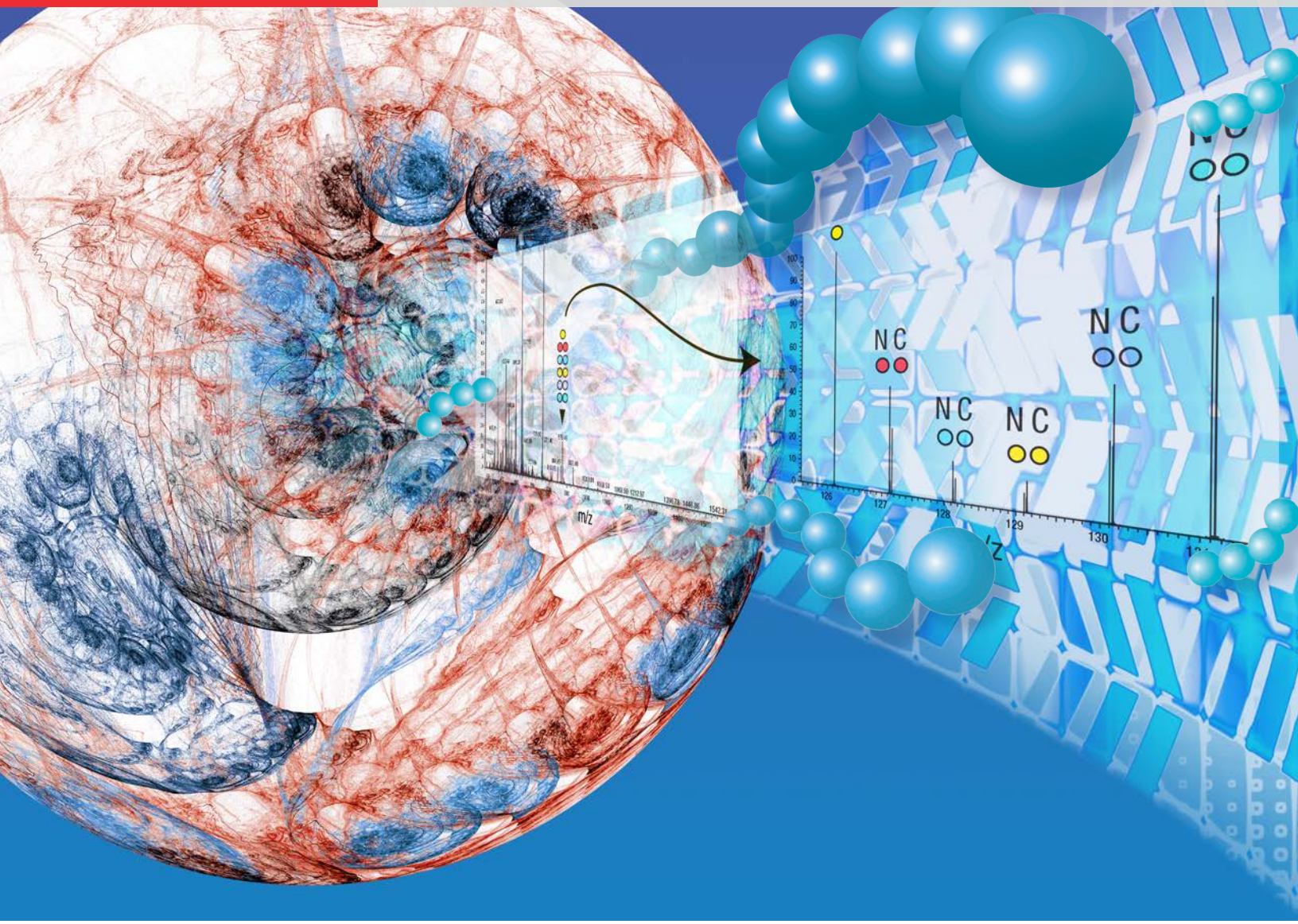


New tools for improved mass spectrometry results

Sample preparation, protein quantitation, and instrument calibration for proteomics research

ThermoFisher SCIENTIFIC

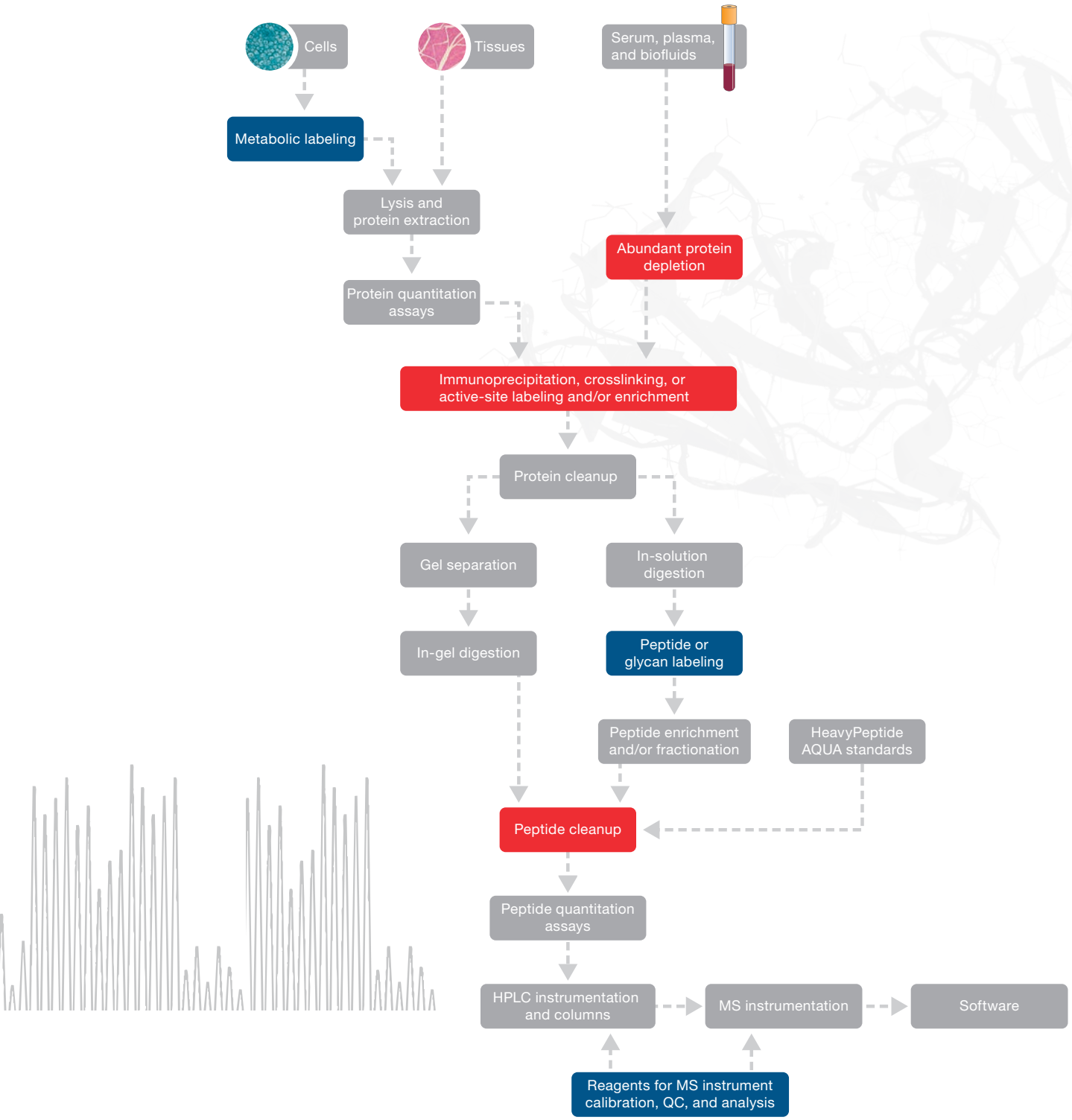
Introduction

We offer a complete portfolio of sample preparation, protein quantitation, and instrument calibration reagents designed for better mass spectrometry (MS) analysis. This portfolio has been developed around the context of biology, and the newest products include improvements to sample preparation (abundant protein depletion spin columns, MS-cleavable crosslinkers, peptide desalting spin columns) and higher multiplexing capabilities for protein quantitation (Thermo Scientific™ NeuCode™ stable isotope labeling with amino acids in cell culture (SILAC™) amino acids and Tandem Mass Tag 11-plex (TMT11plex™) isobaric labeling reagents). In addition, we have developed a new Thermo Scientific™ Pierce™ Intact Protein Standard Mixture as a tool for top-down proteomics applications.

