosphopeptides eluted from TiO_2 and immobilized metal affinity chromatography (IMAC) columns and tips.

Highlights:

- **Convenient** – spin format enables parallel processing of multiple samples
- **High-binding capacity** – excellent recovery of up to 100µg of hydrophilic peptides per column
- **Efficient** – porous graphite resin enables efficient cleaning up phosphopeptide samples before MS analysis

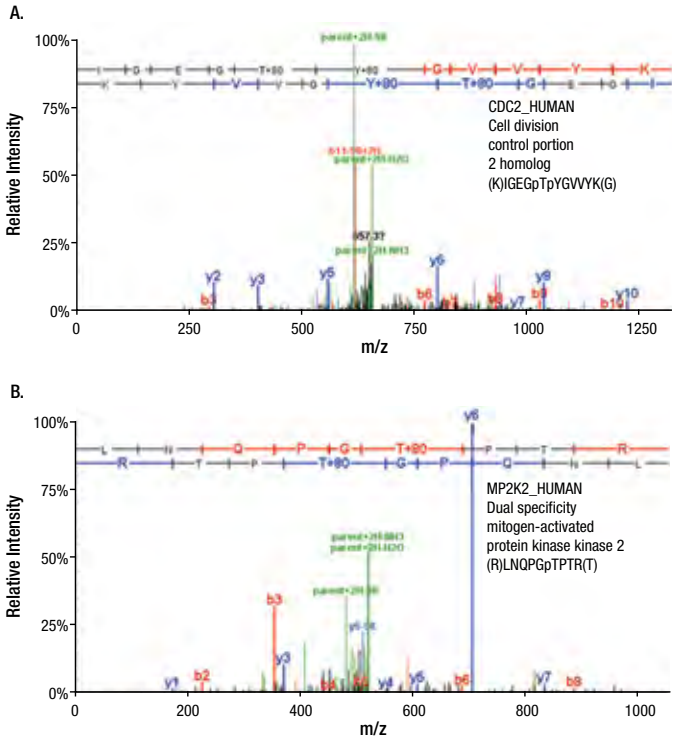


Figure 1. Graphite clean up enables phosphopeptide identification. U2OS human osteosarcoma cells synchronized at the G2/M boundary with nocodazole (200ng/mL, 36 hours) were lysed with 6 M guanidine•HCl. After enzymatic protein digestion (100µg), phosphopeptides were enriched with IMAC and desalted with Pierce Graphite Spin Columns or C18 tips before LC-MS/MS analysis on an LTQ Orbitrap XL Mass Spectrometer. Two representative spectra are shown for two phosphopeptides not observed after C18 clean up. **Panel A**: A novel doubly-phosphorylated peptide was identified within the putative ATP binding site of cyclin dependent kinase cdc2 (CDK1). This phosphopeptide is not present in Phospho.ELM version 8.2 database. **Panel B**: Dual specificity mitogen-activated protein kinase kinase 2 (MP2K2) phosphopeptide.

C18 resins and tips commonly used to desalt peptides do not efficiently capture hydrophilic peptides, like phosphopeptides. The Pierce Graphite Spin Columns improve phosphopeptide analysis by efficiently binding hydrophilic peptides and efficiently removing urea, salts and other contaminants before MS analysis (Figure 2). The spin columns are ideal for matrix-assisted laser desorption ionization (MALDI) or nanoelectrospray ionization techniques.

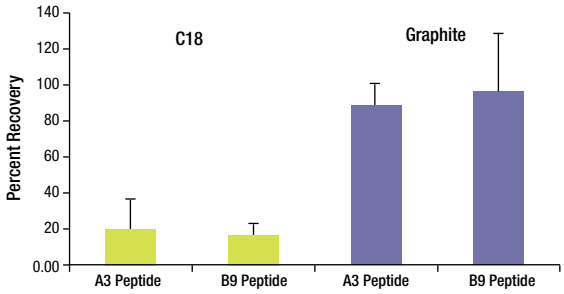


Figure 2. Improved recovery of representative hydrophilic phosphopeptides using graphite spin columns. Stable isotope-labeled A3 and B9 peptides (10pmol) were acidified with 1% trifluoroacetic acid, processed according to instructions for C18 tips or the Pierce Graphite Spin Columns, and eluted with 50% acetonitrile/0.1% formic acid. The corresponding heavy isotope-labeled peptides (5pmol) were spiked in the eluate, dried and resuspended in 0.1% formic acid. Samples were analyzed by targeted LC-MS/MS with the Orbitrap XL Mass Spectrometer to quantitate percent recovery. Peptides: A3= RPRAApTFPFR†, B9 = RTPKDpSPGIPPFR†.
†Position of heavy isotope labeled amino acid used for absolute MS quantitation.

Ordering Information

Product #	Description	Pkg. Size
88302	**Pierce Graphite Spin Columns** Contains 10mg in 0.5mL of slurry.	30 columns

Thermo Scientific Pierce Detergent Removal Products

For samples with protein peptide concentrations > 100µg/mL.

Thermo Scientific™ Pierce™ Detergent Removal Resin works with high concentrations of a broad range of commonly used detergents, while providing exceptional results.

The Thermo Scientific™ Pierce™ Detergent Removal Spin Plates provide a high-throughput method for effectively removing detergents from samples. Thermo Scientific™ Pierce™ Detergent Removal Spin Columns provide flexibility and are available in a variety of sizes to treat both protein and peptide samples ranging from 0.01mL to 1mL.

Highlights:

- **Performance** – remove detergent with > 90% recovery
- **Peptide detergent removal capability** – effectively removes detergents from peptide samples, enabling MS analysis
- **Protein detergent removal capability**
- **Improves MS peptide coverage**
- **Convenient** – available in various formats including 96-well spin plates, spin columns and bulk resin
- **Fast and efficient protocol** – 96 samples can be processed in 15 minutes
- **No sample dilution**

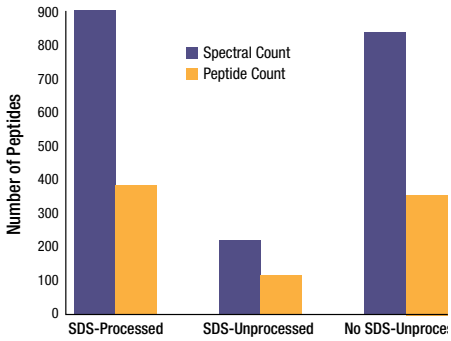
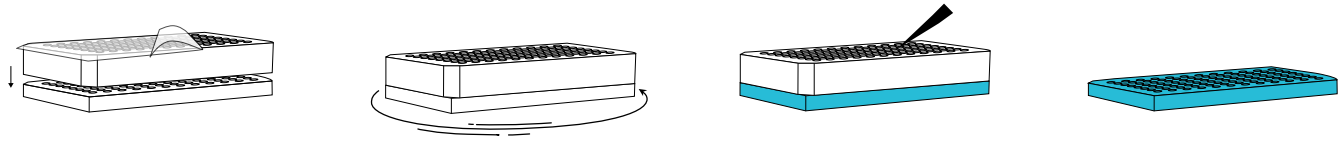


Figure 1. Effective detergent removal enables greater peptide identification. A tryptic digest of HeLa cell lysate (0.1mL, 100µg) containing 1% SDS was processed through 0.5mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. The processed sample allowed similar numbers of identified peptides as digests containing no SDS. Peptide identification is greatly reduced in sample containing SDS.



1. Remove the bottom seal and stack the detergent-removal plate on top of a wash plate. Remove the top seal and centrifuge.†

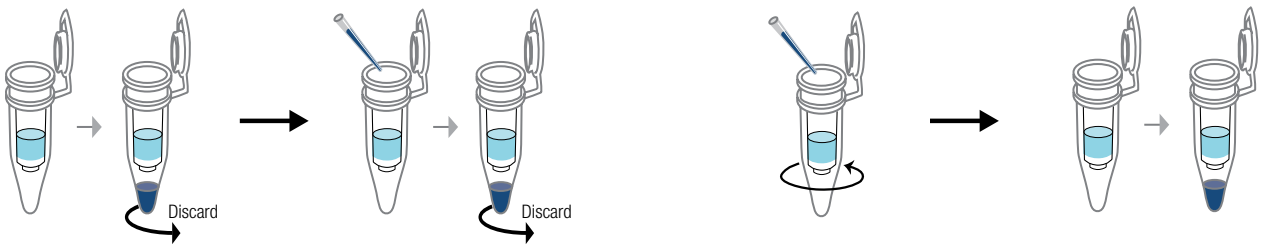
2. Add 300µL of buffer to each well and centrifuge. Discard the flow-through. Repeat this step two times.

