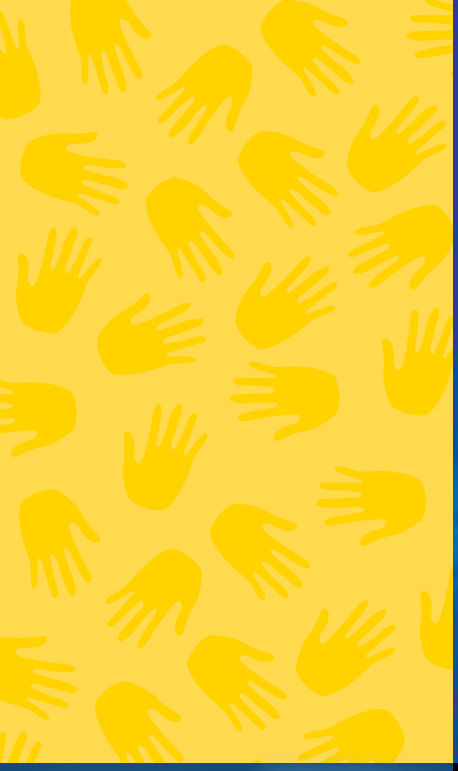
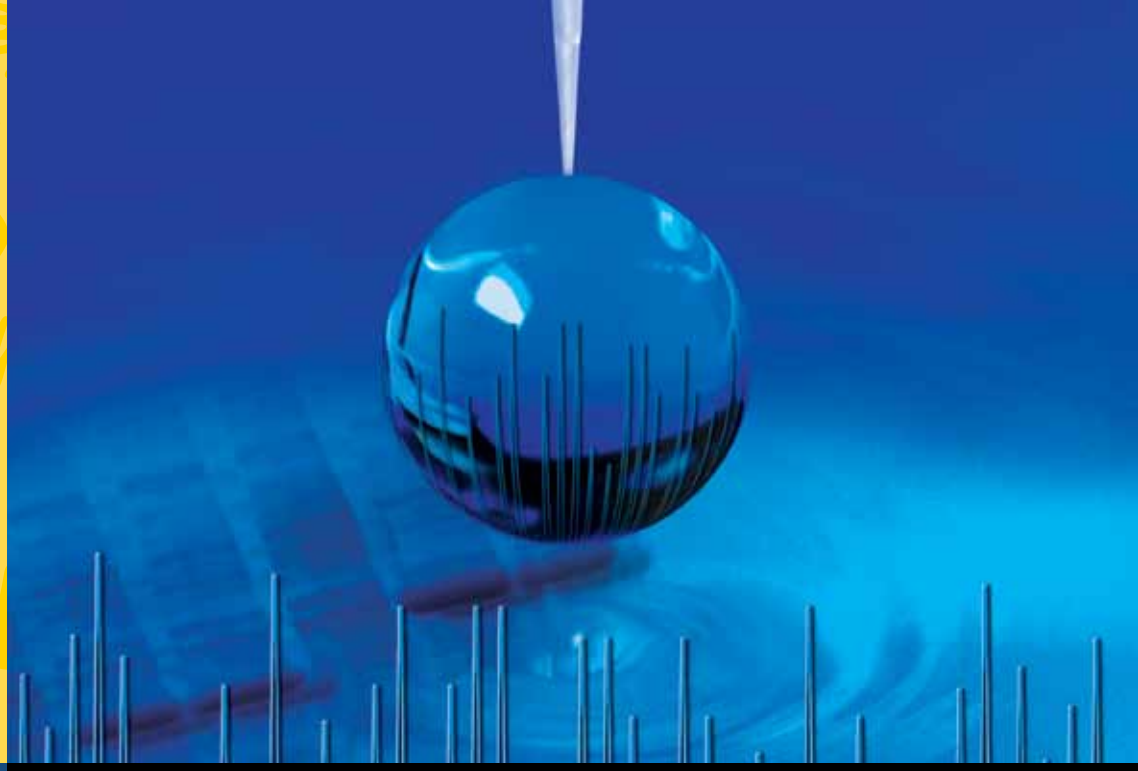




Thermo Scientific Pierce Products for Mass Spectrometry Sample Preparation

Featuring products to extract, digest, enrich, clean up and quantify proteins and peptides

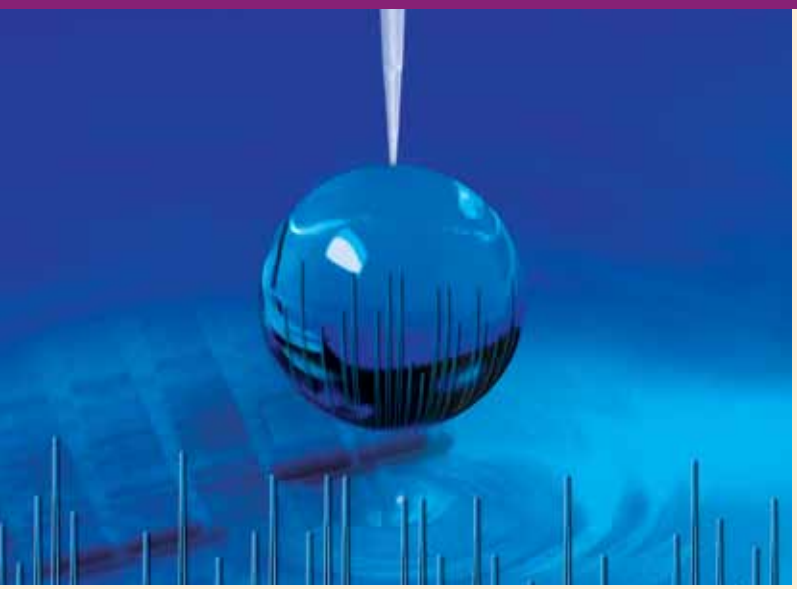
Version 2

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

Table of Contents

Introduction



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 anlaysis. 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 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 protein 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 mass spectrometry 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 is easier to interpret for protein identification. 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 by MALDI-MS or liquid chromatography tandem mass spectrometry (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.

Introduction

To contend with these challenges, we have developed a complete workflow of sample preparation solutions designed for better MS analysis. Our researchers understand the need for workflows that enrich subproteomes based upon the context of biology, which might include subcellular fractionation, enrichment based upon post-translational modifications (e.g. phosphorylation, glycosylation), or co-immunoprecipitation of a target and its associated proteins. We strive to develop products and integrated proteomics solutions that are compatible and supportive of MS analysis. We recognize the need to provide complete solutions and technical support for protein research and analytical characterization using mass spectrometry. These reagents and instructions are well tested and supported to assist the biologist and the mass spectrometrist to succeed in their research. Moreover, they provide consistent results between labs and prevent wasted time spent troubleshooting experimental methods and results. The following workflows highlight reagents and options for preparing samples from a variety of starting materials for successful MS analysis.

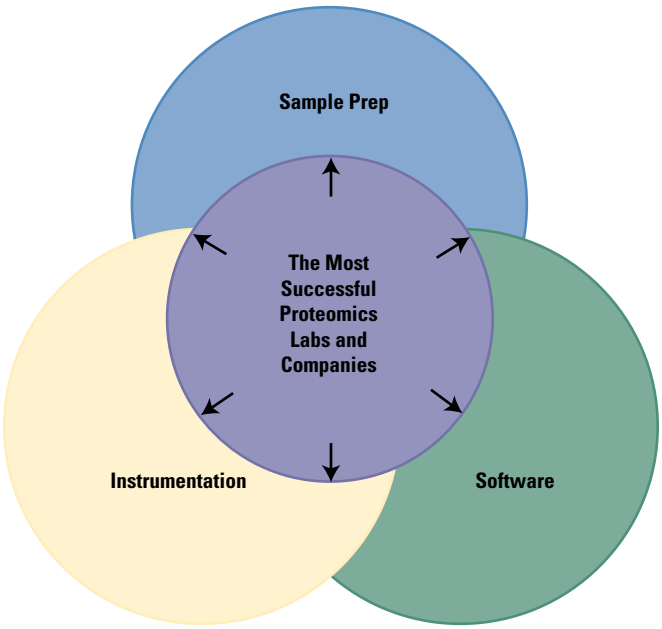


Figure 1. The key to proteomics success. Successful proteomics laboratories and companies recognize the importance of sophisticated sample preparation, instrumentation and software technologies and skills. Workflows designed to maximize the overlap between these complementary technologies are an effective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

Discovery vs Targeted Proteomic Analysis

A successful proteomics experiment requires integration of the right sample prepration, instrumentation and software. These are the experimental tools. A proteomics scientists 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 (Figure 2). 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 proteomics. Discovery proteomics efforts optimize 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 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 fractions (e.g., subcellular compartments or protein complexes), and fractionation to decrease sample complexity (e.g., SDS-PAGE or chromatography). These strategies reduce the dynamic range between components in a fraction 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 2-30 samples. Quantitative discovery proteomics experiments utilize label-free or stable isotope labeling methods to quantify these proteins. Label-free strategies require highly reproducible fractionation and alignment of peptides across LC-MS/MS experiments to compare spectral counts or ion intensities. Stable isotope protein labeling strategies (e.g., SILAC, TMT and cysTMT tags) incorporate ^{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 six samples to be labeled and combined prior to processing and LC-MS/MS analysis. This 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 Velos Mass Spectrometer.

Targeted proteomics experiments are typically designed to quantify less than one hundred 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. 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 analysis on a triple quadrupole mass spectrometer. A triple quadrupole mass spectrometer quantifies peptides by monitoring specific mass windows for peptides of interest, fragmenting the isolated peptide(s), and then quantifying several fragment ions that are specific for the peptide of interest. This selective reaction monitoring (SRM) strategy for targeted quantitation, along with chromatographic retention time information, provides very high sensitivity, specificity, dynamic range and throughput. Targeted quantitative protein studies are typically performed on triple quadrupole mass spectrometers, such as the Thermo Scientific TSQ Vantage Mass Spectrometer.

Targeted quantitative proteomic experiments are increasingly used in pharmaceutical and diagnostic applications to quantify proteins and metabolites in complex samples. To further improve quantitative precision and accuracy, known amounts of synthetic peptides containing heavy stable isotopes, such as Thermo Scientific HeavyPeptides, are added to samples prior to MS analysis. These peptides serve as internal quantitative standards for absolute quantification of the corresponding natural peptides in a biological sample.

We offer a complete line of workflows and reagents for protein identification and quantitation by mass spectrometry. Whether you are conducting a discovery protein identification and profiling experiment or a targeted, high throughput quantitative study, our researchers understand the need for integrated proteomics solutions that are compatible with your MS analysis.

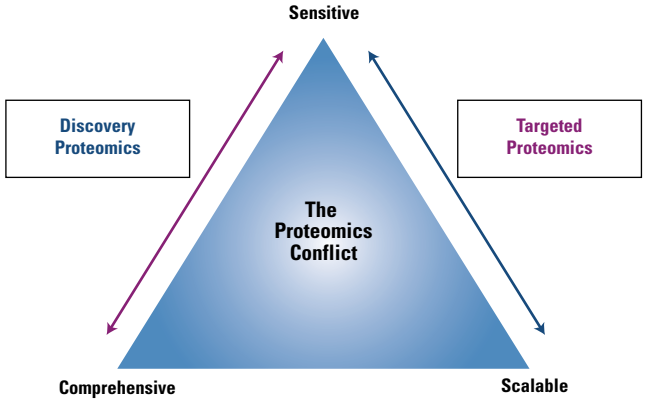


Figure 2. The proteomics conflict. It is impossible to optimize sensitivity, throughput and comprehensiveness simultaneously. Discovery proteomics strategies optimize sensitivity and comprehensiveness with few samples. Targeted proteomics strategies optimize sensitivity and scalability by limiting the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

Discovery Analysis Workflow

Specialized Reagents

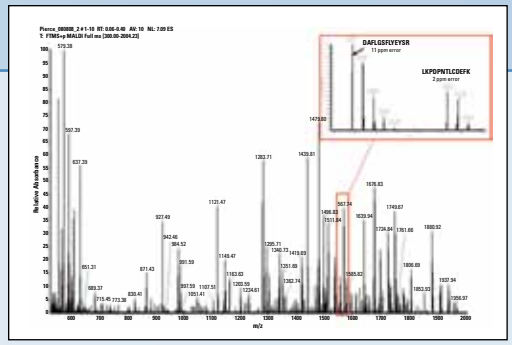
- TMT Mass Tagging Reagents
- cysTMT Mass Tagging Reagents
- SILAC Protein Quantitation Reagents

General Reagents

- Cell lysis and fractionation
- Abundant protein depletion
- Proteases (Trypsin, LysC, Chymotrypsin, GluC, AspN)
- Detergent removal
- Enrichment (Fe-NTA, TiO_2)
- C18 tips, graphite spin columns
- TFA, FA, acetonitrile

Instruments

- LTQ OrbiTrap Velos Mass Spectrometer



Software

- Proteome Discoverer
- SIEVE
- ProSight PC

Targeted Analysis Workflow

Specialized Reagents

- HeavyPeptide Reagents
- TMT Mass Tagging Reagents

General Reagents

- Cell lysis and fractionation
- Abundant protein depletion
- Proteases (Trypsin, LysC, Chymotrypsin, GluC, AspN)
- Detergent removal
- Enrichment (Fe-NTA, TiO_2)
- C18 tips, graphite spin columns
- TFA, FA, acetonitrile

Instruments

- TSQ Triple Quadrupole Mass Spectrometer

Software

- Pinpoint Software

Protein Quantitation Reagents - Discovery Analysis

SILAC Protein Quantitation Kits and Reagents

For quantitative analysis of differential protein expression.

Stable isotope labeling using amino acids in cell culture (SILAC) is a powerful method to identify and quantify relative differential changes in complex protein samples. The SILAC method uses *in vivo* metabolic incorporation of "heavy" ^{13}C- or ^{15}N-labeled amino acids into proteins followed by mass spectrometry (MS) analysis for accelerated comprehensive identification, characterization and quantitation of proteins.

Applications:

- Characterization of proteins involved in stem cell differentiation using stem cell-specific kits
- Quantitative analysis of relative changes in protein abundance from different cell treatments
- Quantitative analysis of proteins for which there are no antibodies available
- Protein expression profiling of normal vs. disease cells
- Identification and quantification of hundreds to thousands of proteins in a single experiment
- Immunoprecipitation of native proteins and protein complexes from multiple conditions

Highlights:

- **Efficient** – 100% label incorporation into proteins of living cells
- **Reproducible** – eliminates intra-experimental variability caused by differential sample preparation
- **Flexible** – media deficient in both L-lysine and L-arginine, allowing for more complete proteome coverage through dual amino acid isotope labeling
- **Versatile** – label proteins expressed in a wide variety of mammalian cell lines adapted to grow in DMEM or RPMI 1640 medium, including HeLa, 293T, COS7, U2OS, A549, A431, HepG2, NIH 3T3, Jurkat and others
- **Compatible** – test human mesenchymal stem cells or murine embryonic stem cells with differentiation media to uncover key proteins regulating development

SILAC requires growing mammalian cells in specialized media supplemented with light or heavy forms of essential amino acids; e.g., $^{12}C_6$ and $^{13}C_6$ L-lysine, respectively. A typical experiment involves growing one cell population in medium containing light amino acids (control), while the other population is grown in the presence of heavy amino acids (experimental). The heavy and light amino acids are incorporated into proteins through natural cellular protein synthesis. After alteration of the proteome in one sample through chemical treatment or genetic manipulation, equal amounts of protein from both cell populations are then combined, separated by SDS-polyacrylamide gel electrophoresis and digested with trypsin before MS analysis. Because peptides labeled with heavy and light amino acids are chemically identical, they co-elute during reverse-phase column prefractionation and, therefore, are detected simultaneously during MS analysis. The relative peak intensities of multiple isotopically distinct peptides from each protein are then used to determine the average change in protein abundance in the treated sample (see Figure 3).

Several different Thermo Scientific Pierce SILAC Kits are available, providing media that are compatible with several different kinds of mammalian cell lines, including human mesenchymal stem cells and mouse embryonic stem cells. Each kit includes all necessary reagents to isotopically label cells, including media, heavy and light amino acid pairs and dialyzed serum. Several isotopes of lysine and arginine are available separately, enabling multiplexed experiments and analysis. When combined with Thermo Scientific Protein/Peptide Sample Enrichment Products, Pierce SILAC Protein Quantitation Kits also enable MS analysis of low-abundance proteins such as cell-surface proteins, organelle-specific proteins and protein post-translational modifications such as phosphorylation or glycosylation.

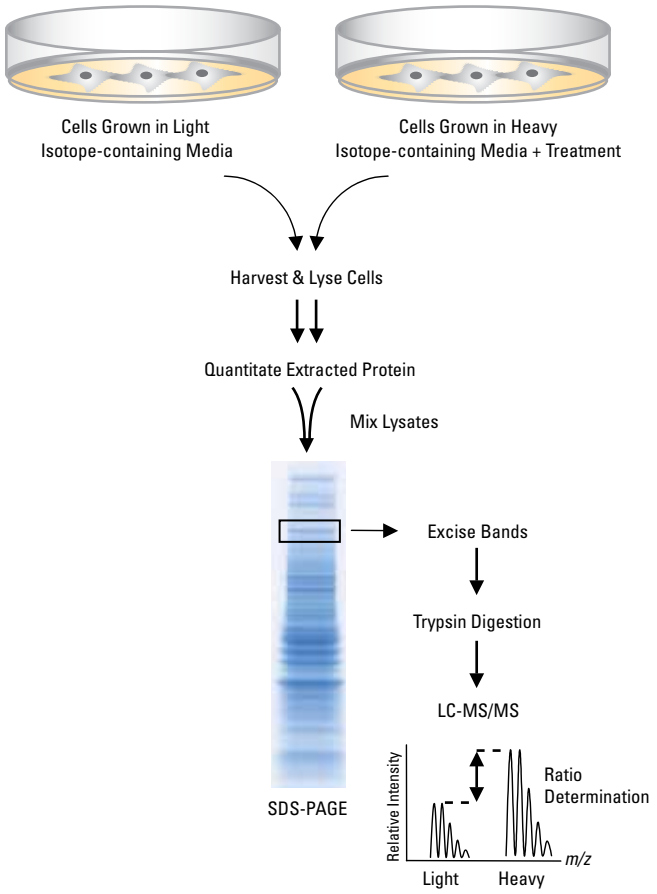


Figure 3. Schematic of SILAC workflow. A549 cells adapted to DMEM were grown for six passages (10 days) using SILAC DMEM (Product # 89983) containing 0.1 mg/ml heavy $^{13}C_6$ L-lysine-2HCl or light L-lysine-HCl supplemented with 10% dialyzed FBS. After 100% label incorporation, $^{13}C_6$ L-lysine-labeled cells were treated with 5 µM camptothecin (Sigma, St. Louis, Product # C9911) for 24 hours. Cells from each sample (light and heavy) were lysed using Thermo Scientific M-PER Mammalian Protein Extraction Reagent (Product # 78501). Samples were normalized for protein concentration using the Thermo Scientific Pierce BCA Protein Assay (Product # 23225), and 50 mg of each sample were equally mixed before 4-20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Gels were stained with Thermo Scientific GelCode Blue Stain Reagent (Product # 24592) and proteins were digested and alkylated using the Thermo Scientific Pierce In-Gel Tryptic Digestion Kit (Product # 89871) before analysis using an LTQ Orbitrap Hybrid Mass Spectrometer.

Example Experiment

Using a Pierce® SILAC Quantitation Kit, A549 cells adapted to grow in Dulbecco's Modified Eagle Medium (DMEM) were labeled with $^{13}C_6$ L-lysine to > 98% isotope incorporation. Heavy-labeled cells treated with camptothecin were lysed, mixed with control lysates, separated by SDS-PAGE and digested with trypsin before MS analysis. More than 350 proteins were successfully identified by MS/MS sequencing using a Thermo Scientific LTQ Orbitrap Mass Spectrometer. Identified peptides were then quantitated using the Thermo Scientific Bioworks Software Suite to generate SILAC ratios corresponding to relative changes in protein abundance.

Most of the proteins identified had no change in abundance level after camptothecin treatment; however, 20% of proteins quantified in heavy-labeled cells had protein levels (SILAC ratios) 1.5-fold higher than control cells. One protein that was identified as being up-regulated in response to camptothecin treatment was proliferating cell nuclear antigen (PCNA), a protein with involvement in DNA repair (see Figure 4). To validate SILAC data, protein levels were separately quantitated by Western blot (see Figure 5). PCNA protein levels increased 1.9-fold; however, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein did not significantly change. The abundance ratios determined by Western blot were comparable to those determined by SILAC.

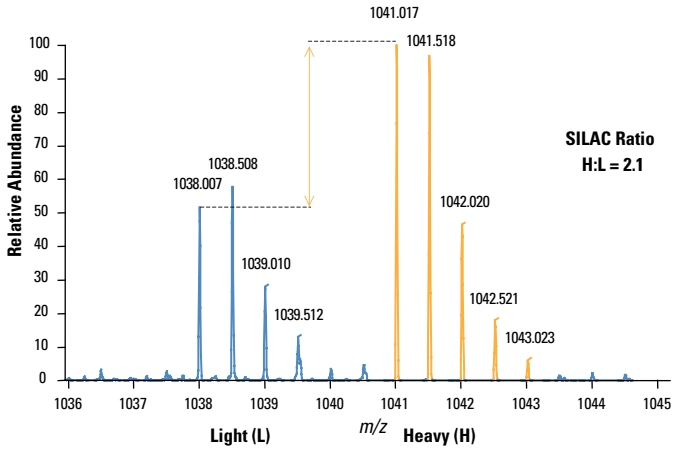


Figure 4. Representative MS spectra generated using SILAC. Light and heavy ($^{13}C_6$) L-lysine-containing peptides (AEDNADTLALVFEAPNQEK) from PCNA were analyzed by MS. Mass spectra of heavy peptides containing $^{13}C_6$ L-lysine have an increased mass of 6 Da and are shifted to the right of light peptide spectra by a mass to charge ratio (m/z) of 3 caused by a +2 ionization of peptides.

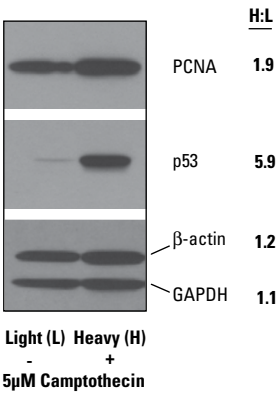


Figure 5. Comparison of A549 protein levels detected by Western blotting after camptothecin treatment. Ten micrograms of each light (L) and heavy (H) sample were analyzed by 4-20% SDS-PAGE and Western blotting using specific antibodies.

References
Bomgarden, R., *et al.* (2008). *Previews.* **11(2):** 24-25.
Everly, P.A., *et al.* (2004). *Mol & Cell Proteomics.* **3.7:** 729-735.
Levine, A.J. (1997). *Cell.* **88:** 323-331.
Mann, M. (2006). *Nature Reviews.* **7:** 952-959.

Ordering Information

Product #	Description	Pkg. Size
89982	Pierce SILAC Protein Quantitation Kit – RPMI 1640	Kit
	Includes: RPMI Media for SILAC†	2 x 500 ml
	Dialyzed FBS	2 x 50 ml
	$^{13}C_6$ L-Lysine•2HCl	50 mg
	L-Lysine•2HCl	50 mg
	L-Arginine•HCl	2 x 50 mg
89983	Pierce SILAC Protein Quantitation Kit – DMEM	Kit
	Includes: DMEM Media for SILAC†	2 x 500 ml
	Dialyzed FBS	2 x 50 ml
	$^{13}C_6$ L-Lysine•2HCl	50 mg
	L-Lysine•2HCl	50 mg
	L-Arginine•HCl	2 x 50 mg
89984	RPMI Media for SILAC†	500 ml
89985	DMEM Media for SILAC†	500 ml
88215	DMEM:F12 (1:1) Media for SILAC†	500 ml
89986	Dialyzed FBS	50 ml
89987	L-Lysine•2HCl	50 mg
89988	$^{13}C_6$ L-Lysine•2HCl	50 mg
89989	L-Arginine•HCl	50 mg
89990	$^{13}C_6$ $^{15}N_4$ L-Arginine•HCl	50 mg
88209	$^{13}C_6$ $^{15}N_2$ L-Lysine•2HCl	50 mg
88210	$^{13}C_6$ L-Arginine•HCl	50 mg
88211	L-Proline	115 mg
88214	Phenol Red Free MEM for SILAC†	500 ml
88200	Human Mesenchymal Stem Cell SILAC Kit	Kit
	Includes: Human Mesenchymal Stem Cell Universal Expansion Media for SILAC†	2 x 500 ml
	$^{13}C_6$ L-Lysine•2HCl	2 x 50 mg
	L-Lysine•2HCl	2 x 50 mg
	L-Arginine	2 x 50 mg
	Stem Cell Screened Dialyzed FBS	2 x 50 ml
88201	Human Mesenchymal Stem Cell Universal Expansion Media for SILAC†	500 ml

Ordering Information

Product #	Description	Pkg. Size
88202	Human Mesenchymal Stem Cell Adipogenic Differentiation Media for SILAC†	450 ml
88203	Human Mesenchymal Stem Cell Chondrogenic Differentiation Media for SILAC†	450 ml
88204	Human Mesenchymal Stem Cell Neural Differentiation Media for SILAC†	450 ml
88205	Human Mesenchymal Stem Cell Osteogenic Differentiation Media for SILAC	450 ml
88206	Mouse Embryonic Stem Cell SILAC Kit	Kit
	Includes: Mouse Stem Cell Expansion DMEM for SILAC†	2 x 500 ml
	$^{13}C_6$ L-Lysine•2HCl	2 x 50 mg
	L-Lysine•2HCl	2 x 50 mg
	L-Arginine	2 x 50 mg
	Stem Cell Screened Dialyzed FBS	2 x 50 ml
88207	Mouse Stem Cell Expansion DMEM for SILAC†	500 ml
88208	Low Osmolarity Mouse Stem Cell DMEM for SILAC†	500 ml
88213	Serum Substitute for Mouse Embryonic Stem Cells	50 ml
88212	Stem Cell Screened Dialyzed FBS	50 ml

† All media for SILAC are minus L-Lysine and L-Arginine.

Products for Cell Culture

High quality classical, serum-free and protein-free media for life science research.

The Thermo Scientific HyClone Classical Media, Reagents and Supplements line covers the most commonly used formulations in both liquid and powder. As a premier supplier of quality sera for over 30 years, you are assured that our classical media, serum-free and protein-free media set the standards for quality and innovation. Our product experts have decades of experience in optimizing cell culture performance and understand your research challenges. With innovations in serum processing, disposable liquid manufacturing, customized media development, buffers, WFI and specialized products for stem cell research, we have the tools you need.

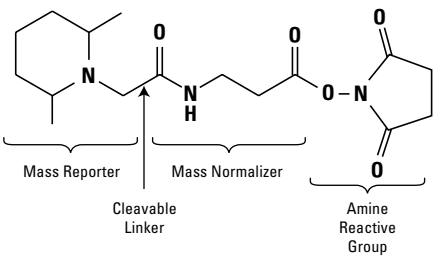
For more information on the HyClone Cell Culture products, please visit *www.thermoscientific.com/hyclone*

Isobaric Mass Tagging Overview

New options for relative and absolute protein quantification for challenging research situations.

Isobaric chemical tags are powerful tools that enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry. There are two sets of chemical tags sharing an identical structure that covalently attaches to the free amino termini of peptides and to lysines residues (Figure 6A) or to cysteine residues of peptides and proteins (Figure 6B), thereby labeling various peptides in a given sample. During the MS/MS analysis, each isobaric tag produces a unique reporter ion signature that makes quantitation possible. In the first MS analysis, the labeled peptides are indistinguishable from each other; however, in the tandem MS mode during which peptides are isolated and fragmented, each tag generates a unique reporter ion. Protein quantitation is then accomplished by comparing the intensities of the six reporter ions in the MS/MS spectra.

A. For lysine attachment



B. For cysteine attachment

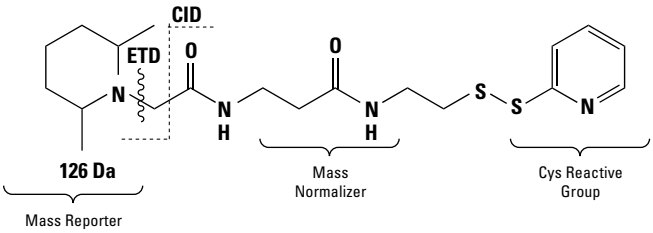
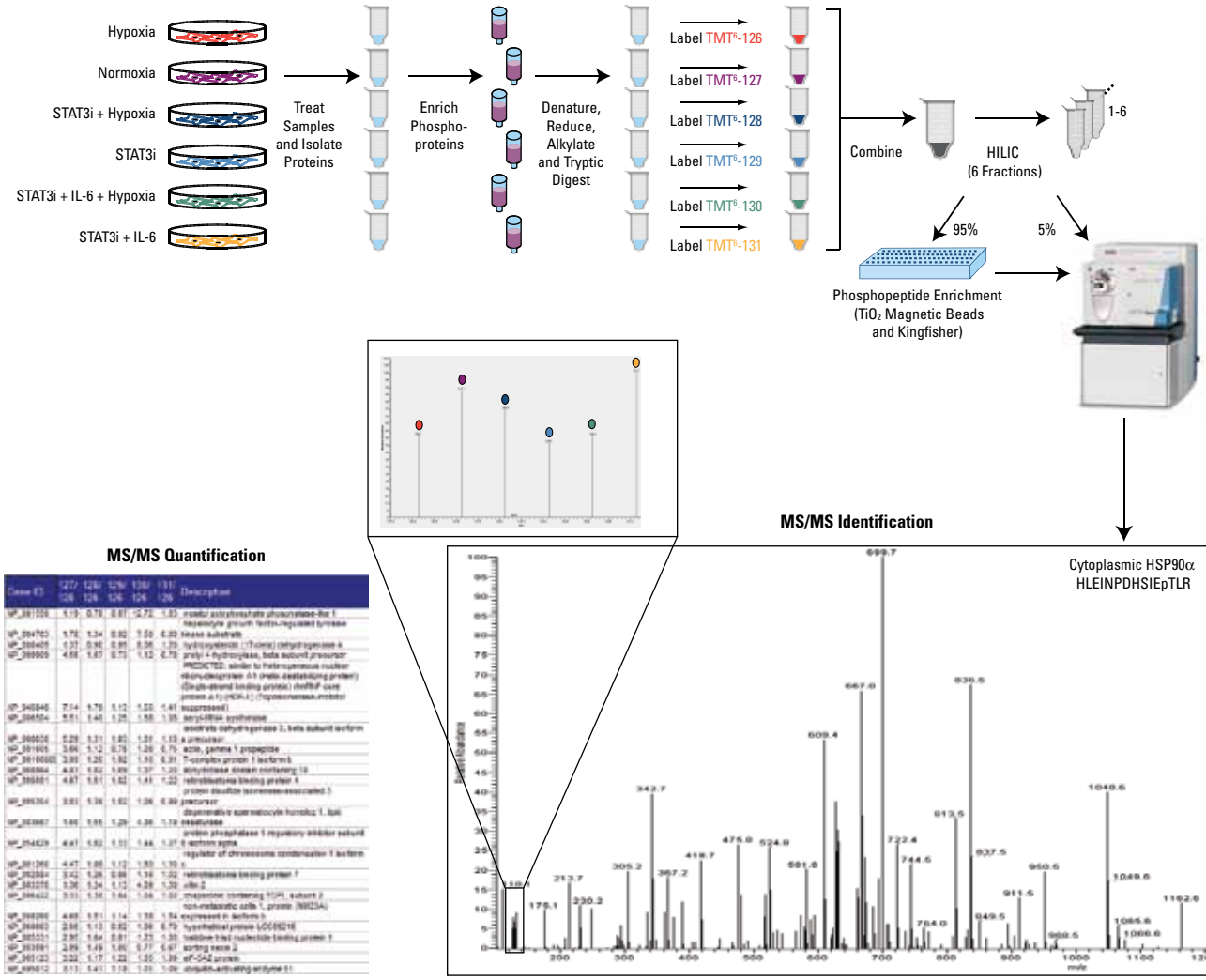


Figure 6. Structural design of tandem mass tags. Mass reporter: Each member has a unique mass and reports sample-specific abundance of a labeled peptide during MS/MS analysis. **Cleavable linker:** Preferentially fragments under typical MS/MS conditions to release the mass reporter. **Mass normalizer:** Each member has a unique mass that balances the mass reporter, ensuring the same overall mass for all tags in a set. **Reactive group:** Reactive NHS ester or thiol reactive pyridyldithiol provide high-efficiency amine-specific or cysteine-specific labeling of proteins/peptides, respectively.