We recognize the need to provide complete solutions and technical support for proteomics research and analytical analysis using MS instrumentation. These new reagents have been verified to assist the biologist and the mass spectrometrist to succeed in their research. Robust, integrated workflows provide consistent results between labs and eliminate time wasted on troubleshooting experimental methods and results.

Contents

Workflows


Cells
Tissues
Serum, plasma, and biofluids
Metabolic labeling
Lysis and protein extraction
Abundant protein depletion
Protein quantitation assays
Immunoprecipitation, crosslinking, or active-site labeling and/or enrichment
Protein cleanup
Gel separation
In-solution digestion
In-gel digestion
Peptide or glycan labeling
Peptide enrichment and/or fractionation
HeavyPeptide AQUA standards
Peptide cleanup
Peptide quantitation assays
HPLC instrumentation and columns
MS instrumentation
Software
Reagents for MS instrument calibration, QC, and analysis

Sample preparation

High-Select HSA/Immunoglobulin and Top14 Abundant Protein Depletion Spin Columns and resin

Deplete abundant plasma proteins quickly and economically

Thermo Scientific™ High-Select™ Abundant Protein Depletion Spin Columns and resins are optimized to decrease the abundant albumin and antibody components of human plasma samples in preparation for MS. Thermo Scientific™ High-Select HSA/Immunoglobulin Depletion Resin uses highly specific, immobilized antibodies to remove HSA (Human Serum Albumin) and all major subclasses of immunoglobulin from serum and plasma. Similarly, the Thermo Scientific™ High-Select Top14 Abundant Protein Depletion Spin Columns are designed to remove HSA, IgG, and 12 other high-abundance proteins from human serum or plasma (Table 1). The resins are provided in two different convenient spin column formats or as bulk resin.

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

HSA/ Immunoglobulin	Top14 columns	
• Albumin	• Albumin	• α1-Acid glycoprotein
• IgG	• IgG	• Fibrinogen
• IgA	• IgA	• Haptoglobin
• IgM	• IgM	• α1-Antitrypsin
• IgD	• IgD	• α2-Macroglobulin
• IgE	• IgE	• Transferrin
• IgG (light chains)	• IgG (light chains)	• Apolipoprotein A-I

Highlights

- **Optimized**—resin is scaled and optimized for treating human plasma samples for MS and/or 1D and 2D electrophoresis
- **Effic ent**—resins are designed to remove >90% of IgG and >95% of albumin, plus other abundant proteins (up to 12)
- **Fast**—spin columns process samples in 5–10 min
- **Economical**—cost-effective spin columns are priced for single use and provide convenience or flexibility with bulk resins
- **Consistent**—one-time use prevents abundant protein carryover and experimental variability
- **Flexible**—choose the system appropriate for your need: HSA/immunoglobulin-specifi or top 14 abundant protein depletion columns

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

The High-Select HSA/Immunoglobulin Depletion Columns can deplete >95% of albumin and immunoglobulins from human serum, while the High-Select Top14 Abundant Protein Depletion Columns remove >95% of the 14 most abundant proteins (Figures 1 and 2). The removal of these high-adundance proteins enables better detection of unique proteins (Figure 3). Each prefilled depletion column can process 10 µL (mini) or 100 µL (midi) of human serum in 5–10 min using a convenient spin format compatible with low-speed centrifugation.

A

Percent depletion	
Protein	**HSA/Immunoglobulin**
Albumin	99.30
Immunoglobulins	99.62

B

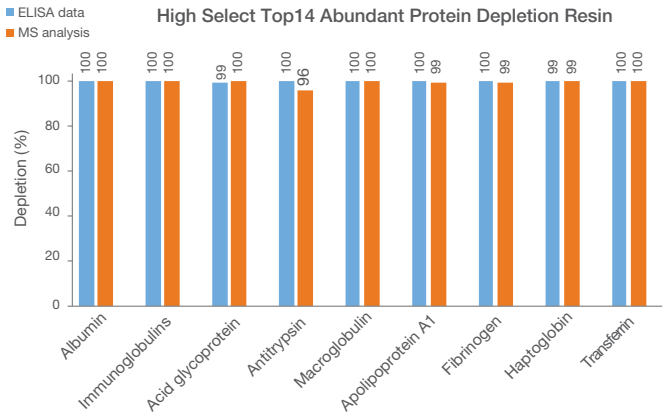


Figure 1. Protein removal achieved using High-Select Abundant Protein Depletion Spin Columns. (A) Values were determined by targeted MS. **(B)** The abundant protein depletion percentage was confirmed by ELISA and was in agreement with >99% removal. Immunoglobulins include IgG (KappaXL and Lambda), IgA, IgM, IgD, and IgE.

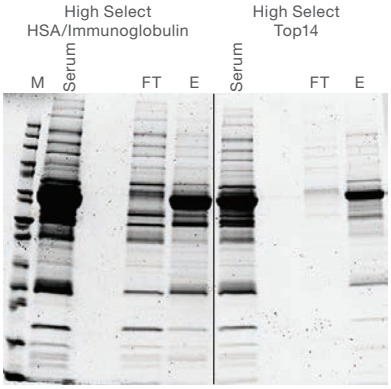


Figure 2. Performance of High-Select Abundant Protein Depletion Spin Columns. Human serum (10–20 µL, Cat. No. PI31876) was loaded onto each resin and processed according to protocols. Total protein in the depleted fractions was estimated using the Pierce BCA Protein Assay Kit (Fisher Scientific, Cat. No. PI23225). The total amount of albumin in the depleted fractions was determined using the AssayMax™ Human Albumin ELISA Kit (Assaypro, Cat. No. EA2201-1). FT = flow-through (i.e., depleted sample); E = eluate (i.e., proteins that were bound by the resin, plus stripped affinity antibodies of the column). With the top 14 proteins removed, low-abundance proteins are now visible in each depleted sample lane (FT).

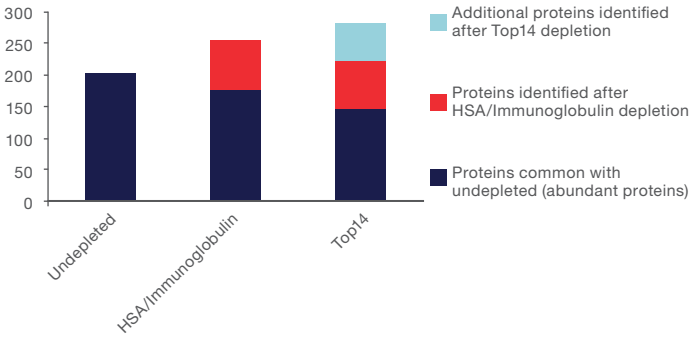


Figure 3. Abundant protein depletion improves identifi ation of unique proteins. Protein group identification profiles for normal human plasma samples which were (a) not depleted, or depleted using the HSA/Immunoglobulin (b) and Top14 (c) depletion resins, are shown. All samples were reduced/alkylated and digested with trypsin. Samples were analyzed by LC-MS on an Orbitrap Fusion™ Tribrid™ mass spectrometer over a 60 minute gradient using 750 ng of sample per injection (performed in triplicate). Raw files were searched against a human protein database using Thermo Scientific™ Proteome Discoverer™ 1.4 software. Non-redundant protein group identification numbers are reported for each sample type.