3. Stack the detergent-removal plate on top of a sample-collection plate. Apply sample and incubate at room temperature for 2 minutes. Centrifuge to remove detergent.

4. Recover the detergent-free sample for downstream analysis.

† Centrifugations are performed for 2 minutes at 1000 x *g*.

Figure 2. Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Plates.



1. Centrifuge for 1 minute at 1500 x *g* to remove the storage buffer.

2. Add 0.4mL equilibration buffer, centrifuge at 1500 x *g* for 1 minute and discard the flow-through. Repeat two additional times.

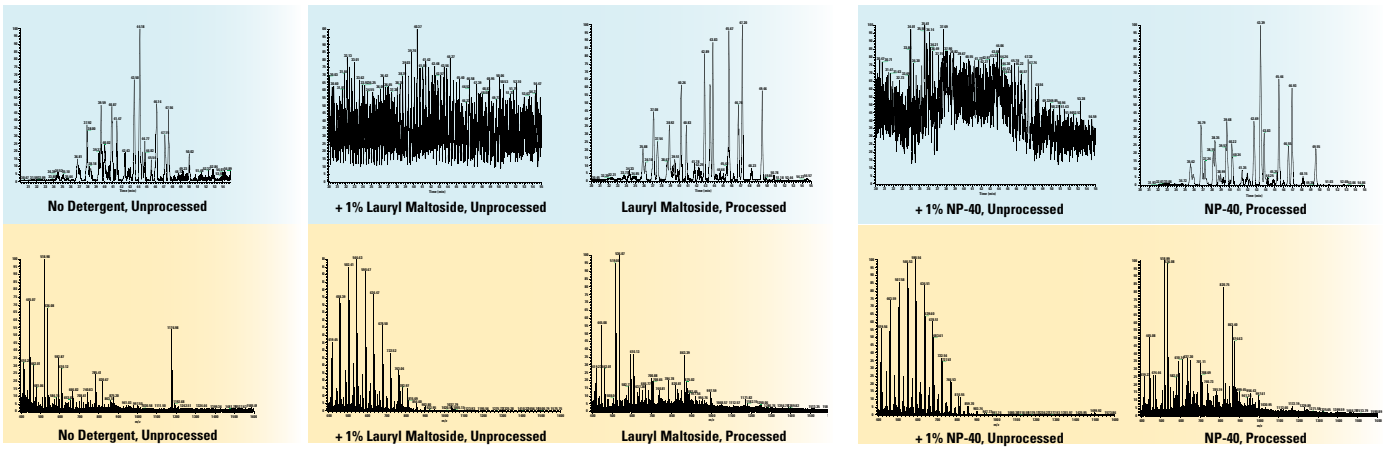
3. Add detergent-containing sample (25-100µL) and incubate for 2 minutes at RT.

4. Centrifuge at 1500 x *g* for 2 minutes to collect the detergent-free sample for downstream applications.

Figure 3. Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Columns (0.5mL).

peptide clean up

Remove contaminants using optimized formats



Base Peak LC-MS Chromatograms

Integrated Mass Spectra

Figure 4. Effective detergent removal eliminates interference and allows high sequence coverage analysis of BSA. Tryptic digests (0.1mL, 100µg) containing detergent were each processed through 0.5mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. **Top row:** Base peak LC-MS chromatograms. **Bottom row:** Integrated mass spectra. Similar results were produced for Brij-35 Detergent, octyl glucoside, octyl thioglucoside and SDS (data not shown).

Table 1. Effectiveness and protein recovery of detergent-removal resins.

Process Format†	Detergent	Detergent Concentration (%)	Detergent Removal (%)	BSA Recovery (%)
96-well Spin Plate	SDS	5	99	89
	Triton X-100	4	99	100
	NP-40	1	95	100
	CHAPS	5	99	100
0.5mL Spin Column	Sodium deoxycholate	5	99	100
	Octyl glucoside	5	99	90
	Octyl thioglucoside	5	99	95
	Lauryl maltoside	1	98	99
	Triton X-114	2	95	100
	Brij®-35	1	99	97
	Tween®-20	0.25	99	87

† *Each plate well and column contained ~550µL of detergent-removal resin slurry and 0.1mL of sample. Similar results were obtained with both process formats.*

Ordering Information

Product #	Description	Pkg. Size
88304	Pierce 96-well Detergent Removal Spin Plates *Sufficient for 25 to 100µL samples per well.*	2 plates
87776	Pierce Detergent Removal Spin Column, 125µL *Sufficient for 10 to 25µL sample per column.*	25 columns
87777	Pierce Detergent Removal Spin Column, 0.5mL *Sufficient for 25 to 100µL sample per column.*	25 columns
87778	Pierce Detergent Removal Spin Column, 2mL *Sufficient for 150 to 500µL sample per column.*	5 columns
87779	Pierce Detergent Removal Spin Column, 4mL *Sufficient for 500 to 1000µL sample per column.*	5 columns
87780	Pierce Detergent Removal Resin	10mL

Thermo Scientific HiPPR (High Protein and Peptide Recovery) Detergent Removal Columns and Plates

For samples with protein/peptide concentrations between 1-100µg/mL.

Thermo Scientific™ HiPPR Detergent Removal Resin in spin column or 96-well filter plate formats improves MS results by efficiently removing detergents from 25 to 200µL samples with low protein or peptide concentrations.

The HiPPR (High Protein and Peptide Recovery) Detergent Removal Resin is optimized for protein or peptide concentrations of 1 to 100µg/mL and removes > 95% of detergents with minimal sample loss. The HiPPR Detergent Removal Resin is ideal for removing commonly used detergents, including SDS, Triton™ X-100, NP-40 and CHAPS at concentrations of 0.5-1% and is available in pre-filled spin columns and 96-well filter spin plates for sample volumes up to 100µL. For other sample sizes, the detergent removal resin slurry is available with empty spin columns that can be used to make custom spin columns for processing sample volumes of 25 to 200µL.

Highlights:

- **Optimized** – removes > 95% of detergent from samples with low-concentrations (1 to 100µg/mL) of proteins or peptides
- **Fast** – sample processing takes less than 15 minutes
- **Effective** – eliminates detergent interference in downstream applications like ELISA, isoelectric focusing and MS

Product Details:

The detergents and surfactants used to prepare protein and peptide samples can interfere with analysis by ELISA, isoelectric focusing and MS. Removing detergents from peptide samples is especially challenging and critical for MS analysis because even low concentrations of detergents will contaminate instruments and interfere with column binding, elution and peptide ionization. The HiPPR Detergent Removal Resin is ideal for rapid detergent removal from tryptic digests to improve the results of LC-MS/MS and MALDI-MS analysis as to help maintain column and instrument performance over time.

Table 1. Detergent removal efficiency and protein recovery. BSA sample (25-200µL) + detergent in 0.15M NaCl, 0.05% sodium azide was mixed with equal volume of detergent removal resin (2x volume for CHAPS removal) and processed as shown in the protocol.

Detergent	Sample Volume (µL)	Protein Quantity (µg)	Detergent Removal (%)	Protein Recovery (%)
SDS (1%)	25	0.375	>99	98
	50	0.75	>99	97
	100	1.5	>99	100
	200	3.0	>99	100
Triton X-100 (1%)	25	0.375	>95	82
	50	0.75	>95	86
	100	1.5	>95	86
	200	3.0	>95	93
NP-40 (0.75%)	25	0.375	95	90
	50	0.75	96	94
	100	1.5	97	91
	200	3.0	97	97
CHAPS (1%)	25	0.375	95	64
	50	0.75	97	70
	100	1.5	98	78
	200	3.0	98	75

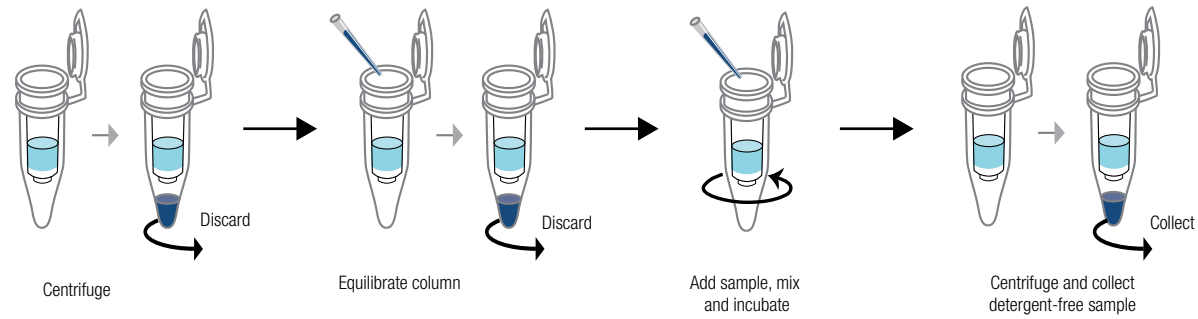


Figure 1. Protocol summary for Thermo Scientific HiPPR Detergent Removal Spin Columns.