Protein Quantitation Reagents - Discovery Analysis

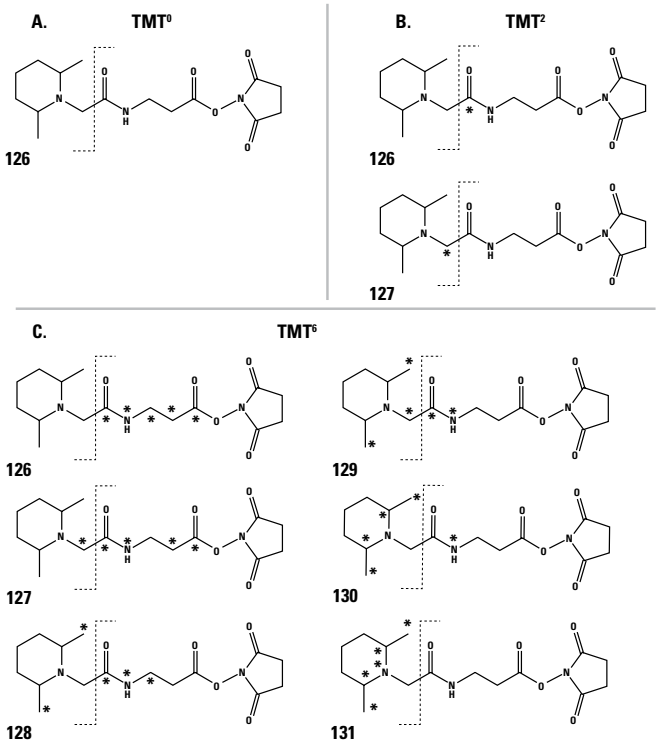


Protein profiling with Thermo Scientific Tandem Mass Tag (TMT) Tags. Proteins from up to six treated samples are: 1. denatured; 2. digested with trypsin; 3. labeled with TMT⁶ Label Reagents; 4. combined; 5. cleaned or fractionated by strong cation exchange; 6. chromatographically separated, isolated and fragmented as peptides by in-line reverse-phase LC-MS/MS; and 7. identified and quantified with Thermo Scientific BioWorks, Proteome Discoverer 1.2 or Matrix Science Mascot® Search Engine.

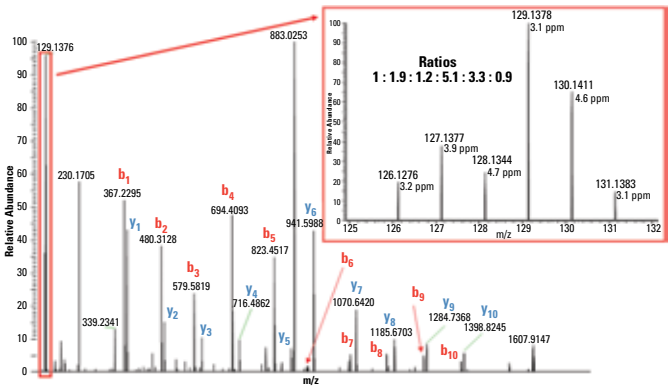
Amine-reactive Tandem Mass Tagging Reagents (TMT)

Thermo Scientific Tandem Mass Tag (TMT®) Kits and Reagents enable a rapid and cost-effective transition from method-development to high-throughput protein quantitation. The tags consist of TMT⁰ (zero), the TMTduplex and the TMTsixplex set. The TMT⁰ Label Reagent allows testing and optimization of sample preparation, labeling, fractionation and MS fragmentation for peptide identification and reporter detection without using the more costly isotope-labeled compounds. The TMTduplex allows duplex protein profiling for small studies. The TMTsixplex allows sixplex protein profiling for multiple conditions, including time courses, dose responses, replicates or multiple sample comparisons. Each isobaric tag is based on the same chemical structure, eliminating the need to modify labeling conditions or HPLC separation conditions between experiments.

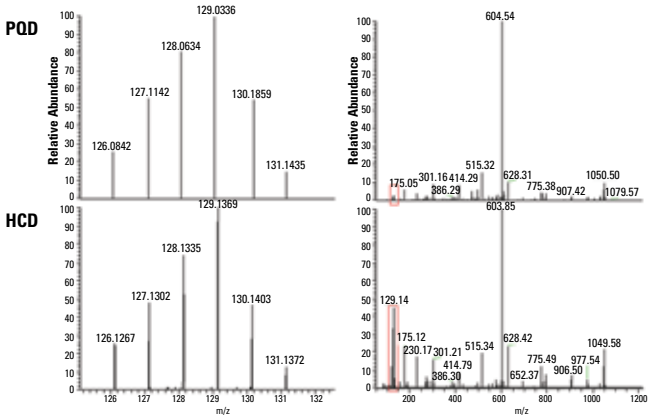
The TMT Label Reagents are provided as standalone sets or in optimized kit formats containing all necessary reagents and controls for maximum flexibility, convenience and reliability. The TMT Reagents combined with Thermo Scientific Instruments and Software provide a complete and integrated solution to perform absolute quantitation of target proteins.



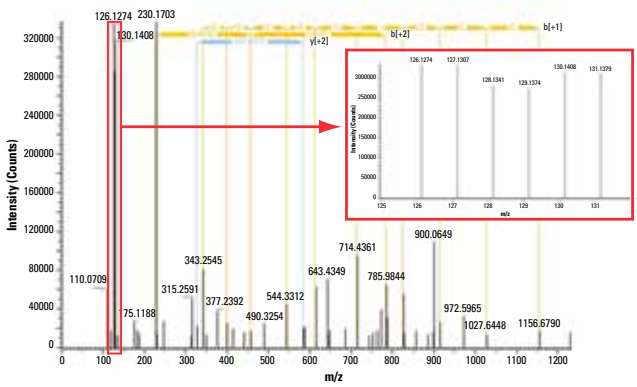
Thermo Scientific TMT Reagent chemistry. Structures of isobaric TMTsixplex Reagents with positions of ^{13}C and ^{15}N heavy isotopes identified (*) and sites of Collisionally Induced Dissociation (dashed lines) and Electron Capture Dissociation (wavy lines). **A.** TMT⁰ has no isotopic substitutions and is used for method development. **B.** A pair of isobaric mass labels with a single isotopic substitution per tag is used for simple pairwise comparisons of relative protein expression. **C.** A sixplex of isobaric mass labels each with five isotopic substitutions per tag is used. Used for complex analyses including multiplex patient screening, time-course analysis or dose escalation studies.



Analysis of a TMTsixplex-labeled mix of bovine serum albumin on a high-resolution Thermo Scientific Orbitrap XL Mass Spectrometer. MS/MS fragment ions in the peptide fragmentation and reporter ion regions for the ♦HLVDEPQNLIK♦ (+2) peptide. BSA tryptic digests were labeled with TMT6 Label Reagents and mixed at a 1:2:1:5:3:1 ratio.



Analysis of a TMTsixplex-labeled peptide by pulsed Q dissociation (PQD) and high energy collision dissociation (HCD). TMTsixplex-labeled A♦ITIFQER (2+) from rabbit glyceraldehyde-3-phosphate dehydrogenase in a 10-protein sample were mixed at a 1:2:3:4:2:0.5 ratio. Shown are the MS/MS spectra for the peptide fragment and reporter ion regions. PQD fragmentation was performed on a Thermo Scientific LTQ XL Ion Trap and by HCD on a high-resolution Orbitrap® XL Mass Spectrometer.



Protein identification and quantitative analysis of a complex mixture. TMTsixplex Reagent-labeled digests of a HeLa cell lysate were mixed at a 1:1:1:1:1:1 ratio and analyzed on a LTQ Orbitrap XL Spectrometer.

Protein Quantitation Reagents - Discovery Analysis

Highlights:

- Tandem mass tagging enables protein identification and quantitation from multiple samples of cells, tissues or biological fluids
- Consistent chemistry allows efficient transition from method development to multiplex quantitation, enabling biomarker discovery research
- Amine-reactive, NHS-ester activated reagents ensure efficient labeling of membrane and post-translationally modified proteins
- Expandable system allows concurrent multiplexing of up to six different samples in a single experiment
- Optimized fragmentation and fully supported quantitation with Protein Discoverer™ 1.0 for all Thermo Scientific LC MS/MS platforms, such as LTQ XL™ and LTQ Orbitrap XL Systems

Applications:

- Protein identification and quantitation from multiple samples of cells, tissue or biological fluids
- Protein expression profiling of normal vs. disease states or control vs. treated samples
- Multiplex up to six different samples concurrently in a single experiment
- Quantitative analysis of proteins for which no antibodies are available
- Identification and quantitation of membrane and post-translationally modified proteins
- Identification and quantification of hundreds to thousands of proteins in a single experiment

Ordering Information

Product #	Description	Pkg. Size
90063	**TMTduplex Isobaric Mass Tagging Kit Labeling Reagents for Multiplexed and Absolute Protein Quantification**	Kit
	Includes: TMT^{0}-126 Label Reagent	5 vials
	TMT^{2}-126 Label Reagent	5 vials
	TMT^{2}-127 Label Reagent	5 vials
	Dissolution Buffer	5 ml
	Denaturing Reagent	1 ml
	Reducing Reagent	1 ml
	Iodoacetamide	12 vials x 9 mg
	Quenching Reagent	1 ml
	Trypsin	2 x 20 µg
	Trypsin Storage Solution	250 µl
	Albumin, Bovine	2.5 mg
90064	**TMTsixplex Isobaric Mass Tagging Kit Labeling Reagents for Multiplexed and Absolute Protein Quantification**	Kit
	Includes: TMT^{0}-126 Label Reagent	5 vials
	TMT^{6}-126 Label Reagent	5 vials
	TMT^{6}-127 Label Reagent	5 vials
	TMT^{6}-128 Label Reagent	5 vials
	TMT^{6}-129 Label Reagent	5 vials
	TMT^{6}-130 Label Reagent	5 vials
	TMT^{6}-131 Label Reagent	5 vials
	Dissolution Buffer	5 ml
	Denaturing Reagent	1 ml
	Reducing Reagent	1 ml
	Iodoacetamide	12 vials x 9 mg
	Quenching Reagent	1 ml
	Trypsin	5 x 20 µg
	Trypsin Storage Solution	250 µl
	Albumin, Bovine	2.5 mg

Ordering Information

Product #	Description	Pkg. Size
90065	**TMTduplex Label Reagent Set Labeling Reagents for Multiplexed and Absolute Protein Quantification**	Kit
	Includes: TMT^{2}-126 Label Reagent	5 vials
	TMT^{2}-127 Label Reagent	5 vials
90066	**TMTsixplex Label Reagent Set Labeling Reagents for Multiplexed and Absolute Protein Quantification TMT^{6}-126 Label Reagent**	Kit
	Includes: TMT^{2}-126 Label Reagent	5 vials
	TMT^{6}-127 Label Reagent	5 vials
	TMT^{6}-128 Label Reagent	5 vials
	TMT^{6}-129 Label Reagent	5 vials
	TMT^{6}-130 Label Reagent	5 vials
	TMT^{6}-131 Label Reagent	5 vials
90067	**TMTzero Label Reagent Labeling Reagent for Multiplexed and Absolute Protein Quantification**	5 vials
	Includes: TMT^{0}-126 Label Reagent	
90060	**TMTduplex Isotopic Label Reagent Set, 5 x 0.8mg**	Kit
	Sufficient reagents for 5 duplex isotopic experiments with controls.	
	Includes: TMT^{0} Label Reagent	5 x 0.8 mg
	TMT^{6}-127 Label Reagent	5 x 0.8 mg
90061	**TMTsixplex Label Reagent Set, 0.8mg**	Kit
	Sufficient reagents for 1 sixplex isobaric experiment.	
	Includes: TMT^{6}-126 Label Reagent	1 x 0.8 mg
	TMT^{6}-127 Label Reagent	1 x 0.8 mg
	TMT^{6}-128 Label Reagent	1 x 0.8 mg
	TMT^{6}- 129 Label Reagent	1 x 0.8 mg
	TMT^{6}-130 Label Reagent	1 x 0.8 mg
	TMT^{6}-131 Label Reagent	1 x 0.8 mg
90062	**TMTsixplex Label Reagent Set, 2 x 0.8mg**	Kit
	Sufficient reagents for 2 sixplex isobaric experiments.	
	Includes: TMT^{6}-126 Label Reagent,	2 x 0.8 mg
	TMT^{6}- 127 Label Reagent	2 x 0.8 mg
	TMT^{6}-128 Label Reagent	2 x 0.8 mg
	TMT^{6}-129 Label Reagent	2 x 0.8 mg
	TMT^{6}-130 Label Reagent	2 x 0.8 mg
	TMT^{6}-131 Label Reagent	2 x 0.8 mg
90068	**TMTsixplex Label Reagent Set, 2 x 5mg**	Kit
	Sufficient reagents for 12 sixplex isobaric experiments with controls.	
	Includes: TMT^{6}-126 Label Reagent	2 x 5 mg
	TMT^{6}-127 Label Reagent	2 x 5 mg
	TMT^{6}-128 Label Reagent	2 x 5 mg
	TMT^{6}-129 Label Reagent	2 x 5 mg
	TMT^{6}-130 Label Reagent	2 x 5 mg
	TMT^{6}-131 Label Reagent	2 x 5 mg

Cysteine-reactive Tandem Mass Tagging Reagents (cysTMT)

Multiplexed cysteine-reactive mass tags reduce sample complexity for better sensitivity and dynamic range.

The Thermo Scientific Tandem Mass Tag (TMT) Reagents enable concurrent identification and multiplexed quantitation of proteins in different samples using tandem mass spectrometry (MS).[1,2] Isobaric TMT Reagents share identical structures, including a peptide-reactive labeling group, a spacer arm and an MS/MS quantitative reporter (Figure 7). During MS/MS analysis of peptides, each isobaric tag produces a unique reporter ion that makes relative quantitation possible.

Complementing our amine-reactive TMT Reagents, we have developed cysteine-reactive TMT (cysTMT™) Reagents and Immobilized Anti-TMT Antibody Resin to specifically label, enrich, and quantitate cysteine-containing peptides. This approach of thiol-targeted labeling, affinity enrichment and quantitation is similar to isotope-coded affinity tags (ICAT); however, the specificity, higher plexing, and unique capture mechanism of cysTMT Reagents offer several advantages. These reagents improve the dynamic range of detection and quantitation by reducing sample complexity. Furthermore, they enable functional studies of cysteine accessibility, oxidation or S-nitrosylation state.[3-6]

Highlights:

- Specific labeling of free thiols with UV absorption to monitor reaction progress
- Flexible duplex isotopic (MS) or sixplex isobaric (MS/MS) quantitation options
- Improved dynamic range with targeted enrichment of labeled peptides

The new Thermo Scientific cysTMTzero and cysTMTsixplex Reagents contain a thiol-reactive pyridyldithiol functional group for mass tagging of cysteine-containing peptides (Figure 7). Unlike iodoacetamide labeling reagents, pyridyldithiols are not light-sensitive and react specifically with reduced thiols. Upon labeling, pyridine-a-thione is released and can be used to monitor reaction progress and efficiency (Figure 8). The cysTMT Reagents are compatible with urea or detergents and allow labeling and mixing of intact protein samples before or after digestion (Figure 9).

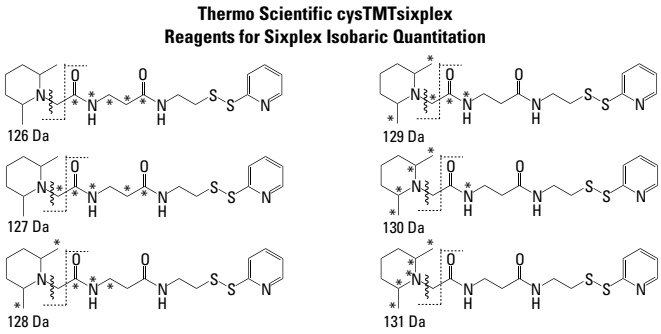


Figure 7. Thermo Scientific cysTMT Reagent chemistry. Structures of isobaric cysTMTsixplex Reagents with positions of ^{13}C and ^{15}N heavy isotopes identified (*) and sites of Collisionally Induced Dissociation (dashed lines) and Electron Capture Dissociation (wavy lines). Sixplex quantitation of 126-131 MS/MS reporter ions is used for samples labeled with sixplex isobaric reagents. Duplex isotopic MS quantitation is performed on peptides labeled with cysTMTzero or cysTMTsixplex-127 Reagent.

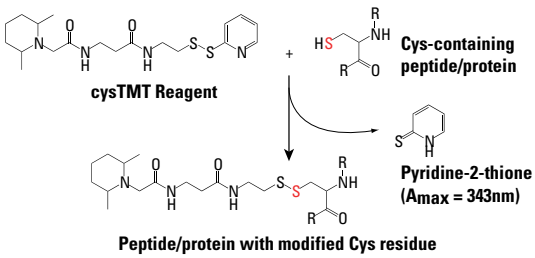


Figure 8. Specific cysteine labeling. The cysTMT Reagents specifically react with free thiols. Reaction progress can be monitored by UV absorbance at 343nm.

Protein labeling with cysTMT Reagents allows samples to be mixed early in workflows for improved quantitation. Unincorporated tags are easily removed using gel electrophoresis, size-exclusion chromatography or acetone precipitation. Alternatively, unincorporated tags can be removed from labeled peptides using strong-cation exchange chromatography.

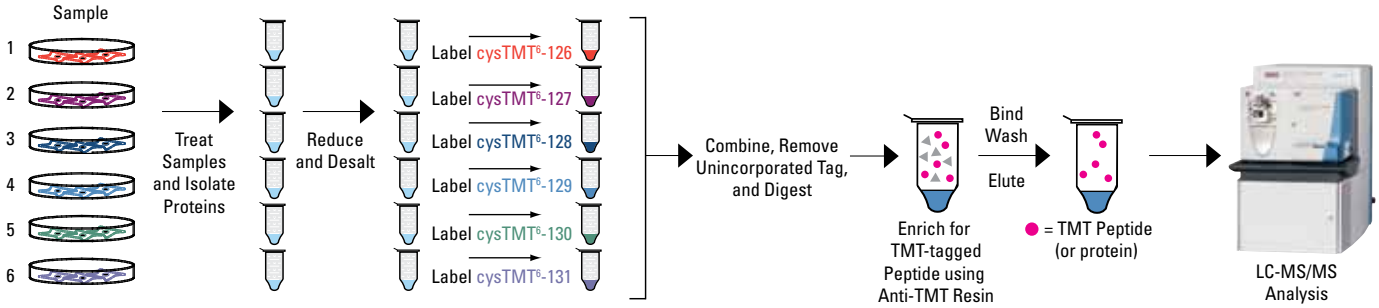


Figure 9. Cysteine-reactive reagents have flexible labeling, enrichment, and quantitation workflows. Reduced protein samples are labeled before digestion. After mixing, excess tags are removed with standard techniques such as gel electrophoresis, acetone precipitation, gel-filtration chromatography or strong cation exchange. Peptides labeled with cysTMT Reagents are enriched and then analyzed by LC-MS/MS.

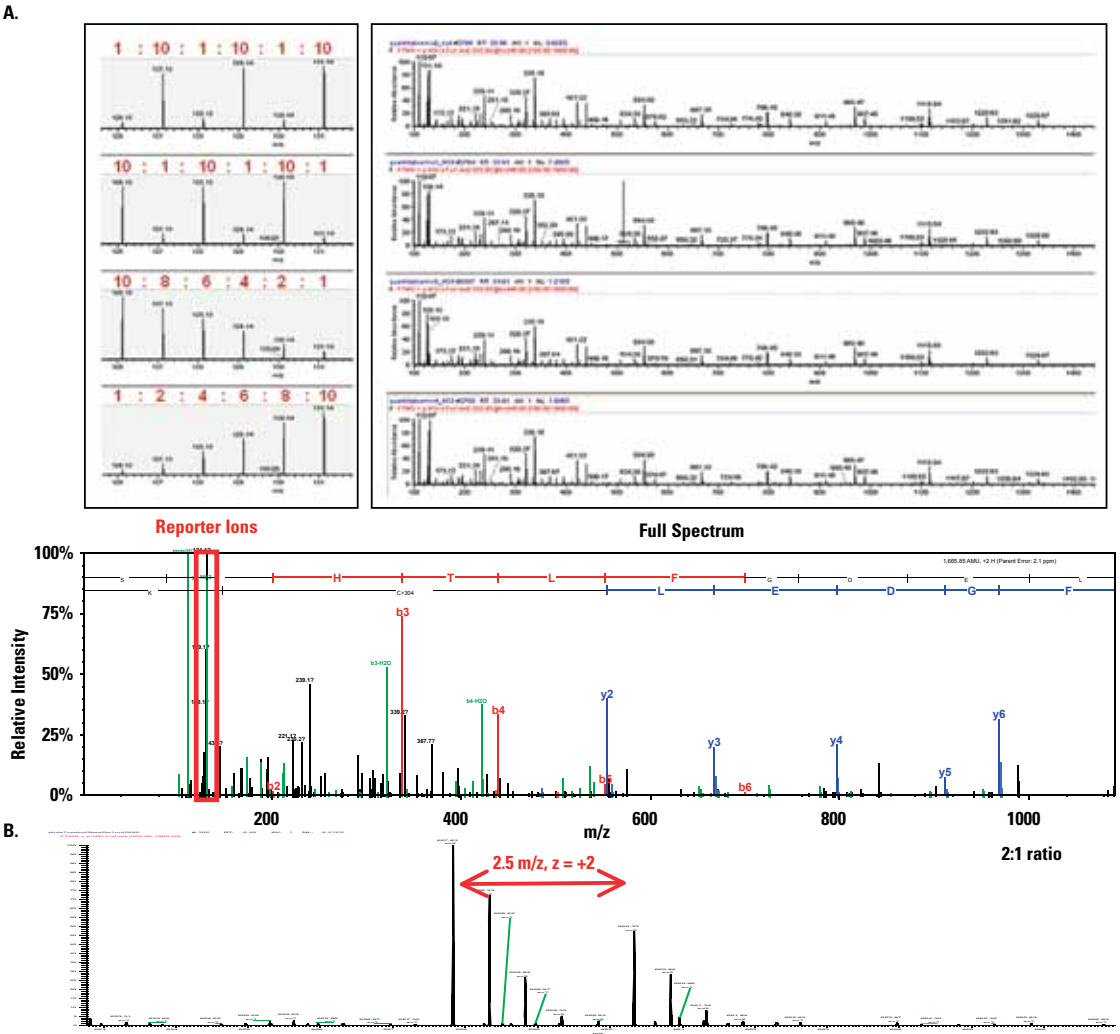


Figure 10. Robust isobaric and isotopic quantitation with Thermo Scientific cysTMT Reagents. To prepare the labeled protein, BSA was solubilized at 1mg/mL in 6M urea/50mM Tris/5mM EDTA and reduced with 1mM Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH (Product # 77720) for 1 hour at 37°C. Reductant was removed and each sample was labeled with a cysTMT Reagent (cysTMTzero or cysTMTsixplex 126-131) for 2 hours at room temperature. Labeled samples were combined and processed with Thermo Scientific Pierce Detergent Removal Spin Columns (Product # 87777) to remove excess label. Proteins were digested at 37°C overnight with MS-Grade Trypsin (Product # 90055) and desalted using Thermo Scientific Pierce C18 Tips (Product # 87784). Peptides were separated with a NanoLC-2D HPLC (Eksigent Technologies, Inc.) with a ProteoPep II™ C18 Column 75µm ID x 20cm (New Objective) using a 4-40% gradient (A: water, 0.1% formic acid; B: acetonitrile, 0.1% formic acid) at 500nl/min for 60 minutes. A Thermo Scientific LTQ Orbitrap XL ETD Mass Spectrometer was used to detect peptides using a top four experiment consisting of single-stage MS and acquisition of four MS/MS spectra using the HCD fragmentation mode. Data were analyzed with the Thermo Scientific Proteome Discoverer 1.1, Matrix Science Mascot 2.2 (Matrix Science), or Scaffold 2.6 (Proteome Software.) **Panel A:** MS/MS spectra of BSA peptides labeled with isobaric cysTMTsixplex Reagents mixed in different ratios and quantified using the reporter ions (126-131 m/z). **Panel B:** BSA peptides labeled with cysTMTzero or cysTMT6-127 Reagent and mixed 2:1.

To demonstrate the flexibility of the cysTMT Reagents for peptide quantitation, proteins were labeled with either cysTMTzero or cysTMTsixplex Reagent, combined at predetermined ratios, digested, and quantified by LC-MS or LC-MS/MS (Figure 10). Depending on the researcher's preference, the cysTMT Reagent can be used as light versus heavy isotopic pairs or as sixplex isobaric tags, providing flexible quantitation options (Figures 7, 10). Because the reporter ions of the cysTMT Reagents are identical to the amine-reactive TMT Reagents, quantitative data analysis is supported by existing software.

To enrich labeled proteins or peptides, we have also developed an immobilized anti-TMT monoclonal antibody against the reporter region of the TMT Reagent. Characterization of the antibody interaction with TMT and cysTMT Reagent labeled proteins and peptides by surface plasmon resonance (not shown) and Western blot detection indicates subnanomolar affinity (K_d < 100pM) for the reporter region and high specificity (Figure 11 A, B). The Thermo Scientific Immobilized Anti-TMT Antibody Resin can be used for multiplexed quantitative immuno-enrichment of proteins (not shown) or peptides labeled with TMT or cysTMT Reagents. To evaluate enrichment, a peptide labeled with cysTMT Reagent was spiked intoa BSA digest and then enriched using the Anti-TMT Resin (Figure 11C).

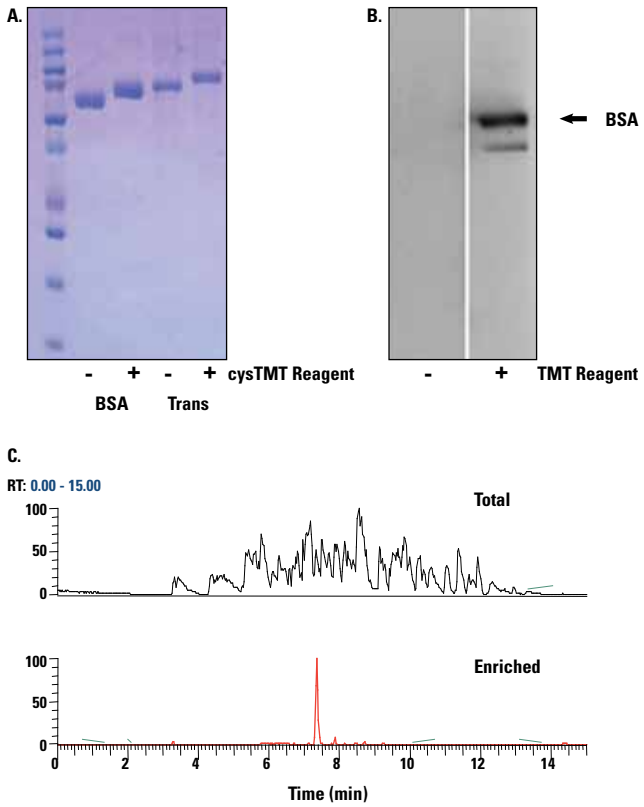


Figure 11. Sensitive detection and specific enrichment of labeled proteins and peptides. Panel A: Labeling of bovine serum albumin (BSA) and transferrin (Trans) with cysTMTzero Reagent results in an increase in protein molecular weight. **Panel B:** Chemiluminescent Western blot analysis of intact BSA that is unlabeled or labeled with amine-reactive TMT^0 Label Reagent (Product # 90067) according to instructions. The Anti-TMT Antibody (Product # 90075) was used for detection. **Panel C:** A cysteine-containing peptide [GLP-1 (7-17)-Cys, AnaSpec, Inc.] (1mg/mL) was reduced with immobilized TCEP for 45 minutes at room temperature and labeled for 2 hours with 0.85 equivalents of cysTMTzero Reagent. Labeling efficiency was assessed by UV absorbance at 343nm; the absence of free tag was confirmed by LC-MS/MS analysis. A peptide digest (100µg) was resuspended in TBS at 0.5µg/µl and a synthetic peptide (1nmol) labeled with cysTMT Reagent was added to the digest. This sample was processed with the Thermo Scientific TMT Enrichment Kit (Product # 90077) and vacuum-dried before LC-MS/MS analysis. Base peak chromatograms of sample before (upper) and after (lower) processing with the Anti-TMT Resin demonstrate enrichment.

To evaluate enrichment in a complex sample, 100µg of cell lysate was labeled, digested, and processed to generate equivalent amounts of duplicate unenriched and enriched labeled samples. Enrichment of labeled peptides resulted in fewer peptides identified per protein (1.9 versus 5.2 peptides per protein), but nearly twice as many unique protein identifications (Figure 12). This reduction in peptide sample complexity allows identification of lower abundance proteins and easier quantification.

Figure 12. Thermo Scientific cysTMT Reagents enable enrichment of more proteins from equivalent samples. HeLa cell lysates were labeled with cysTMT Reagents, desalted with Thermo Scientific Zeba Spin Desalting Columns 7K (Product # 89890) to remove unincorporated tags, and enriched with the cysTMT Enrichment Kit. Total and enriched labeled peptide samples giving equivalent LC-MS/MS maximum peak intensities corresponded to 12-fold peptide enrichment. **Panel A:** The number of unique, common and total proteins identified in the total and enriched samples. **Panel B:** The number of unique, common and total distinct peptides identified in the total and enriched samples.

The combination of cysTMT Reagents with anti-TMT resin enrichment has several advantages over cleavable isotope-coded affinity tags (cICAT). To test these two reagents side by side, two proteins were labeled with the cICAT or cysTMT Reagents and enriched before MS analysis (Figure 13). The cysTMT Reagent and workflow results were as good or better than the cICAT reagent, but with the following advantages: 1) more specific sulfhydryl group labeling; 2) a choice of sixplex isobaric multiplexing or duplex isotopic quantitation using the same chemistry; 3) isobaric tagging and quantitation for more efficient MS data analysis; and 4) a simpler workflow that does not require tag cleavage.

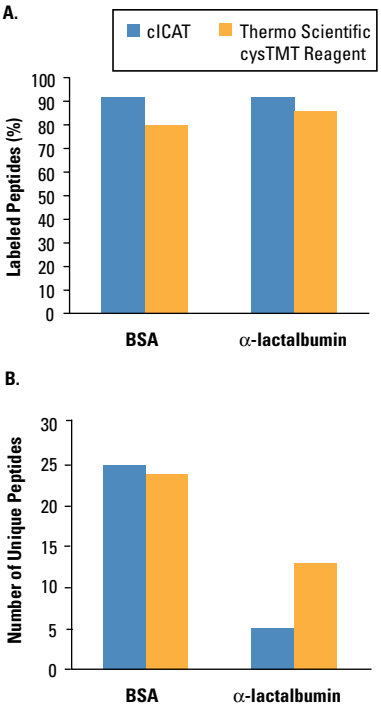


Figure 13. The Thermo Scientific cysTMT Reagents yielded labeling and enrichment results comparable to the cICAT reagents. Bovine serum albumin and α-lactalbumin were labeled with cysTMTzero Reagent, digested, and enriched as described above. An equal amount of the same proteins were labeled with cICAT, digested, and enriched according to the manufacturer's protocol. Samples were analyzed by LC-MS/MS, and data was analyzed with Thermo Scientific Proteome Discoverer 1.1 or Mascot 2.2 (Matrix Science) and Scaffold® 2.6 (Proteome Software). **Panel A:** Percent enrichment of peptides labeled with cICAT or cysTMT Reagents and enriched according to instructions. **Panel B:** Number of unique labeled peptides identified after cICAT or cysTMT Reagent labeling and enrichment.