Learn more at **thermofisher.com/msdepletion**

MS-cleavable crosslinkers

High-quality reagents in convenient packaging for protein characterization studies

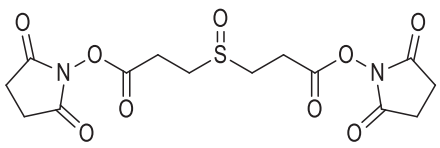
Thermo Scientific™ DSSO (disuccinimidyl sulfoxide) and DSBU (disuccinimidyl dibutyric urea, also known as BuUrBu [Note: BuUrBu stands for dibutyric urea]) are high-quality MS-cleavable crosslinkers that contain an amine-reactive *N*-hydroxysuccinimide (NHS) ester at each end of a 7-atom and 11-atom spacer arm (Figure 4). These products are offered in convenient single-use packaging (10 x 1 mg).

Chemical crosslinking in combination with mass spectrometry is a powerful method to characterize proteins. This method has been applied to recombinant and native protein complexes and, more recently, to whole-cell lysates or intact unicellular organisms in efforts to identify protein–protein interactions on a global scale.

Highlights

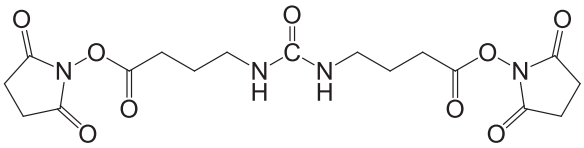
- **High quality**—products manufactured in ISO 9001–certified facilities
- **Convenience**—reagents available in Thermo Scientific™ Pierce™ No-Weigh™ format
- **Cleavable**—enable distinctive fragmentation patterns for MS identifi ation
- **MS-verified**—tested utilizing different types of fragmentation patterns on Thermo Scientific™ mass spectrometers

A



DSSO (disuccinimidyl sulfoxide)
Molecular weight: 388.35
Spacer arm: 10.3 Å

B



DSBU (disuccinimidyl dibutyric urea)
Molecular weight: 426.38
Spacer arm: 12.5 Å

Figure 4. Chemical structures of crosslinkers: (A) DSSO and (B) DSBU.

Both traditional noncleavable and MS-cleavable crosslinkers provide insight into the identifi ation of protein–protein interaction sites, but MS-cleavable crosslinkers are advantageous due to their ability to be cleaved using different types of gas-phase fragmentation methods (e.g., CID, HCD, ETD, and EtHCD) and levels of tandem mass spectrometry (MS^2 and MS^3), improving identification of protein–protein interaction sites (Figure 5).

Features of DSSO and DSBU

- Amine-reactive NHS ester (at both ends) reacts rapidly with any molecule containing a primary amine
- Membrane-permeant, allowing for intracellular crosslinking
- High-purity crystalline reagent can be used to create high-purity conjugates
- MS-cleavable
- Water-insoluble (dissolve first in DMF or DMSO)

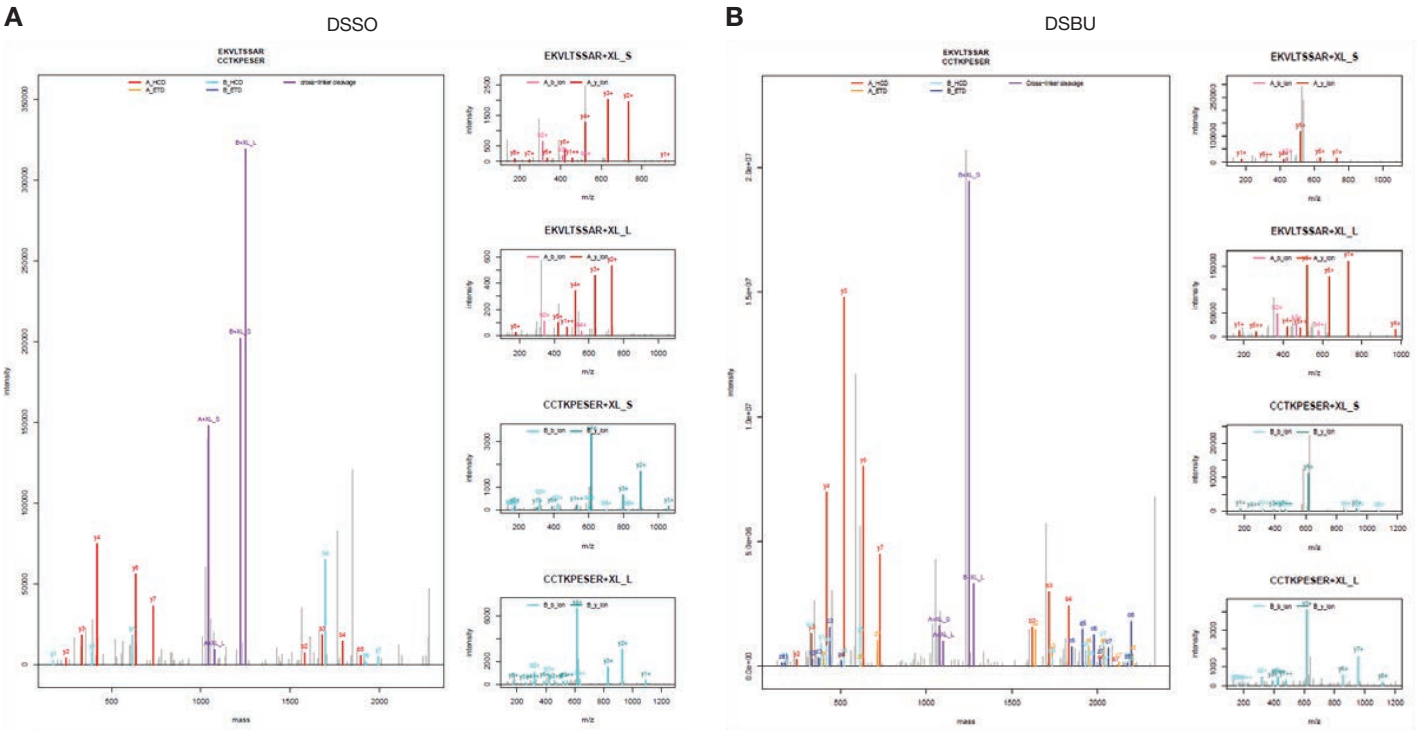


Figure 5. Spectra of BSA-crosslinked peptide identified by MS^2/MS^3 method and XLinkX software* using (A) DSSO and (B) DSBU crosslinkers. XLinkX software uses unique fragment patterns of MS-cleavable crosslinkers (purple annotation) to detect and filter crosslinked peptides for a database search.

* Licensed from the Heck group, University of Utrecht, The Netherlands.

Learn more at **thermofisher.com/mscrosslinkers**

Pierce Peptide Desalting Spin Columns

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

Thermo Scientific™ Pierce™ Peptide Desalting Spin Columns provide a convenient and reproducible way to desalt and remove contaminants from peptide samples for a variety of applications. The spin column format and simple protocol allow processing of multiple samples (10–300 µL each) in parallel in approximately 30 minutes (Figure 6). Pierce Peptide Desalting Spin Columns efficiently bind peptides and remove high concentrations of a wide variety of contaminants that are commonly used during sample processing (Table 2).