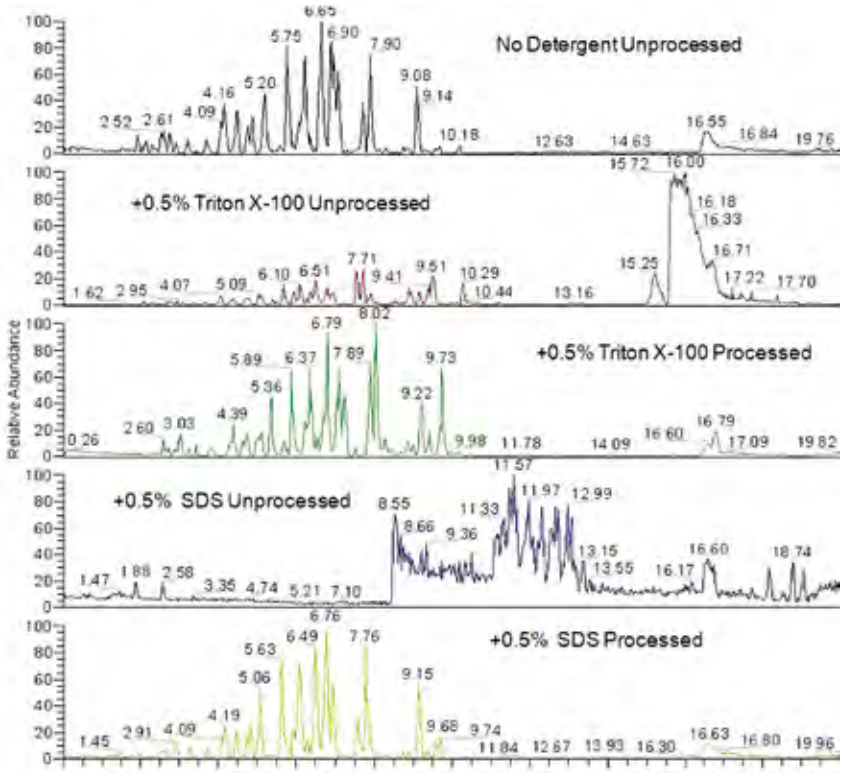


Figure 2. Thermo Scientific HiPPR Detergent Removal Resin improves LC-MS/MS analysis of enzymatically digested BSA. BSA (100µg/mL) tryptic digests were prepared without detergent, in the presence of 0.5% Triton X-100 or spiked with 0.5% SDS following enzymatic digestion. Samples (0.1mL) containing detergent were processed with the HiPPR Detergent Removal Resin and compared to unprocessed or detergent-free samples by LC-MS/MS. Results demonstrate that detergent removal is effective and produces results similar to those observed for samples containing no detergent.

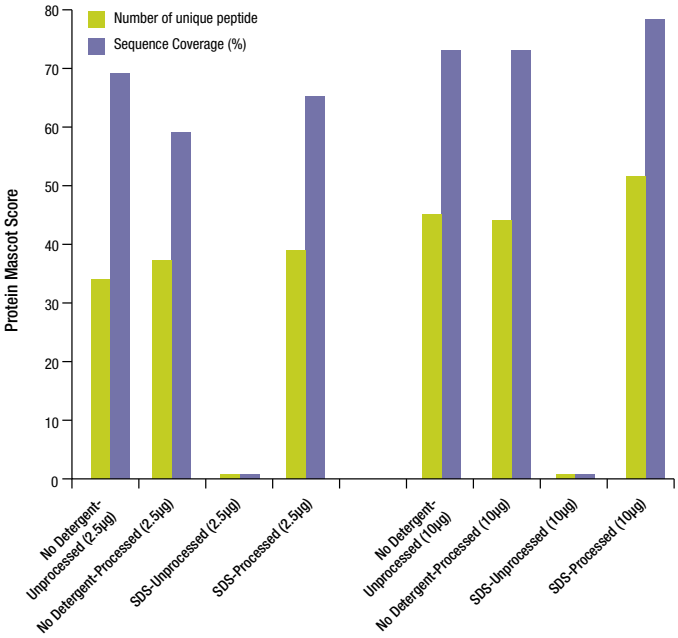
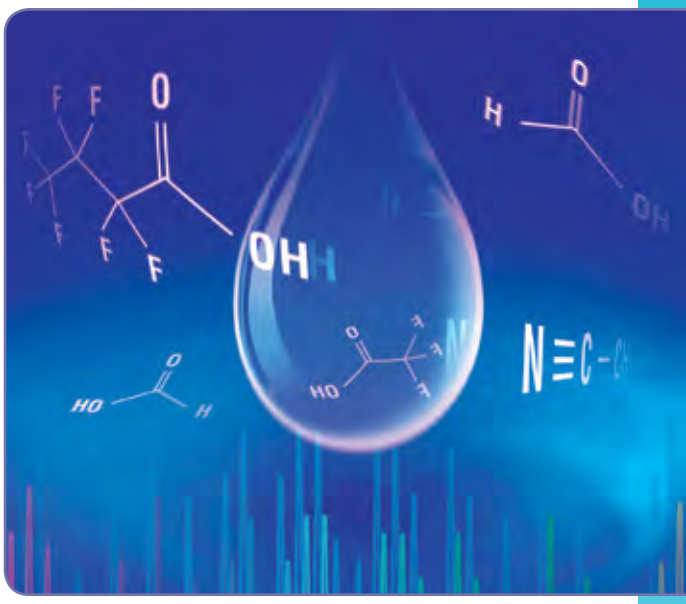


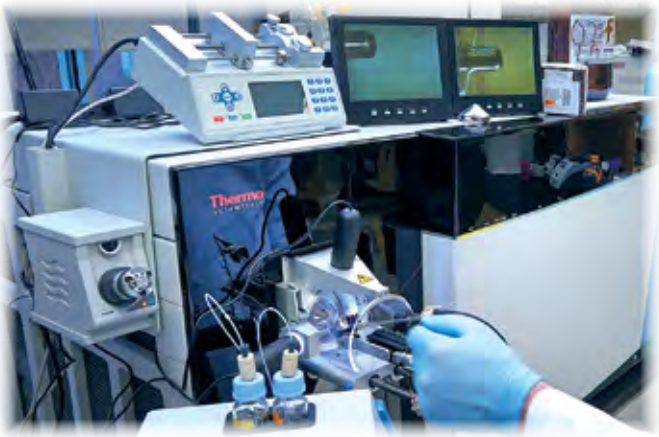
Figure 3. Effective detergent removal enables greater peptide identification. BSA (25 and 100µg/mL) was digested in the presence and absence of detergents and the samples were processed for LC-MS/MS analysis. Effective detergent removal resulted in greater peptide identification and high MASCOT scores.

Ordering Information

Product #	Description	Pkg. Size
88305	**HiPPR Detergent Removal Spin Column Kit** Includes: Detergent Removal Resin Spin Columns Accessory Pack	5mL kit 5mL 54 columns
88306	**HiPPR Detergent Removal Spin Columns, 0.1mL** *Sufficient for 24 samples of 100µL each.*	24 columns
88307	**HiPPR Detergent Removal 96-well Spin Plates, 0.1mL** *Sufficient for 192 samples of 100µL each.* Includes: HiPPR Detergent Removal 96-well Filter Spin Plate 96-well Deep-well Collection Plate 96-well Collection Plate	2 plates 2 plates 2 plates 2 plates

The proper choice of mobile phases and acidic ion-pairing agents is essential for achieving effective and reproducible liquid chromatography (LC) separation of peptides for electrospray ionization (ESI) MS. The most commonly used solvents or solvent blends include LC/MS grade water and acetonitrile, with ion-pairing agents such as trifluoroacetic acid (TFA), formic acid (FA) or heptafluorobuteric acid (HFBA). For matrix-assisted laser-induced dissociation ionization (MALDI), peptides are combined with specific matrices of crystalline energy absorbing dyes. All Thermo Scientific MALDI matrices are available in a convenient single-use format.