Conclusions

Cysteine-reactive Tandem Mass Tag (cysTMT) Reagents are effective for targeted labeling of cysteine residues pre- or post-digestion. The cysTMT Reagents can be used as either isotopic pairs or as an isobaric sixplex set for MS- or MS/MS-based multiplexed quantitation. Proteins and peptides labeled on amines or cysteines with TMT or cysTMT Reagents, respectively, can be detected and enriched with the anti-TMT antibody. These reagents can be used to study cysteine accessibility and modifications or to simply reduce proteome complexity for improved dynamic range and sensitivity.

References

1. Thompson, A., *et al.* (2003). Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. *Anal Chem* **75**:1895-204.
2. Dayon, L., *et al.* (2008). Relative quantification of protein in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. *Anal Chem* **80**(8):2921-31.
3. Gygi, S.P., *et al.* (1999). Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotech.* **17**:994-9.
4. Sethuraman, M., *et al.* (2004). Isotope-coded affinity tag approach to identify and quantify oxidant-sensitive protein thiols. *Mol Cell Proteomics* **3**:273-8.
5. Meza, J.E., *et al.* (2003). Essential cysteine-alkylation strategies to monitor structurally altered estrogen receptor as found in oxidant-stressed breast cancers. *Anal Biochem* **20**(1):21-31.
6. Chen, Y-Y., *et al.* (2008). Cysteine S-nitrosylation protects protein-tyrosine phosphatase 1B against oxidation-induced permanent inactivation. *J Biol Chem* **283**:35265-72.

Ordering Information

Product #	Description	Pkg. Size
90069	**cysTMTzero Analysis Label Reagent Set** *Sufficient reagents for 5 samples.*	5 x 0.2 mg
90071	**cysTMTsixplex Isobaric Label Reagent Set** *Sufficient reagents for 1 sixplex experiment.*	
	Includes: cysTMT^6-126 Label Reagent	1 x 0.2 mg
	cysTMT^6-127 Label Reagent	1 x 0.2 mg
	cysTMT^6-128 Label Reagent	1 x 0.2 mg
	cysTMT^6-129 Label Reagent	1 x 0.2 mg
	cysTMT^6-130 Label Reagent	1 x 0.2 mg
	cysTMT^6-131 Label Reagent	1 x 0.2 mg
90072	**cysTMTsixplex Isobaric Label Reagent Set** *Sufficient reagents for 5 sixplex experiments.*	
	Includes: cysTMT^6-126 Label Reagent	5 x 0.2 mg
	cysTMT^6-127 Label Reagent	5 x 0.2 mg
	cysTMT^6-128 Label Reagent,	5 x 0.2 mg
	cysTMT^6-129 Label Reagent	5 x 0.2 mg
	cysTMT^6-130 Label Reagent	5 x 0.2 mg
	cysTMT^6-131 Label Reagent	5 x 0.2 mg
90073	**cysTMTsixplex Isobaric Label Reagent Set** *Sufficient reagents for 10 sixplex experiments.*	
	Includes: cysTMT^6-126 Label Reagent	1 x 2 mg
	cysTMT^6-127 Label Reagent	1 x 2 mg
	cysTMT^6-128 Label Reagent	1 x 2 mg
	cysTMT^6-129 Label Reagent	1 x 2 mg
	cysTMT^6-130 Label Reagent	1 x 2 mg
	cysTMT^6-131 Label Reagent	1 x 2 mg
90074	**cysTMT0/cysTMT6 Isotopic Set** *Sufficient reagents for 5 isotopically (heavy/light) duplex experiments.*	5 x 0.2 mg
	Includes: cysTMTzero Label Reagent	5 x 0.2 mg
	cysTMT^6-127 Label Reagent	
90075	**Anti-TMT Antibody**	0.1 mg/ml
90076	**Immobilized Anti-TMT Antibody Resin**	12 ml of a 50% slurry
90077	**TMT Enrichment Kit**	Kit
	Includes: Immobilized Anti-TMT Antibody Resin	6ml
	Tris Buffered Saline (20 X)	10ml
	Urea	12g
	CHAPS	255mg
	Elution Buffer (50% acetonitrile/ 0.4 % TFA)	10ml
	Reducing Reagent (0.5 M TCEP)	1ml
	Zeba Spin Desalting Columns, 7K MWCO	0.5ml, 30

Protein Quantitation Reagents - Targeted Analysis

HeavyPeptide AQUA Standards

Reliable solutions for quantitative proteomics.

Protein Quantitation by Mass Spectrometry

One of the key challenges in proteomics is the quantitation of proteins at very low concentrations in complex protein mixtures. Assays that may exist lack absolute specificity and are difficult to multiplex. This is particularly true for disease biomarkers used for diagnostics, treatment development and monitoring[1].

Quantitative mass spectrometry for small molecules[2] is based on the well established method of isotopic dilution. Due to its absolute specificity, sensitivity[3] and high multiplexing potential[4] this technique is quickly adopted for peptide quantitation[5,6,7] and absolute protein quantitation[8,9] in complex matrices.

STEP 1:

Proteotypic peptide selection

Starting with a software assisted decision[11] e.g., Thermo Scientific PinPoint Software *(www.thermoscientific.com/pinpoint)*, it is common to get multiple proteotypic peptide candidates per protein. Software selected peptides are tested on the mass spectrometer equipment to establish SRM/MRM protocols.

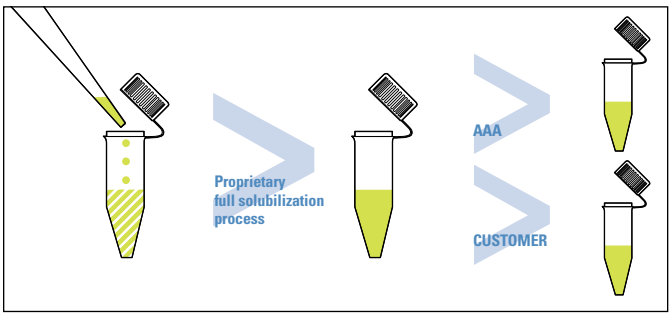
STEP 2:

Quantitation

Protease digested samples are spiked with known quantities of synthetic stable-isotope labeled peptides –HeavyPeptides – as internal standards. Multiplexing potential is very high and recent equipment and software developments are further increasing that unique ability.

Based on years of experience and 1,000s of Thermo Scientific HeavyPeptide standards successfully prepared we developed various standard grades for quantitative proteomics, provided FULLY solubilized, with various concentration precision so the choice is yours.

To achieve absolute quantitative proteomics, proteins are digested with a protease like trypsin and proteotypic[10] peptides are used as stoichiometric surrogate. Accurate absolute quantitation is achieved by spiking the sample with isotopic labeled standards, also known as HeavyPeptides.



Thermo Scientific HeavyPeptide Standards preparation process.

Assay development booster: FasTrack Service

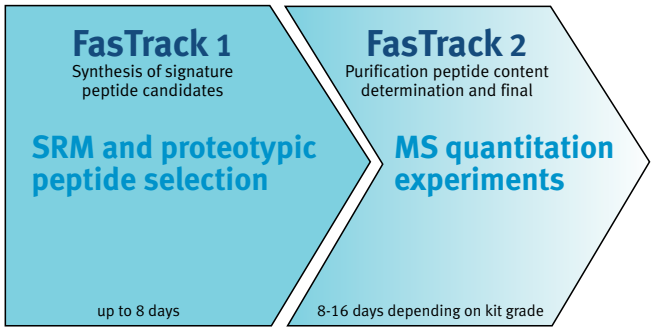
Available for AQUA® Ultimate and QuantPro grade. The FasTrack service is designed for accelerating assay development within a controlled budget environment. FasTrack service is available for both HeavyPeptides AQUA Ultimate and QuantPro grade and offers a 2-step-approach:

FasTrack 1:

Crude HeavyPeptide is synthesized within 8 days. 100ug are shipped for proteotypic peptide selection and assay development. The rest of the peptide is kept in stock for the optional FasTrack 2.

FasTrack 2 (optional):

The crude peptide from FasTrack 1 is purified to reach a minimum purity of 97%, followed by full solubilization and concentration measurement. This second step is optional and normally will only be ordered for some of the peptides of FasTrack 1.



Protein Quantitation Reagents - Targeted Analysis

HeavyPeptide Grades

Within our portfolio you will find the HeavyPeptide Grade meeting your precision and budget requirements for absolute and relative quantitation.

	AQUA Ultimate	AQUA QuantPro	AQUA Basic	AQUA Crude
Thermo Scientific HeavyPeptide and LightPeptide Standards	Provided fully solubilized with a concentration precision equal or better than ±5%. Best choice for biomarker validation and for experiments demanding ultimate quantitative precision and reproducibility from batch to batch.	Provided fully solubilized with a concentration precision equal or better than ±25%. Ideal solution for biomarker verification.	Provided lyophilized and are more adequate for relative quantitation. The batch to batch consistency is difficult to predict.	Provided non purified in 96 well plate format. They are designed to assist the proteotypic peptide selection during SRM/MRM setup.
Includes	One isotopic labeled peptide (HeavyPeptide) or one non-labeled control peptide (LightPeptide)	One isotopic labeled peptide (HeavyPeptide) or one non-labeled control peptide (LightPeptide)	One isotopic labeled peptide (HeavyPeptide) or one non-labeled control peptide (LightPeptide)	One isotopic labeled peptide (HeavyPeptide) or one non-labeled control peptide (LightPeptide)
Formulation	5 pmol/µl in 5% v/v Acetonitrile/H_2O	5 pmol/µl in 5% v/v Acetonitrile/H_2O	lyophilized	dry
Actual concentration	measured by AAA	measured by AAA	measured by AAA	
Concentration precision	±5%	±25%		
Peptide purity	>97%	>97%	>95%	crude
Isotopic enrichment	>99%	>99%	>99%	>99%
Length	up to 15 amino acids	up to 15 amino acids	up to 15 amino acids	up to 15 amino acids
Quality control	mass spectrometry, analytical HPLC	mass spectrometry, analytical HPLC	mass spectrometry, analytical HPLC	mass spectrometry
Production time*	~15 working days	~15 working days	~15 working days	~8 working days
Shipment	in solution on wet ice	in solution on wet ice	dry at room temperature	dry at room temperature

** Production time estimates: These timelines are for information only. Depending on the number of kits the delivery time may vary and we will be specified on demand.*

Options

- Additional light amino to extend peptide length
- Additional heavy amino
- Other heavy amino acid
- Other solvent, concentration aliquot size
- Peptide in various vessel material and shape (i.e., 96-well plate format with or without detachable tubes in glass or plastic, 2D bar code etc.)

Modifications

- Single phosphorylation (pY, pT or pS)
- Double phosphorylation (pY, pT or pS)
- CAM (carbamidomethylation on cysteine)*
- Chloro-L-Thyrosine
- pyro-Glutamic acid
- Met[O] (Oxidation on Methionine)
- tryptic digest site extension on C and/or N terminal
- other modifications on request

**CAM tends to cyclisation at N-term; fully cycled form can be provided, please inquire.*

Applications

- Biomarker discovery, verification, validation
- Functional quantitative proteomics[13]
- Quantitation of post-translational modified proteins
- RNAi results confirmation
- Pharmacokinetics
- Metabolomics
- Clinical biochemistry for drug and metabolite monitoring
- Anti-doping testing
- Protein expression monitoring
- Pathways validation
- Cell signaling profiling
- Allergen quantitation

Optional heavy amino acids.

Amino acid	Mass difference to standard AA	Isotope	Isotopic enrichment
Alanine / A	+ 4 Da	U-$^{13}C_3$; ^{15}N	>99%
Arginine / R	+ 10 Da	U-$^{13}C_6$; $^{15}N_4$	>99%
Isoleucine / I	+ 7 Da	U-$^{13}C_6$; ^{15}N	>99%
Leucine / L	+ 7 Da	U-$^{13}C_6$; ^{15}N	>99%
Lysine / K	+ 8 Da	U-$^{13}C_6$; $^{15}N_2$	>99%
Phenylalanine / F	+ 10 Da	U-$^{13}C_9$; ^{15}N	>99%
Proline / P	+ 6 Da	U-$^{13}C_5$; ^{15}N	>99%
Valine / V	+ 6 Da	U-$^{13}C_5$; ^{15}N	>99%

Other amino acids on request.

References

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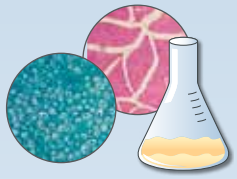
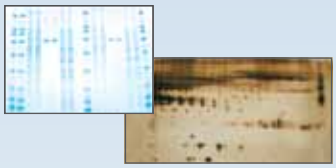
Overview - Workflows for Mass Spec Sample Preparation

Workflow 1 – Gel-based



Complex protein lysate

M-PER Cell Lysis Reagent
NE-PER Cell Lysis Reagent
Subcellular Fractionation Kit



1D/2D gel plug

Pierce Protein Gels

Wash, reduce, alkylate, digest, extract

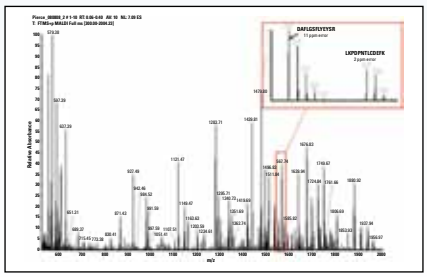
In-gel Digestion Kit
Proteases

Concentrate or prepare for LC-MS/MS clean up

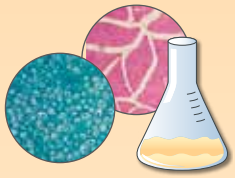
Pierce C18 Spin Columns
Pierce C18 Tips
Pierce Graphite Spin Columns
Detergent Removal Columns
MALDI Matrices
TFA, FA, HFBA

MS

LTQ Orbitrap Velos
Mass Spectrometer



Workflow 2 – In Solution



Complex protein lysate

- M-PER Cell Lysis Reagent
- NE-PER Cell Lysis Reagent
- Subcellular Fractionation Kit
- Halt Protease and Phosphatase Inhibitors
- Pierce IP/Co-IP Kits
- SCX Spin Columns

Reduce, alkylate, digest

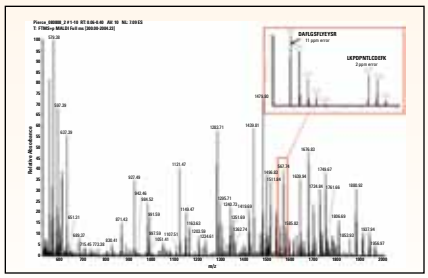
- In-solution Digestion Kit
- Proteases

Peptide Clean Up

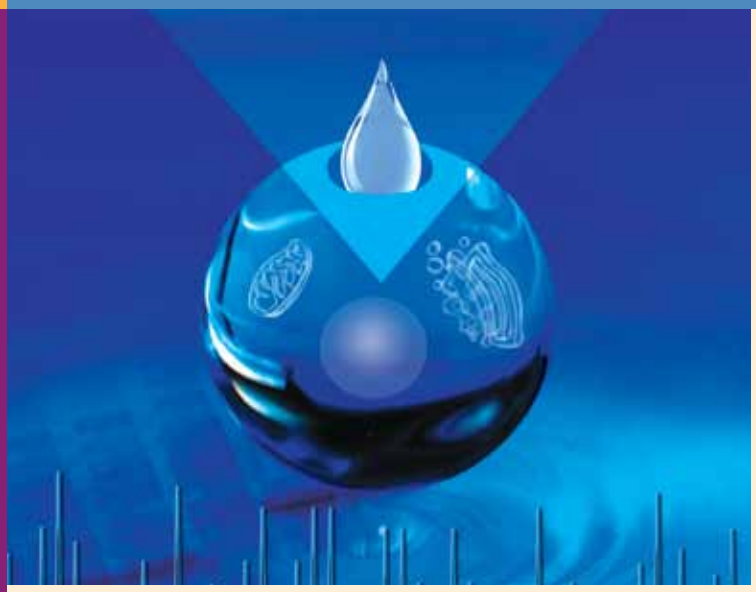
- C18 Tips and Spin Columns
- Detergent Removal Columns
- Nanoflow HPLC Columns

RPLC-MS/MS

- LTQ Orbitrap Velos Mass Spectrometer



Lysate Preparation



Cell Lysis and Protein Extraction

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 or tissue), subcellular structures or specific proteins. Both physical and reagent-based methods may be required to extract cellular proteins because of the diversity of cell types and cell membranes composition (or cell walls).

Physical lysis is a common method of cell disruption and extraction of cellular contents. However, it requires specialized equipment and protocols which are 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, 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 which can be used to isolate intact nuclei, mitochondria and other organelles before protein solubilization.

Cell lysis disrupts cellular compartments which can activate endogenous proteases and phosphatases. To prevent extracted proteins from degradation or artifactual modification by the activities of these enzymes, it is necessary to add protease and/or phosphatase inhibitors to the lysis reagents.

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 detergent removal in order to study proteins of interest or maintain long term stability of the extracted protein.

Halt Protease Inhibitor Cocktails

Stops proteolysis fast with a cocktail that is delivered to your sample within seconds.

Waiting for an inhibitor tablet to dissolve leaves your lysate unprotected and at risk for protein degradation. This vulnerability is especially critical when the protein target is in low abundance. The single-use solution format allows you to deliver the appropriate amount of inhibitor for the volume of lysate in an instant. With the Thermo Scientific Halt Inhibitor Cocktails there is no waiting for a tablet to dissolve, no sample vortexing required, no imprecise tablet splitting and, most importantly, no potential loss of valuable target while waiting for the tablet to dissolve.

The Halt™ Inhibitor Cocktail is simply withdrawn from the tube and added directly to the lysate sample within seconds. Comparatively, it takes approximately two minutes to fully dissolve a tablet-based cocktail, exposing your valuable protein to proteolytic attack. When tested on a pancreatic tissue lysate, the ready-to-use formulation significantly out-performed the tablet in proteolytic activity knockdown and sample delivery speed (Figure 1).

Highlights:

- **Robust** – effective for suppressing proteolytic activity in detergent-based cell lysis reagents
- **Flexible** – same high-quality protease inhibitor components available separately
- **Versatile** – prepare a custom cocktail or increase the concentration of a specific protease inhibitor in the formulation
- **Compatible** – EDTA-free formulation inhibits proteolytic activity in applications in which EDTA may interfere with protein stability, subsequent assays or purification methods such as immobilized metal chelate affinity chromatography (IMAC)

Halt Protease Inhibitor Cocktails are provided in ready-to-use formulations that inhibit serine, cysteine and aspartic acid proteases and metalloproteases. We have demonstrated outstanding performance with Thermo Scientific M-PER Mammalian, B-PER Bacterial, T-PER Tissue and Y-PER Yeast Protein Extraction Reagents (Figure 2). Add the appropriate volume of inhibitor cocktail to the sample (10 µl per 1 ml of cell extract) and proceed directly to protein purification. The EDTA-free formulation is ideal for preparing samples that will be analyzed by 2D gel electrophoresis. As a 100X concentrate in DMSO, the cocktail contains reversible and irreversible inhibitors of serine, cysteine, metalloproteases, and aspartic acid proteases in addition to aminopeptidases present in virtually all cell lysate samples.

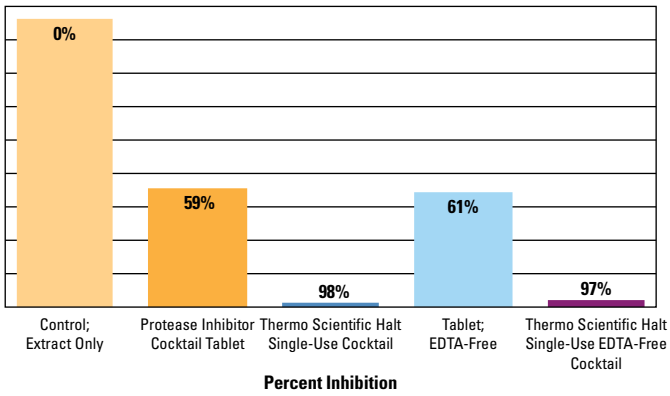


Figure 1. Thermo Scientific Halt Protease Inhibitor Single-use Cocktails are more effective than tablet-format cocktails. Using a validated protease assay and 1.0 mg/ml of rat pancreatic extract, the Halt Protease Inhibitor Single-Use Cocktails, with and without EDTA added, were tested against commercially available tablet-format protease inhibitor cocktails under the same conditions. A 1X final concentration of each inhibitor was added. The single-use formulation resulted in ≥ 97% inhibition compared to ≥ 59% inhibition for the tablet.

Table 1. Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail formulation and concentrations.

Protease Inhibitor	MW	Protease Family Targeted	Inhibitor Type	Solubility (Solvent system)	Concentration of each inhibitor in the protease inhibitor cocktails (100X in DMSO) (Product # 78430 and 78425)
AEBSF•HCl	239.5	Serine proteases	Irreversible	200 mg/ml (H_2O)	100 mM
Aprotinin	6511.5	Serine proteases	Reversible	10 mg/ml (H_2O)	80 µM
Bestatin	308.38	Amino-peptidases	Reversible	5 mg/ml (methanol)	5 mM
E-64	357.4	Cysteine proteases	Irreversible	20 mg/ml (1:1 EtOH/H_2O)	1.5 mM
EDTA (not included in Product # 78425)	372.24	Metalloproteases *(Chelates divalent cations)*	Reversible	10 g/100 ml (H_2O)	0.5 M
Leupeptin	475.6	Serine and cysteine proteases	Reversible	1 mg/ml (H_2O)	2 mM
Pepstatin A	685.9	Aspartic acid proteases	Reversible	1 mg/ml (MeOH)	1 mM

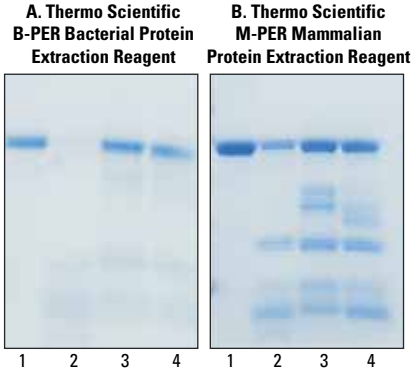


Figure 2. Thermo Scientific Halt Protease Inhibitor Single-Use Cocktails are compatible with Thermo Scientific M-PER Mammalian Protein Extraction and B-PER Bacterial Protein Extraction Reagents. Bovine serum albumin (BSA) was incubated overnight at 37°C in the presence of 0.1 mg/ml trypsin in B-PER® Extraction Reagent and M-PER® Extraction Reagent, respectively. Under extreme time and temperature conditions, substantial protection from degradation was observed as marginal to barely detectable cleavage products of BSA. BSA only **(Lane 1)**; Trypsin (0.1 mg/ml) (+) BSA and no Halt Protease Inhibitor Single-Use Cocktail present **(Lane 2)**; BSA (+) Trypsin with Halt Protease Inhibitor Single-Use Cocktail with EDTA **(Lane 3)**; and BSA (+) Trypsin with Halt Protease Inhibitor Single-Use Cocktail **(Lane 4)**.

Ordering Information

Product #	Description	Pkg. Size
78425	**Halt Protease Inhibitor Single-Use Cocktail EDTA-Free (100X)** *Each 100 µl microtube contains sufficient cocktail to treat 10 ml of lysate. Protease inhibitors prepared in DMSO solution.* Protease Inhibitor Cocktail, 100 µl microtubes	24 x 100 µl
87786	**Halt Protease Inhibitor Cocktail (100X)** *Sufficient to treat 100 ml of sample.* Protease Inhibitor Cocktail (100X), 1 ml 0.5M EDTA Solution (100X), 1 ml	1 ml
87785	**Halt Protease Inhibitor Cocktail, EDTA-Free (100X)** *Sufficient to treat 100 ml of sample.*	1 ml
78430	**Halt Protease Inhibitor Single-Use Cocktail (100X)** *Each 100 µl microtube contains sufficient cocktail to treat 10 ml of lysate. Protease inhibitors prepared in DMSO solution.* Protease Inhibitor Cocktail, 100 µl microtubes 0.5 M EDTA Solution (100X), 2.5 ml	24 x 100 µl
78429	**Halt Protease Inhibitor Cocktail**	5 ml
78437	**Halt Protease Inhibitor Cocktail, EDTA Free**	5 ml
78438	**Halt Protease Inhibitor Cocktail**	10 ml
78439	**Halt Protease Inhibitor Cocktail, EDTA Free**	10 ml

Halt Phosphatase Inhibitor Cocktails

Safeguards against serine, threonine and tyrosine phosphatase activities.

Phosphorylation and dephosphorylation is a molecular on/off switch that regulates a number of key biological pathways within the cell, including signal transduction, cell division and apoptosis. The Thermo Scientific Halt Phosphatase Inhibitor Cocktail preserves protein phosphorylation in cell lysates and tissue extracts.

The cocktail contains a mixture of four phosphatase inhibitors of broad specificity, including sodium fluoride, sodium orthovanadate, sodium pyrophosphate and β-glycerophosphate. Unlike other commercially available phosphatase inhibitor cocktails that protect against either serine/threonine phosphatases or protein tyrosine phosphatases, the Halt Phosphatase Inhibitor Cocktail protects phosphoproteins from both families of phosphatases (Figure 3).

Highlights:

- **Complete protection from phosphatases** – inhibits both serine/threonine and protein tyrosine phosphatases
- **Compatible with standard protein assays** – quantify treated samples with the Thermo Scientific Pierce BCA Protein Assay Kit, Coomassie Plus (Bradford) Assay and Pierce 660 nm Protein Assay
- **Efficient** – 1 ml protects up to 100 ml of sample

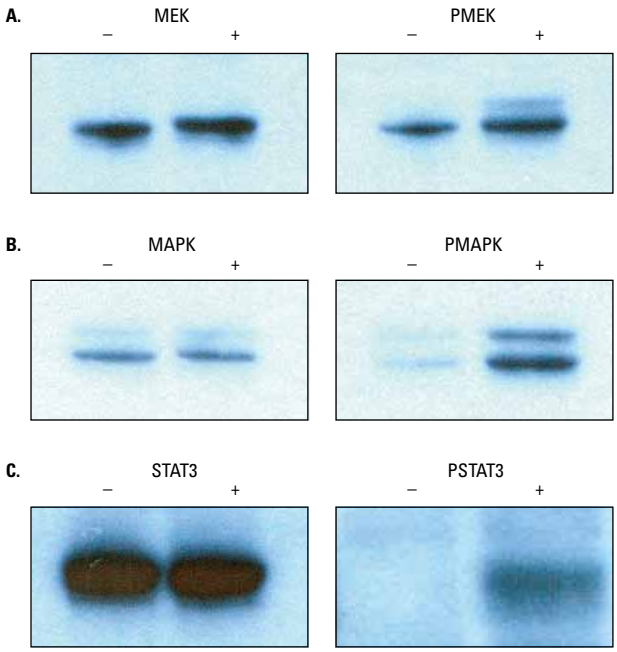


Figure 3. Halt Phosphatase Inhibitor Cocktail preserves phosphorylation of MEK, MAP Kinase 42/44 and STAT3 in HeLa cell lysate. HeLa cells were lysed in the absence (-) and presence (+) of a 1X concentration of the Halt Phosphatase Inhibitor cocktail. Cell lysates were analyzed by Western blot for total and phosphorylated protein as indicated. **Panel A.** MEK and phosphorylated MEK (PMEK), **Panel B.** MAP Kinase and PMAP Kinase and **Panel C.** STAT3 and PSTAT3. The proteins are phosphorylated on serine, threonine/tyrosine and tyrosine, respectively.

Ordering Information

Product #	Description	Pkg. Size
78420	**Halt Phosphatase Inhibitor Cocktail** *Sufficient reagent to protect up to 100 ml of sample.*	1 ml
78426	**Halt Phosphatase Inhibitor Cocktail**	5 x 1 ml
78427	**Halt Phosphatase Inhibitor Cocktail**	10 ml
78428	**Halt Phosphatase Inhibitor Single-Use Cocktail** *Each 100 µl microtube contains sufficient cocktail to treat 10 ml of lysis.*	24 x 100 µl

Halt Combined Protease and Phosphatase Inhibitor Cocktails

Provides complete protection from proteases and phosphatases in one reliable cocktail.

The all-in-one Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail provides single-solution convenience with full sample protection. This broad-spectrum inhibitor cocktail is specifically optimized to protect proteins from degradation during extraction and purification. The cocktail contains inhibitors against the major classes of proteases and phosphatases, targeting aminopeptidases, cysteine and serine proteases, and serine/threonine and tyrosine phosphatases (Table 2).

This combined cocktail is the first of its kind. The aqueous-based format allows for convenient cold-room storage without freezing and is much easier to use than tablets that require tedious splitting with a razor blade to treat samples < 10 ml. More importantly, the cocktail formulation is more effective than the tablet format (Figure 4) at inhibiting proteases in lysates and preserving phosphorylation during cell harvest. The formulation is also mass spectrometry-compatible because it does not contain AEBSF, which can cause peaks to shift.

Highlights:

- **Easy to use** – convenient all-in-one cocktail contains both protease and phosphatase inhibitors
- **Versatile** – compatible with Pierce Cell Lysis Reagents and mass spectrometry
- **Complete** – identity of each component of the cocktail is listed

Table 2. Inhibitors included in the Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail formulation and their targeted enzyme class.

Inhibitor	Target
Sodium Fluoride	Ser/Thr and Acidic Phosphatases
Sodium Orthovanadate	Tyr and Alkaline Phosphatases
β-glycerophosphate	Ser/Thr Phosphatases
Sodium Pyrophosphate	Ser/Thr Phosphatases
Aprotinin	Ser Proteases
Bestatin	Amino-peptidases
E64	Cysteine Proteases
Leupeptin	Ser/Cys Proteases
EDTA (optional)	Metalloproteases

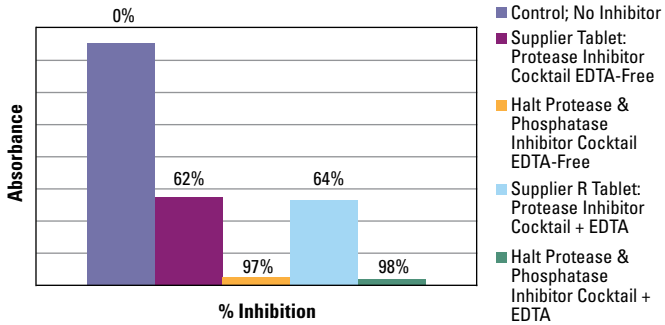


Figure 4. Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktails are more effective than tablet-format protease inhibitor cocktails. Using a general protease assay with a rat pancreatic extract (1 mg/ml), the Halt Protease and Phosphatase Inhibitor Cocktails (± EDTA) were compared with the tablet-based protease inhibitor cocktails (± EDTA) of Supplier R Tablet. The same experimental conditions were used for all samples tested. A 1X final concentration of each inhibitor cocktail was added to the extract.

Ordering Information

Product #	Description	Pkg. Size
78440	**Halt Protease and Phosphatase Inhibitor Cocktail**	1 ml
78441	**Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free**	1 ml
78442	**Halt Protease and Phosphatase Single-Use Inhibitor Cocktail** *Each 100 µl microtube contains sufficient cocktail to treat 10 ml of lysate.*	24 x 100 µl
78443	**Halt Protease and Phosphatase Single-Use Inhibitor Cocktail, EDTA-free** *Each 100 µl microtube contains sufficient cocktail to treat 10 ml of lysate.*	24 x 100 µl
78444	**Halt Protease and Phosphatase Inhibitor Cocktail**	5 x 1 ml
78445	**Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free**	5 x 1 ml
78446	**Halt Protease and Phosphatase Inhibitor Cocktail**	10 ml
78447	**Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free**	10 ml
Related Products		
28340	**Surfact-Amps Detergent Sampler** Kit Contents: Brij®-35 Surfact-Amps Solution (10%) Brij-58 Surfact-Amps Solution (10%) NP-40 Surfact-Amps Solution (10%) Triton® X-100 Surfact-Amps Solution (10%) Triton X-114 Surfact-Amps Solution (10%) Tween®-20 Surfact-Amps Solution (10%) Tween-80 Surfact-Amps Solution (10%) Octyl beta-Glucoside Octyl beta-Thioglucopyranoside (OTG) CHAPS	10-vial kit 10 mL 10 mL 10 mL 10 mL 10 mL 10 mL 10 mL 100 mg 100 mg 100 mg
28300	**CHAPS** Formulation: 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate	5 g
28351	**Octylthioglucoside (OTG)** Formulation: *n*-Octyl-β-D-thioglucopyranoside	5 g
28324	**NP-40 Surfact-Amps Detergent Solution** Formulation: 10% (w/v) aqueous solution of NP-40	6 x 10 mL
28316	**Brij-35 Surfact-Amps Detergent Solution** Formulation: 10% (w/v) aqueous solution of Brij-35	6 x 10 mL
89904	**Sodium Deoxycholate** Formulation: Sodium deoxycholic acid	5 g

For more selections, visit thermoscientific.com/pierce.