Highlights

- **Removes MS-interfering contaminants**— resin helps to reduce signal suppression and improves signal-to-noise ratios and sequence coverage; works on a variety of reversed-phase–compatible contaminants
- **Sensitive**—special hydrophobic polymer-based resin provides excellent recovery of peptide loads—from 5 µg to as high as 5 mg
- **Easy to use**—resin provided in single-use spin column format that fits many common 2 mL tubes
- **Compatible**—resin has been tested using a variety of complex samples, including TMT-labeled peptides, and is stable under extreme pH conditions

Table 2. Effective cleanup of peptide samples using Pierce Peptide Desalting Spin Columns. The columns were challenged with 300 µL of the buffers listed below. Samples were tested for contaminant removal after the recommended number of column washes.

Contaminant	Concentration	Removal efficiency (%)
NaCl	500 mM	>95
Ammonium bicarbonate	500 mM	>95
Tris-HCl, pH 8.0	500 mM	>95
Triethylammonium bicarbonate (TEAB)	500 mM	>95
HEPES-triethylamine	500 mM	>95
Quenched TMT reagent	1 mg	>95

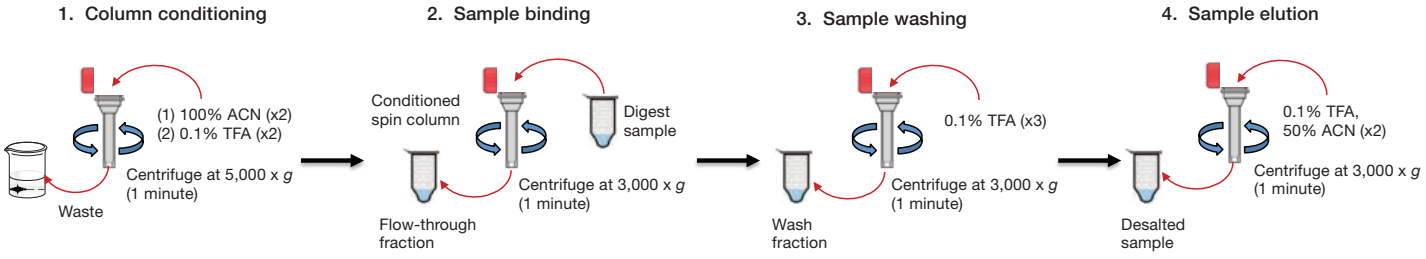


Figure 6. Procedure for using Pierce Peptide Desalting Spin Columns. Peptides are bound to the hydrophobic resin under aqueous conditions and desalted by washing the column with water by low-speed centrifugation. Peptides are eluted from the column in high-organic solvent (50% ACN). ACN = acetonitrile; TFA = trifluoroacetic acid

Traditionally, after isolation of peptides, salts and buffers are removed using reversed-phase (RP) resins, which capture the hydrophobic peptides. The peptides bind to RP columns in high-aqueous mobile phase; salts and buffers are washed off; and the peptides are eluted using a high-organic mobile phase. Pipette tips containing C18 material are the commonly used format for salt removal. This tip design requires many repetitive pipette steps, and sample volumes are limited to 10–20 µL with binding capacities up to 5 µg. The Pierce Peptide Desalting Spin Columns contain an alternative resin and provide a convenient and reproducible way to desalt and remove contaminants from peptide samples up to 300 µL and up to 5 mg of peptides. The peptide spin columns perform similar to comparable C18 columns but with greater flexibility (Figure 7). Each spin column can bind 5 µg to 5 mg of native and/or TMT-labeled peptides, providing greater flexibility for larger peptide samples (Figures 8 and 9).

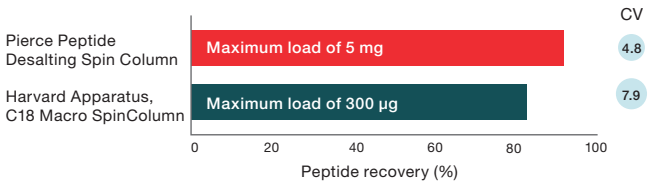


Figure 7. Pierce Peptide Desalting Spin Columns enable efficient recovery of desalted peptide samples. The polymer-based hydrophobic resin contained in each column provides excellent binding and recovery characteristics for peptide samples in preparation for MS and other methods, compared to equivalent products from other suppliers. Digested HeLa extract (300 µg or 5 mg) was loaded onto each column and processed according to the supplier's protocol, using the maximum recommended load capacity (Harvard Apparatus, Cat. No. 74-4107). Total desalted peptide recovered was estimated using the Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay (Fisher Scientific, Cat. No. PI23275).

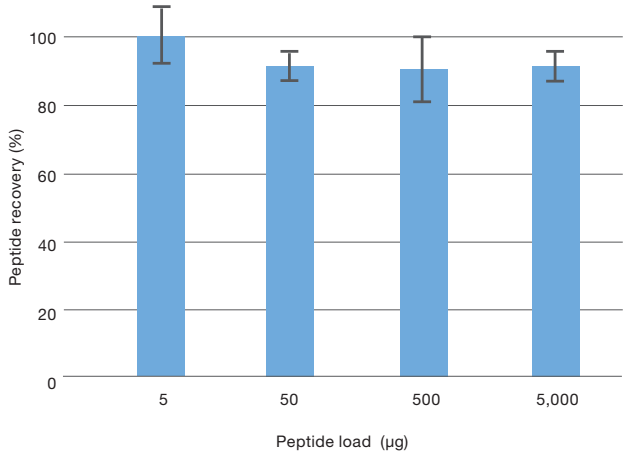


Figure 8. Peptide recovery of native peptides at various loads using Pierce Peptide Desalting Spin Columns. Digested HeLa extracts (5–5,000 µg) were loaded onto Pierce Peptide Desalting Spin Columns and processed according to the protocol. Total recovered desalted peptides were estimated using the Pierce Quantitative Colorimetric Peptide Assay (Fisher Scientific, Cat. No. PI23275).

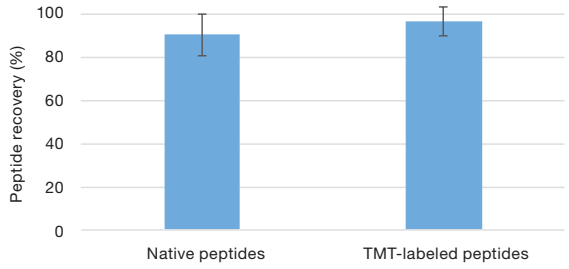


Figure 9. Peptide recovery of native and TMT-labeled peptides. Pierce Peptide Desalting Spin Columns provide excellent binding and recovery characteristics for native and TMT-labeled peptide samples in preparation for MS. The columns effectively remove excess unreactive TMT label along with contaminating salts. Digested HeLa extract (500 µg) was labeled with TMT isobaric tags and loaded onto a spin column and processed according to the protocol. Total desalted peptide recovered was estimated using the Pierce Quantitative Colorimetric Peptide Assay (Fisher Scientific, Cat. No. PI23275).

Learn more at **thermofisher.com/peptidecleanup**

Protein quantitation

SILAC metabolic labeling kits and NeuCode amino acids

Complete kits for stable isotope labeling with amino acids in cell culture (SILAC)

SILAC is a powerful method to identify and quantify relative differential changes in complex protein samples. The SILAC method uses metabolic incorporation of "heavy" ^{13}C- or ^{15}N-labeled amino acids into proteins followed by MS analysis for accelerated, comprehensive identification, characterization, and quantitation of proteins (Figure 10).