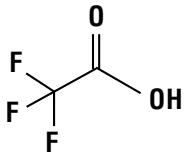
Thermo Scientific Pierce Trifluoroacetic Acid (TFA)

Convenient, high-quality packaging to prepare 0.1% TFA solutions in seconds.

Thermo Scientific™ Pierce™ Trifluoroacetic Acid (TFA) is manufactured and tested to meet strict specifications that ensure superior performance for use as an ion-pairing agent in reverse-phase peptide separations. TFA is the most commonly used ion-pairing agent for use in reverse-phase HPLC peptide separations because it sharpens peaks and improves resolution, is volatile and easily removed, has low absorption within detection wavelengths, and has a proven history of use.

Highlights:

- **High purity and exceptional clarity** – allows sensitive, nondestructive peptide detection at low UV wavelengths in reverse-phase HPLC protein and peptide separation systems
- **High-performance packaging** – TFA packaged under nitrogen in amber glass ampules or bottles with protective PTFE-lined fluorocarbon caps to ensure TFA integrity
- **Economical convenience** – choose the TFA format that works best for your application in just a few seconds, 1mL ampules can be used to prepare 1L of fresh 0.1% v/v trifluoroacetic acid solution for the mobile phase in reverse-phase chromatography



TFA
Trifluoroacetic acid
M.W. 114.02

Preparing 0.1% TFA solutions

For complex peptide separations, the key to success can be to vary selectivity. Varying mobile phase composition on the same column can change selectivity enough to resolve peptides that would otherwise overlap. TFA is the most frequently used modifier for peptide separations in reverse-phase HPLC. The TFA concentration usually specified is 0.1%. For reproducible separations from run-to-run or from lab-to-lab, it is essential to make TFA concentrations the same.

TFA concentration can and should be specified as either "w/v" (weight/volume) or as "v/v" (volume/volume). The w/v specification designates that the TFA is to be weighed and added to a volume of mobile phase (e.g., 0.1% TFA w/v requires 1g of TFA per liter). The v/v specification designates that the TFA is to be measured by volume (e.g., 0.1% TFA v/v requires 1mL of TFA per liter).

Because the density of TFA is 1.53g/mL the difference between 0.1% TFA (w/v) and 0.1% TFA (v/v) is more than 50%. For the sake of reproducibility, it is essential for authors of a method to specify, and for users of a method to know, whether the TFA concentration is given as "w/v" or "v/v".

Applications:

- Ion pair reagent for reverse-phase HPLC
- Protein/peptide sequencing
- Protein/peptide solubilizing agent
- Solid-phase peptide synthesis
- Amino acid analysis
- Making 0.1% solutions of trifluoroacetic acid (w/v vs. v/v)

Table 1. General properties of TFA.

Alternative Names	Perfluoroacetic acid, Trifluoroethanoic acid, Trifluoracetic acid
Molecular Formula	CF_3COOH
Molecular Weight	114.02
Density	1.53g/mL, 20°C
Melting/Boiling Point	-15°C / 72°C
CAS Number	76-05-01

Table 2. Specifications of Thermo Scientific Pierce TFA.

- TFA purity: >=99.5%
- Water content: <=0.1%
- Chain length: <=99.5% C2
- Ninhydrin positives: A570 <=0.02 above blank
- Tollen's test (aldehydes): Negative
- UV Absorbance (0.1% aqueous)
  - A280 <=0.002
  - A254 <=0.005
  - A230 <=0.090
- UV Absorbance (neat)
  - A300 <=0.03
  - A275 <=0.04
  - Cut-off <=262nm

References

1. Che, F.-Y., *et al.* (2011). Comprehensive proteomic analysis of membrane proteins in toxoplasma gondii. *Mol. Cell. Proteomics.* **10**:M110.000745.
2. Agnes, J. T., *et al.* (2011). Identification of Anaplasma marginale outer membrane protein antigens conserved between A. marginale sensu stricto strains and the live A. Marginale subsp. centrale vaccine. *Infect. Immun.* **79**:1311-8.
3. Warner, A. H., *et al.* (2010). Evidence for multiple group 1 late embryogenesis abundant proteins in encysted embryos of artemia and their organelles. *J. Biochem.* **148**:581-92.
4. Blouin, C. M., *et al.* (2010). Lipid droplet analysis in caveolin-deficient adipocytes: Alterations in surface phospholipid composition and maturation defects. *J. Lipid Res.* **51**:945-56.

Ordering Information

Product #	Description	Pkg. Size
28904	**Pierce Trifluoroacetic Acid (TFA), Sequencing grade** *Sufficient for sequencing and liquid chromatography applications.*	10 x 1mL
28902	**Pierce Trifluoroacetic Acid (TFA), Sequencing grade** *Sufficient for sequencing and liquid chromatography applications.*	10 x 1g
85183	**Pierce Trifluoroacetic Acid (TFA), LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	50mL
28903	**Pierce Trifluoroacetic Acid (TFA), Sequencing grade** *Sufficient for sequencing and liquid chromatography applications.*	100g
28901	**Pierce Trifluoroacetic Acid (TFA), Sequencing grade** *Sufficient for sequencing and liquid chromatography applications.*	500mL
28904B	**Pierce Trifluoroacetic Acid (TFA), Sequencing grade** *Sufficient for sequencing and liquid chromatography applications.*	Custom

Thermo Scientific Pierce Trifluoroacetic Acid (0.1%) in Acetonitrile

Convenient, pre-diluted solvent blend for MS applications.

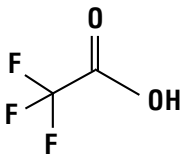
Thermo Scientific™ Pierce™ 0.1% Trifluoroacetic Acid (v/v) in Acetonitrile is an LC/MS-grade preparation with high purity and low-UV absorptivity that is ideal for HPLC and MS applications.

This 0.1% TFA in Acetonitrile is specially purified by a proprietary method and tested to ensure lot-to-lot consistency with a low UV absorbance, providing the most sensitive detection across wavelengths and prolonging equipment life. The chromatography- and spectrometry-grade 0.1% TFA in acetonitrile is 0.2-micron filtered, packaged in solvent-rinsed amber glass bottles and sealed under nitrogen with PTFE-lined fluorocarbon caps for ultimate protection.

Highlights:

- **Validated** – tested more than 20 ways to ensure maximal LC/MS sensitivity
- **Purified** – processed, filtered and sealed to extend LC/MS column life
- **Convenient** – packaged to eliminate variability and reduce handling

For complex peptide separations, the key to success can be to vary selectivity. Varying mobile phase composition on the same column can change selectivity enough to resolve peptides that would otherwise overlap. TFA is the most frequently used modifier for peptide separations in reverse-phase HPLC. The TFA concentration usually specified is 0.1%. For reproducible separations from run-to-run or from lab-to-lab, it is essential to make TFA concentrations the same.



TFA
Trifluoroacetic acid
M.W. 114.02

Table 1. General properties of TFA.

Alternative Names	Perfluoroacetic acid, Trifluoroethanoic acid, Trifluoracetic acid
Molecular Formula	CF_3COOH
Molecular Weight	114.02
Density	1.53g/mL, 20°C
Melting/Boiling Point	-15°C / 72°C
CAS Number	76-05-01

Table 2. Specifications of Thermo Scientific Pierce 0.1% Trifluoroacetic Acid (v/v) in Acetonitrile.