Lysate Preparation

Name	Description	Organisms/Samples
B-PER - Bacterial Protein Extraction Reagent	Efficient, gentle lysis and extraction of soluble proteins from *E. coli* and other bacterial cells. Uses mild nonionic detergents to disrupt cells and solubilizing proteins without denaturation, eliminating the need for harsh mechanical procedures like sonication.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α >M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER II	Similar to B-PER, but optimized for low cell density, or for proteins with low expression levels.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER PBS	Similar to B-PER, but in Phosphate Buffer. This amine free formulation is ideal for amine-reactive labeling and/or crosslinking applications.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER with Enzymes	Similar to B-PER, but kit contains DNAse I and lysozyme, which improve cell membrane and DNA digestion for increased yields, and imcreases the recovery of large molecular weight proteins and insoluble proteins from inclusion bodies.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
B-PER Direct with Enzymes	Similar to B-PER with Enzymes, but bacteria can be lysed directly in cell culture media; ideal for screening 96-well microplate samples.	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.
Y-PER - Yeast Protein Extraction Reagent	Easy-to-use solution gently disrupts the tough yeast cell wall in less than 20 minutes at room temperature, using a mild detergent. No mechanical disruption needed; yields more than twice as much protein as glass bead methods.	*S. cerevisiae, Schizo-saccharomyces pombe, C. albicans, B. subtilis, E. coli, P. pastoris, Strep. avidinii* and *Acinetobacter* sp.
Y-PER Plus	More stringent than Y-PER, but entire formulation (including detergent) are dialyzable.	Yeast (*S. cerevisiae*) and *Acinetobacter* sp.
M-PER - Mammalian Protein Extraction Reagent	Highly efficient total protein extraction from cultured mammalian cells; extracts proteins in nondenatured state, enabling protein to be directly immunoprecipitated; amine-free and fully dialyzable; adhered cells can be directly lysed in plate or after scraping and washing in suspension.	Cultured mammalian cells, COS-7, NIH 3T3, Hepa 1-6, 293, CHO, MDA, MB231 and FM2
P-PER - Plant Protein Extraction Reagent	Contains organic lysing reagent and two aqueous reagents, which, in conjuction with mild mechanical agitation, effectively extract high quality protein extracts from plant leaves, stem, root, seed and flower cells without liquid nitrogen or harsh mechanical aids, such as mortar and pestle.	Multiple plant organs (leaf, stem, root, seed and flowers); multiple plant species (*Arabidopsis*, tobacco, maize, soybeans, peas, spinach, rice and other plant tissues); and fresh, frozen and dehydrated plant tissues
T-PER - Tissue Protein Extraction Reagent	Simple, easy to use reagent for extracting total protein from tissue in 1:20 (w/v) of tissue to T-PER, using centrifugation to pellet cell/tissue debris. Mild detergent is dialyzable.	Heart, liver, kidney and brain.
I-PER - Insect Cell Protein Extraction Reagent	Optimized mild nonioinic detergent formulation provides maximum extraction of soluble proteins from insect cells; better yield than sonication; can be used for both suspended or adherent insect cells	Baculovirus-infected insect cells grown in suspension or monolayer culture.
NE-PER - Nuclear and Cystoplasmic Extraction Kit	Obtain functional concentrated nuclear extracts and cytoplasmic fractions from mammalian cells and tissues in less than two hours, eliminating the need for freeze/thaw cycles, Dounce homogenization, lengthy centrifugation times and cold-room work.	Tissue: calf liver. Tissue: mouse heart, kidney, lung and liver; Cultured cells: epithelial (HeLa), fibroid (COS-7), kidney (NIH 3T3), liver (Hepa 1) and brain (C6).
Mem-PER - Eukaryotic Membrane Protein Extraction Kit	Efficient, gentle reagents that solubilize and isolate membrane proteins from mammalian and yeast cells, as well as soft and hard tissues, in less than an hour. Minimal cross contamination (less than 10%) of hydrophilic proteins into the hydrophobic (membrane protein) fraction	Cultured cells: brain (C6), epithelial (HeLa), fibroblasts (NIH 3T3) and yeast (*S. cerevisiae*).
Subcellular Protein Fractionation Kit	Includes a combination of reagents for stepwise lysis of mammalian cells into functional cytoplasmic, membrane, soluble nuclear, chromatin-bound, and cytoskeletal protein fractions in a single kit; includes a stabilized nuclease and protease inhibitors. Extracts from each compartment have less than 15% contamination between fractions, with sufficient purity for studying protein localization and redistribution.	Cultured mammalian cells.
Mitochondrial Isolation Kit for Cultured Cells	Isolate intact mitochondria from cultured mammalian cells in approximately 40 minutes, with an optional Dounce homogenization protocol for increased yield.	Mammalian cells.
Mitochondrial Isolation Kit for Tissues	Isolate intact mitochondria from soft or hard tissue in less than 60 minutes, with an optional Dounce homogenization protocol for increased yield.	Heart, liver, kidney and brain.
Lysosome Enrichment Kit for Tissues and Cells	Uses density gradient centrifugation to separate lysozyme from contaminating celluar structures in both mammalian cells and soft and hard tissue.	Tissues and cultured cells.
Peroxisome Enrichment Kit for Tissues	Uses density gradient centrifugation to separate peroxisome from contaminating celluar structures in both soft and hard tissue.	Heart, liver, kidney and brain.
Nuclei Enrichment Kit for Tissue	Uses density gradient centrifugation to separate nuclei from contaminating celluar structures in both soft and hard tissue	Heart, liver, kidney and brain.
Pierce RIPA Buffer	Extracts cystoplasmic, membrane, and nuclear proteins from cultured mammalian cells; can be used for both plated cells and cells pelleted from suspension cultures. Protease and phosphatase inhibitors are compatible with this formulation.	Cultured mammalian cells and cytoplasmic, membrane and nuclear proteins.
Pierce IP Lysis Buffer	Gently extracts cytoplasmic, membrane and nuclear proteins while maintaining protein complexes for immunoprecipitation (IP), Pulldowns, and co-IP; does not liberate DNA which can cause high viscosity.	Cultured mammalian cells.

[1] *For 2D sample prep, add 1:9 of lysis extract to 8M Urea, 4% Chaps. If sample has low protein concentration, buffer exchange sample directly into 8M Urea, 4% CHAPS using Zeba desalting columns, pp 36-37.*

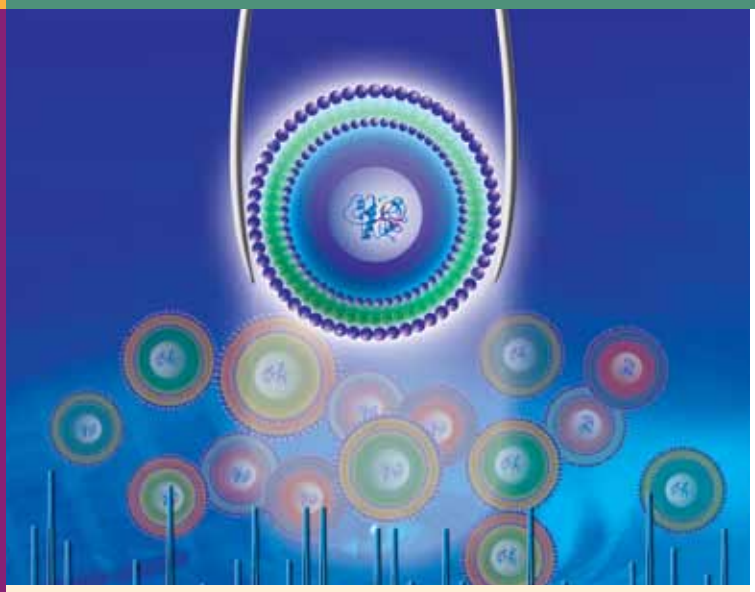
[2] *See pages 54-56 for Detergent Removal Resin product information and pricing.*

Thermo Scientific Protein Assay Compatibility	Mass Spec Workflow Compatibility Notes
Pierce BCA Assay and Coomassie Plus Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay and Coomassie Plus Assay after Compat-Able™ Protein Assay Reagent Set (Product # 23215) or dilute two to four times; Pierce 660 nm Protein Assay after 2-fold dilution	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay and Coomassie Plus Assay after Compat-Able Protein Assay Reagent Set (Product # 23215) or dilute two to four times	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay and Coomassie Plus Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay and Coomassie Plus Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay	In-solution enzymatic digest, 1D and 2D1 electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] but not dialysis.
Pierce BCA Assay and Coomassie Plus Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay and Coomassie Plus Assay; Pierce 660 nm Protein Assay after 2-fold dilution	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay, Reducing Agent-Compatible; Not compatible with Bradford, Coomassie or the original Pierce BCA Assay; Pierce 660 nm Protein Assay after 2-fold dilution	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2], and salt can be removed by dialysis[3] or desalting columns[4].
Pierce BCA Assay (dilute 1:1) and Coomassie Plus Assay; Pierce 660 nm Protein Assay after 2-fold dilution	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] or dialysis[3].
Pierce BCA Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. If required, detergents can be removed by Detergent Removal columns[2] but not dialysis.
Pierce BCA Assay and Coomassie Plus Assay (dilute CER Reagent mixture four times); Pierce 660 nm Protein Assay after 2-fold dilution	In-solution enzymatic digest, 1D and 2D electrophoresis. If required detergents can be removed by Detergent Removal columns[2] but not dialysis. For 2D electrophoresis, buffer exchange directly into 7M Urea, 2M Thiourea, 4% CHAPS, using Zeba Desalting columns[4].
Pierce BCA Assay and Coomassie Plus Assay; hydrophobic phase needs to be dialyzed first; see instruction book; Pierce 660 nm Protein Assay after 2-fold dilution	In-solution enzymatic digest, 1D and 2D electrophoresis. Membrane fractions require dilution (1:5) before 1D electrophoresis. 2D electrophoresis requires detergent removal[2], and buffer exchange directly into 7M Urea, 2M Thiourea, 4% CHAPS, using Zeba Desalting columns[4].
Pierce BCA Assay; Pierce 660 nm Protein Assay	In-solution enzymatic digest, 1D and 2D electrophoresis. MEB detergent can be removed by Detergent Removal columns[2] but not dialysis. Nuclear fractions require desalting[4] before 2D electrophoresis. PEB detergent requires detergent removal[2] or dialysis[3] before 2D electrophoresis.
Pierce BCA Assay (after lysis)	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. Detergent removal is not required for dounce homogenization protocol. Sample prepared using the reagent protocol can remove detergent using dialysis[3].
Coomassie Plus – The Better Bradford Assay Kit	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. Detergent removal is not required.
Coomassie Plus – The Better Bradford Assay Kit	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. Detergent removal is not required.
Coomassie Plus – The Better Bradford Assay Kit	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. Detergent removal is not required.
Pierce BCA Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. Deterent removal required before 2D electrophroresis. Detergents can be removed by Detergent Removal columns[2] but not dialysis.
Pierce BCA Assay; Pierce 660 nm Protein Assay	In-solution enzymatic digest, 1D and 2D[1] electrophoresis. Detergents can be removed by Detergent Removal columns[2] but not dialysis.

[3] *See page 38 for Slide-A-Lyzer G2 Dialysis Cassette product information and pricing.*

[4] *See pages 36-37 for Zeba Desalting Spin Columns product information and pricing.*

Protein Enrichment



Protein Enrichment

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 which share a common post-translational modification 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 have been be used. Other techniques use metabolic or enzymatic incorporation of modified amino acids or PTMs to introduce unique protein chemistry which can be used for enrichment. Finally, proteins can also be enriched using various enzyme class specific compounds or cell-impermeable labeling reagents which selectively label cell surface proteins.

Phosphoprotein Enrichment Kits

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

Phosphorylation is one of the most frequently occurring post-translational modifications 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 mass spectrometry technology in combination with phosphoprotein enrichment using immobilized metal affinity chromatography (IMAC) have resulted in greater resolution of the phosphoproteome.

The new 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 of 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 mass spectrometry, Western blotting and 2D-PAGE

Phospho-specific antibodies recognizing key regulatory proteins involved in growth factor signaling were used to monitor binding specificity of our 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 Pierce Phosphoprotein Enrichment Column as evidenced by their absence in the elution fraction. Conversely, all three proteins were present in the elution fraction from non-treated HeLa extract (Figure 2). Our Phosphoprotein Enrichment Kit provided superior and efficient phosphoprotein enrichment yields when compared to competitors' products (Table 1). It also effectively enriched phosphoproteins from homogenized mouse liver tissue (Figure 3).

Table 1. The Thermo Scientific Pierce Phosphoprotein Enrichment Kit provides higher phosphoprotein yields in less time than competitors' 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 1 mg load per manufacturer's protocol.*

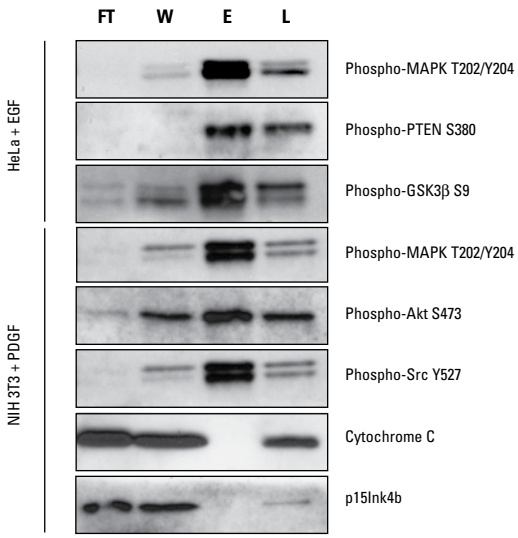


Figure 1. Highly pure phosphoprotein enrichment from complex biological samples. Serum-starved HeLa and NIH 3T3 cells were stimulated with EGF and PDGF, respectively. Cell lysate (2 mg) was used for enrichment. Concentrated flow-through, wash and elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10 µg/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.

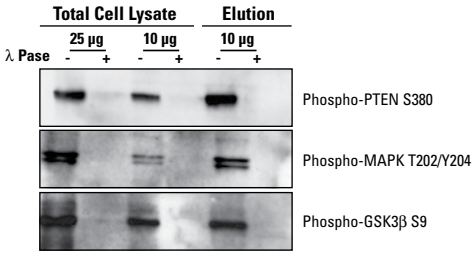


Figure 2. Highly specific phosphoprotein purification from lambda phosphatase-treated cells. Non-treated and lambda dephosphorylated HeLa cell extract (2 mg) was loaded onto separate Thermo Scientific Pierce Phosphoprotein Enrichment Columns. Concentrated elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10 µg/lane). To determine enrichment, 10 µg and 25 µg 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.

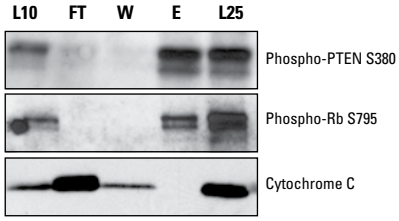


Figure 3. Efficient enrichment of phosphoproteins from mouse liver extract. Homogenized mouse liver extract (~2 mg) was loaded onto a Thermo Scientific Pierce Phosphoprotein Enrichment Column. Concentrated flow-through, wash and elution fractions were resolved by SDS-PAGE. Gel lanes were normalized by protein concentration (10 µg/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 (10 µg), FT = flow-through fraction, W = wash fraction, E = elution fraction and L25 = non-enriched total cell extract (25 µg).

Ordering Information

Product #	Description	Pkg. Size
90003	**Pierce Phosphoprotein Enrichment Kit**	Kit
	Includes: Phosphoprotein Enrichment Column Resin Bed (1 ml)	10 ea.
	Lysis/Binding/Wash Buffer	325 ml
	Elution Buffer	60 ml
	CHAPS	1 g
	White Column Caps	10 caps
	Pierce Protein Concentrator 7 ml/9K MWCO	10 Devices

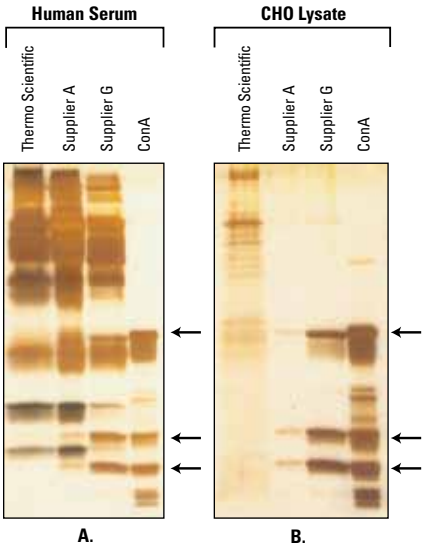
Glycoprotein Isolation Kits

Isolate glycoproteins from complex protein mixtures.

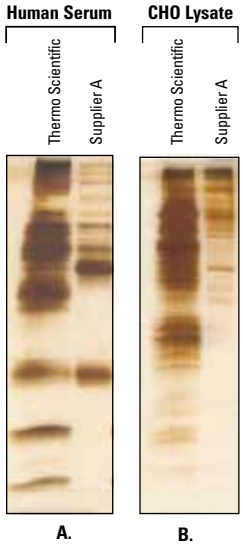
Two lectin-based Thermo Scientific Glycoprotein Isolation Kits, concanavalin A (ConA) and wheat germ agglutinin (WGA), allow isolation of glycoproteins from complex protein mixtures, including serum, tissue and cultured cell lysates, thus enabling downstream analysis. ConA lectin recognizes α-linked mannose and terminal glucose residues, while WGA lectin selectively binds to N-acetyl glucosamine (GlcNAc) groups and to sialic acid.

Highlights:

- **High recovery** – equivalent or greater glycoprotein recovery vs. competitor kits and lectin resins
- **Fast** – glycoprotein purification in less than one hour
- **Versatile** – isolate glycoproteins from various sample types; e.g., human serum and cell lysate
- **Robust** – lectin does not leach from resin when processing sample
- **Convenient** – complete kit contains lectin resins and spin columns with all necessary reagents
- **Compatible with Bradford-based protein assays** – dialysis or protein precipitation of recovered glycoproteins is not required before protein assay



Glycoprotein isolation from human serum and cell lysate: performance comparison of kits using ConA resin. Human serum and CHO lysate samples were processed with the Thermo Scientific Glycoprotein Isolation Kit, ConA and with other commercially available ConA resins. An equivalent amount of total protein was applied to each resin. Eluted glycoprotein fractions were compared with ConA Resin boiled in SDS-PAGE Buffer to release lectins. All fractions were normalized by volume and resolved on 8-16% polyacrylamide gels. Gels were silver-stained. **A.** Eluted glycoprotein fractions from applied human serum and **B.** eluted glycoprotein fractions from applied CHO lysate. Arrows identify protein bands that result from ConA leaching from the resin during the elution process.



Glycoprotein isolation from human serum and cell lysate: performance comparison of kits using WGA resin. Human serum and CHO lysate samples were processed with the Thermo Scientific Glycoprotein Isolation Kit, WGA and with other commercially available WGA resins. An equivalent amount of total protein was applied to each resin. Eluted glycoprotein fractions were normalized by volume and resolved on 8-16% polyacrylamide gels. **A.** Eluted glycoprotein fraction from applied human serum and **B.** eluted glycoprotein fraction from applied CHO lysate.

Ordering Information

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

Ubiquitin Enrichment Kit

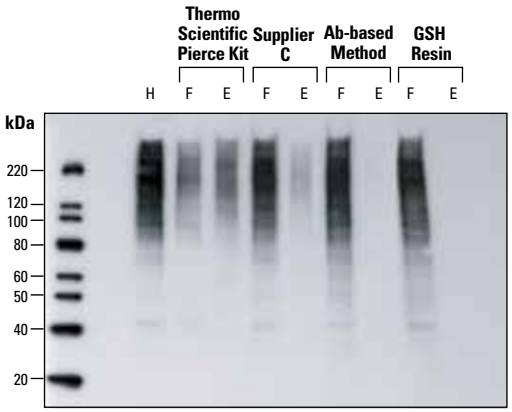
Recover ubiquitin modified protein in < 45 minutes.

The ubiquitin proteasome pathway is the principal non-lysosomal pathway that controls the proteolysis of proteins. This pathway is significantly involved in a variety of cellular processes, including DNA repair, transcriptional regulation, signal transduction, cell metabolism and morphogenesis. Differences in total ubiquitination or the ubiquitination of specific proteins affect numerous pathological conditions, including malignancies, certain genetic diseases and neurodegenerative diseases.[1]

The Thermo Scientific Ubiquitin Enrichment Kit isolates polyubiquitin protein conjugates from cultured cells and tissue samples. The enriched fraction is analyzed to determine the amount of general ubiquitin conjugates present or to identify a specific protein by Western blotting. The assay protocol is fast, straightforward and allows isolation of polyubiquitinated proteins and the fractionation of monoubiquitinated species in the resin flow-through. The Ubiquitin Enrichment Kit outperforms other suppliers' kits and provides a clean and specific preparation of proteins when compared to a control resin.

Highlights:

- **Fast** – less than 45 minutes hands-on time
- **Complete** – includes all reagents needed for ubiquitin-modified protein enrichment from cultured cells and tissue samples, including spin columns and ubiquitin antibody
- **Flexible** – sample incubation from 2 hours to overnight allows assay to be completed in several hours or in less than 30 minutes after overnight incubation
- **Robust** – compatible with all Thermo Scientific Cell Lysis Solutions and standard RIPA formulations
- **Multiple-sample format** – easily processes 1-15 samples concurrently



The Thermo Scientific Ubiquitin Enrichment Kit recovers more ubiquitin-modified proteins than any other method. Epoxomicin-treated HeLa cell lysates (EHeLa, 150 µg) were enriched. After elution, all samples were normalized to the initial load (EHeLa load). The flow-through and elution obtained using the Thermo Scientific Ubiquitin Enrichment Kit are shown first. The results using another supplier's enrichment kit are shown for comparison (Supplier C, manufacturer's instructions for this kit were followed). Additionally, the results obtained using an anti-ubiquitin monoclonal antibody-based enrichment scheme (antibody-based) and a negative control resin (GSH resin) are shown. The elution from each resin shows the amount of ubiquitin-modified protein that was captured using that method.

Reference

1. Ciechanover, A. (1998). The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J.* **17**(24), 7151-1760.

Ordering Information

Product #	Description	Pkg. Size
89899	**Ubiquitin Enrichment Kit** *Contains sufficient materials for enriching up to 15 lysate samples containing ~0.15 mg total protein per sample.* **Pack 1** Polyubiquitin Positive Control (1,000X), 50 µl, 2 mg/ml Anti-ubiquitin Antibody, 50 µl rabbit antiserum **Pack 2** Polyubiquitin Affinity Resin, 300 µl, supplied as a 25% slurry Binding Capacity: ~1 µg per 20 µl of slurry BupH™ Tris Buffered Saline Pack, 1 ea., makes 500 ml of 0.025 M Tris, 0.15 M NaCl; pH 7.2 Spin Columns and Accessories, 18 columns with top and bottom caps	Kit

Protein Enrichment

Cell Surface Protein Isolation Kit

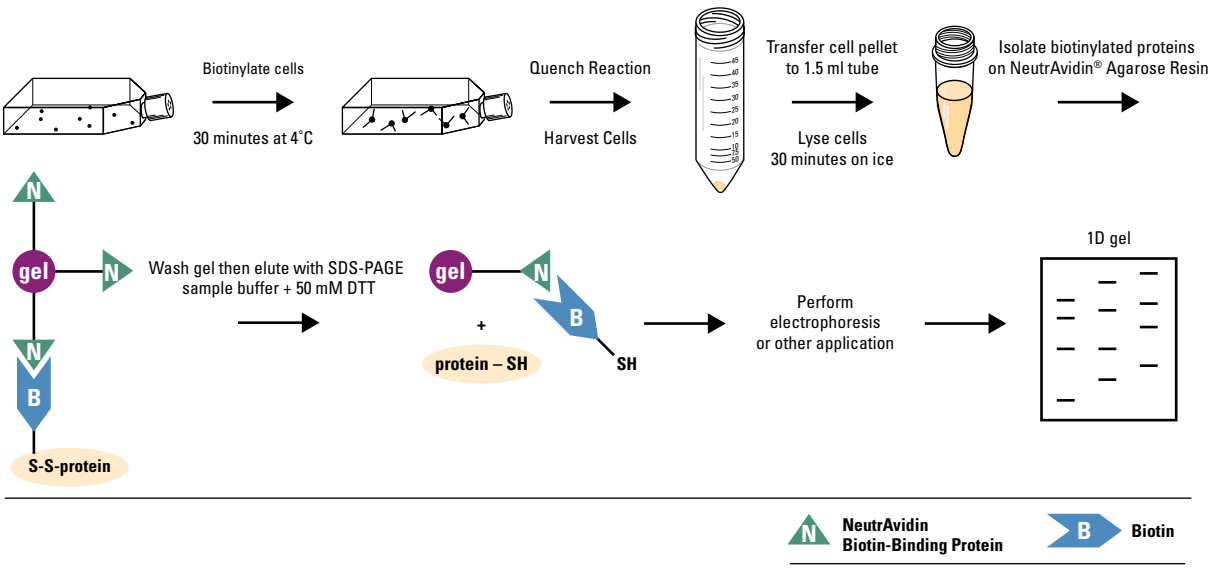
Convenient biotinylation and isolation of cell surface proteins for Western blot analysis.

The Thermo Scientific Pierce Cell Surface Protein Isolation Kit is a complete kit for the convenient biotinylation and isolation of mammalian cell surface proteins, specifically targeting cell surface proteins to the exclusion of intracellular proteins. The kit efficiently labels proteins with accessible lysine residues and sufficient extracellular exposure.

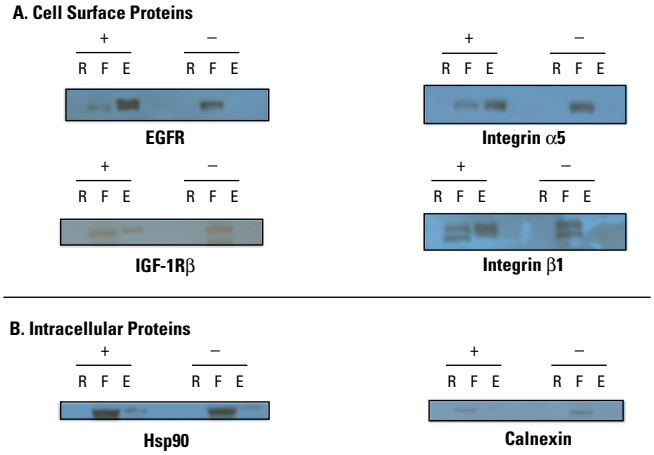
The isolation procedure uses a cell-impermeable, cleavable biotinylation reagent (Sulfo-NHS-SS-Biotin) to label surface proteins at exposed primary amines. Cells are then harvested and lysed, and the labeled surface proteins are affinity-purified using Thermo Scientific NeutrAvidin Agarose Resin. The isolated cell surface proteins contain a small, nonreactive tag of the originally labeled primary amines but are no longer biotinylated (biotin remains bound to the resin).

Highlights:

- **Isolates cell surface proteins** – reduces complexity of total cellular protein
- **Efficiently recovers labeled proteins** – cleavable biotin allows for nearly 100% recovery of isolated cell surface proteins
- **Convenience** – all reagents are provided in one kit, along with complete instructions for labeling, cell lysis and purification of cell surface membrane proteins
- **Western blotting applications** – proteins recovered in SDS-PAGE buffer are loaded directly onto polyacrylamide gels
- **Robust system** – protocol designed for diverse cell lines, including NIH 3T3, HeLa, C6 and A431



Protocol summary for the Thermo Scientific Pierce Cell Surface Protein Isolation Kit.



Protein isolation is specific to cell surface proteins. Panels are Western blot results for known cell surface proteins **(Panel A)** and intracellular proteins **(Panel B)** from HeLa cells tested with the Cell Surface Protein Isolation Kit. Plus symbol (+) denotes results for cells treated with the Sulfo-NHS-SS-Biotin reagent; minus symbol (-) denotes results for cells that were not treated with the biotin reagent but were otherwise carried through the kit procedure. Lanes are no-sample resin-control (R), flow-through (F) and eluted (E) fractions. Presence of target cell surface proteins in the plus-E and minus-F conditions indicate successful isolation with the kit. Presence of intracellular proteins in F condition of both plus and minus conditions indicates that the labeling and purification procedure is specific to cell surface proteins.

Ordering Information

Product #	Description	Pkg. Size
89881	**Cell Surface Protein Isolation Kit** *Sufficient reagents and accessories for eight experiments, each involving four T75 flasks of confluent cells.*	Kit
	Includes: EZ-Link® Sulfo-NHS-SS-Biotin	8 x 12 mg vials
	Quenching Solution	16 ml
	Lysis Buffer	4.5 ml
	NeutrAvidin Agarose	2.25 ml
	Wash Buffer	34 ml
	Dithiothreitol (DTT)	8 x 7.7 mg microtubes
	BupH Phosphate Buffered Saline	2 packs
	BupH Tris Buffered Saline	1 pack
	Spin Columns and Accessories	

Direct IP Kit

Immunoprecipitate using any antibody species or subclass! Eliminate antibody band contamination of IP products.

The Thermo Scientific Pierce Direct IP Kit represents a significant advancement in immunoprecipitation (IP) technology by replacing the use of immobilized Protein A or Protein G with a method for direct covalent attachment of antibodies to the beaded agarose resin.

The primary benefits resulting from this method are the opportunity to use any species or subclass of purified antibody (not just types that bind to Protein A or G) and the ability to purify target protein without contamination by the antibody. The method also makes it possible to immunoprecipitate antigens from serum samples without co-purifying non-target immunoglobulins. Finally, the kit uses microcentrifuge spin cups to effectively wash and separate samples from the beaded agarose resin.

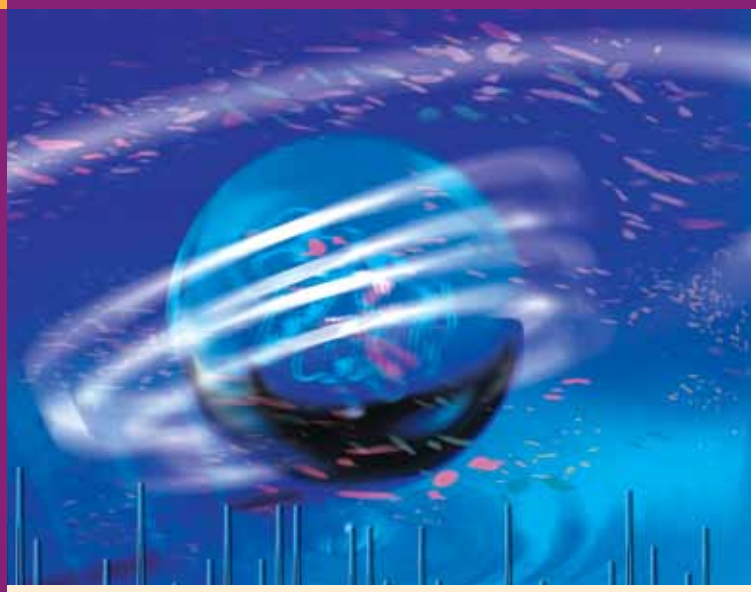
Highlights:

- Immobilize any antibodies independent of isotype or species
- Improved antibody coupling protocol
- Purify target protein without antibody contamination
- Activated resin for directly coupling antibodies to the support resin
- Eliminate antibody contamination in the eluate

Ordering Information

Product #	Description	Pkg. Size
26148	**Pierce Direct IP Kit** *Sufficient reagents to perform 50 reactions.*	Kit
	Includes: AminoLink® Plus Coupling Resin	2 ml
	20X Coupling Buffer	25 ml
	Quenching Buffer	50 ml
	Wash Solution	50 ml
	5M Sodium Cyanoborohydride Solution	0.5 ml
	IP Lysis/Wash Buffer	2 x 50 ml
	100X Conditioning Buffer	5 ml
	20X Tris-Buffered Saline	25 ml
	Elution Buffer	50 ml
	5X Lane Marker Sample Buffer	5 ml
	Pierce Spin Columns – Screw Cap	50 each
	Microcentrifuge Collection Tubes	2 ml, 100 each
	Microcentrifuge Sample Tubes	1.5 ml, 50 each
	Pierce Control Agarose Resin	2 ml

Protein Clean Up



Whether they are simple or complex, samples often need to be processed in several ways to ensure they are compatible and optimized for analysis by mass spectrometry. There are a wide range of methods and products for isolating and fractionating specific portions of the proteome (see protein enrichment pages 28-33). In addition, we also offer a range of general protein processing products (see pages 34-45).