Highlights

- **Effic ent**—100% label incorporation into proteins of living cells
- **Reproducible**—minimizes intra-experiment variability caused by differential sample preparation
- **Compatible**—label proteins expressed in a wide variety of mammalian cell lines, including HeLa, 293T, COS-7, U2OS, A549, NIH 3T3, Jurkat, and others
- **High-quality supplements**—heavy amino acids with >98% isotope purity; dialyzed FBS tested to help ensure that it is sterile, endotoxin-free, and cell culture compatible

General applications

- 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 cells vs. abnormal cells
- Identifi ation and quantifi ation of hundreds to thousands of proteins in a single experiment
- Simultaneous immunoprecipitation of labeled, native proteins and protein complexes from multiple conditions

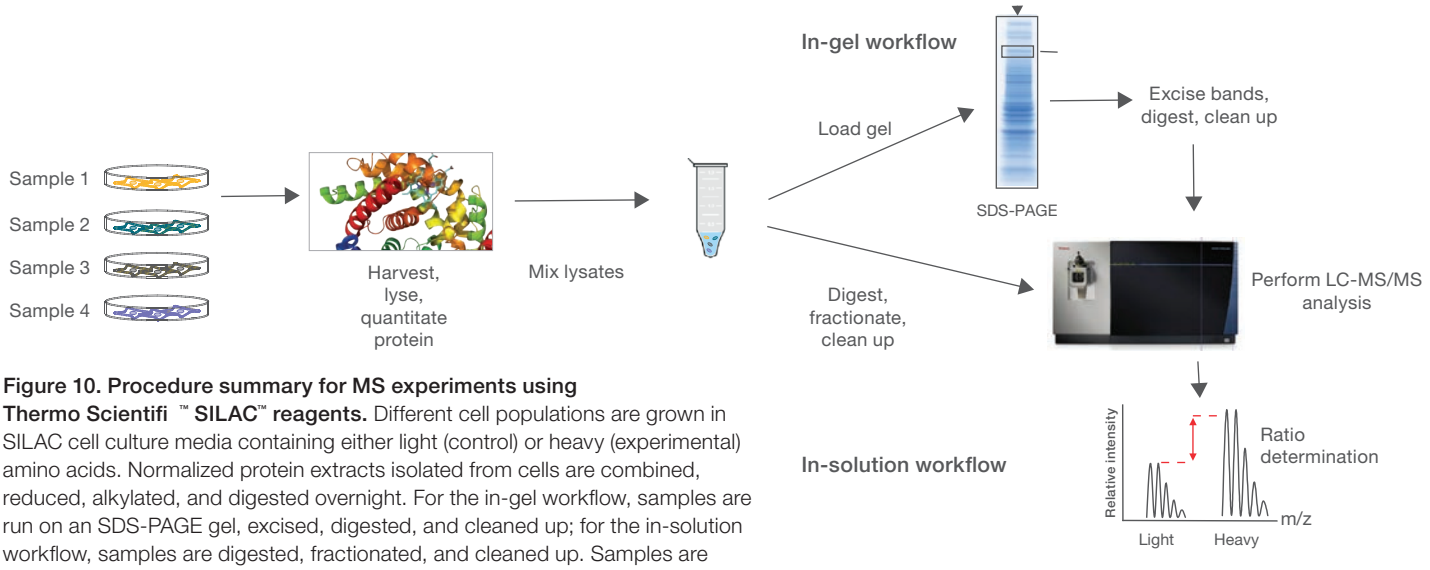


Figure 10. Procedure summary for MS experiments using Thermo Scientifi ™ SILAC™ reagents. Different cell populations are grown in SILAC cell culture media containing either light (control) or heavy (experimental) amino acids. Normalized protein extracts isolated from cells are combined, reduced, alkylated, and digested overnight. For the in-gel workflow, samples are run on an SDS-PAGE gel, excised, digested, and cleaned up; for the in-solution workflow, samples are digested, fractionated, and cleaned up. Samples are then analyzed by high-resolution Thermo Scientific™ Orbitrap™ LC-MS/MS.

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 a 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-PAGE, and digested with trypsin before MS analysis. Because peptides labeled with heavy and light amino acids are chemically identical, they coelute during reversed-phase column prefractionation and 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.

Multiple SILAC kits are available, providing media that are compatible with different mammalian cell lines. Each kit includes all necessary reagents to isotopically label cells, including media, heavy and light amino acid pairs, and dialyzed serum. A wide range of isotopes of lysine and arginine are available separately (Table 3), enabling multiplex experiments and analysis. Dialyzed FBS and other stand-alone media are also available for additional mammalian cell lines. When combined with Thermo Scientific™ protein or peptide enrichment kits, the Thermo Scientific™ SILAC™ Protein Quantitation Kits enable MS analysis of low-abundance proteins such as cell surface proteins, organelle-specific proteins, and proteins with posttranslational modifications such as phosphorylation or glycosylation.

NeuCode amino acids

Thermo Scientific™ NeuCode™ amino acids augment the level of multiplexing achievable in metabolic labeling of proteins for MS analysis. NeuCode metabolic labeling is similar to SILAC but differs in that the labeling only utilizes heavy amino acids. The increased multiplexing capability of NeuCode amino acids is possible through the use of mass defects from extra neutrons in the stable isotopes (Tables 3 and 4). These small mass differences may be resolved on high-resolution mass spectrometers (Thermo Scientific™ Orbitrap Elite™, Orbitrap Fusion™ Tribrid™, and Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometers). Use of only heavy amino acids eliminates the need for 100% incorporation of amino acids used for SILAC (both heavy and light) and may be especially useful for studies with primary cells.

NeuCode amino acids have the same nominal mass and structure but are labeled with different combinations of ^{2}H, ^{13}C, and ^{15}N stable isotopes (Figure 11), which can be resolved using high-resolution MS (Figure 12).

Highlights

- **Labeling effic ency**—100% label incorporation into proteins of living cells, without toxicity
- **Compatibility**—may be multiplexed with existing SILAC amino acids
- **Time savings**—not necessary to label to 100% incorporation if only using heavy amino acids
- **High-quality supplements**—heavy amino acids with >98% isotope purity

Table 3. SILAC isotopes of amino acids available to enable multiplex experiments and analysis.

Amino acid	Light	D_4	$^{13}C_6$	D_8	$^{13}C_6\ ^{15}N_2$	$^{13}C_6\ ^{15}N_4$
Mass shift	0 Da	+4 Da	+6 Da	+8 Da	+8 Da	+10 Da
			Cat. No.			
L-Arginine-HCl	PI89989 (50 mg) PI88427 (500 mg)	N/A	PI88210 (50 mg) PI88433 (500 mg)	N/A	N/A	PI89990 (50 mg) PI88434 (500 mg)
L-Leucine	PI88428 (500 mg)	N/A	PI88435 (50 mg) PI88436 (500 mg)	N/A	N/A	N/A
L-Lysine-2HCl	PI89987 (50 mg) PI88429 (500 mg)	PI88437 (50 mg) PI88438 (500 mg)	PI89988 (50 mg) PI88431 (500 mg)	PIA33613 (50 mg) PIA33614 (500 mg)	PI88209 (50 mg) PI88432 (500 mg)	N/A
L-Proline	PI88211 (115 mg) PI88430 (500 mg)	N/A	N/A	N/A	N/A	N/A

Table 4. Thermo Scientific™ NeuCode™ isotopes of L-lysine-2HCl available to enable multiplex experiments and analysis.