- Trifluoroacetic Acid content: 0.095 to 0.105%
- Water content <=0.01%
- Residue after evaporation: <=1ppm
- Color: <=10 ALPHA
- LC-MS gradient suitability: Passes
- Identification: Passes
- UV Absorbance (au):
  - 210nm <=0.6
  - 220nm <=0.55
  - 230nm <=0.4
  - 254nm <=0.03
- Trace ionic impurities:
  - Aluminum (Al) <=25ppb
  - Calcium (Ca) <=50ppb
  - Copper (Cu) <=10ppb
  - Iron (Fe) <=10ppb
  - Lead (Pb) <=10ppb
  - Magnesium (Mg) <=10ppb
  - Manganese (Mn) <=10ppb
  - Nickel (Ni) <=10ppb
  - Potassium (K) <=20ppb
  - Silver (Ag) <=10ppb
  - Sodium (Na) <=50ppb
  - Zinc (Zn) <=20ppb

Ordering Information

Product #	Description	Pkg. Size
85176	**Pierce 0.1% Trifluoroacetic Acid (v/v) in Acetonitrile, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	1L
85177	**Pierce 0.1% Trifluoroacetic Acid (v/v) in Acetonitrile, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	4 x 1L

Thermo Scientific Pierce Trifluoroacetic Acid (0.1%) in Water

Convenient, pre-diluted solvent blend for MS applications.

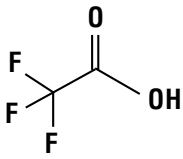
Thermo Scientific™ Pierce™ 0.1% Trifluoroacetic Acid (v/v) in Water is an LC/MS-grade preparation with high purity and low-UV absorptivity that is ideal for HPLC and MS applications.

This 0.1% TFA in Water is specially purified by a proprietary method and tested to ensure lot-to-lot consistency with a low UV absorbance, providing the most sensitive detection across wavelengths and prolonging equipment life. The chromatography- and spectrometry-grade 0.1% TFA in water is 0.2-micron filtered, packaged in solvent-rinsed amber glass bottles and sealed under nitrogen with PTFE-lined fluorocarbon caps for ultimate protection.

Highlights:

- **Validated** – tested more than 20 ways to ensure maximal LC/MS sensitivity
- **Purified** – processed, filtered and sealed to extend LC/MS column life
- **Convenient** – packaged to eliminate variability and reduce handling

For complex peptide separations, the key to success can be to vary selectivity. Varying mobile phase composition on the same column can change selectivity enough to resolve peptides that would otherwise overlap. TFA is the most frequently used modifier for peptide separations in reverse-phase HPLC. The TFA concentration usually specified is 0.1%. For reproducible separations from run-to-run or from lab-to-lab, it is essential to make TFA concentrations the same.



TFA
Trifluoroacetic acid
M.W. 114.02

Table 1. General properties of TFA.

Alternative Names	Perfluoroacetic acid, Trifluoroethanoic acid, Trifluoracetic acid
Molecular Formula	CF_3COOH
Molecular Weight	114.02
Density	1.53g/mL, 20°C
Melting/Boiling Point	-15°C / 72°C
CAS Number	76-05-01

Table 2. Specifications of Thermo Scientific Pierce 0.1% Trifluoroacetic Acid (v/v) in Water.

- Protease: Not Detected
- Trifluoroacetic Acid content: 0.095 to 0.105%
- Residue after evaporation: <=1ppm
- Color: <=10 ALPHA
- LC-MS gradient suitability: Passes
- Identification: Passes
- UV Absorbance (au):
  - 210nm <=0.55
  - 220nm <=0.18
  - 230nm <=0.06
  - 254nm <=0.003
- Trace ionic impurities:
  - Aluminum (Al) <=20ppb
  - Calcium (Ca) <=50ppb
  - Copper (Cu) <=10ppb
  - Iron (Fe) <=10ppb
  - Lead (Pb) <=10ppb
  - Magnesium (Mg) <=10ppb
  - Manganese (Mn) <=10ppb
  - Nickel (Ni) <=10ppb
  - Potassium (K) <=20ppb
  - Silver (Ag) <=10ppb
  - Sodium (Na) <=50ppb
  - Zinc (Zn) <=20ppb

Ordering Information

Product #	Description	Pkg. Size
85172	**Pierce 0.1% Trifluoroacetic Acid (v/v) in Water, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	1L
85173	**Pierce 0.1% Trifluoroacetic Acid (v/v) in Water, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	4 x 1L

Thermo Scientific Pierce Formic Acid

Well-suited for HPLC and MS applications.

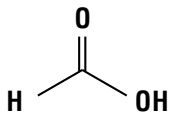
Thermo Scientific™ Pierce™ Formic Acid is high-purity solvent supplied in bottles or ampules as a convenient, contamination-free alternative for preparing elution solvents for HPLC separations of protein and peptides.

Pierce Formic Acid is sealed in amber glass ampules under a dry nitrogen atmosphere. A pre-measured aliquot of acid greatly simplifies preparation of liter quantities of mobile phases at the standard 0.1% formic acid concentration. The quality of this formic acid coupled with either glass-ampule or bottle packaging provides reliability and convenience that adds value to both the chromatographic and MS results.

Highlights:

- **Greater than 99% pure formic acid** – consistent LC baselines, no potential interference introduced in LC or MS applications, and no signal suppression in the mass spectrometer
- **High-performance packaging** – choose bottles or amber glass, pre-scored, nitrogen-flushed ampules to protect formic acid from light, moisture and contamination
- **Convenient format** – ampule packaging simplifies the preparation of gradient and isocratic mobile phases containing 0.1% (v/v) formic acid in water or acetonitrile; the contents of a single vial in a final volume of 1L of solvent yields a mobile phase of the most common formic acid concentration

Formic acid is a common component of reverse-phase mobile phases that provide protons for LC/MS analysis. The presence of a low concentration of formic acid in the mobile phase is also known to improve the peak shapes of the resulting separation. Unlike TFA, formic acid is not an ion-pairing agent and it does not suppress MS ionization of polypeptides when used as a mobile-phase component.



Formic Acid
M.W. 46.03

Table 1. General properties of formic acid.

Molecular Formula	HCOOH (CH_2O_2)
Molecular Weight	46.02
Density	1.22g/mL
CAS Number	64-18-6
Refractive Index	1.3701-1.3721 (20ºC)
Flash Point	69ºC
Freezing Point	8ºC

Table 2. Specifications of Thermo Scientific Pierce Formic Acid.

Visual	Clear liquid, free of particulate matter
Identity (IR)	Must show only peaks characteristic for the compound
Purity	> 99%
Refractive Index	1.3701 to 1.3721 (20°C, 589nm)

Ordering Information

Product #	Description	Pkg. Size
28905	**Pierce Formic Acid, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	10 x 1mL
85178	**Pierce Formic Acid, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	50mL

Thermo Scientific Pierce 0.1% Formic Acid (v/v) in Acetonitrile

Convenient, optimized solvent blend for MS applications.

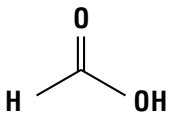
Thermo Scientific™ Pierce™ 0.1% Formic Acid (v/v) in Acetonitrile is an LC/MS-grade preparation with high purity and low-UV absorptivity that is ideal for HPLC and MS applications.

This 0.1% Formic Acid in Acetonitrile is specially purified by a proprietary method and tested to ensure lot-to-lot consistency with a low UV absorbance, providing the most sensitive detection across wavelengths and prolonging equipment life. The chromatography- and spectrometry-grade 0.1% Formic Acid in acetonitrile is 0.2-micron filtered, packaged in solvent-rinsed amber glass bottles and sealed under nitrogen with PTFE-lined fluorocarbon caps for ultimate protection.

Highlights:

- **Validated** – tested more than 20 ways to ensure maximal LC/MS sensitivity
- **Purified** – processed, filtered and sealed to extend LC/MS column life
- **Convenient** – packaged to eliminate variability and reduce handling

Formic acid is a common component of reverse-phase mobile phases that provide protons for LC/MS analysis. The presence of a low concentration of formic acid in the mobile phase is also known to improve the peak shapes of the resulting separation. Unlike TFA, formic acid is not an ion-pairing agent and it does not suppress MS ionization of polypeptides when used as a mobile-phase component.



Formic Acid
M.W. 46.03

Table 1. General properties of formic acid.

Molecular Formula	HCOOH (CH_2O_2)
Molecular Weight	46.02
Density	1.22g/mL
CAS Number	64-18-6
Refractive Index	1.3701-1.3721 (20ºC)
Flash Point	69ºC
Freezing Point	8ºC

Table 2. Specifications of Thermo Scientific Pierce 0.1% Formic Acid (v/v) in Acetonitrile.