Dialysis and desalting products allow buffer exchange, desalting, or small molecule removal to prevent interference with downstream processes. Protein assays help monitor protein concentration for consistent control of experimental loading or yield. SDS-PAGE and mass spectrometry compatible stains allow visualization of experimental results or further fractionation and isolation of proteins of interest.

This section will cover sample clean up techniques for intact proteins. Some of these methods can be used with peptides. For more complete information on peptide clean up techniques, see pages 54-66.

Albumin/IgG Removal Kits

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

The Thermo Scientific Pierce Albumin/IgG Removal Kits are optimized to decrease the abundant albumin and antibody components of human serum samples in preparation for 2D electrophoresis and other protein profiling methods. By eliminating most of these two proteins, which account for nearly 70% of total serum proteins, other less abundant proteins of interest can be more easily detected and studied. Both the Thermo Scientific Antibody-based and Dye/Protein A-based Albumin/IgG Removal Kits are scalable and sufficient to process 25 samples, each containing 600 µg of total serum protein (about 10 µl of serum).

The Antibody-Based Kit (Product # 89876) uses specific antibodies against human serum albumin (HSA) and human gamma immunoglobulin (IgG) immobilized to beaded agarose resin. When samples are incubated with the affinity resin, both HSA and IgG bind. Centrifugation to separate the supernatant from the resin results in sample in which HSA and IgG concentrations have been decreased to negligible levels. The Antibody-Based Kit provides the most specific method for removing HSA and IgG, and it is suitable only for human samples.

The Dye/Protein A-Based Kit (Product # 89875) uses a mixture of Cibacron® Blue Dye and Protein A agarose affinity resin. The dye binds to albumin with relatively good specificity, and Protein A binds to many species and subclasses of IgG. The kit is more economical than the Antibody-Based Kit and can be used to treat samples from other species besides human (e.g., monkey, swine, rabbit).

Highlights:

- Optimized for 2D sample prep – microcentrifuge spin cup method is scaled and optimized for treating 10 µl human serum samples for 2D electrophoresis
- Efficient – kits remove nearly all HSA and IgG, enabling low abundance proteins to be detected in 2D gels
- Fast – kits process samples in less than 40 minutes
- Scalable – use different amounts of affinity resin to match sample volume and concentration
- Two kit options – choose the system appropriate for your application: highest specificity (Antibody-Based Kit) or greater ecomony (Dye/Protein A-Based Kit)

Ordering Information

Product #	Description	Pkg. Size
89876	**Antibody-Based Albumin/IgG Removal Kit** *Sufficient material for up to 12 samples of 600 µg of serum.* Includes: Antibody-Based Resin (Anti-HSA/anti-IgG agarose) Spin Columns and Caps	Kit 4.5 ml 12
89875	**Albumin/IgG Removal Kit** *Sufficient material for up to 25 samples of 600 µg of serum.* Includes: Dye/Protein A-Based Resin (Cibacron-Blue/Protein A Agarose) Spin Columns and Caps	Kit 2 ml 12

Strong Cation and Anion Exchange Columns

Thermo Scientific Pierce Strong Cation and Anion Ion Exchange Spin Columns use adsorbent chromatographic matrices to fractionate proteins based on charge differences in a spin column format. The membrane-based adsorbents have a highly porous structure with pores larger than 3,000 nm, providing proteins with easy access to the membrane's charged ligands. These features provide for high efficiencies and flow rates when fractionating large biomolecules with small diffusivities. Columns are available in mini and maxi sizes in anionic and cationic formats.

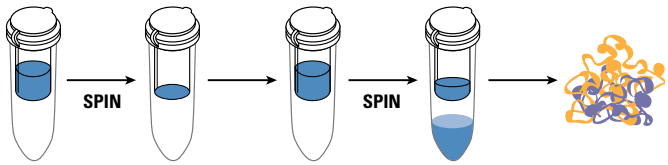
Ion exchange chromatography separates molecules based on differences in their accessible surface charges. This technique is widely used in the pre-fractionation or purification of a target protein from crude biological samples. Novel membrane-based ion exchange chromatography is attracting attention because of its advantages over resin-based column chromatography. Membrane-based ion exchange chromatography has shorter diffusion times than resin-based chromatography. Interactions between molecules and active sites on the membrane occur in a convective manner through pores, which shortens the diffusion time compared with fluid inside the pores of a resin particle. Also, the relatively mild binding and eluting conditions of this separation method produce high protein recovery with intact biological activity.

Highlights:

- **Fast and simple** – membrane-based spin format eliminates column packing
- **Convenient and expandable** – centrifugal format enables convenient parallel processing of multiple samples
- **Robust** – membrane adsorber spin columns do not crack or run dry
- **Low bed volume** – small membrane adsorber bed volumes make working with low buffer volumes possible, leading to concentrated elution fractions
- **Anion and cation formats** – choose either negatively charged or positively charged columns
- **Two column sizes** – choose either Mini size (4 mg, 500 µl capacity) or Maxi size (80 mg, 20 ml capacity)

Applications:

- Pre-fractionation of proteins in lysate
- Scouting purification conditions for new protein preparation protocols
- Removal of endotoxins from monoclonal antibodies
- Polishing His-tagged proteins after metal chelate chromatography
- Purification and concentration of proteins and viral particles
- Purification of antibodies from serum, ascites or tissue culture supernatant
- Removal of detergent from protein solutions
- Sample preparation before 1D- or 2D-PAGE
- Purification of phosphopeptides before MS analysis



Procedure summary.

Ordering Information

Product #	Description	Pkg. Size
90008	Pierce Strong Cation Exchange Spin Column, Mini	24 Spin Columns
90009	Pierce Strong Cation Exchange Spin Column, Maxi	8 Spin Columns
90010	Pierce Strong Anion Exchange Spin Column, Mini	24 Spin Columns
90011	Pierce Strong Anion Exchange Spin Column, Maxi	8 Spin Columns

Zeba Desalt Spin Columns

Quickly desalt sample volumes ranging from 2 µl to 4 ml with exceptional protein recovery

Thermo Scientific Zeba Spin Desalting Columns, 7K and 40K MWCO, are polypropylene centrifuge columns containing high-performance Zeba™ Desalting Resin, which offers rapid and exceptional protein desalting with high recovery.

The easy-to-use spin column format, 96-well spin plates and chromatography cartridges dramatically improve results over standard drip-column methodologies and provide a wide variety of sample processing formats, allowing multiple samples to be processed in as little as 8 minutes.

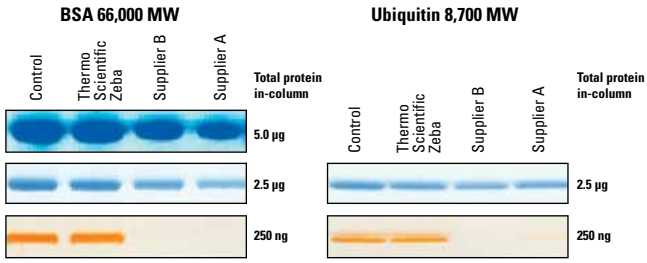
Highlights:

- Exceptional protein recovery
- No screening fractions for protein or waiting for protein to emerge by gravity-flow
- Wide product offering accommodates samples 2 µl to 4 ml (Table 1)
- Easy-to-use with no cumbersome column preparation or equilibration
- Minimal sample dilution

Table 1. Column sizes and recommended sample volumes.

Spin Column Size	Resin Bed	Sample Size
Micro	75 µl	2-12 µl
0.5 ml	0.5 ml	30-130 µl
2 ml	2 ml	200-700 µl
5 ml	5 ml	500-2,000 µl
10 ml	10 ml	1,500-4,000 µl

The high-performance desalting resins contained in the Zeba Desalting Products offer exceptional desalting and protein recovery characteristics that outperform other commercially available resins (Figure 1 and Table 2). Zeba Desalting Products provide > 95% retention of salts and other small molecules. The Zeba 7K MWCO is recommended for processing proteins > 7 kDa, and the Zeba 40K MWCO is recommended for processing proteins > 40 kDa.



Protein	Resin	% Recovery
BSA (66K MW)	Zeba	98
	Supplier B	47
	Supplier A	37
Ubiquitin (8.7K MW)	Zeba	76
	Supplier B	47
	Supplier A	50

Figure 1. Obtain high protein recovery with Thermo Scientific Zeba 7K Desalt Micro Spin Columns. Samples of bovine serum albumin (BSA) or ubiquitin at a variety of concentrations were desalted with our high-performance desalting resin or another supplier's resin in Pierce Spin Columns. In all cases, sample volume was 10 µl plus a 3 µl buffer stacker placed over the sample. Significantly less protein was recovered using the other supplier's resin. Recovery percentages of BSA or ubiquitin at 250 ng/µl (2.5 µg total protein load) after the desalting process were analyzed by the Thermo Scientific Pierce BCA Protein Assay (Product # 23225).

Table 2. The Thermo Scientific Zeba 40K and 7K Desalt Resin performance. Sample volumes of 100 µl of different compounds were processed with 0.5 ml of two Zeba Resins, as well as with two 30K-50K MWCO resins from Suppliers G and B. The Zeba Resins removed 85-100% of DTT, NaCl and biotin.

		Percent Removal			
Component	**Concentration Loaded**	**Zeba 40K Resin**	**Zeba 7K Resin**	**Supplier G Resin**	**Supplier B Resin**
NaCl (58.44 Da)	1 M	100	100	95	100
Dithiothreitol (154 Da)	0.5 M	100	99	76	97
Sulfo-NHS-LC-biotin (557 Da)	0.27 mM	88	87	72	77
Bacitracin (1,200 Da)	0.5 mg/ml	97	79	80	74
Vitamin B12 (1,386 Da)	0.5 mg/ml	99	93	67	86

Ordering Information

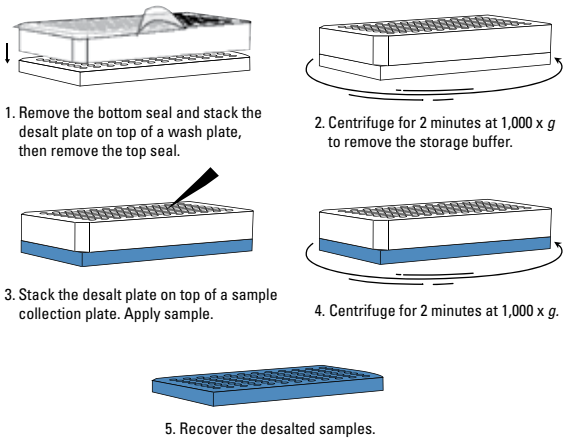
Product #	Description	Pkg. Size
89877	Zeba Micro Desalt Spin Columns 7K MWCO	25 columns
89878	Zeba Micro Desalt Spin Columns 7K MWCO	50 columns
89882	Zeba Desalt Spin Columns, 0.5 ml 7K MWCO	25 columns
89883	Zeba Desalt Spin Columns, 0.5 ml 7K MWCO	50 columns
89889	Zeba Desalt Spin Columns, 2 ml 7K MWCO	5 columns
89890	Zeba Desalt Spin Columns, 2 ml 7K MWCO	25 columns
89891	Zeba Desalt Spin Columns, 5 ml 7K MWCO	5 columns
89892	Zeba Desalt Spin Columns, 5 ml 7K MWCO	25 columns
89893	Zeba Desalt Spin Columns, 10 ml 7K MWCO	5 columns
89894	Zeba Desalt Spin Columns, 10 ml 7K MWCO	25 columns
87764	Zeba Micro Desalt Spin Columns 40K MWCO	25 columns
87765	Zeba Micro Desalt Spin Columns 40K MWCO	50 columns
87766	Zeba Desalt Spin Columns, 0.5 ml 40K MWCO	25 columns
87767	Zeba Desalt Spin Columns, 0.5 ml 40K MWCO	50 columns
87768	Zeba Desalt Spin Columns, 2 ml 40K MWCO	5 columns
87769	Zeba Desalt Spin Columns, 2 ml 40K MWCO	25 columns
87770	Zeba Desalt Spin Column, 5 ml 40K MWCO	5 columns
87771	Zeba Desalt Spin Column, 5 ml 40K MWCO	25 columns
87772	Zeba Desalt Spin Column, 10 ml 40K MWCO	5 columns
87773	Zeba Desalt Spin Column, 10 ml 40K MWCO	25 columns
Related Products		
89934	Pierce Desalting Chromatography Cartridges	5 x 1 ml
89935	Pierce Desalting Chromatography Cartridges	5 x 5 ml

Zeba 96-well Desalt Spin Plates

Thermo Scientific Zeba 96-well Spin Desalting Plates provide high-throughput removal of salt and small molecules from samples thus preparing them for an array of downstream analyses such as mass spectrometry, HPLC, capillary electrophoresis, metabolite screening, and assay development. Each well in the plate contains ~550 µl resin slurry and can process 20-100 µl samples.

Table 3. Protein recovery and desalting efficiency.

Protein	% Protein Recovery	% NaCl Removed
BSA (66 kDa)	98.5	> 95
α-Lactalbumin (14.1 kDa)	91.5	> 95
Ubiquitin (8.6 kDa)	85	> 95



Thermo Scientific Zeba 96-well Desalt Spin Plates are easy to use.

Table 4. Efficiency of small molecule removal at different volumes using the spin desalting plates.[1]

	% Removal		
Sample Volume (ml)	NaCl (54 Da)	Vitamin B12 (1.36 kDa)	DTT (154 Da)
20	99.5	100	100
40	99.4	100	100
80	99	98	99
100	99	98	96
150	94	93	95
200	87	52	90

[1]NaCl (1M stock) levels were determined using conductivity. Vitamin B12 (0.5mg/ml stock) levels were determined by measuring absorbance at 360 nm. DTT (500mM stock) levels were determined using Thermo Scientific Ellman's Reagent (Product # 22582)

Table 5. Recovery of different samples and volumes using the spin desalting plates.[2]

	% Recovery				
Sample Volume (ml)	Bacitracin (1.2 kDa)	HGH Fragment (1.8 kDa)	Ubiquitin (8.7 kDa)	α-lactal bumin (14.1 kDa)	BSA (66 kDa)
20	0	0	41.5	81	100
40	0	0	34.1	55	100
80	0	0	63	78	100
100	0.3	0.5	65	77	100
150	8.2	8.6	72	78	100
200	27	17	72	77	100

[2]Samples were prepared at 0.5mg/ml. Concentration was determined using the Thermo Scientific Pierce BCA Protein Assay (Product # 23225).

Ordering Information

Product #	Description	Pkg. Size
89807	Zeba 7K 96-well Desalt Spin Plates	2 plates
89808	Zeba 7K 96-well Desalt Spin Plates	4 plates
87774	Zeba 40K 96-well Desalt Spin Plates	2 plates
87775	Zeba 40K 96-well Desalt Spin Plates	4 plates

**The two-plate kits contain two wash plates and two collection plates. The four-plate kits contain two wash plates and four collection plates.*

Slide-A-Lyzer G2 Dialysis Cassettes

Simple, effective dialysis without the hassle of conventional tubing.

Join the thousands of researchers worldwide who save time and preserve their valuable samples by using Thermo Scientific Slide-A-Lyzer Dialysis Cassettes. Not only are the new Slide-A-Lyzer G2 Cassettes flexible and easy-to-use – they're pipette-accessible, making it easy to add and remove your samples!

Applications:

- Removing low-molecular weight contaminants
- Performing buffer exchange
- Desalting

Highlights:

- Twist-off cap is pipette-accessible for easy sample loading and retrieval
- Self-floating chambers for buoyancy and vertical orientation during dialysis
- Designed to maintain the highest possible sample integrity and protection
- Fast and consistent dialysis with maximum sample recovery
- Rigorous quality testing for maximum consistency

We still offer the original Slide-A-Lyzer Dialysis Cassettes in 2K, 3.5K, 7K, 10K and 20K MWCO in a variety of different sample capacities. To see the complete listing of these Cassettes, visit www.thermoscientific.com/dialysis

Table 6. Traditional Tubing vs. Cassette

	Traditional Tubing	Cassette
Sample Handling	Slippery When Wet Flat tubing is difficult to handle and fill when wet.	Easy Handling and Secure Sample Delivery No mess, no fuss.
Sample Recovery	Sample Loss Sample can easily be lost when tubing leaks or clamps slip off.	Sample Protection Highest possible sample protection with > 95% sample recovery.
Sample Integrity	Frequent Leaking Leaking into dialysate can compromise sample.	No Leaking! Sample remains intact with no contamination from surrounding dialysate.
Speed	Time-Consuming Typically dialyze overnight. Difficult to recover sample from wet tubing.	Fast and Efficient High surface area/sample volume ratio will dialyze twice as fast as conventional tubing.

Ordering Information

Product Capacity*	0.1-0.5 ml	0.5-3 ml	15 ml	30 ml	70 ml
Description	10/pkg.	10/pkg.	8/pkg.	6/pkg.	6/pkg.
2K MWCO (Blue)	**87717**	**87718**	**87719**	**87720**	**87721**
3.5 K MWCO (Pink)	**87722**	**87723**	**87724**	**87725**	**87726**
7K MWCO (Green)	**87727**	**87728**	**N/A**	**N/A**	**N/A**
10K MWCO (Orange)	**87729**	**87730**	**87731**	**87732**	**87733**
20K MWCO (Purple)	**87734**	**87735**	**87736**	**87737**	**87738**

**Cassette size indicates maximum sample volume. Refer to instructions for proper use and determination of minimum sample volume when using pipette or syringe.*

High-Performance Dialysis, Desalting and Detergent Removal Technical Handbook

Dialysis and desalting tools have dramatically changed in the past decade. This 29-page handbook features the new Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassettes, which are pipette-accessible and have a built-in buoy for easy flotation. The handbook also covers our original Slide-A-Lyzer® Cassettes, dialysis tubing, Thermo Scientific Zeba Desalting Columns and Plates, Thermo Scientific Pierce Protein Concentrators and Pierce Detergent Removal Columns. 1601823

Protein Concentrators

Disposable ultrafiltration centrifugal devices for concentrating proteins

Protein concentration is a commonly performed and essential procedure for sample preparations. There are a variety of devices available, all containing ultrafiltration membranes with a range of molecular-weight cutoffs (MWCO). The type of device used can significantly affect protein recovery, especially with low-concentration samples that are often lost or damaged in the process.

The new Thermo Scientific Pierce Protein Concentrators are disposable ultrafiltration centrifugal devices for concentration and diafiltration/buffer-exchange of biological samples such as enzymes, antigens or antibodies. Because of their unique design, the Pierce Concentrators avoid the problems commonly associated with protein concentration. These concentrators consist of a high-performance regenerated cellulose membrane welded to a conical device and are compatible with swinging-bucket and fixed-angle rotors. The design enables a high degree of concentration in a single centrifugation step, while minimizing polarization and adsorption at the membrane surface. Additionally, researchers can accurately control the dead-stop volume and final concentration factor for reliable and consistent sample processing. Concentration factors of > 110-fold are achieved in 45 minutes with the 20 ml devices (Table 7). The membranes are accurately rated and routinely provide > 90% recovery of proteins larger than the membrane MWCO, and > 85% with low-concentration samples (Tables 7 and 8).

Highlights:

- **Superior protein concentration and recovery** – achieve > 110-fold protein concentration in 45 minutes with > 90% protein recovery
- **Convenient** – concentrate 1-20 ml of sample in a fast spin format
- **Instrument-compatible** – use swinging-bucket or fixed-angle rotors; collect sample without invert spinning
- **Versatile** – the 150K MWCO concentrator is ideal for samples containing microorganisms such as viral particles

Table 7. Thermo Scientific Pierce Protein Concentrators provide exceptional recovery with low-concentration samples. Samples of Cytochrome C and bovine serum albumin (BSA) at approximately 0.2 and 0.01 mg/ml, and IgG at 0.35 mg/ml starting concentration were centrifuged in Pierce Concentrators at 2-3,000 x *g* for 15-45 minutes. Samples were recovered without membrane washing and protein recovery was determined by the Thermo Scientific Pierce BCA Protein Assay.

Protein	Concentrator Unit	Time (2-3,000 x *g*)	Starting Concentration	Recovery	Fold Concentration
Cytochrome C 12 kDa	7ml/9K	35 min	0.2 mg/ml	96%	137
			0.01 mg/ml	88%	129
	20ml/9K	45 min	0.2 mg/ml	100%	121
			0.01 mg/ml	96%	117
BSA 66 kDa	7ml/20K	35 min	0.2 mg/ml	97%	81
			0.01 mg/ml	87%	65
	20ml/20K	45 min	0.2 mg/ml	98%	137
			0.01 mg/ml	95%	118
IgG 150 kDa	7ml/150K	15 min	0.35 mg/ml	95%	325
	20ml/150K	15 min	0.35 mg/ml	97%	109

Table 8. Typical retention of proteins using the 150 MWCO concentrator.*

Protein	Concentration (mg/ml)	Recovery (%)
IgG (150 kDa)	0.5	93
Aldolase (160 kDa)	0.25	87

**Protein solutions (13.5 ml) in phosphate-buffered saline were concentrated in a fixed-angle rotor at 2,000 x g at 22°C. The concentration was determined by Pierce BCA Protein Assay.*

Protein Clean Up

Pierce Concentrators effectively combine speed, capacity and recovery for high performance concentration, purification and separation of proteins even with dilute samples. We compared the Pierce Concentrators to ultrafiltration centrifugal devices from the other suppliers. Using a variety of test proteins and starting concentrations, protein recovery was monitored by Thermo Scientific Pierce BCA Protein Assay (Product #23225). For samples above the rated MWCO, high levels of protein was recovered (approximately 90% or greater) with Pierce Concentrators with a starting concentration as low as 0.02 mg/ml (Table 3). Significantly lower or no protein was recovered with devices from supplier V, indicating inappropriate molecular weight ratings or significant binding of protein to the device membrane.

Applications:

- Protein concentration with tissue culture media, antiserum or monoclonal antibody preparations
- 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 9. Thermo Scientific Pierce Concentrators perform better than units from other suppliers.*

Concentrator (MWCO)	Recovery (%) BSA (66,000 MW)	Lysozyme (14,000 MW)	Ubiquitin (8,700 MW)
Pierce Concentrator (9K)	91	98	96
Supplier M (10K)	90	93	91
Supplier V (10K)	76	5	5

**Proteins samples (~0.02 mg/ml) were centrifuged in concentrators at 3,000 x g until a 15- to 25-fold decrease in sample volume was achieved. Samples were recovered without membrane washing. Recovery was determined using the Pierce BCA Protein Assay. Results were unaffected by centrifugation rate and similar for the 7 and 20 ml Pierce Concentrators. Similar or higher recovery values were obtained with the Pierce Concentrators at higher protein loads (data not shown).*

Ordering Information

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

Protein Detection

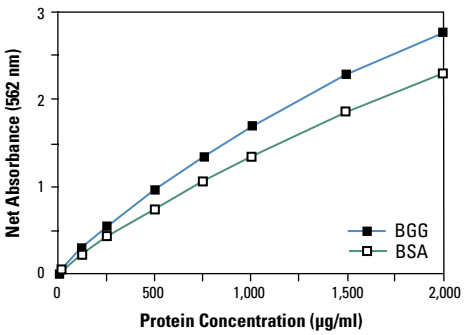
BCA Protein Assay

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

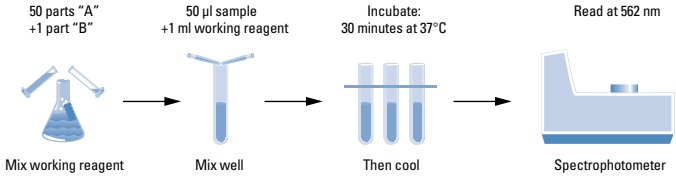
The Thermo Scientific Pierce BCA Protein Assay is useful for studying protein:protein interactions, measuring column fractions after affinity chromatography, estimating percent recovery of membrane proteins from cell extracts and performing high-throughput screening of fusion proteins.

Highlights:

- Colorimetric method; read at 562 nm
- Compatible with most ionic and nonionic detergents
- Four times faster and easier than the classical Lowry method
- All reagents stable at room temperature for two years
- Working reagent stable for 24 hours
- Linear working range for BSA from 20-2,000 µg/ml
- Minimum detection level of 5 µg/ml with the enhanced protocol
- Convenient microplate or cuvette format
- Less protein:protein variation than dye-binding methods



Color response curves for the Thermo Scientific Albumin Standard (BSA, Product # 23209) and Bovine Gamma Globulin Standard (BGG, Product # 23212) assayed with the Thermo Scientific Pierce BCA Protein Assay.



Procedure summary for the Thermo Scientific Pierce BCA Protein Assay.

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Ordering Information

Product #	Description	Pkg. Size
23225	**BCA Protein Assay Kit** *Sufficient reagents to perform 500 standard tube assays or 5,000 microplate assays.* Includes: Reagent A Reagent B Albumin Standard (2 mg/ml)	Kit 2 x 500 ml 25 ml 10 x 1 ml ampules
23227	**BCA Protein Assay Kit** *Sufficient reagents to perform 250 standard tube assays or 2,500 microplate assays.* Includes: Reagent A Reagent B Albumin Standard (2 mg/ml)	Kit 2 x 500 ml 25 ml 10 x 1 ml ampules
23229	**BCA Compat-Able Protein Assay Kit** *Sufficient reagents to perform 250 standard tube assays or 2,500 microplate assays.* Includes: Reagent A Reagent B Albumin Standard (2 mg/ml) Product # 23215, Compat-Able Protein Assay Preparation Reagent Kit	Kit 500 ml 25 ml 10 x 1 ml ampules
23221	**BCA Protein Assay Reagent A**	250 ml
23228	**BCA Protein Assay Reagent A**	500 ml
23223	**BCA Protein Assay Reagent A**	1,000 ml
23222	**BCA Protein Assay Reagent A**	3.75 L
23224	**BCA Protein Assay Reagent B** 4% $CuSO_4 \cdot 5H_2O$	25 ml
23230	**BCA Solid** *Bicinchoninic acid as recrystallized purified powder sufficient to make 2.5 L of Reagent A.*	25g

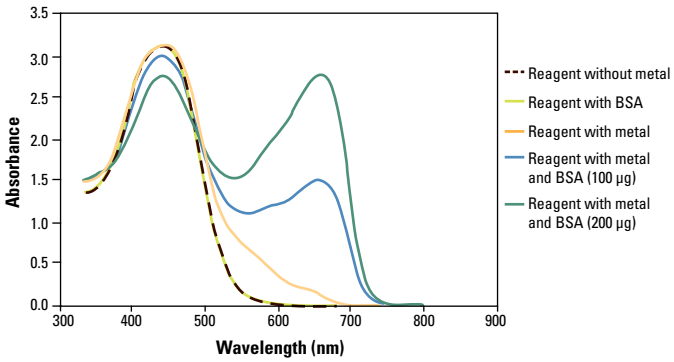
Pierce 660 nm Protein Assay

Fast and simple like Bradford assays, but with better linearity and detergent compatibility!

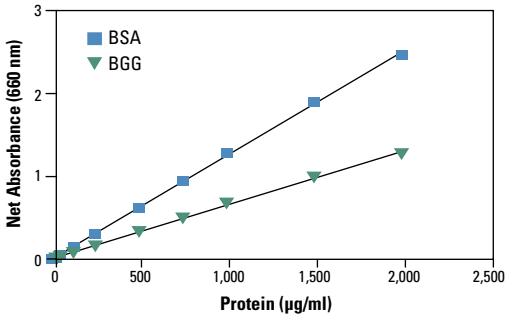
The Thermo Scientific Pierce 660 nm Protein Assay is a quick, ready-to-use colorimetric method for total protein quantitation. The assay is reproducible, rapid and more linear compared to coomassie-based Bradford assays and compatible with higher concentrations of most detergents, reducing agents and other commonly used reagents. The accessory Thermo Scientific Ionic Detergent Compatibility Reagent provides for even broader detergent compatibility. Although the Pierce 660 nm Protein Assay produces a higher level of protein-to-protein variation (~37%) than the other assays, such as the Thermo Scientific Pierce BCA Protein Assay, the simpler single-reagent format and broader substance compatibility make the 660 nm Assay more convenient for many routine applications. The Pierce 660 nm Protein Assay can be performed in either a test tube or a microplate, in which protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions assayed alongside the unknown samples.

Highlights:

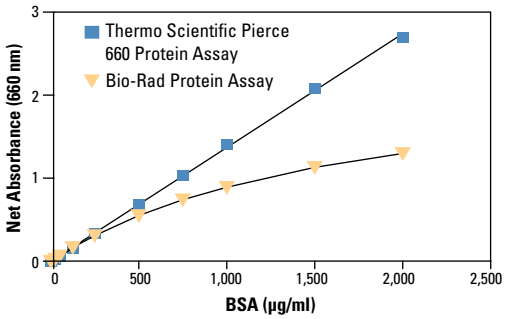
- Detects protein concentrations from 25-2,000 µg/ml
- Single, ready-to-use reagent with a simple mix-and-read assay
- Fast, 5-minute color development for measurement at 660 nm
- Compatible with commonly used detergents and reducing agents
- Compatible with ionic detergents and even SDS-PAGE sample loading buffer containing bromophenol blue when used with the Ionic Detergent Compatibility Reagent
- Convenient cuvette or microplate format
- Supplied with set of pre-diluted standards for utmost convenience and accuracy
- Room temperature storage means no waiting for the reagent to warm before use



The absorption maximum of the reagent-metal complex shifts proportionally upon binding to BSA. The absorption spectra were recorded for the Pierce 660 nm Protein Assay Reagent from 340 to 800 nm using a Varian Cary® Spectrophotometer. The assay reagent is a proprietary dye-metal complex that binds to protein in acidic conditions, which shifts the dye's absorption maximum.



Typical color response curves using the test tube procedure. The linear detection ranges are 25-2,000 µg/ml for bovine serum albumin (BSA) and 50-2,000 µg/ml for bovine gamma globulin (BGG). The average absorbance for the blank replicates (control) was subtracted from the absorbance for individual standard replicates.



Performance comparison of Bradford Protein Assay versus the Thermo Scientific Pierce 660 nm Protein Assay. Assays were performed according to the standard test-tube procedure using 100 µl of BSA. The Pierce 660 nm Protein Assay has a greater linear range of 25-2,000 µg/ml, compared with the Bradford Assay, which has a linear range of only 125-1,000 µg/ml.