	+4 Da		+8 Da				
Amino acid	K202	K040	K080	K521	K341	K440	K602
Mass shift	4.00078	4.02511	8.01420	8.02637	8.03221	8.03853	8.05021
Cat. No.	PIA36754 (25 mg)	PI88437 (50 mg) PI88438 (500 mg)	PIA36750 (25 mg) PIA33613 (50 mg) PIA33614 (500 mg)	PIA36753 (25 mg)	PIA36851 (25 mg)	PIA36752 (25 mg)	PIA36751 (25 mg) PI88209 (50 mg) PI88432 (500 mg)

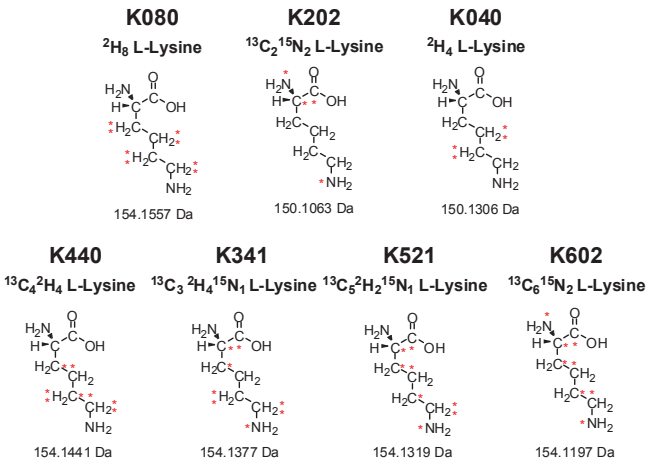


Figure 11. Structures and masses of NeuCode amino acids. Stable isotope labels are indicated by red asterisks.

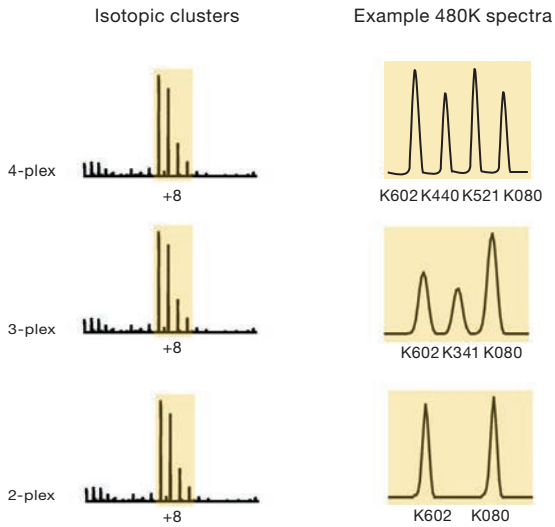


Figure 12. Different combination of lysine isotopologs can be used to increase multiplexing from 2-plex to 4-plex at high resolution.

Learn more at **thermofisher.com/silac**

Isobaric amine-reactive tandem mass tag labeling reagents

Higher multiplex quantitation of up to 11 samples

Thermo Scientific™ Tandem Mass Tag™ (TMT) labeling kits and reagents enable multiplex relative quantitation by MS. All of the mass tagging reagents within a set have the same nominal mass (i.e., isobaric) and chemical structure composed of an amine-reactive NHS ester group, a spacer arm, and a mass reporter (Figure 13). The reagent set can be used to label up to 11 different peptide samples prepared from cells or tissues. For each sample, a unique reporter mass (i.e., 126–131 Da) in the low-mass region of the MS/MS spectrum is used to measure relative protein expression levels during peptide fragmentation.

Previously, we expanded isobaric TMT-labeled multiplexing from 6-plex to 10-plex using high-resolution MS (>50K at m/z 200) to separate ^{15}N and ^{13}C stable isotope variants. Using the same principle, we synthesized the full ^{13}C isotope variant of the TMT-131 reporter, called TMT11-131C. This tag increases isobaric tag multiplex quantitation to 11 samples in a single liquid chromatography (LC)-MS analysis without any changes in reagent structure or LC-MS analysis (Figure 14). The procedure using the TMT11plex reagents is described in Figure 15. To demonstrate the capability of using an 11th tag for relative quantitation, TMT11-131C was used to successfully measure changes in *Bacillus subtilis* during a protein A/G expression time course showing differential expression of key metabolic proteins over time (Figure 16).

Highlights

- **Powerful**—concurrent MS analysis of multiple samples increases sample throughput and enables relative quantitation of up to 11 different samples derived from cells, tissues, or biological fluids
- **Consistent**—identical reagent structure and performance among TMTzero™, TMTduplex™, TMTsixplex™, TMT10plex™, and TMT11plex™ reagents allow efficient transition from method development to multiplex quantitation
- **Robust**—increased multiplex capability results in fewer missing quantitative values
- **Effic ent**—amine-reactive, NHS ester–activated reagents enable efficient labeling of all peptides regardless of protein sequence or proteolytic enzyme specificity
- **Compatible**—optimized for use with high-resolution MS/MS platforms such as Thermo Scientific™ Orbitrap Fusion Lumos, Velos Pro™, Orbitrap Elite™, and Q Exactive™ instruments, with data analysis fully supported by Proteome Discoverer 2.1 software

Applications

- Protein identification and quantitation from multiple samples of cells, tissues, or biological fluids
- Protein expression profiling of normal vs. abnormal states, or control vs. treated cells
- Quantitative analysis of proteins for which no antibodies are available
- Identifi ation and quantifi ation of hundreds to thousands of proteins in a single experiment

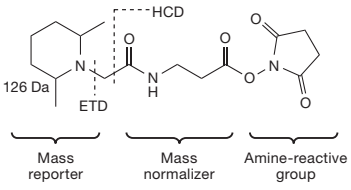


Figure 13. Functional regions of the TMT reagent's chemical structure, including MS/MS sites of fragmentation by HCD and ETD.

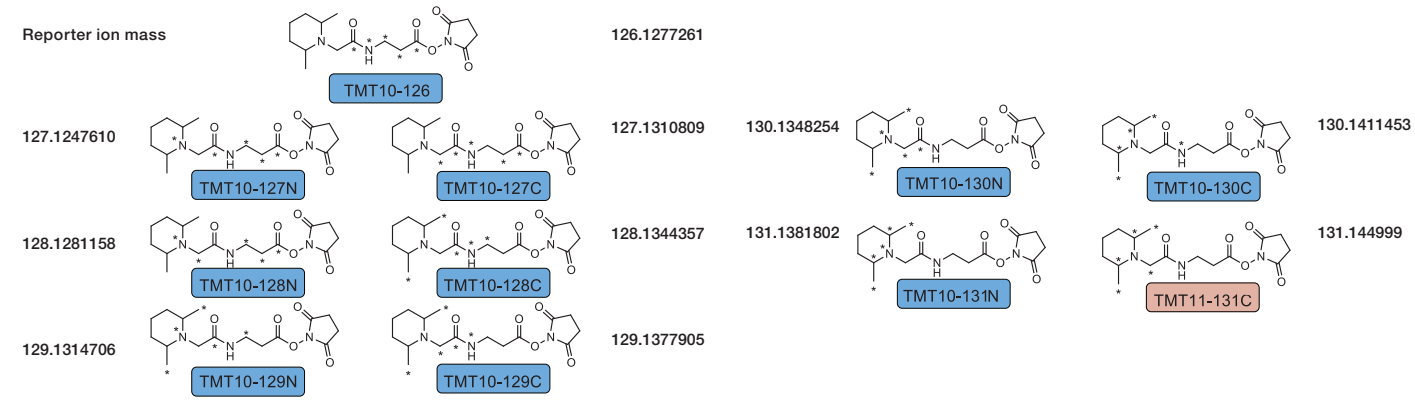


Figure 14. Chemical structures of TMT11plex reagents with ^{13}C and ^{15}N heavy-isotope positions (asterisks).