- Formic Acid content: 0.095 to 0.105%
- Water content <=0.01%
- Residue after evaporation: <=1ppm
- Color: <=10 ALPHA
- LC-MS gradient suitability: Passes
- Identification: Passes
- UV Absorbance (au):
  - 210nm <=1.3
  - 220nm <=1.25
  - 230nm <=0.75
  - 254nm <=0.03

- Trace ionic impurities:
  - Aluminum (Al) <=25ppb
  - Calcium (Ca) <=50ppb
  - Copper (Cu) <=10ppb
  - Iron (Fe) <=10ppb
  - Lead (Pb) <=10ppb
  - Magnesium (Mg) <=10ppb
  - Manganese (Mn) <=10ppb
  - Nickel (Ni) <=10ppb
  - Potassium (K) <=20ppb
  - Silver (Ag) <=10ppb
  - Sodium (Na) <=50ppb
  - Zinc (Zn) <=20ppb

Ordering Information

Product #	Description	Pkg. Size
85174	**Pierce 0.1% Formic Acid (v/v) in Acetonitrile, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	1L
85175	**Pierce 0.1% Formic Acid (v/v) in Acetonitrile, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	4 x 1L

Thermo Scientific Pierce 0.1% Formic Acid (v/v) in Water

Convenient, optimized solvent blend for MS applications.

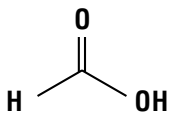
Thermo Scientific™ Pierce™ 0.1% Formic Acid (v/v) in Water is an LC/MS-grade preparation with high purity and low UV-absorptivity that is ideal for HPLC and MS applications.

This 0.1% Formic Acid in Water is specially purified by a proprietary method and tested to ensure lot-to-lot consistency with a low UV absorbance, providing the most sensitive detection across wavelengths and prolonging equipment life. The chromatography- and spectrometry-grade 0.1% Formic Acid in Water is 0.2-micron filtered, packaged in solvent-rinsed amber glass bottles and sealed under nitrogen with PTFE-lined fluorocarbon caps for ultimate protection.

Highlights:

- **Validated** – tested more than 20 ways to ensure maximal LC/MS sensitivity
- **Purified** – processed, filtered and sealed to extend LC/MS column life
- **Convenient** – packaged to eliminate variability and reduce handling

Formic acid is a common component of reverse-phase mobile phases that provide protons for LC/MS analysis. The presence of a low concentration of formic acid in the mobile phase is also known to improve the peak shapes of the resulting separation. Unlike TFA, formic acid is not an ion-pairing agent and it does not suppress MS ionization of polypeptides when used as a mobile-phase component.



Formic Acid
M.W. 46.03

Table 1. General properties of formic acid.

Molecular Formula	HCOOH (CH_2O_2)
Molecular Weight	46.02
Density	1.22g/mL
CAS Number	64-18-6
Refractive Index	1.3701-1.3721 (20ºC)
Flash Point	69ºC
Freezing Point	8ºC

Table 2. Specifications of Thermo Scientific Pierce 0.1% Formic Acid (v/v) in Water.

- Protease: Not detected
- Formic Acid content: 0.095 to 0.105%
- Residue after evaporation: <=1ppm
- Color: <=10 ALPHA
- LC-MS gradient suitability: Passes
- Identification: Passes
- UV Absorbance (au):
  - 210nm <=1.25
  - 220nm <=0.85
  - 230nm <=0.55
  - 254nm <=0.01

- Trace ionic impurities:
  - Aluminum (Al) <=20ppb
  - Calcium (Ca) <=50ppb
  - Copper (Cu) <=10ppb
  - Iron (Fe) <=10ppb
  - Lead (Pb) <=10ppb
  - Magnesium (Mg) <=10ppb
  - Manganese (Mn) <=10ppb
  - Nickel (Ni) <=10ppb
  - Potassium (K) <=20ppb
  - Silver (Ag) <=10ppb
  - Sodium (Na) <=50ppb
  - Zinc (Zn) <=20ppb

Ordering Information

Product #	Description	Pkg. Size
85170	**Pierce 0.1% Formic Acid (v/v) in Water, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	1L
85171	**Pierce 0.1% Formic Acid (v/v) in Water, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	4 x 1L

Thermo Scientific Pierce Acetonitrile, LC/MS Grade

Ideal formulation for HPLC and MS applications.

Thermo Scientific™ Pierce™ Acetonitrile (ACN) is an LC/MS-grade preparation with high purity and low-UV absorptivity that makes it suitable for HPLC and MS applications.

Pierce LC/MS Grade Acetonitrile is specially purified using a proprietary method and tested to ensure lot-to-lot consistency with a low UV absorbance to provide the most sensitive detection across all wavelengths. Pierce Acetonitrile is 0.2-micron filtered, packaged in solvent-rinsed amber glass bottles and sealed under a nitrogen atmosphere with PTFE-lined fluorocarbon caps for ultimate protection.

Highlights:

- **Validated** – 37 quality tests ensure low, stable baselines and lot-to-lot consistency
- **Sensitive** – low UV absorbance yields low baselines and high detection sensitivity
- **Purified** – low impurities protects columns and simplifies analysis by eliminating extraneous peaks

Pierce LC/MS Grade Acetonitrile is specially purified and tested to the highest specifications to ensure the integrity of your data, maximize sensitivity in your assay and to prolong the life of your equipment. These Acetonitrile specifications also meet ACS standards.

$$N \equiv C - CH_3$$

Acetonitrile
M.W. 41.05

Table 1. General properties of acetonitrile.

Alternative Names	Methyl cyanide, cyanomethane, ethanenitrile
Molecular Formula	CH_3CN
Molecular Weight	41.05
Density	0.780g/mL
CAS Number	75-05-8

Table 2. Specifications of Thermo Scientific Pierce Acetonitrile.

- Purity (by GC): >=99.9%
- Water content: <=0.01%
- Residue after evaporation: <=0.8ppm
- Titratable Acid, mEQ/g: <=0.008
- Titratable Acid, mEQ/g: <=0.0006
- LC-MS gradient suitability: Passes
- LC-MS at positive mode as Reserpine: <=50ppb
- LCMS at negative mode as Aldicarb: <=50ppb
- LC gradient suitability at 254 and 210nm: Passes
- Optical absorbance (au):
  - 190nm <=1
  - 195nm <=0.15
  - 200nm <=0.05
  - 205nm <=0.04
  - 210nm <=0.03
  - 215nm <=0.025
  - 220nm <=0.015
  - 225nm <=0.015
  - 230nm <=0.01
  - 254nm <=0.005
  - 280nm <=0.005
- Trace ionic impurities:
  - Aluminum (Al) <=25ppb
  - Barium (Ba) <=5ppb
  - Cadmium (Cd) <=5ppb
  - Calcium (Ca) <=25ppb
  - Chromium (Cr) <=5ppb
  - Cobalt (Co) <=5ppb
  - Copper (Cu) <=5ppb
  - Iron (Fe) <=5ppb
  - Lead (Pb) <=5ppb
  - Magnesium (Mg) <=10ppb
  - Manganese (Mn) <=5ppb
  - Nickel (Ni) <=5ppb
  - Potassium (K) <=10ppb
  - Silver (Ag) <=5ppb
  - Sodium (Na) <=50ppb
  - Tin (Sn) <=5ppb
  - Zinc (Zn) <=10ppb

Ordering Information

Product #	Description	Pkg. Size
51101	**Pierce Acetonitrile (ACN), LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	1L
85188	**Pierce Acetonitrile (ACN), LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	4 x 1L

Water, LC/MS Grade

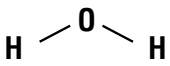
Ultrapure water for formulation of solvents for HPLC.

Thermo Scientific™ Pierce™ Water is an ultrapure, LC/MS-grade preparation with low-UV absorptivity that makes it suitable and trustworthy for use in HPLC and MS applications.

Pierce LC/MS Grade Water is specially purified by a proprietary method and tested to ensure lot-to-lot consistency with a low UV absorbance to provide you with the most sensitive detection across all wavelengths. Pierce Water is 0.2-micron filtered, packaged in solvent-rinsed, amber glass bottles and sealed under a nitrogen atmosphere with TFE-lined fluorocarbon caps for ultimate protection.

Highlights:

- **Validated** – 30 tests to ensure purity and quality for use in LC/MS applications
- **High-performance packaging** – 0.2-micron filtered, packed in solvent-rinsed amber glass bottles and sealed under nitrogen atmosphere to eliminate absorption of unknown atmospheric gases
- **High purity** – ensures high lot-to-lot consistency and reliability



Water
M.W. 18.02

Table 1. General properties of water.