Ordering Information

Product #	Description	Pkg. Size
22660	**BCA Protein Assay Kit** *Sufficient reagent for 500 standard assays and 5,000 microplate assays.*	750 ml
22662	**Pierce 660 nm Protein Assay Kit** *Sufficient reagents to perform 300 standard assays and 3,000 microplate assays.* Includes: Pierce 660 nm Protein Assay Reagent Pre-Diluted Protein Assay Standards, Bovine Serum Albumin (BSA) Set	Kit 450 ml 7 x 3.5 ml
22663	**Ionic Detergent Compatibility Reagent** *5 pouches, each sufficient for addition to 20 ml Pierce 660 nm Protein Assay Reagent.*	5 x 1 g

Protein Gels

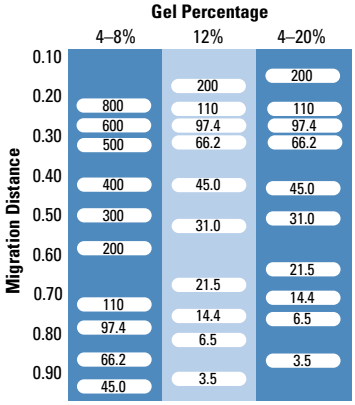
Thermo Scientific Pierce Protein Gels take ease-of-use to new levels. The gels use a special formulation to produce stronger, more resilient gels, making handling during and after electrophoresis easier. The extra stability of Pierce Protein Gels combined with the Tris-HEPES-SDS Running Buffer offers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

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

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

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

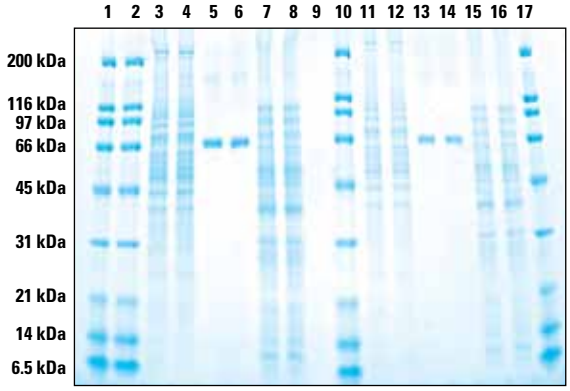
Table 10. Protein migration table of the various Thermo Scientific Pierce Protein Gels.



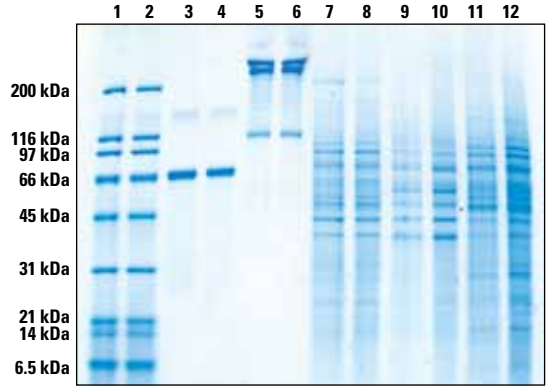
Highlights:

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

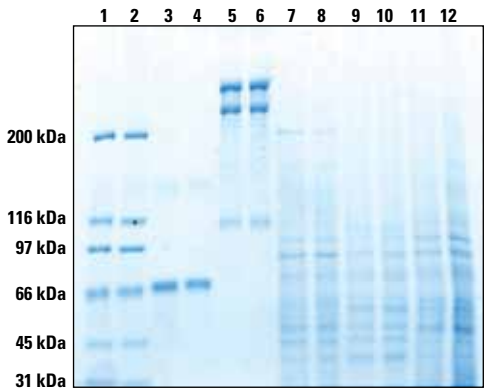
Protein Detection



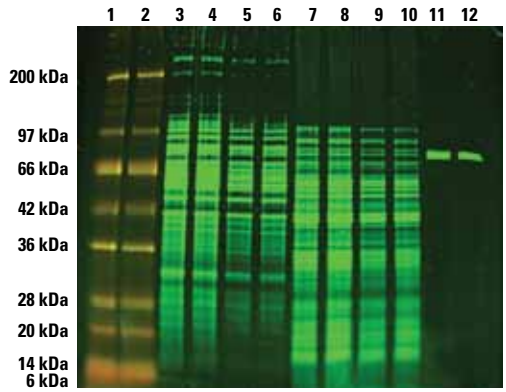
Thermo Scientific Pierce Protein Gel, 4-20%, stained with Thermo Scientific GelCode Blue Stain. Proteins were separated on 4-20% 17-well Pierce Protein Gel (Product # 84714), washed 30 minutes with water, stained for 60 minutes with GelCode Blue Stain (Product # 24592) and destained for 60 minutes (3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



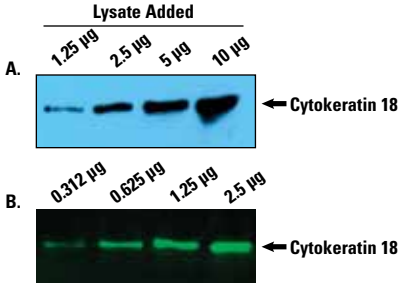
Thermo Scientific Pierce Protein Gel, 12%, stained with Thermo Scientific GelCode Blue Stain. Proteins were separated on 12% 12-well Pierce Protein Gel (Product # 84711), washed three times for 10 minutes each with water, stained for 60 minutes with GelCode Blue Stain (Product # 24592) and destained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



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



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



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

Gel Specifications

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

Compatible Gel Tanks

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

Ordering Information

Thermo Scientific Pierce Protein Gels

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

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

Silver Stain for Mass Spectrometry

Silver stains offer exceptional detection characteristics, enabling detection of bands containing < 0.25 ng of protein. However, silver stain formulations are often incompatible with MS applications because of fixation and crosslinking that can occur during staining. We recognized the need for a silver stain that is not only compatible with MS applications, but is truly optimized to provide the best results. Thermo Scientific Pierce Silver Stain for Mass Spectrometry (Product # 24600) provides superior reliability, sensitivity and robustness for MS-target applications.

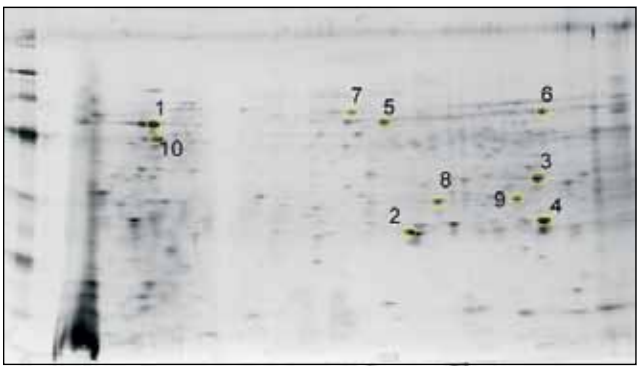


Figure 2. 2-D gel analysis of rat mitochondrial preparation (stained with Thermo Scientific Pierce Silver Stain for MS.) A preparation of rat mitochondria was extracted, loaded and separated using identical 2-D gel conditions. All gels were electrophoresed in a pH 5-8 gradient. Each gel was stained with Pierce Silver Stain for Mass Spectrometry (shown), Supplier I MS compatible stain and Thermo Scientific GelCode Blue Stain Reagent, respectively. Ten spots were identified that stained well for all staining conditions. These spots were picked for in-gel digestion and MS analysis.

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

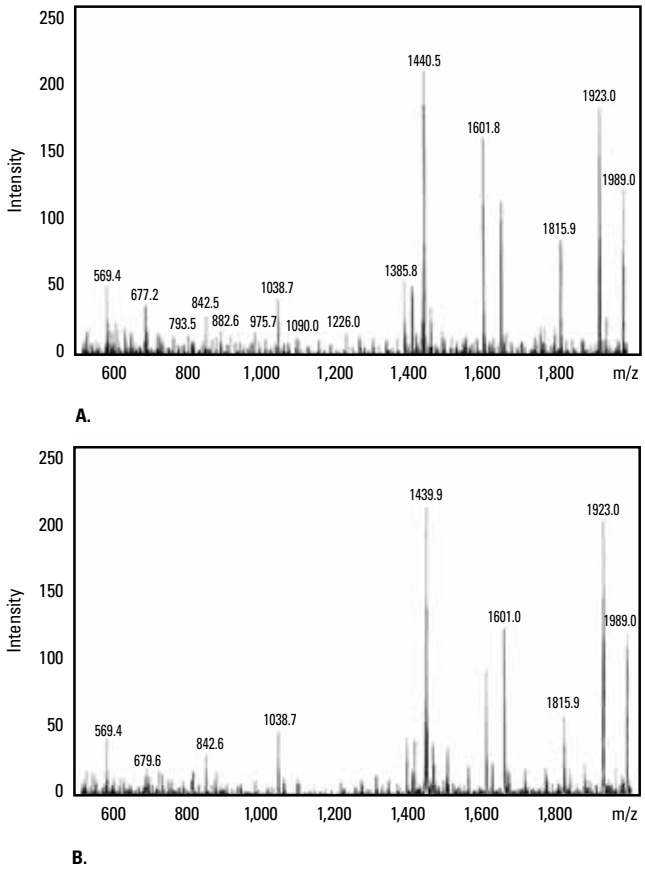


Figure 3. Comparative peptide fingerprint analysis of 2-D spot #1 by MS. MALDI ion trap MS was performed on an Agilent Technologies LC/MSD Trap XCT. Indicated spots (Figure 2) were picked from 2-D gels run identically with mitochondrial extract. Spots from both the **A.** Thermo Scientific Pierce Silver Stain for MS- and **B.** Thermo Scientific GelCode Blue Reagent-stained gels were reduced, alkylated and trypsinized using the Thermo Scientific In-Gel Tryptic Digestion Kit (Product # 89871). Peptide mass fingerprinting was performed with ProteinProspector. Both analyses identified the same mitochondrial protein: ATP Synthase, H^+ Transporting, Mitochondrial F1 Complex.

Ordering Information

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

Imperial Protein Stain

MS can be effectively combined with staining for protein identification. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein separation, the individual protein bands are stained to detect low quantities of proteins adequate for downsteam MS analysis. We offer an exceptional line of stains for detecting proteins in gels. These stains not only offer superior performance for standard detection, but they are directly compatible with MS applications. Each stain produces little to no protein fixation or modification for clean and accurate mass spectral analysis.

Thermo Scientific Imperial Protein Stain, a ready-to-use coomassie stain for detecting protein bands in SDS-polyacrylamide and 2-D gels, was designed for researchers who require maximum sensitivity and speed. The stain is a unique formulation of coomassie R-250 that delivers substantial improvements in protein-staining performance compared with homemade or other commercial stains (Figure 4). Multiple staining protocols are provided to meet demanding time and sensitivity requirements. Bands containing as low as 6 ng protein can be detected in as little as 20 minutes (Figure 6). Imperial Stain does not require a fixation step and uses a simple water wash to yield a clear background.

Highlights:

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

Convenience:

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

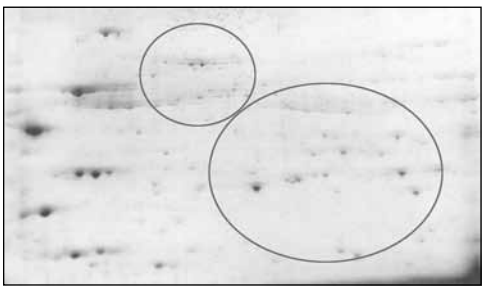


Figure 4. Thermo Scientific Imperial Protein Stain reveals spots that are faint or not detected with other Coomassie stains. Mitochondrial protein extract was prepared from heart tissue of six-week-old Sprague-Dawley rat. Processed protein extract (72 µg) was focused on a pH 5-8 IPG strip followed by 8-16% SDS-PAGE. The gels were stained for 1 hour and destained overnight following manufacturer-recommended protocols.

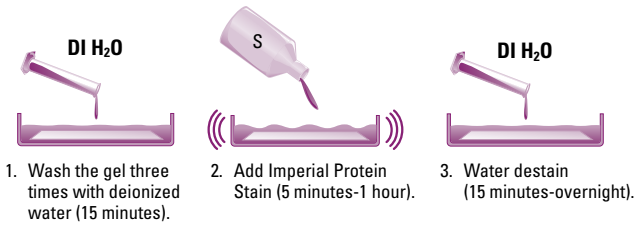


Figure 5. Thermo Scientific Imperial Protein Stain protocol.

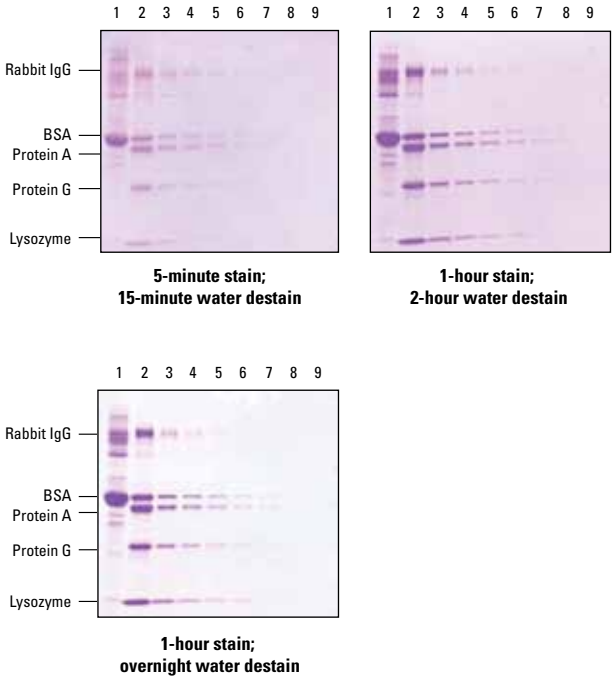


Figure 6. Enhanced sensitivity and crystal-clear background using Thermo Scientific Imperial Protein Stain. For even greater sensitivity and reduced background, gels are stained with Imperial Protein Stain for 1 hour and destained in water from 1 hour to overnight. **Lane 1.** BSA only (6 µg), Lanes 2-9 contained the indicated proteins at the following concentrations: **Lane 2.** 1,000 ng, **Lane 3.** 200 ng, **Lane 4.** 100 ng, **Lane 5.** 50 ng, **Lane 6.** 25 ng, **Lane 7.** 12 ng, **Lane 8.** 6 ng and **Lane 9.** 3 ng.

Ordering Information

Product #	Description	Pkg. Size
24615	Imperial Protein Stain	1 L
24617	Imperial Protein Stain	3 x 1 L

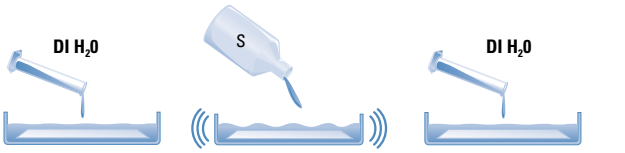
GelCode Blue Safe Protein Stain

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

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

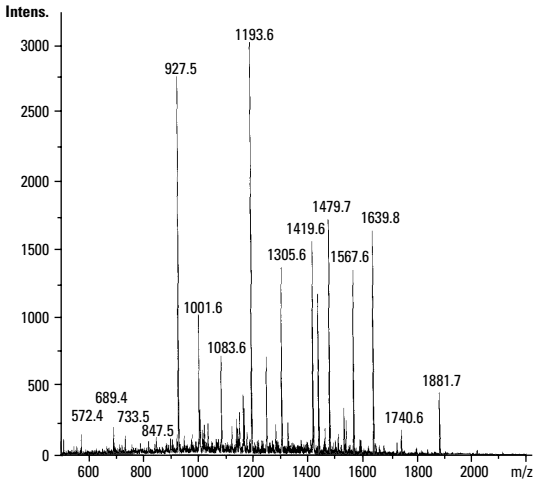
Highlights:

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



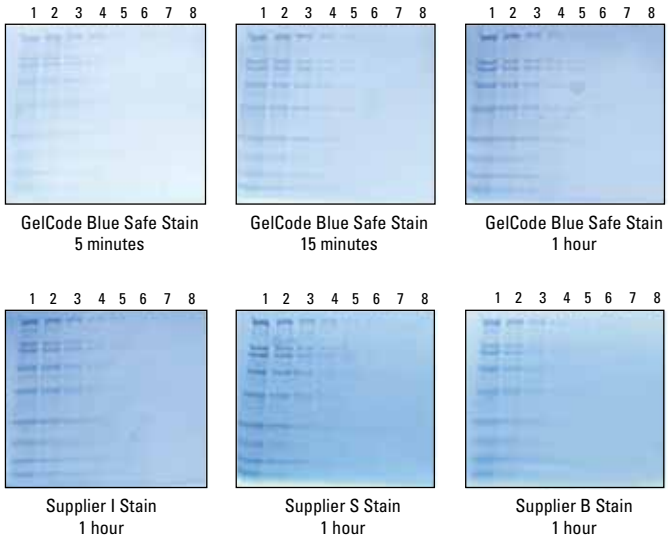
1. Wash the gel three times with deionized water (15 minutes).
2. Add activated GelCode Blue Safe Protein Stain (5 minutes-1 hour).
3. Destain in ultrapure water (1-2 hours or overnight).

Thermo Scientific GelCode Blue Safe Protein Stain protocol.

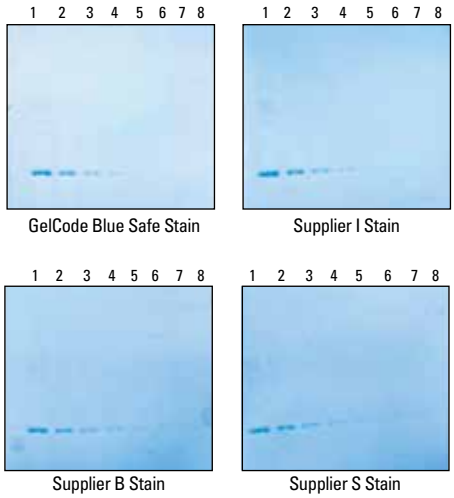


Mass spectrometry (MS)-compatible. BSA (2 µg) was electrophoresed on a 4-20% Thermo Scientific Precise Protein Gel (Product # 25224) and stained with GelCode Blue Safe Stain for one hour. BSA bands were excised from the gel and prepared for MALDI-MS analysis using the Thermo Scientific In-Gel Tryptic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

Panel A.



Panel B.

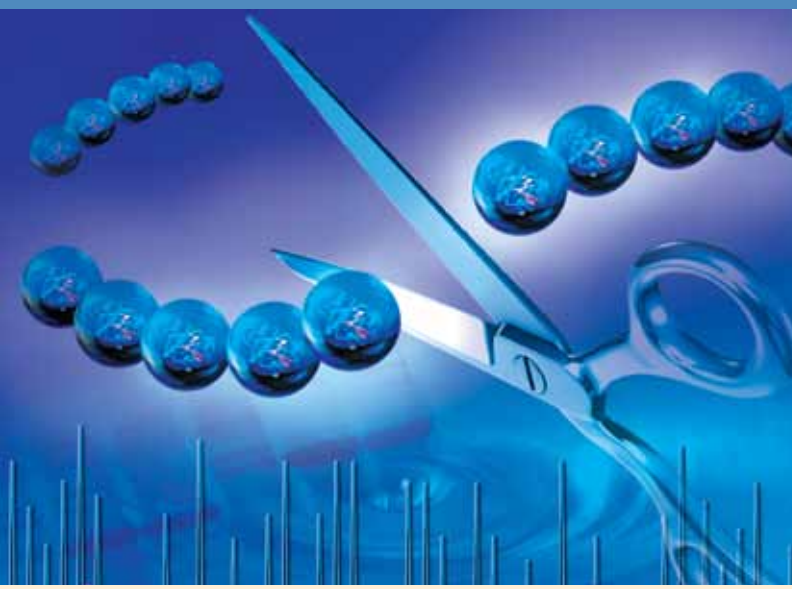


Thermo Scientific GelCode Blue Safe Protein Stain is fast and sensitive. Panel A. A mixture of proteins (myosin, β-galactosidase, phosphorylase B, BSA, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme and aprotinin) was electrophoresed on 4-20% Precise Protein Gels (Product # 25224).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

Ordering Information

Product #	Description	Pkg. Size
24594	**GelCode Blue Safe Protein Stain** *Sufficient reagent to stain up to 40-50 mini-gels.*	1 L
24596	**GelCode Blue Safe Protein Stain** *Sufficient reagent to stain up to 140-175 mini-gels.*	3.5 L

Protein Digestion



Following SDS-PAGE isolation and detection using Coomassie, fluorescence or silver stains, protein bands are reduced with either TCEP or DTT, followed by alkylation of cysteine residues with alkylating reagents like iodoacetamide or iodoacetic acid. A wide variety of endoproteinases, (e.g., trypsin, chymotrypsin, Glu-C, and Lys-C) are used to prepare peptides for MS/MS-based sequencing and protein identification. These proteolytic reagents also apply, seamlessly, to solution digest preparations as well.

Proteases for MS

High-purity, individual proteases for MS applications.

Effective protein characterization and identification by mass spectrometry (MS) begins with protein digestion. Trypsin is the protease of choice for accomplishing this task; however, digestion with alternative proteases, such as GluC, LysC, AspN or chymotrypsin, can improve sequence coverage. These Thermo Scientific Pierce Proteases are purified and optimized for protein digestion and analysis by sequencing or mass spectrometry.

Highlights:

- **Specific** – proteases offer specific cleavage at predictable sites, enabling exploration of primary protein structure
- **Increased sequence coverage** – better protein characterization results from overlapping peptides with complementary chromatographic, ionization and fragmentation properties
- **Compatible** – protocols are designed for MS applications
- **Robust** – streamlined data processing and analysis improves interpretation and confidence

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 LysC 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.

Table 1. Percent sequence coverage for Erk1.

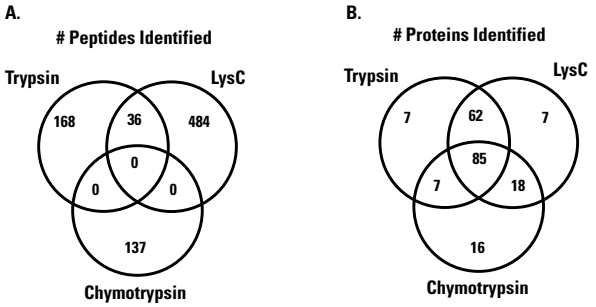
Results were obtained by digestion with individual proteases, MS/MS analysis with CID or ETD fragmentation methods, and pair-wise combination of search results in Thermo Scientific Proteome Discoverer MultiConsensus Reports for Erk1.

	Sequence Coverage (%)	
	CID	ETD
Trypsin	87	51
LysC	45	52
GluC	47	43
Trypsin alone + LysC alone	93	74
Trypsin alone + GluC alone	93	71

Table 2. Cleavage sites of various proteases.

Protease	Cleavage Specificity
Trypsin	Carboxyl side of arginine and lysine residues
LysC	Carboxyl side of lysine residues
GluC	Carboxyl side of glutamate or glutamate
AspN	Amino side of aspartate residues
Chymotrypsin	Carboxyl side of tyrosine, phenylalanine, tryptophan

Protein Digestion



Peptide and protein identification from in-gel digestion of HeLa lysate is improved when treated separately with different proteases; i.e., trypsin, chymotrypsin and LysC.

References

Biringer, R.G., *et al.* (2006). Enhanced sequence coverage of proteins in human cerebrospinal fluid using multiple enzymatic digestion and linear ion trap LC-MS/MS. *Brief Func Genom and Prot* **5**:144-153.

Schlosser, A., *et al.* (2005). Mapping of phosphorylation sites by a multi-protease approach with specific enrichment and nano *Anal Chem* **77**:5243-5250.

Wu, S-L., *et al.* (2005). Extended range proteomic analysis (ERPA): A new and sensitive LC-MC platform for high sequence coverage of complex protein with extensive post-translational modifications. *J Proteome Res* **4**:1155-1170.

Ordering Information

Product #	Description	Pkg. Size
90055	Trypsin	5 x 20 µg
90051	LysC	1 x 20 µg
90053	AspN	2 µg
90054	GluC	5 x 10 µg
90056	Chymotrypsin	4 x 25 µg

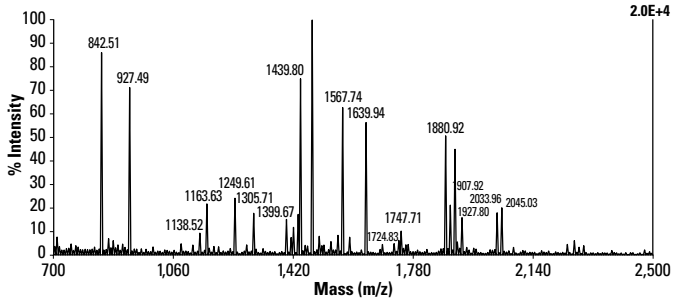
In-Gel Tryptic Digestion Kit

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:

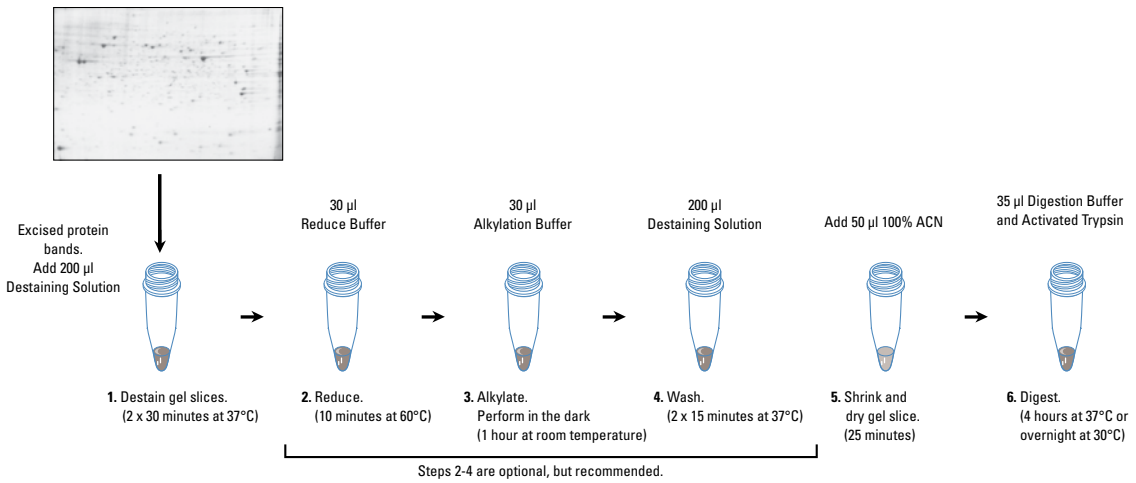
- **Convenient** – includes all necessary reagents for destaining Coomassie- or fluorescent dye-stained proteins, reduction and alkylation of cystines, and tryptic digestion
- **Robust** – the procedure and reagents produce reliable digestions and data generation using a wide range of conditions without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



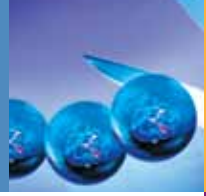
MALDI-TOF MS analysis of bovine serum albumin (BSA) digest. Ten nanograms (150 fmol) of BSA was separated by SDS-PAGE and stained with Thermo Scientific GelCode Blue Stain Reagent and then processed with the In-Gel Tryptic Digestion Kit. The resulting digest was treated with Thermo Scientific Pierce C-18 Spin Columns (Product # 89870) 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 *Sufficient for approximately 150 in-gel digestions.*	Kit
	Includes: Trypsin, Modified	20 µg
	Trypsin Storage Solution	40 µl
	Acetonitrile	70 ml
	Ammonium Bicarbonate	300 mg
	Tris[2-carboxyethyl]phosphine (TCEP)	500 µl
	Iodoacetamide	500 mg



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



In-Solution Tryptic Digestion and Guanidination Kit

Analyze proteins by mass spec with confidence.

Accurate identification of proteins and analysis of post-translational modifications by mass spectrometry (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 mass spectrometry (MS) provides a powerful tool for identifying proteins or analyzing post-translational modifications. Reliable mass spectral analysis requires accurate and complete digestion of the target proteins as well as modification of peptides to optimize ionization and detection. The In-Solution Tryptic Digestion and Guanidination Kit contains optimized procedures and reagents for reduction, alkylation, digestion and guanidination to provide reliable MS analysis of approximately 90 protein samples containing 0.025-10 µg of protein.

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

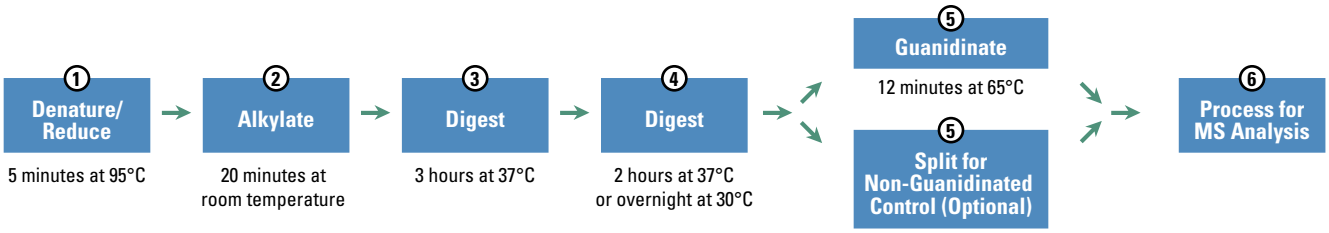
Table 3. Sequence coverage data for tryptic digestions with and without guanidination for three proteins.

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

† High levels of sequence coverage 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 600-2,000 m/z.

Ordering Information

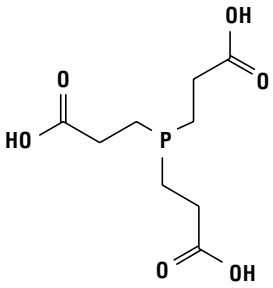
Product #	Description	Pkg. Size
89895	**In-Solution Tryptic Digestion and Guanidination Kit**	Kit
	Sufficient reagents for preparing 90 digests.	
	Includes: Trypsin, Modified	20 µg
	Trypsin Storage Solution	50 µl
	Ammonium Bicarbonate	50 mg
	No-Weigh™ DTT	7.7 mg
	Iodoacetamide (IAA)	500 mg
	O-Methylisourea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



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

Bond-Breaker TCEP Solution, Neutral pH

Thermo Scientific TCEP Solution is the efficient, odor-free alternative to sample reduction prior to SDS-PAGE analysis.

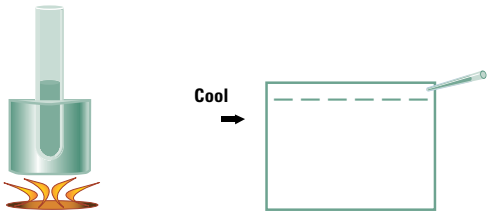


TCEP
M.W. 250.15

Highlights:

- Ready-to-use, odorless, stable and neutral 0.5 M TCEP solution
- Eliminates TCEP•HCl stock solution preparation and neutralization prior to SDS-PAGE gel loading
- Convenient, time-saving format for TCEP reductions
- Neutral pH minimizes possibility of amide bond cleavage during reduction
- Room temperature-stable, saves valuable refrigerator space
- Contributes to more pleasant, safer laboratory environment

1. Prepare Reducing Sample Buffer: Bond-Breaker TCEP Solution, 1:10 dilution in 2X Sample Buffer.
2. Mix equal volumes of Sample and 2X TCEP Reducing Sample Buffer.



3. Heat to 95°C, 5 minutes.
4. Cool and load for SDS-PAGE analysis.

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

Ordering Information

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

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

TCEP•HCl

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

Highlights:

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

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

Ordering Information

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

No-Weigh DTT

Don't waste your talents at the balance!

Thermo Scientific No-Weigh DTT is dithiothreitol supplied as dry, room temperature-stable, single-use aliquots in foil-sealed microtubes. Each No-Weigh Microtube contains 7.7 mg of DTT, the exact amount needed to make a 500 mM solution by addition of 100 µl of water or buffer. With this convenient format, there is no more waste and hassle making and attempting to store stock solutions of more reagent than is needed for each use.

Highlights:

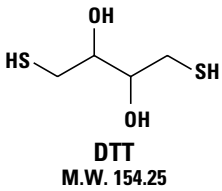
- **Saves time** – puncture foil, add 100 µl of water and remove the volume needed
- **Eliminates waste** – easily prepare a single-use amount (100 µl) of 500 mM DTT
- **Eliminates worry** – no more concerns about stock solution stability and degradation; make only what you need for each day, keeping the remainder as stable, dry, unopened product

Ordering Information

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

DTT

A water-soluble reagent that reduces disulfide bonds.