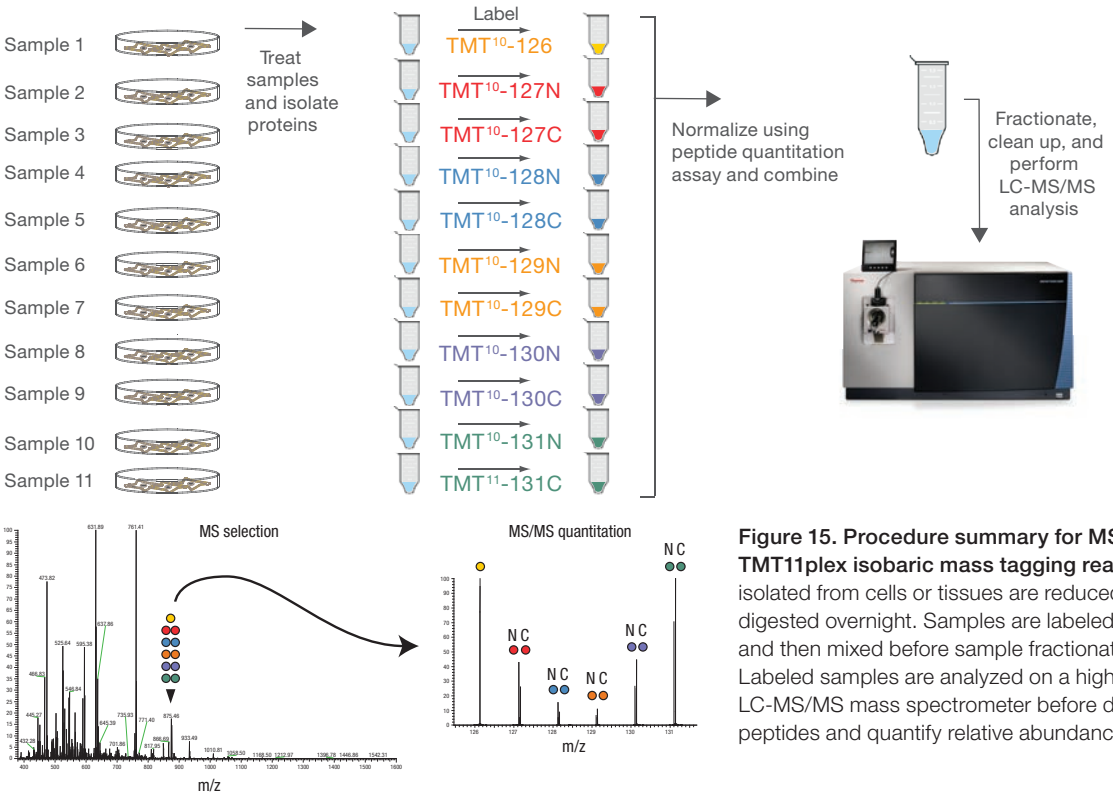


Figure 15. Procedure summary for MS experiments with TMT11plex isobaric mass tagging reagents. Protein extracts isolated from cells or tissues are reduced, alkylated, and digested overnight. Samples are labeled with the TMT reagents, and then mixed before sample fractionation and cleanup. Labeled samples are analyzed on a high-resolution Orbitrap LC-MS/MS mass spectrometer before data analysis to identify peptides and quantify relative abundance of reporter ions.

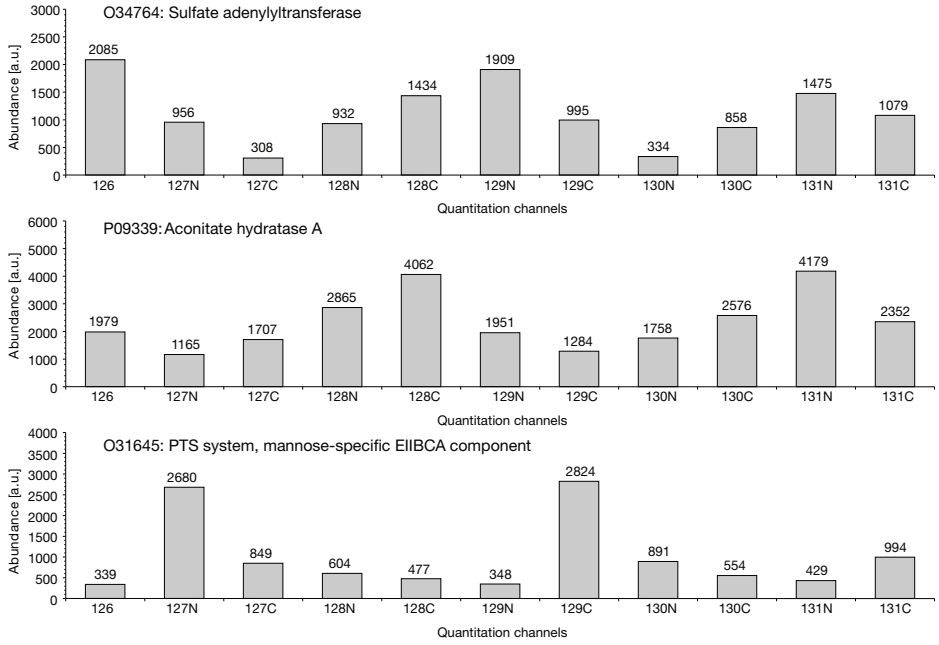


Figure 16. Differential expression of key proteins in *Bacillus subtilis* expressing recombinant protein A/G over time, as determined by TMT reporter ion quantitation using the Orbitrap Fusion Tribrid mass spectrometer.

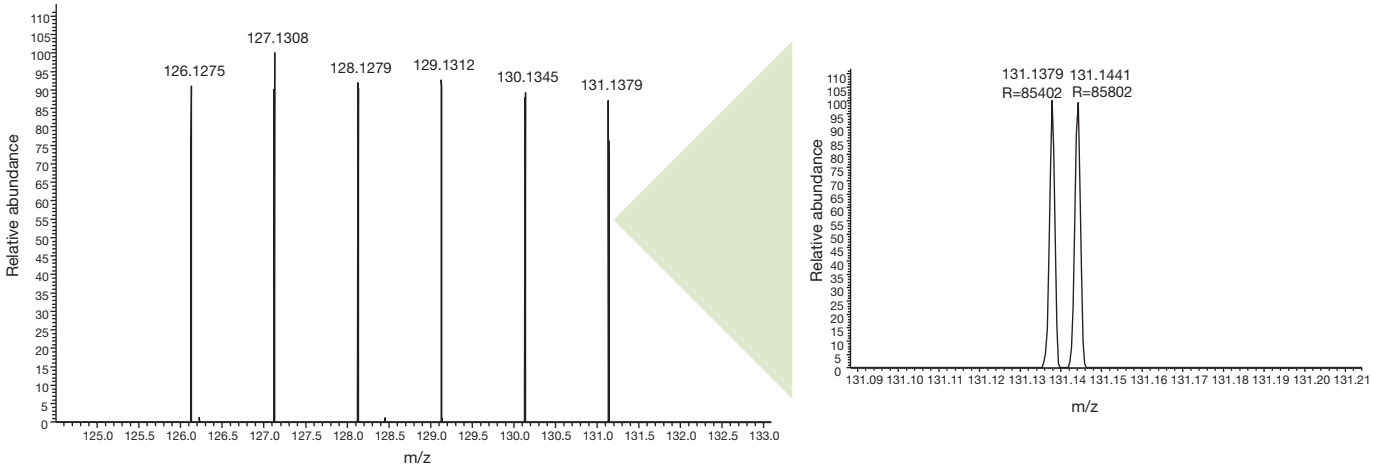


Figure 17. Representative TMT reporter ions in the low-mass region of a MS² spectra from an equimolar (1:1) peptide sample mix. Zoom shows high-resolution separation (60K at m/z 200) of N and C variants of TMT-131 reporter ions.

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Pierce Intact Protein Standard Mix

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- **Wide coverage**—mixture of six recombinant proteins with wide m/z distribution (600–1,500 m/z) and mass range (10–70 kDa)
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Using the intact protein standard mixture routinely before the analysis of samples makes it possible to monitor and normalize LC-MS performance between samples and over time.