Molecular Formula	H_2O
Molecular Weight	18.01
CAS Number	7732-18-5

Table 2. Specifications of Thermo Scientific Pierce Water.

- Protease: Not detected
- Total halogens (as chloride): Not detected
- Residue after evaporation: <=1ppm
- LC-MS gradient suitability: Passes
- LC-MS at positive mode as Reserpine: <=50ppb
- LC-MS at negative mode as Aldicarb: <=50ppb
- LC gradient suitability at 254 and 205nm: Passes
- Optical absorbance (au):
  - 210nm <=0.01
  - 220nm <=0.01
  - 230nm <=0.01
  - 240nm <=0.01
  - 254nm <=0.005
  - 260nm <=0.005
  - 280nm <=0.005
- Trace ionic impurities:
  - Aluminum (Al) <=10ppb
  - Barium (Ba) <=10ppb
  - Cadmium (Cd) <=10ppb
  - Calcium (Ca) <=20ppb
  - Chromium (Cr) <=10ppb
  - Cobalt (Co) <=10ppb
  - Copper (Cu) <=10ppb
  - Iron (Fe) <=10ppb
  - Lead (Pb) <=10ppb
  - Magnesium (Mg) <=10ppb
  - Manganese (Mn) <=10ppb
  - Nickel (Ni) <=10ppb
  - Potassium (K) <=10ppb
  - Silver (Ag) <=10ppb
  - Sodium (Na) <=20ppb
  - Tin (Sn) <=10ppb
  - Zinc (Zn) <=10ppb

Ordering Information

Product #	Description	Pkg. Size
51140	**Pierce Water, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	1L
85189	**Pierce Water, LC-MS Grade** *Sufficient for liquid chromatography and MS applications.*	4 x 1L

Thermo Scientific Pierce Heptafluorobutyric Acid (HFBA)

Ion-pair reagent for the reverse-phase HPLC separation of proteins and peptides.

Thermo Scientific™ Pierce™ Heptafluorobutyric Acid (HFBA) is manufactured and tested to meet strict specifications that ensure superior performance for use as an ion-pairing agent in reverse-phase peptide separations.

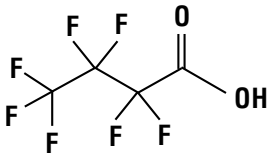
Pierce HFBA is highly purified and stably packaged heptafluorobutyric acid that is tested and prepared for use as an ion-pairing reagent in high-pressure liquid chromatography (HPLC) methods and similar applications. The solution is tested for overall purity, chain-length purity, water content, sulfate content and absorptivity at three different UV-wavelengths. The liquid reagent is offered in convenient 1mL ampules and 100mL bottles.

Highlights:

- **Greater than 99.5% purity** – allows sensitive, nondestructive peptide detection at low UV wavelengths in reverse-phase HPLC protein and peptide separation systems
- **High-performance packaging** – HFBA packaged under nitrogen in amber glass ampules or bottles
- **Economical convenience** – in just a few seconds, a 1mL ampule can be used to prepare 1 liter of fresh 0.1% heptafluorobutyric acid solution for the mobile phase in reverse-phase chromatography

Applications:

- Ion pair reagent for reverse-phase HPLC
- Protein/peptide sequencing
- Protein/peptide solubilizing agent
- Solid-phase peptide synthesis
- Amino acid analysis



HFBA
Heptafluorobutyric Acid
2,2,3,3,4,4,4-heptafluorobutanoic acid
M.W. 214.04

Table 1. Properties of heptafluorobutyric acid.

Molecular Formula	$C_4HF_7O_2$
Molecular Weight	214.04
Density	1.64g/mL
CAS Number	375-22-4

Table 2. Specifications of Thermo Scientific Pierce HFBA.

Purity	> 99.5%
Water Content	< 0.1%
Chain Length	> 99.5% C4
UV (neat)	A280 < 0.004 A254 < 0.006 A230 < 0.150
Sulfate	Negative

References

1. Hermann, P.M., *et al.* (2000). *J. Neurosci.* **20**:6355-6364.
2. Hearn, M.T.W. and Hancock, W.S. (1979). *Trends in Biochemical Sciences* **4**:N58-N62.
3. Bennett, H.P.J., *et al.* (1980). J. *Liquid Chromatogr.* **3**:1353-1365.
4. Bennett, H.P.J., *et al.* (1981). *Biochem.* **20**:4530-4538.

Ordering Information

Product #	Description	Pkg. Size
25003	**Pierce Heptafluorobutyric Acid (HFBA), Sequencing Grade** *Sufficient for sequencing and liquid chromatography applications.*	100mL
53104	**Pierce Heptafluorobutyric Acid (HFBA), HPLC Grade** *Sufficient for sequencing and liquid chromatography applications.*	10 x 1mL

Thermo Scientific Single-Use MALDI Matrices

Ready-to-use, high-quality MS reagents.

Thermo Scientific™ Single-Use MALDI Matrices are highly purified, recrystallized reagents supplied in a convenient, single-use, microtube format for use in MS. Three different popular matrices (CHCA, SA and DHB) are offered individually as packages of 24 single-use microtubes and as a sample pack containing 8 single-use microtubes of each matrix.

MALDI matrices provide the cleanest MS spectra when recrystallized and prepared in 60% acetonitrile/0.1% TFA. Traditional recrystallization and preparation of milligram amounts of matrix produces significant waste and is inconvenient. Our exclusive packaging technology provides purified, recrystallized alpha-cyano-4-hydroxy-cinnamic acid (CHCA), sinapinic acid (SA) and 2,5-dihydroxybenzoic acid (DHB) MALDI matrices in just the right amounts for individual experiments, making it easy to prepare high-quality MALDI reagents in minutes with decreased waste.

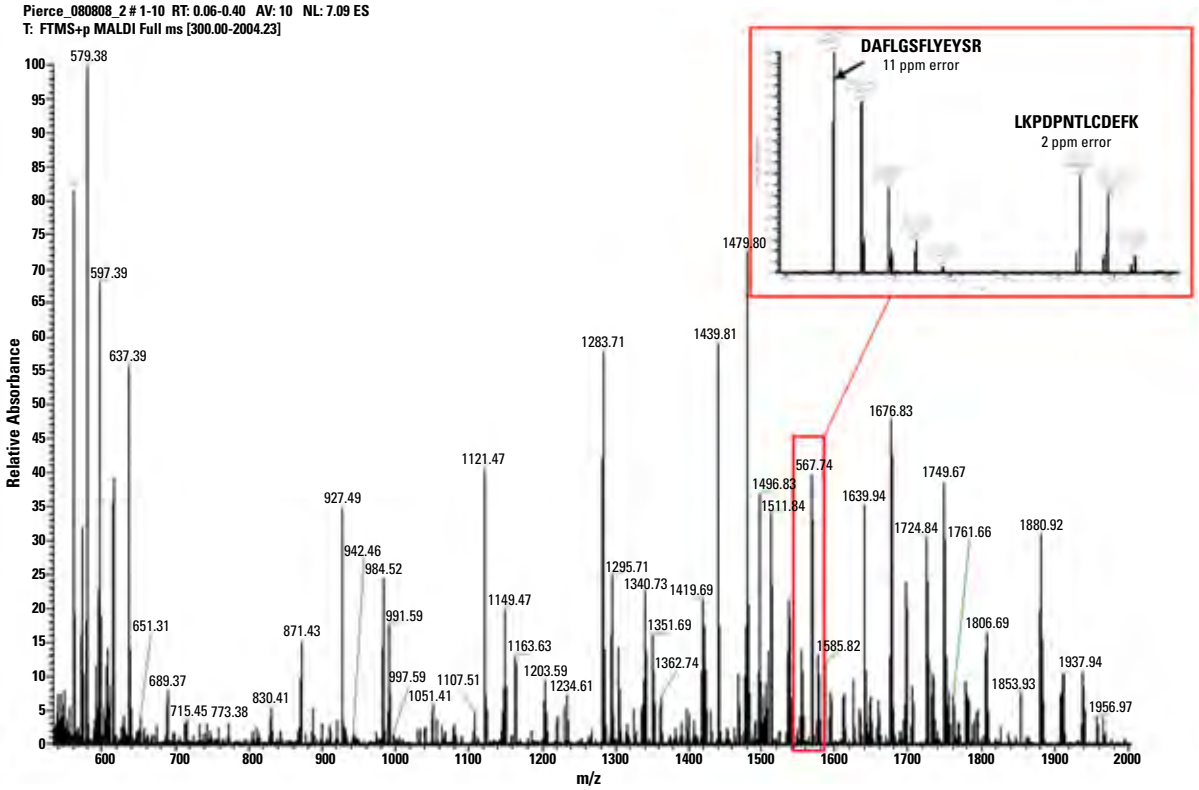


Figure 1. Thermo Scientific Single-Use MALDI Matrices produce better spectra. MALDI-MS spectra from a MALDI-LTQ Orbitrap XL Mass Spectrometer of a tryptic digest of bovine serum albumin co-crystallized with typical reagent quality and Single-Use CHCA or DHB Recrystallized MALDI Matrices.