DTT
M.W. 154.25

Highlights:

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

Ordering Information

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

Alkylating Reagents

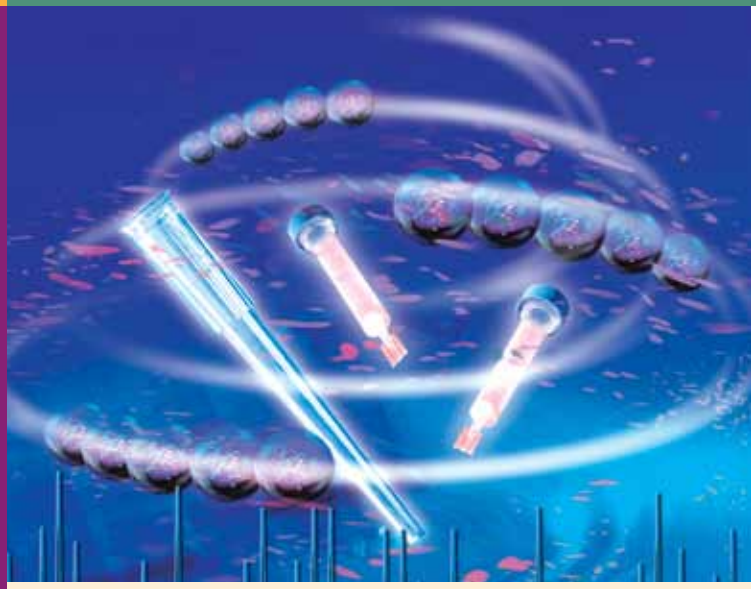
Protein modification reagents for mass spectrometry.

Thermo Scientific Single-Use Iodoacetamide (IAM) is MS-grade IAM that is packaged in single-use quantities. Because dissolved iodoacetamide is unstable in light, it must be prepared immediately before alkylation of reduced proteins and protease digestion for MS analysis. Each dried, stabilized iodoacetamide aliquot is easily dissolved to yield just the right amount for individual experiments without the usual concerns about waste and storage of the excess. The Single-Use Iodoacetamide formulation is convenient and works well with other products for MS workflows, including Thermo Scientific Halt Phosphatase Inhibitor Cocktails, DTT, TCEP, MS-grade proteases and MALDI matrices. Additional alkylating reagents and protein modification reagents are listed here.

Ordering Information

Product #	Description	Pkg. Size
90034	Iodoacetamide (IAM), Single-Use Each tube yields 375 mM solution when dissolved in 132 µl of ammonium biocarbonate.	24 x 9.3 mg
35603	Iodoacetic Acid (IAA)	500 mg
23011	Methyl methanethiosulfonate (MMTS)	200 mg
23030	N-Ethylmalemide (NEM)	25 g

Peptide Enrichment and Clean Up



Successful analysis of low abundant proteins and identification of post-translationally modified (PTM) peptides requires at least two steps: enrichment and cleanup. 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 this purpose: Thermo Scientific Phosphoprotein Kit for proteins or Thermo Scientific Pierce Fe-NTA Phosphopeptide and Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kits for phosphopeptides. After isolation of phosphorylated peptides, salts and buffers can be removed using either the graphite or C-18 spin columns. In addition to providing convenient tools for removing salts and buffers, the Thermo Scientific Pierce Detergent Removal Columns remove detergents that commonly interfere with MS analysis.

Proper choice of mobile phases and acidic ion-pairing reagents is a prerequisite for achieving good results from LC-MS/MS (liquid chromatography with tandem mass spectrometry) analysis. The Thermo Scientific Pierce product portfolio has the solvents (Water and acetonitrile) and ion-pairing agents (TFA, Formic Acid, HFBA) needed for analysis of peptides by MS/MS. The analysis of peptide digests by MALDI-TOF, requires specific matrices (crystalline energy absorbing dye molecules). All of the common matrices are available in our convenient single-use format.

Detergent Removal Spin Columns

Quickly remove detergents from protein samples

Detergents or surfactants are important for solubilizing, stabilizing and disaggregating proteins; however, detergents interfere with many downstream analysis methods. Therefore, it is often crucial to remove non-bound detergents before using proteins samples for ELISA, isoelectric focusing or mass spectrometry (MS). Unfortunately, typical sample clean-up methods, such as dialysis and size-exclusion chromatography, are often ineffective at removing detergents. We developed an efficient and rapid spin-column method (Figure 1) for removing detergents from protein and peptide solutions. The Thermo Scientific Pierce Detergent Removal Resin efficiently removes high concentrations of detergents from 0.01-1 ml samples with minimal sample loss.

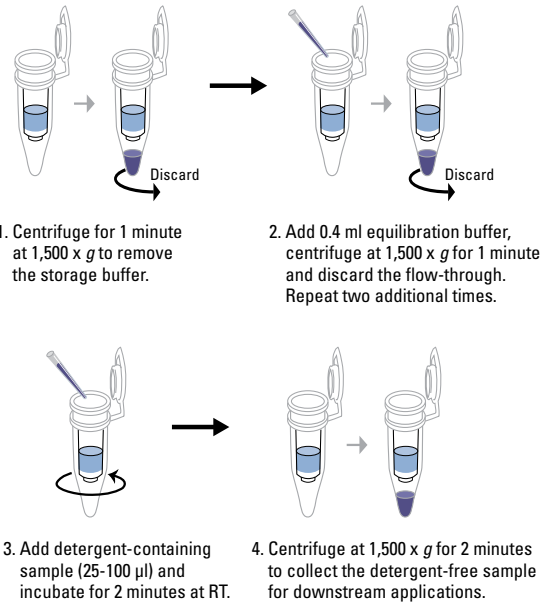


Figure 1. Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Columns (0.5 ml).

Results and Discussion

We processed protein samples containing a wide range of detergents with the Pierce Detergent Removal Resin. Detergents at concentrations from 1 to 5% were effectively removed with generally > 90% protein recovery (Table 1, on the following page).

Table 1. Detergents are effectively removed with high protein recovery.*

Detergent and Removable Concentration (%)	Detergent Removal (%)	BSA Recovery (%)
SDS (2.5)	99	95
Sodium deoxycholate (5)	99	100
CHAPS (3)	99	90
Octyl glucoside (5)	99	90
Octyl thioglucoside (5)	99	95
Lauryl maltoside (1)	98	99
Triton X-100 (2)	99	87
Triton X-114 (2)	95	100
NP-40 (1)	95	91
Brij-35 (1)	99	97

**Samples (0.1 ml containing 100 µg BSA and detergent) were processed through 0.5 ml of Pierce Detergent Removal Resin and the percent detergent removed was determined. Similar results were produced for insulin (5.7 kDa), α–lactalbumin (14.2 kDa) and carbonic anhydrase (29 kDa) (data not shown).*

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. We used the Pierce Detergent Removal Resin to remove a variety of detergents from BSA and HeLa cell lysate tryptic digests followed by LC-MS/MS and MALDI-MS analysis (Figures 2 and 3).

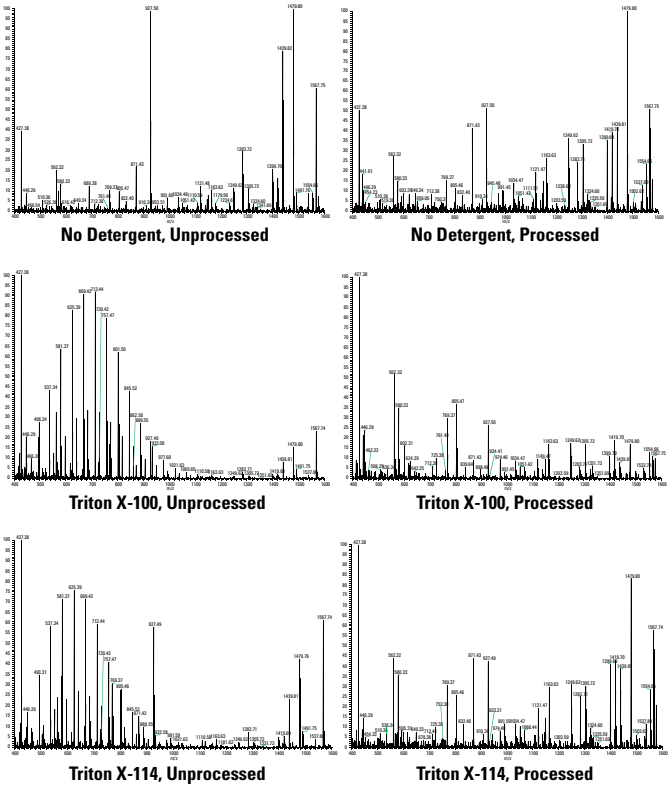


Figure 2. Peaks corresponding to detergents are eliminated in processed samples, allowing reliable peptide/protein identification. BSA tryptic digest (0.1 ml, 100 µg) containing a detergent was processed through 0.5 ml of Pierce Detergent Removal Resin and subjected to MALDI-MS analysis on a MALDI-Orbitrap Mass Spectrometer. Similar results were produced for samples containing CHAPS, NP-40 and SDS (data not shown).

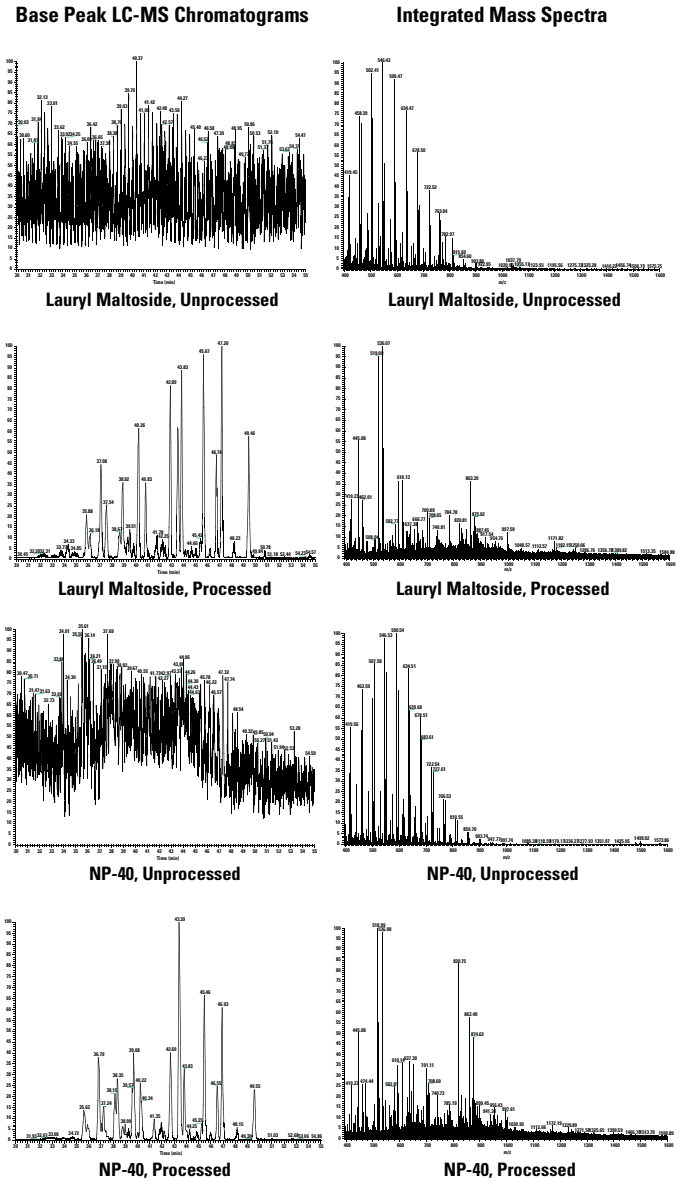


Figure 3. Effective detergent removal eliminates interference and allows high sequence coverage analysis of BSA. Tryptic digests (0.1 ml, 100 µg) containing detergent were each processed through 0.5 ml of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. **Left Column:** Base peak LC-MS chromatograms. **Right Column:** Integrated mass spectra. Similar results were produced for Brij-35, octyl glucoside, octyl thioglucoside and SDS (data not shown).

Peptide Enrichment and Clean Up

After processing samples, the high baseline caused by detergents is reduced or eliminated. Analysis of digested HeLa cell lysates by LC-MS/MS resulted in an approximate four-fold increase of identified peptides compared to a contaminated sample and equivalent numbers of peptides compared to a control sample, indicating minimal losses of peptides (Figure 4).

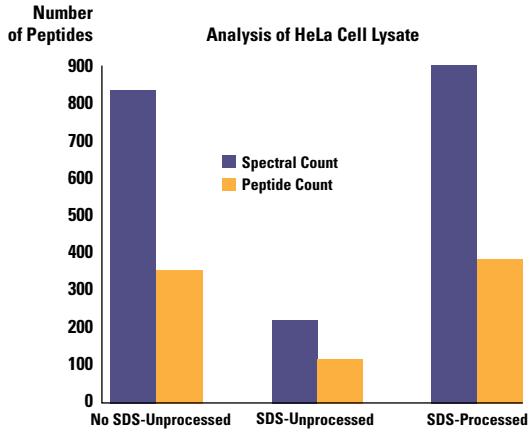


Figure 4. Effective detergent removal enables greater peptide identification. A tryptic digest of HeLa cell lysate (0.1 ml, 100 µg) containing 1% SDS was processed through 0.5 ml of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. The processed sample allowed similar numbers of identified peptides as digests containing no SDS. Peptide identification is greatly reduced in sample containing SDS.

Methods

Detergent removal analysis: Protein samples (1 mg/ml) containing detergent in 0.15 M NaCl and 0.05% sodium azide were processed through 0.5 ml of Pierce Detergent Removal Resin. Residual SDS was measured using Stains-All (Sigma Aldrich);[1] Triton X-100, Triton X-114 and NP-40 were measured by absorbance at 275 nm (protein absorbance was subtracted); sodium deoxycholate, CHAPS, octyl glucoside, octyl thioglucoside and lauryl maltoside were measured using concentrated sulfuric acid and phenol.[2] Removal of Brij-35 was monitored by LC-MS/MS and MALDI-MS analysis. Protein concentration was determined with the Thermo Scientific Pierce BCA Protein Assay (Product # 23225).

LC-MS/MS and MALDI-MS analysis: BSA and HeLa lysate (1 mg/ml) in 50 mM ammonium bicarbonate buffer, pH 8.0 were digested overnight with trypsin at 37°C (enzyme-to-protein ratio, 1:50) in the presence of 1% of each detergent except SDS, which was added after trypsin digestion. Each sample (0.1 ml) was processed through 0.5 ml of Pierce Detergent Removal Resin. Control samples (unprocessed) were not processed. Samples were diluted and loaded (~1.5 pmol) directly onto a C18 column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer. For MALDI-MS analysis, samples were diluted 1:15 (1 pmol) and analyzed using a Thermo Scientific MALDI-Orbitrap Mass Spectrometer. The matrix was alpha-cyano 4-hydroxy cinnamic acid (5 mg/ml) with acetonitrile/water/0.1% TFA as a co-solvent.

References

1. Rusconi, F., *et al.* (2001). Quantitation of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal. Biochem.* **295**:31-37.
2. Urbani, A. and Warne, T. (2005). A colorimetric determination for glycosidic and bile salt-based detergents: applications in membrane protein research. *Anal. Biochem.* **336**:117-124.

Ordering Information

Product #	Description	Pkg. Size
87776	Pierce Detergent Removal Spin Column, 125 µl	25 columns
87777	Pierce Detergent Removal Spin Column, 0.5 ml	25 columns
87778	Pierce Detergent Removal Spin Column, 2 ml	5 columns
87779	Pierce Detergent Removal Spin Column, 4 ml	5 columns
87780	Pierce Detergent Removal Resin	10 ml

Fe-NTA Phosphopeptide Enrichment Kit

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

The new Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit enables fast and efficient enrichment of phosphorylated peptides. These spin columns are easy to use and require less than 1 hour to process protein digests or strong cation-exchange peptide fractions for analysis by mass spectrometry (MS).

Highlights:

- Convenient spin format for parallel processing of multiple samples
- High-binding capacity resin for enriching up to 150µg of phosphopeptides per column
- Excellent enrichment and recovery of phosphopeptides

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.

Mass spectrometry 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 new 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.

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 Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail (Product # 78440). Protein concentration was determined with Thermo Scientific Pierce 660nm Protein Assay (Product # 22660). Proteins were reduced with Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH (Product # 77720), alkylated with single-use iodoacetamide, (Product # 90034) digested overnight with MS-grade trypsin (Product # 90055), 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 (Eksigent) with a ProteoPep II C18 Column (75µm ID x 20cm, New Objective) 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 (Matrix Science) and Scaffold 2.6 (Proteome Software).

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 new Thermo Scientific 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 5 and Table 2).

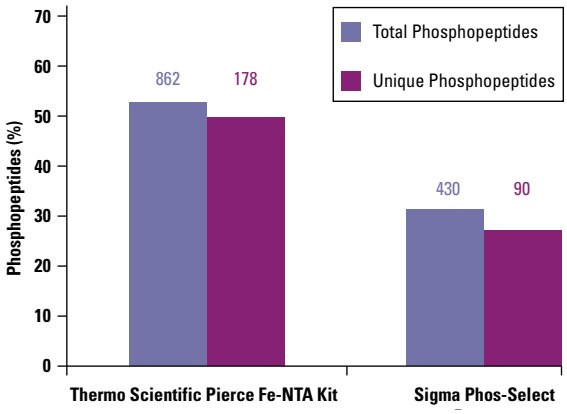


Figure 5. Our kit enriched a greater percentage of total and unique phosphopeptides from U2-OS cell lysate. Numbers refer to the total and unique number of phosphopeptides identified in each condition. A summary of results is listed in Table 1.

Table 2. Average phosphopeptide enrichment results from duplicate experiments.§

	Thermo Scientific Pierce Fe-NTA	Sigma Phos-Select
Total phosphopeptides	862	430
Total peptides	1,753	1,665
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®.

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 6). Phosphopeptide enrichment greatly reduces sample complexity and enables effective identification and characterization of phosphorylated peptides by MS (Figure 7).

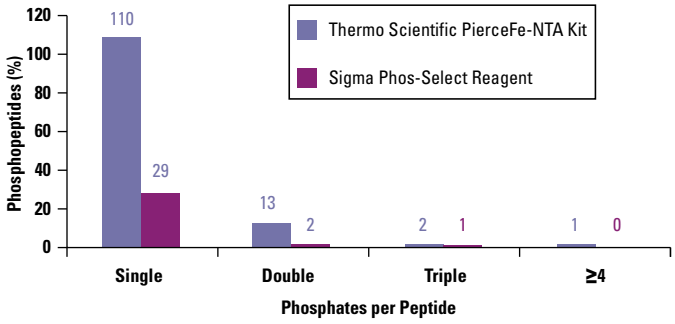


Figure 6. The Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit effectively captures phosphopeptides with multiple phosphates.

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).

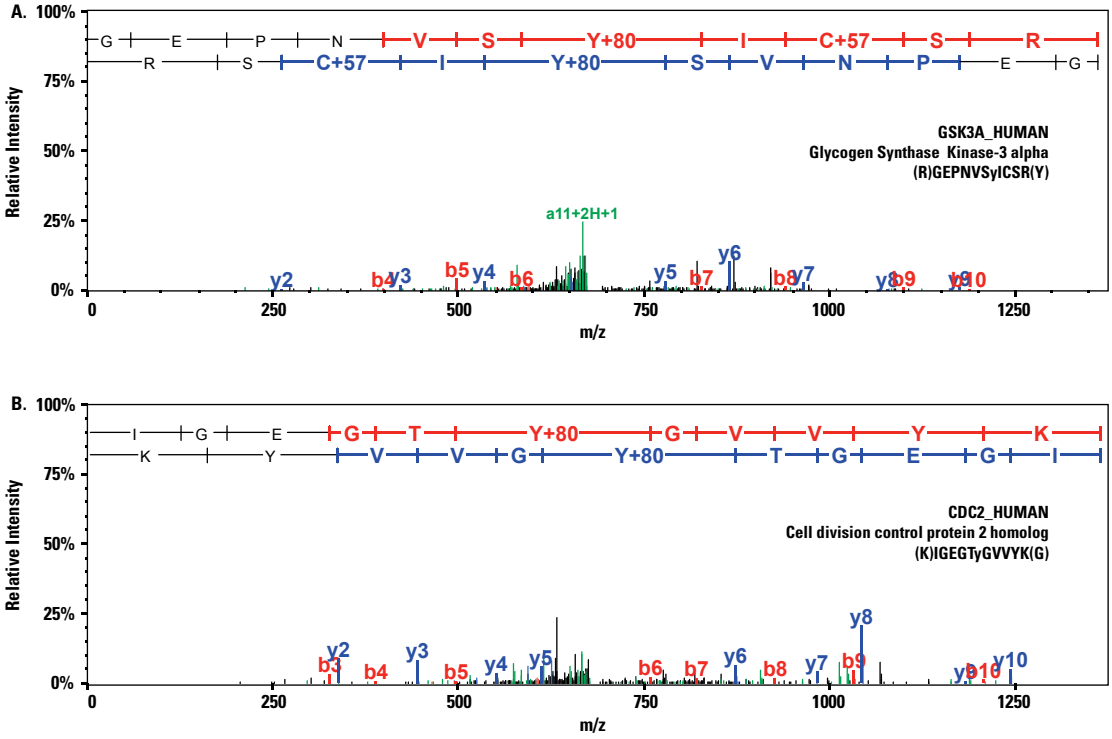


Figure 7. Example MS/MS spectra from enriched phosphopeptides. Lowercase letters indicate the position of tyrosine phosphorylation in enriched peptides. **Panel A:** Glycogen synthase-3 alpha. **Panel B:** cdc2/cyclin dependent kinase 1.

Ordering Information

Product #	Description	Pkg. Size
88300	Pierce Fe-NTA Phosphopeptide Enrichment Kit *Sufficient for 30 samples*	Kit
Related Products		
88302	Pierce Graphite Spin Columns	30 columns

Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit

TiO_2 magnetic particles for high throughput phosphopeptide isolation.

The Thermo Scientific Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit is 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 mass spectrometry (Table 3).

The high-performance, iron oxide, superparamagnetic particles are validated and optimized for use with high-throughput magnetic platforms, such as the Thermo Scientific 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 mass spectrometry
- **Optimized for HTS** – 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 non-specific 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 100 fmol of phosphoprotein

Table 3. Phosphopeptide enrichment improves MS-identification of phosphoproteins. Two milligrams of a tryptic digest prepared from periferal blood mononuclear cells (lymphocytes) with and without phosphopeptide enrichment were analyzed by MS. Enrichment was performed with the Thermo Scientific Pierce Titanium Dioxide Phosphopeptide Enrichment Kit using the Thermo Scientific KingFisher 96 Instrument. Samples were analyzed on a Thermo Scientific 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 Titanium Dioxide Phosphopeptide Enrichment Kit**	Kit
	Includes: TiO_2 Magnetic Beads (20X)	1 ml
	Binding Buffer	100 ml
	Washing Buffer	25 ml
	Elution Buffer	3 ml
	Thermo-Fast 96 Robotic PCR Plate (0.2 ml wells)	2 plates
88812	**Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit, Trial Size**	Kit
	Includes: TiO_2 Magnetic Beads (20X)	0.25 ml
	Binding Buffer	100 ml
	Washing Buffer	25 ml
	Elution Buffer	3 ml
	Thermo-Fast 96 Robotic PCR Plate (0.2 ml wells)	2 plates

C18 Tips

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

The Thermo Scientific Pierce C18 Tips enable efficient purification of peptides and small proteins before mass spectrometry, 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 100 µl bed volumes processing up to 8 or 80 µg 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

Improve protein analysis results with Pierce C18 Tips by removing urea, salts and other contaminants before MS analysis (Figures 8 and 9). The tips are ideal for matrix-assisted laser desorption ionization (MALDI) or nanoelectrospray ionization techniques. They are available in convenient 10 and 100 µl tips with binding capacities of 8 and 80 µg, respectively.

Ordering Information

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

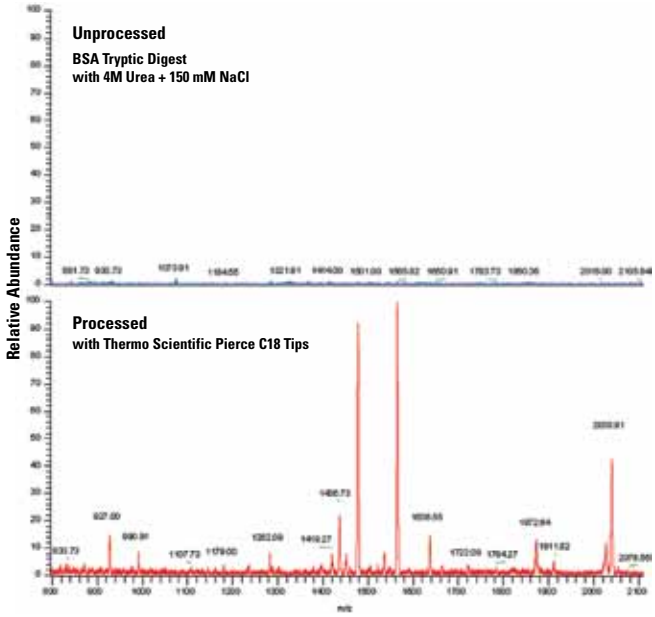


Figure 8. Removing urea and NaCl eliminates interference in MS chromatograms. A bovine serum albumin (BSA) tryptic digest was analyzed on a Thermo Scientific MALDI-Orbitrap XL Hybrid Mass Spectrometer. Digests and samples containing 150 mM NaCl and 4 M urea were analyzed with or without processing with Pierce C18 Tips (10 µl).

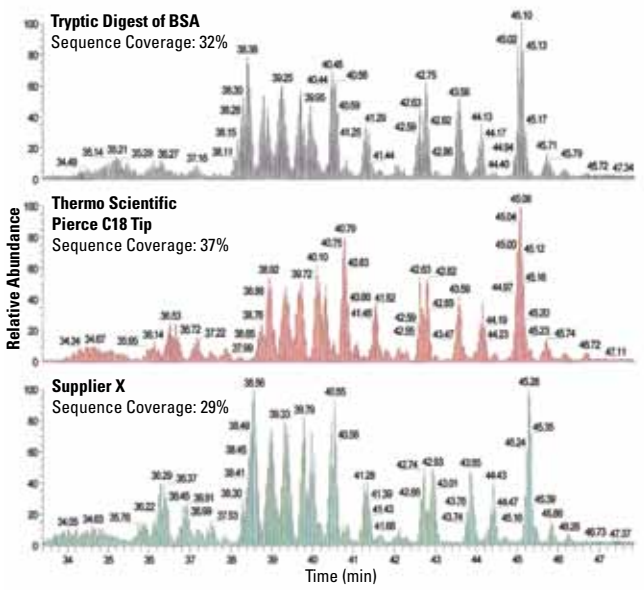


Figure 9. The Thermo Scientific Pierce C18 Tips outperform other suppliers' tips. BSA tryptic digests were analyzed either directly on a Thermo Scientific LTQ-XL Ion Trap Mass Spectrometer or after processing with Pierce C18 Tips (100 µl) or supplier X tips. Base peak chromatograms of the peptide elution 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.

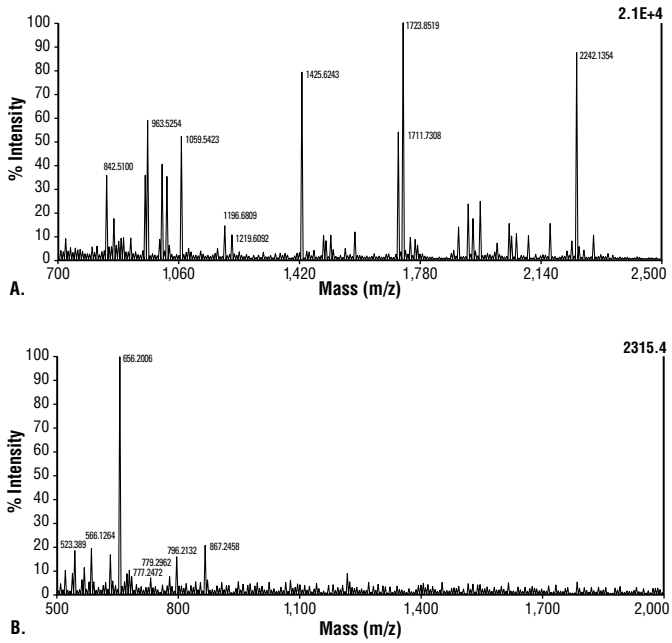
C18 Spin Columns

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 Thermo Scientific 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 (10-150 µl) in approximately 30 minutes without laborious repeat pipetting or specialized equipment. Pierce C18 Spin Columns can be used effectively for processing peptides derived from 10 ng to 30 µg of protein. Sensitivity and detection limits are dependent on the downstream application.

Highlights:

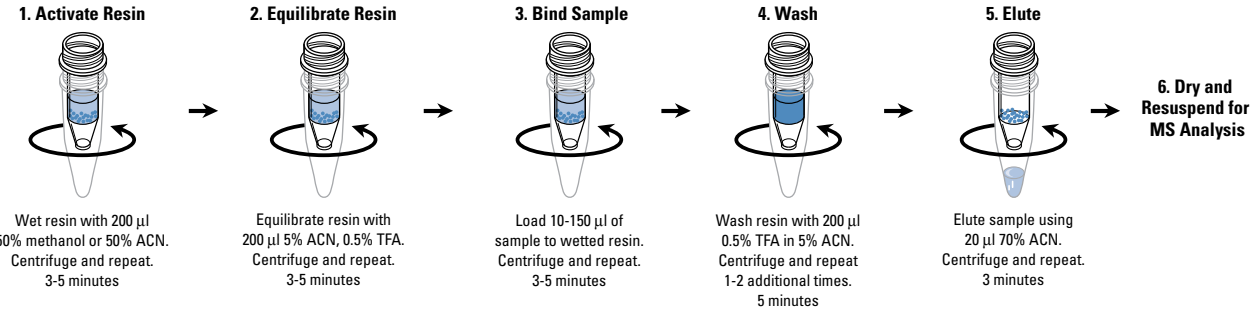
- **Removes MS-interfering contaminants** – significantly reduces signal suppression and improves signal-to-noise ratios and sequence coverage. Works on a variety of reverse-phase-compatible contaminants
- **Robust** – works with a wide variety of load volumes (10-150 µl) and concentrations. No need 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



Effective clean up of mass spectrometry sample with Thermo Scientific Pierce C18 Spin Columns. 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. **B.** MALDI-TOF MS analysis of an identical digest that has not been C18 processed.

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 C18 Spin Column protocol summary.

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 (MS) analyses of samples from protein digests (Figure 10), strong-cation exchange fractions, and enriched phosphopeptides eluted from TiO_2 and immobilized metal affinity chromatography (IMAC) columns and tips.

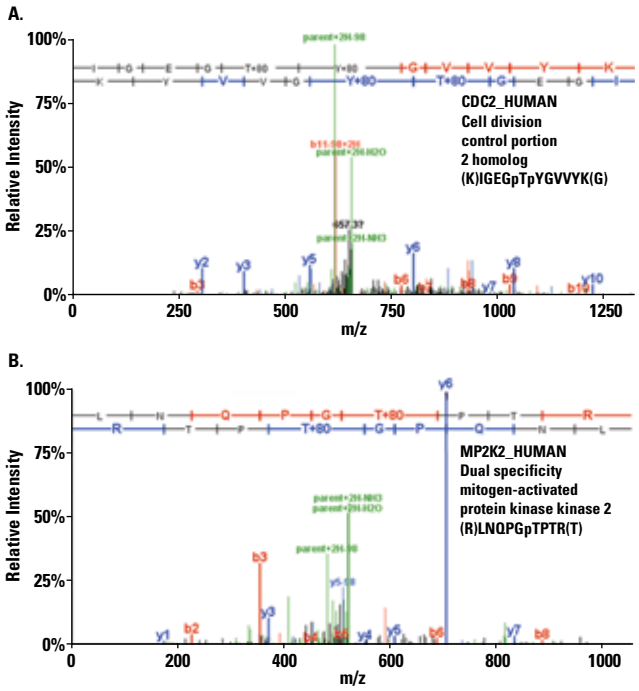


Figure 10. Graphite clean-up enables phosphopeptide identification. U2OS human osteosarcoma cells synchronized at the G2/M boundary with nocodazole (200 ng/ml, 36 hours) were lysed with 6 M guanidine•HCl. After enzymatic protein digestion (100 µg), phosphopeptides were enriched with IMAC and desalted with Pierce Graphite Spin Columns or C18 tips before LC-MS/MS analysis on a Thermo Scientific Orbitrap XL Mass Spectrometer. Two representative spectra are shown for two phosphopeptides not observed after C18 cleanup. **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.