Table 5. Pierce Intact Protein Standard Mix. The theoretical masses include known sequence variants and disulfide bonds. UniProt™ database accession numbers correspond to the original protein sequences.

Protein name	Protein accession number(s)	Theoretical average mass (Da)	Theoretical monoisotopic mass (Da)
Human IGF-I LR3*	P05019 (49–119)	9,111.47	9,105.34872
Human thioredoxin	Q99757 (60–166)	11,865.52	11,858.04393
Streptococcus dysgalactiae protein G	P06654 (221–413)	21,442.61	21,429.75915
Bovine carbonic anhydrase II*	P00921	28,981.29	28,963.6881
Streptococcus protein AG (chimeric)	P02976, P19909	50,459.74	50,429.84641
Escherichia coli Klenow fragment	P00582 (324–928)	68,001.15	67,959.42515

* Proteins may undergo partial deamidation under acidic conditions.

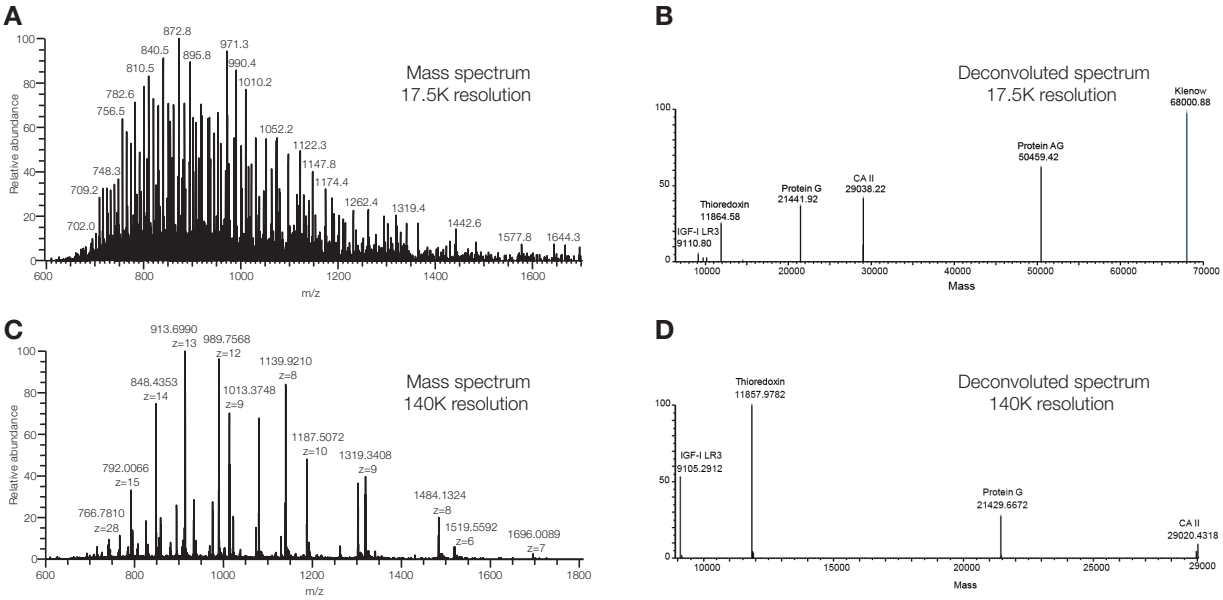

Figure 18. Representative ESI-MS spectra of Pierce Intact Protein Standard Mix (0.38 µg/µL) reconstituted in a 1:1 (v/v) mixture of 0.1% formic acid in 50% acetonitrile and LC-MS–grade water. The sample was analyzed using a Thermo Scientific™ Q Exactive™ Plus mass spectrometer at 17.5K resolution at m/z 200 **(A, B)** and 140K resolution at m/z 200 **(C, D)** in protein mode. Deconvoluted spectra at each resolution were obtained using Thermo Scientific™ BioPharma Finder™ 2.0 software.

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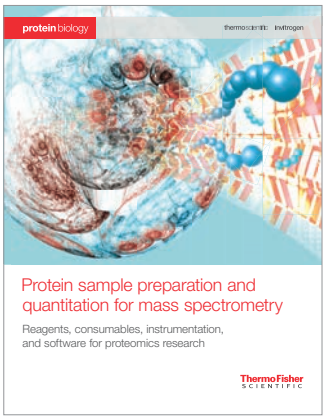
Ordering information

Product	Quantity	Cat. No.
Abundant protein depletion		
High-Select HSA/Immunoglobulin Depletion Mini Spin Columns	6 columns	PIA36365
	24 columns	PIA36366
High-Select HSA/Immunoglobulin Depletion Midi Spin Columns	10 columns	PIA36367
High-Select HSA/Immunoglobulin Depletion Resin	50 mL	PIA36368
High-Select Top14 Abundant Protein Depletion Mini Spin Columns	6 columns	PIA36369
	24 columns	PIA36370
High-Select Top14 Abundant Protein Depletion Midi Spin Columns	10 columns	PIA36371
High-Select Top14 Abundant Protein Depletion Resin	50 mL	PIA36372
MS-cleavable crosslinkers		
DSSO (disuccinimidyl sulfoxide)	10 x 1 mg	PIA33545
DSBU (disuccinimidyl dibutyric urea)	10 x 1 mg	PIA35459
Peptide cleanup		
Pierce Peptide Desalting Spin Columns	25 columns	PI89852
Pierce Peptide Desalting Spin Columns	50 columns	PI89851
Protein quantitation reagents—SILAC		
Pierce SILAC Protein Quantitation Kit (Lys-C)—RPMI 1640	1 kit	PIA33971
Pierce SILAC Protein Quantitation Kit (Lys-C)—DMEM	1 kit	PIA33969
Pierce SILAC Protein Quantitation Kit (Lys-C)—DMEM:F-12	1 kit	PIA33970
NeuCode Lysine-080 (3,3,4,4,5,5,6,6-D_8 L-Lysine-2HCl)	25 mg	PIA36750
NeuCode Lysine-602 ($^{13}C_6$ $^{15}N_2$ L-lysine-2HCl)	25 mg	PIA36751
NeuCode Lysine-440 (L-Lysine:2HCl (3,4,5,6-$^{13}C_4$, 5,5,6,6-D_4, 98%)	25 mg	PIA36752
NeuCode Lysine-521 (L-Lysine:2HCl)	25 mg	PIA36753
NeuCode Lysine-341 ($^{13}C_3$ $^{2}H_4$ $^{15}N_1$ L-Lysine-2HCl)	25 mg	PIA36851
NeuCode Lysine-202 ($^{13}C_2$ $^{15}N_2$ L-Lysine-2HCl)	25 mg	PIA36754
NeuCode 4-plex Bundle (NeuCode Lysine-080, NeuCode Lysine-602, NeuCode Lysine-440, NeuCode Lysine-521)	1 x 25 mg	PIA36755
RPMI 1640 Medium for SILAC	500 mL	PI88365
	6 x 500 mL	PIA33823
DMEM for SILAC	500 mL	PI88364
	6 x 500 mL	PIA33822
DMEM:F-12 (1:1) for SILAC	500 mL	PI88370
MEM for SILAC	500 mL	PI88368
IMDM for SILAC	500 mL	PI88367
Protein quantitation reagents—amine-reactive mass tag reagents		
TMT10plex Isobaric Label Reagent Set plus TMT11-131C Label Reagent	1 x 5 mg/tag	PIA34808
TMT11-131C Label Reagent	1 x 5 mg	PIA34807
Calibration solutions and standards		
Pierce Intact Protein Standard Mix	1 x 76 µg	PIA33526
	5 x 76 µg	PIA33527

Mass spectrometry digital resources



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