Ordering Information

Product #	Description	Pkg. Size
90031	**CHCA MALDI Matrix, Single-Use** (alpha-Cyano-4-hydroxy-cinnamic acid)	24 x 1mg
90032	**DHB MALDI Matrix, Single-Use** (Sinapinic Acid)	24 x 1mg
90033	**DHB MALDI Matrix, Single-Use** (2,5-Dihydroxybenzoic acid)	24 x 4mg
90035	**MALDI Matrix Sampler Pack, Single-Use** Contains CHCA, SA and DHB matrices	8 microtubes of ea. matrix

related Thermo Scientific products

Ordering Information

Calibration Solutions

Product #	Description	Pkg. Size
88320	Pierce™ Peptide Retention Time Calibration Mixture, 0.5pmol/µL	50µL
88321	Pierce™ Peptide Retention Time Calibration Mixture, 5pmol/µL	200µL
88322	Pierce™ LTQ ESI Positive Ion Calibration Solution	10mL
88323	Pierce™ LTQ Velos ESI Positive Ion Calibration Solution	10mL
88324	Pierce™ ESI Negative Ion Calibration Solution	10mL
88325	Pierce™ Triple Quadrupole Calibration Solution	10mL
88326	Reserpine Standard for LC-MS	5 x 1mL
88328	Pierce™ HeLa Protein Digest Standard	20µg
88329	Pierce HeLa Protein Digest Standard	5 x 20µg

Protein Quantitation Reagents – SILAC

Product #	Description	Pkg. Size
89982	Pierce™ SILAC Protein Quantitation Kit -RPMI 1640	Kit
89983	Pierce SILAC Protein Quantitation Kit - DMEM	Kit
89989	L-Arginine-HCl	50mg
88427	L-Arginine-HCl	500mg
88210	$^{13}C_6$ L-Arginine-HCl	50mg
88433	$^{13}C_6$ L-Arginine-HCl	500mg
89990	$^{13}C_6$ $^{15}N_4$ L-Arginine-HCl	50mg
88434	$^{13}C_6$ $^{15}N_4$ L- Arginine-HCl	500mg
89987	L-Lysine-2HCl	50mg
88429	L-Lysine-2HCl	500mg

Protein Quantitation Reagents – SILAC (continued)

Product #	Description	Pkg. Size
89988	$^{13}C_6$ L-Lysine-2HCl	50mg
88431	$^{13}C_6$ L-Lysine-2HCl	500mg
88209	$^{13}C_6$ $^{15}N_2$ L-Lysine-2HCl	50mg
88432	$^{13}C_6$ $^{15}N_2$ L-Lysine-2HCl	500mg
88437	L-Lysine-2HCl (4,4,5,5-D_4)	50mg
88438	L-Lysine-2HCl (4,4,5,5-D_4)	500mg
88428	L-Leucine	500mg
88435	$^{13}C_6$ L-Leucine	50mg
88436	$^{13}C_6$ L-Leucine	500mg
88211	L-Proline	115mg
88430	L-Proline	500mg
89984	RPMI Media for SILAC	500mL
88421	RPMI Media for SILAC	6 x 500mL
89985	DMEM Media for SILAC	500mL
88420	DMEM Media for SILAC	6 x 500mL
88215	DMEM:F12 (1:1) Media for SILAC	500mL
89986	Dialyzed FBS for SILAC	50mL
88212	Dialyzed FBS for SILAC	100mL
88440	Dialyzed FBS for SILAC	500mL
88422	MEM for SILAC	500mL
88423	IMDM for SILAC	500mL
88424	Ham's F12 for SILAC	500mL
88425	Powdered DMEM Media for SILAC *Sufficient to prepare 10L of medium.*	135g
88426	Powdered RPMI Media for SILAC *Sufficient to prepare 10L of medium.*	104g
88439	SILAC Protein Quantitation Kit - DMEM:F12	Kit
88441	McCoy's 5A Media for SILAC	500mL

Protein Quantitation Reagents – Amine-Reactive Tandem Mass Tag™ Reagents

Product #	Description	Pkg. Size
90063	TMTduplex™ Isobaric Mass Tagging Kit	15-rxn kit
90064	TMTsixplex™ Isobaric Mass Tagging Kit	35-rxn kit
90065	TMTduplex™ Isobaric Label Reagent Set	10-rxn kit
90066	TMTsixplex™ Isobaric Label Reagent Set	30-rxn kit
90067	TMTzero™ Label Reagent	5 x 0.8mg
90060	TMTduplex™ Isotopic Label Reagent Set	10-rxn kit
90061	TMTsixplex Isobaric Label Reagent Set	6-rxn kit
90062	TMTsixplex Isobaric Label Reagent Set	12-rxn kit
90068	TMTsixplex Isobaric Label Reagent Set	72-rxn kit
90110	TMT10plex™ Label Reagent Set	10-rxn kit
90111	TMT10plex Label Reagent Set	30-rxn kit
90113	TMT10plex™ Isobaric Mass Tag Labeling Kit	30-rxn kit
90406	TMT10plex™ Isobaric Label Reagent Set	60-rxn set

Protein Quantitation Reagents – Cysteine-Reactive Tandem Mass Tag™ Reagents

Product #	Description	Pkg. Size
90100	iodoTMTzero™ Label Reagent, 5 x 0.2mg	5-rxn set
90101	iodoTMTsixplex™ Label Reagent Set	6-rxn set
90102	iodoTMTsixplex Label Reagent Set	30-rxn set
90103	iodoTMTsixplex™ Isobaric Mass Tag Labeling Kit	30-rxn kit

Protein Quantitation Reagents – Carbonyl-Reactive Tandem Mass Tag Reagents

Product #	Description	Pkg. Size
90400	aminoxyTMTzero™ Label Reagent	6 x 0.2mg
90401	aminoxyTMTsixplex™ Label Reagent Set	1 x 0.2mg
90402	aminoxyTMTsixplex Label Reagent Set	5 x 0.2mg

TMT™ Accessories and Reagents

Product #	Description	Pkg. Size
90075	Anti-TMT Antibody	0.1mL
90076	Immobilized Anti-TMT Antibody Resin	6mL
90104	TMT™ Elution Buffer	20mL
90114	1M Triethylammonium Bicarbonate (TEAB)	50mL
90115	50% Hydroxylamine	5mL

Protein Quantitation Reagents – Peptides for Targeted Quantitation

Product #	Description	Pkg. Size
300206	HeavyPeptide™ AQUA BASIC, Custom Peptide, up to 15 aa, >95% purity unmodified	1 x 30nmol
300207	HeavyPeptide AQUA BASIC, Custom Peptide, up to 15 aa, >95% purity unmodified	1 x 30nmol
300204	HeavyPeptide™ AQUA QuantPro Custom Peptide Kit, up to 15 aa, >97% purity unmodified	10 x 1nmol
300205	LightPeptide™ AQUA QuantPro Custom Peptide Kit, up to 15 aa, >97% purity unmodified	10 x 1nmol
300210	HeavyPeptide™ AQUA Ultimate Custom Peptide Kit, up to 15 aa, >97% purity unmodified	10 x 1nmol
300215	LightPeptide™ AQUA Ultimate Custom Peptide Kit, up to 15 aa, >97% purity unmodified	10 x 1nmol
500116	PEPotec™ SRM Peptide Library: custom crude peptide, 6-25 aa, unmodified	0.1mg
500550	PEPotec SRM Peptide Library: custom crude peptide, 6-25 aa, heavy labeled	0.1mg
300322	HeavyPeptide™ IGNIS Prime Quantitation Kit: 95% purity, up to 20 aa plus calibration mixture	10 x 1µg
88330	1-Step Heavy Protein IVT Kit	8-rxn kit
88331	1-Step Heavy Protein IVT Kit	40-rxn kit

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