Highlights:

- Convenient spin format for parallel processing of multiple samples
- High-binding capacity with excellent recovery of up to 100 µg of hydrophilic peptides per column
- Porous graphite resin for cleaning up phosphopeptide samples before MS analysis

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 11). The spin columns are ideal for matrix-assisted laser desorption ionization (MALDI) or nanoelectrospray ionization techniques.

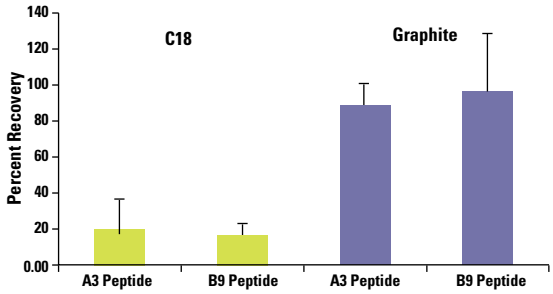


Figure 11. Improved recovery of representative hydrophilic phosphopeptides using graphite spin columns. Stable isotope-labeled A3 and B9 peptides (10 pmol) were acidified with 1% trifluoroacetic acid, processed according to instructions for C18 tips or the Pierce Graphite Spin Columns and eluted with 50% acetonitrile/0.1% formic acid. The corresponding heavy isotope-labeled peptides (5 pmol) 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 10 mg in 0.5 ml of slurry*	30 columns

Trifluoroacetic Acid (TFA)

High-purity TFA in convenient, high-performance packaging.

Thermo Scientific Trifluoroacetic Acid (TFA) is manufactured to the highest specifications to ensure the integrity of your data, maximize sensitivity in your assay and prolong the life of your equipment. Each lot of trifluoroacetic acid is tested to meet strict specifications that are critical to HPLC and MS methods. TFA is the most commonly used ion pairing agent in reverse-phase 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.

Highlights:

- **Purity** – superior purity (≥ 99.5%) and exceptional clarity, allowing sensitive, nondestructive peptide detection at low UV wavelengths in reverse-phase HPLC protein and peptide separation systems[1]
- **Versatility** – performs incomparably in protein sequencing applications[2,3] and solid-phase peptide synthesis[4] and as a protein/peptide solubilizing agent[2,3]
- **High-performance packaging** – packaged in amber glass with protective PTFE-lined fluorocarbon caps or ampuled under nitrogen
- **Economical convenience** – choose the package format that works best for your specific applications; e.g., the 1 ml ampules provide a simple way to prepare liter quantities of 0.1% TFA for stationary and mobile phases in reverse-phase chromatography

Properties of Trifluoroacetic Acid

- Molecular Formula: CF_3COOH
- MW: 114.03 g/mol
- Density: 1.53 g/ml, 20°C
- Melting/boiling point: –15°C / 72°C
- CAS # 76-05-01

Applications for Trifluoroacetic Acid

- Ion pair reagent for reverse-phase HPLC[1-3]
- Protein/peptide sequencing[4-7]
- Protein/peptide solubilizing agent[4-7]
- Solid-phase peptide synthesis
- Amino acid analysis

References

1. Chicz, R.M. and Regnier, F.E. (1990). *Methods Enzymol.* **182,** 392-421.
2. Zarember, K.A., *et al.* (2002). *Infect. Immun.* **70,** 569-576.
3. Lassy, R.A. and Miller, C.G. (2000). *J. Bacteriol.* **182,** 2536-2543.
4. Smith, B.J. (1997). *Protein Sequencing Protocols.* Humana Press.
5. Allen, G. (1989). Sequencing of Proteins and Peptides, Second Revised Addition. Elsevier.
6. Backstrom, J.R., *et al.* (1996). *J. Neurosci.* **16,** 7910-7919.
7. Hermann, P.M., *et. al.* (2000). *J. Neurosci.* **20,** 6355-6364.

Ordering Information

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

Heptafluorobutyric Acid

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

$CF_3CF_2CF_2CO_2H$

Heptafluorobutyric Acid
M.W. 214.04

Highlights:

- Clear, colorless liquid
- Purity is ≥ 99.5% by GC; ≤ 0.1% water, no sulfate
- Sequencing reagent for classical and automated Edman degradation of peptides and proteins
- Density: 1.645
- Boiling Point: 120°C

References
Bennett, H.P., *et al.* (1981). *Biochemistry* **20,** 4530-4538.
Bennett, H.P.J., *et al.* (1980). *J. Liquid Chromatogr.* **3,** 1353-1366.
Hearn, M.T.W. and Hancock, W.S. (1979). *Trends Biochem. Sci.* **4,** N58-N62.

Ordering Information

Product #	Description	Pkg. Size
25003	Heptafluorobutyric Acid	100 ml
53104	Heptafluorobutyric Acid	10 x 1 ml

Acetonitrile and Water (LC/MS Grade)

Ideal for HPLC and mass spectrometry applications.

Highlights:

- Distilled in glass and filtered through 0.2 micron PTFE membranes
- Packed in solvent-rinsed glass bottles, sealed with PTFE-lined screw cap bottles for ultimate protection

Ordering Information

Product #	Description	Pkg. Size
51101	Acetonitrile	1 L
51140	Water	1 L

Formic Acid

Use our 1 ml ampules and have confidence in the integrity of your mobile-phase.

Thermo Scientific Formic Acid Ampules are a convenient, contamination-free alternative for preparing elution solvents for HPLC separations of proteins and peptides. Open and use the vial when you need it, ensuring that the formic acid has not been compromised. The high-purity 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 ampule packaging provides reliability and convenience that adds value to both the chromatographic and MS results.

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 trifluoroacetic acid (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.

Properties of Formic Acid

- Molecular Formula: HCOOH (CH_2O_2)
- MW: 46.02 g/mol
- Density: 1.22 g/ml
- CAS # 64-18-6
- Refractive Index: 1.3701-1.3721(20°C)
- Freezing/Flash Point: 8°C/69°C

Highlights:

- **> 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** – amber glass, pre-scored, nitrogen-flushed ampules 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 1 L of solvent yields a mobile phase of the most common formic acid concentration

Ordering Information

Product #	Description	Pkg. Size
28905	Formic Acid, 99+%	10 x 1 ml

Single-Use MALDI Matrices

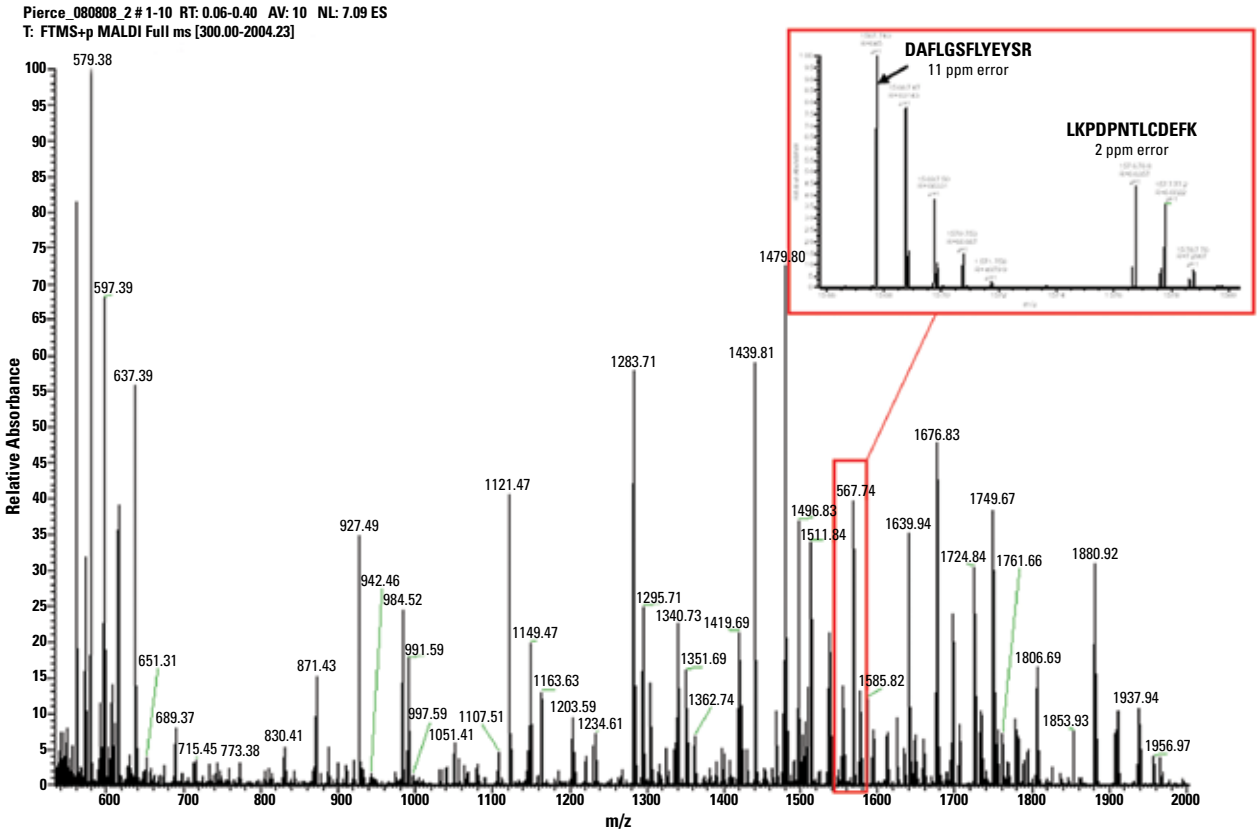
Ready-to-use, high-quality mass spectrometry reagents.

Thermo Scientific Single-Use MALDI Matrices are highly purified, recrystallized reagents supplied in a convenient, single-use, microtube format for use in mass spectrometry (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.

Ordering Information

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



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

Nanoflow Columns

High sensitivity, flexible silica columns.

Highlights:

- 75 µm and 150 µm ID for greater sensitivity
- Wide choice of stationary phases for alternative selectivity options
- Ideal for LC/MS applications

Thermo Scientific Chromatography Columns are available in nanobore formats for nanospray LC/MS applications. At flow rates of nL/min versus mL/min, they offer higher sensitivity with greater signal-to-noise ratio than traditional capillary columns used with electrospray.

Column Hardware

IntegraFrit™ Columns have an integral highporosity frit, behind which is the packed chromatography bed. The frit end of the fused-silica column is polished flat to ensure a clean connection to the emitter of choice. PicoFrit® Columns eliminate post-column performance losses by spraying directly from the column tapered end, boosting MS performance compared to that provided by a column attached to a tip.

High Sensitivity Analysis

Analytes can be detected, identified and quantified at femtomole levels and below. Figure 12 shows base peak chromatograms for only 200 amol of a yeast 5 protein digest mixture using 1.9 µm Hypersil GOLD™ and 5 µm BioBasic™ 18 materials packed into 50 mm x 0.075 mm PicoFrit columns.

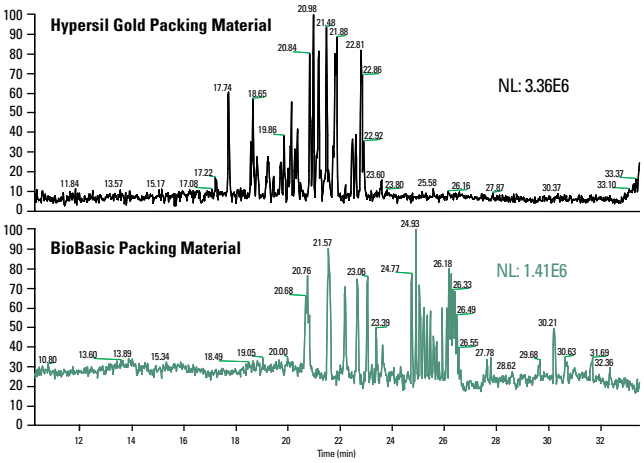


Figure 12. Base peak chromatograms for 200 amol of a yeast 5 protein digest on 1.9 mm Hypersil GOLD and BioBasic PicoFrit Columns.

Stationary Phases

Thermo Scientific Nanobore Columns are available in a wide variety of packing materials to suit the wide diversity of biomolecules.

- BioBasic Columns offer silica in C18, C8 and C4 plus SCX phase, all with a 300Å pore size for larger biomolecules.
- Hypersil GOLD Columns offer outstanding peak shape plus 1.9 µm particles for enhanced speed and efficiency.
- Hypersil GOLD aQ Columns are a polar endcapped C18 stationary phase that can be used with 100% aqueous mobile phases.
- Hypersil GOLD PFP Columns offer alternative selectivity, particularly where an analyte contains an aromatic ring.
- Hypercarb Columns offer retention and separation without derivatization.

For very polar molecules such as oligosaccharides, nucleotides, nucleosides and hydrophilic peptides, Hypercarb offers retention and separation without derivatization.

For more information on these and other Thermo Scientific Chromatography Columns, please visit www.thermoscientific.com/columns

Instrumentation

Successful proteomic analyses require optimum technology in all phases of the workflow, including effective sample preparation; robust, reproducible, and sensitive data acquisition; and powerful data analysis. We can provide complete liquid chromatography/mass spectrometry workflow solutions for a wide range of proteomic analyses, from qualitative discovery to quantitative discovery to targeted quantitative verification. Thermo Scientific tandem mass tag (TMT) technology and SILAC kits enhance relative protein quantitation, while custom AQUA HeavyPeptides provide standards for absolute protein quantitation. A wide range of Thermo Scientific ion trap, Orbitrap, FT, and triple quadrupole mass spectrometers (Table 1) ensure exactly the right technology and level of performance is available for every proteomic application. Specialized Thermo Scientific software – Proteome Discoverer, SIEVE, ProSightPC, Pinpoint, and more – ensures as much valuable information as possible is extracted from the acquired data.

Table 1. Thermo Mass Spectrometry Systems and Capabilities

Instrument	SILAC/ICAT/Mass Tags	Isobaric MassTags	Selective Reaction Monitoring (Targetted quantitation)	Discovery Proteomics (Data Dependent Mass Spectrometry – Protein Identification)
LTQ Velos	No	Yes (PQD)*	Yes (but limited)**	Good
LTQ XL ETD	No	Yes (PQD)	Yes (but limited)	Good
LTQ XL	No	Yes (PQD)	Yes (but limited)	Good
MALDI LTQ XL	No	Yes (PQD)	Yes (but limited)	Good
LTQ Orbitrap Velos	Yes	Yes (PQD and HCD)	Yes (but limited)	Excellent
LTQ Orbitrap XL ETD	Yes	Yes (PQD and HCD)	Yes (but limited)	Excellent
LTQ Orbitrap XL	Yes	Yes (PQD and HCD)	Yes (but limited)	Excellent
MALDI LTQ Orbitrap XL	Yes	Yes (PQD and HCD)	Yes (but limited)	Fair
LTQ Orbitrap Discovery	Yes	Yes (PQD only) no HCD cell	Yes (but limited)	Excellent
LTQ FT Ultra	Yes	No	Yes (but limited)	Excellent
TSQ Vantage	No	No	Yes	Not recommended
TSQ Quantum Ultra	No	No	Yes	Not recommended
TSQ Access max	No	No	Yes	Not recommended

**PQD option is included in all instruments via the Xcalibur software.*
***Only practical if you have small target lists (10 or less).*
****Unless you see the word MALDI, the instrument requires (usually) an LC interface.*

LTQ FT Ultra Mass Spectrometer

The Thermo Scientific LTQ FT Ultra Mass Spectrometer combines the most advanced Ion Trap and Fourier Transform Ion Cyclotron Resonance (FT-ICR) technologies into a single instrument with unprecedented analytical power and versatility. Ultra-high resolution and sensitivity coupled with sub ppm mass accuracy, and MS^n are universally available for the touhest applications demanding rigorous structural characterization.

The LTQ FT Ultra mass spectrometer features a novel ICR cell design, the Ultra Cell. This cell design produces enhanced sensitivity and greater dynamic range. The linear excitation characteristics of the cell allow larger cyclotron radii which significantly reduces space charge effects. These performance gains are achieved without the costs and difficulties associated with a higher field magnet.

Highlights:

- Attomole sensitivity
- Widest dynamic range
- ppb mass accuracy
- Resolution > 750,000

For more information, please visit www.thermoscientific.com/ms

LTQ Orbitrap family of hybrid mass spectrometers

For more information on the complete line of Thermo Scientific LTQ Orbitrap Mass Spectrometers, please visit www.thermoscientific.com/ms

LTQ Orbitrap Discovery Mass Spectrometer

Breakthrough technology for every lab.

Based on the fast and highly sensitive LTQ XL linear ion trap and the patented Thermo Scientific Orbitrap technology, the Thermo Scientific LTQ Orbitrap Discovery is a robust, powerful and cost-effective system ideally suited for general proteomics and metabolism applications. Its excellent mass accuracy, mass resolution and reliable, high sensitivity MS^n performance make it a clear alternative to Q-TOF systems.

LTQ Orbitrap XL Mass Spectrometer

Unrivaled performance and flexibility.

Thermo Scientific LTQ Orbitrap XL Hybrid Mass Spectromter features the new HCD collision cell for ultimate flexibility in fragmentation applications for advanced proteomics and small molecule research, enabling the most challenging analysis of low level components in complex mixtures. The LTQ Orbitrap XL can also be further upgraded to include the powerful Electron Transfer Dissociation (ETD) and MALDI capabilities.

LTQ Orbitrap

LTQ Orbitrap Velos Mass Spectrometer

Ultimate confidence for structural elucidation.

The Thermo Scientific LTQ Orbitrap Velos Mass Spectrometer combines the industry-leading Orbitrap Mass Analyzer, a new Higher-Energy Collisional Dissociation (HCD) cell and the new LTQ Velos™ Ion Trap, the world's fastest and most sensitive ion trap mass spectrometer, in one instrument to routinely deliver ultra-high resolution and accurate mass data.

With the LTQ Orbitrap Velos Mass Spectrometer, you can identify more unique proteins in a single experiment and rapidly quantify low level isobarically-labeled peptides. The multiple fragmentation techniques deliver optimal structural characterization.

LTQ family of linear ion trap mass spectrometers

For more information on the complete line of Thermo Scientific LTQ Mass Spectrometers, please visit www.thermoscientific.com/ms

LTQ XL Mass Spectrometer

Redefining performance.

The LTQ XL Linear Ion Trap Mass Spectrometer extends the legendary MS^n performance of the LTQ Linear Ion Trap with powerful technologies to generate extensive structural information for the most demanding proteomics and metabolism applications.

The LTQ XL Ion Trap Mass Spectrometer offers multiple dissociation techniques: CID, HCD, PQD, and optional ETD. Pulsed Q Collision Induced Dissociation (PQD) is a patented technique that eliminates the low mass cut-off concern inherent in all ion traps. This technique results in extensive coverage for predicted and unpredicted metabolites, and the ability to perform peptide quantification using isobaric mass taggs. In addition, fast polarity switching for high-sensitivity analysis of unknowns, high resolution isolation (HRI) and intelligent Data Dependent™ acquisition are all tools to help the researcher gain confident structural identification routinely.

LTQ XL

LTQ Velos Mass Spectrometer

Accelerate your discoveries.

The Thermo Scientific LTQ Velos Ion Trap Mass Spectrometer features the new S-Lens ion guide technology, a revolutionary dual-pressure linear ion trap, and predictive automatic gain control, making it the fastest, most-sensitive ion trap available today. The LTQ Velos Mass Spectrometer enables the identification and quantification of low-abundance compounds in half the time and provides absolute confidence in every result. It can easily be upgraded with the ultra-high resolution and mass accuracy of Orbitrap technology.

TSQ Series Triple Stage Quadrupole Mass Spectrometers

For more information on the complete line of Thermo Scientific TSQ Triple Stage Quadrupole Mass Spectrometers, please visit www.thermoscientific.com/ms

TSQ Quantum Access MAX Mass Spectrometer

Unsurpassed price to performance.

The Thermo Scientific TSQ Quantum Access MAX Triple Stage Quadrupole Mass Spectrometer is an excellent choice for a wide variety of applications, including pharmaceutical, environmental, food safety, clinical research, and forensics, providing unmatched specificity at an affordable price.

The TSQ Quantum Access MAX™ Mass Spectrometer features Quantitation-Enhanced Data-Dependent MS/MS (QED-MS/MS) that allows for simultaneous quantitative and qualitative analysis. The specificity provided by Highly Selective Reaction Monitoring (H-SRM) followed by QED-MS/MS provides uncompromised quantitative performance at low levels followed by a fast, highly specific full MS/MS scan for confirmation.

TSQ Quantum Ultra Triple Stage Quadrupole Mass Spectrometer

Ultimate productivity for bioanalytical analysis.

The Thermo Scientific TSQ Quantum Ultra Mass Spectrometer provides faster method development at the highest possible specificity than is routinely achievable. Featuring the proprietary Thermo Scientific HyperQuad Quadrupole Mass Analyzer System, the TSQ Quantum Ultra MS performs high-resolution selected reaction monitoring (H-SRM), which provides greater analytical selectivity.

Also available, Thermo Scientific TSQ Quantum Ultra EMR offers higher resolution precursor selection and an extended mass range of up to 3000 daltons for analysis of peptides, polysaccharides, intact proteins and oligonucleotides. The Thermo Scientific TSQ Quantum Ultra AM has the additional capability of routine accurate mass measurements on the chromatographic timescale.

TSQ Quantum Ultra MS

TSQ Vantage Mass Spectrometer

Highest sensitivity, lowest noise.

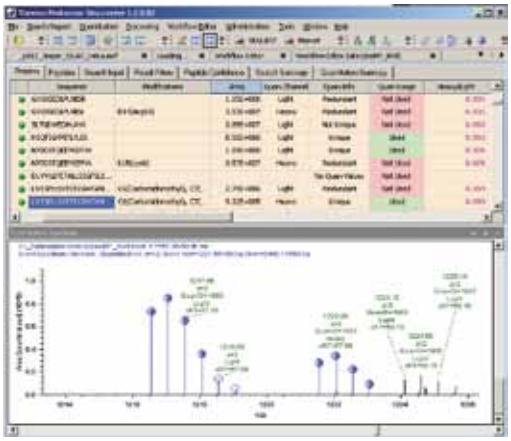
Whether you are analyzing small molecules or biomolecules, the Thermo Scientific TSQ Vantage Mass Spectrometer gives you precise, consistent, and reproducible results at amazingly low levels of quantitation. The TSQ Vantage™ feature a combined with S-lens ion guide technology, second generation (G2) ion optics and hyperbolic quadrupoles that delivers the highest sensitivity with the lowest chemical noise. The S-lens design is a significant advance over high pressure, skimmer-based ion source designs as it eliminates mass discrimination, and lowers the gas load on the turbomolecular pumps. This innovation keeps the ion optical path cleaner, longer, while maintaining sensitivity.

Software

Proteome Discoverer 1.1 Software

Software mass informatics platform for protein scientists.

Thermo Scientific Proteome Discoverer Software is our latest comprehensive proteomics software solution. It is designed to address the need for flexibility by offering a wide array of bio-software tools and customizable workflows for every proteomics experiment using an easy, wizard-driven interface.



Highlights:

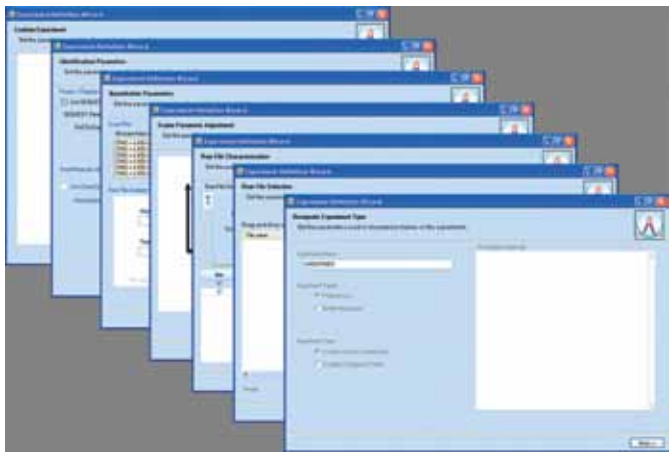
- Improved Tandem Mass Tags (TMT) quantitation including statistical analysis of the results
- Flexible handling of peptide identification for improved coverage and relative quantitation
- Compatibility between Proteome Discoverer Daemon and Thermo Scientific Xcalibur instrument control software that allows automated data processing
- Includes industry-standard database search algorithms SEQUEST® and Mascot (optional) for confident and comprehensive protein identification and post-translational modification characterization
- Z-Core database search algorithm for quick and easy searching of ETD data
- Ability to easily combine results of multiple search engines and dissociation techniques (CID, HCD, ETD) in a single consensus report
- False Discovery Rate (FDR) for each search for validation of protein IDs
- Simple wizards and customizable workflows to analyze MSn data from raw spectra through protein annotation
- Support for data standards developed by the HUPO Proteomics Standards Initiative (PSI)
- Annotation capability
- Exclusion list generation for Xcalibur™ Software, allowing intelligent iterative interrogation of samples
- SRF file import wizard for seamless transition from older Thermo Scientific BioWorks software

SIEVE Software

Automated label-free differential expression analysis.

Thermo Scientific SIEVE Software provides label-free quantitative differential expression analysis of proteins and peptides from the comparison of multiple LC/MS datasets. SIEVE Software is a statistically rigorous tool for analyzing the data acquired in protein biomarker discovery experiments, and has the power to compare as many as 100 LC/MS data files at one time (i.e., 50 sample vs. 50 control experiments). It can also perform a simple comparison of two sample files.

The SIEVE platform performs differential analysis by comparing spectral information from LC/MS analyses of control and treated samples to determine any differential protein expression between samples. Statistically significant results undergo a SEQUEST database search to determine peptide and protein identification. The use of SIEVE Software pre-filter data greatly reduces the number of spectra to search, significantly decreases the time spent on protein identification, and increases the throughput of complex biomarker discovery experiments.



Highlights:

- Employs the novel ChromAlign™ algorithm for chromatographic alignment prior to a proprietary Recursive Base Peak Framing iterative process to find statistically meaningful differences
- Provides a p-value for the expression ratio of each putative biomarker for more confidence
- Supplied with a license to Spotfire® DecisionSite™ software, a powerful interactive visual environment, and includes templates customized for differential expression data review.

ProSightPC 2.0 Software

High-throughput accurate mass protein analysis.

Thermo Scientific ProSightPC Software, the first stand-alone software for analyzing top-down proteomics data, has been enhanced to support all high-mass-accuracy, high-resolution MS/MS proteomics experiments. ProSightPC™ 2.0 Software enables high-throughput processing of accurate-mass MS^2 data, whether from top-down, middle-down or bottom-up experiments, and facilitates the characterization of proteins with known post-translational modifications.

ProSightPC 2.0 software includes five different search modes (Accurate Mass, Biomarker, Sequence Tag, Single Protein, and Gene Restricted) that can be used to determine exact protein sequences, including modifications and alternative splicing. The software can process fragmentation from ECD, IRMPD or CID to determine the protein sequence. The proprietary ProSight Warehouse allows users to import FASTA databases and shotgun annotate these databases with all possible modifications.

Highlights:

- Enables high-throughput processing of all accurate-mass MS^2 data, whether from top-down, middle-down or bottom-up experiments
- Includes the characterization of proteins with known post-translational modification
- Top-down sequencing provides information on exact protein modification sites, truncated proteins, splice variants and biomarkers
- Exact confirmation of bottom-up experiments
- Offers multiple search modes and supports ECD, IRMPD, and CID fragmentation to determine the protein sequence
- Search for post-translational modifications and splice variants using the proprietary ProSight Warehouse
- Uses shotgun annotation to find all possible post-translational modification
- Can import any FASTA databases
- Sequence Gazer helps users probe their data manually and add, remove or change modifications to look for better data fit

Xcaliber Instrument Contriol Software

The universal mass spectrometry data system.

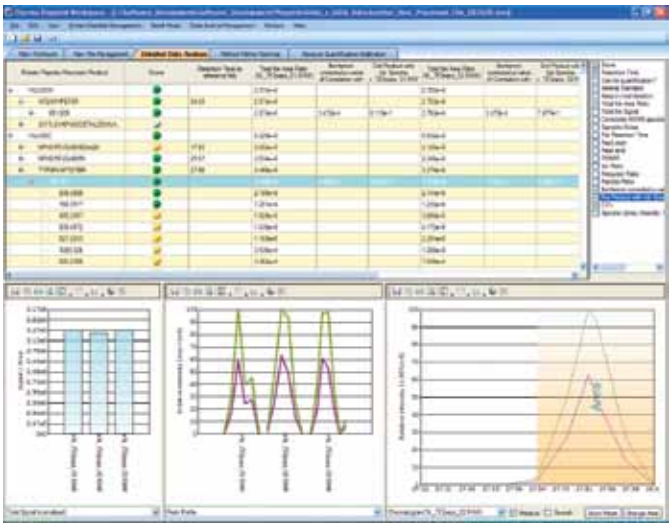
Thermo Scientific Xcalibur Software is a flexible Windows®-based data system that provides instrument control and data analysis for the entire family of Thermo Scientific mass spectrometers and related instruments. The wide range of functionality and ability to integrate third-party control provides the tools to perform a wide range of applications. Several specialized software modules work with Xcalibur™ data system to meet the needs of specific applications.

The easy-to-use interface enables quick and efficient acquisition, data processing and results delivery. Xcalibur Instrument Control Software integrates instrument setup, acquisition, data processing and reporting. Results can be quickly reviewed with the Qual, Quan and Library Browsers. Xcalibur 2.0 Software allows you to operate in a 21 CFR part 11 compliant fashion, providing complete security functions and tracking features, as well as compliant reporting if the new reporting package, XReport 1.0, is used.

Pinpoint Software

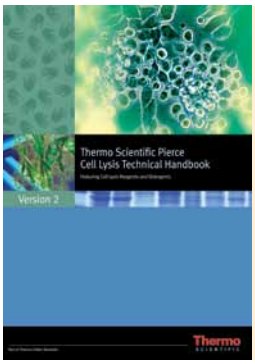
Automated targeted peptide quantitation platform.

Thermo Scientific Pinpoint software is designed to simplify the transition from early-stage biomarker discovery to larger-scale, quantitative verification of putative biomarkers and general quantitative proteomics. It takes advantage of the power of Thermo Scientific TSQ triple quadrupole mass spectrometers to generate high-confidence quantitative data.



Highlights:

- Easy import and processing of panels of targeted proteins/peptides selected from discovery experiments
- Automated searching of user-created and public (e.g., Peptide Atlas) MS/MS spectral libraries, or *in-silico* prediction of target peptides
- Automated selection of the most abundant precursor and product ions, with highly accurate, automated determination of optimal collision energies for SRM transitions
- Flexible method design that can integrate high-resolution precursor selection, timed acquisition, and full MS/MS scans (QED) into SRM assays
- Spectral matching capabilities between experimental and library data provides unique correlation analysis with probability scoring for targeted peptide verification with high confidence.
- Customizable reports that can integrate discovery results with relative and absolute quantitation results
- Easy result export for method refinement for subsequent qualitative and quantitative experiments



Cell Lysis Technical Handbook

This 49-page handbook provides protocols, technical tips and product information to help maximize results for Protein/Gene Expression studies. The handbook provides background, helpful hints and troubleshooting advice for cell lysis, protein purification, cell fractionation, protease inhibitors and protein refolding. The handbook is an essential resource for any laboratory studying Protein/Gene Expression.

RED Device Brochure

Rapid Equilibrium Dialysis (RED) Device is a transforming technology for plasma protein binding assays. Learn more about this technology and how it can accelerate lead optimization and reduce attrition rate in this brochure. Also learn about applications in drug partition studies and protein binding in liver microsomes.

Dialysis and Desalting Technical Handbook

This updated 28-page handbook features the popular Thermo Scientific Slide-A-Lyzer Dialysis Cassettes, SnakeSkin Dialysis Tubing and Zeba Protein Desalt Products. The handbook presents numerous tips to improve usage of these products, as well as helpful selection criteria to choose the most appropriate tool for your application.

Protein Purification Technical Handbook

This updated 80-page handbook provides technical and product information to help maximize results for affinity purification procedures. The handbook provides background, helpful hints and troubleshooting advice for covalent coupling of affinity ligands to chromatography supports, avidin:biotin-binding, affinity purification of antibodies, immunoprecipitation and co-immunoprecipitation assays, affinity procedures for contaminant removal, and related procedures.

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