Thermo Scientific Pierce Reagents for Quantitative Proteomics

protein quantitation
using mass spectrometry

Discovery Analysis Reagents • Targeted Analysis Tools

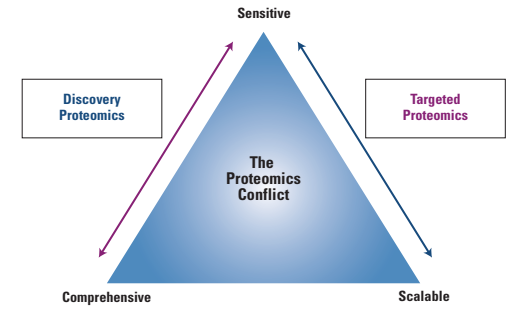
Thermo SCIENTIFIC

proteomics
discovery vs. targeted analysis

an overview

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

For example, if the scope of a project is increased, it is understood that it will take more time or cost more money. Similarly, proteomics researchers must recognize the conflict of scalability, sensitivity and comprehensive analysis. It is impossible to achieve all three simultaneously. Strategies to improve sensitivity and comprehensiveness generally require large sample quantities and multi-dimensional fractionation, which sacrifices throughput. Alternatively, efforts to improve the sensitivity and throughput of protein quantification necessarily limit the number of features that can be monitored. For this reason, proteomics research is typically divided into two categories: discovery and targeted 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.



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.

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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.

discovery proteomic analysis

Identifiy many proteins across a broad dynamic range.

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 and Tandem Mass Tags™ methods) 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 proteomic analysis

Quantify proteins and metabolites in complex samples.

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/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.

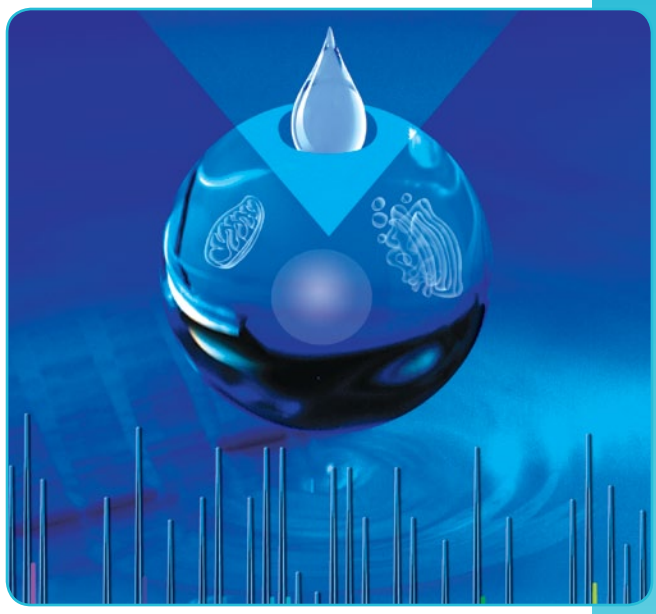
Targeted quantitative proteomic experiments are increasingly used in pharmaceutical and diagnostic applications to quantify proteins and metabolites in complex samples. To further improve quantitative precision and accuracy, known amounts of synthetic peptides containing heavy stable isotopes, such as Thermo Scientific HeavyPeptide Reagents, 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.

discovery analysis

For quantitative analysis of differential protein expression in cultured cells

Thermo Scientific SILAC Protein Quantitation Kits and Reagents

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

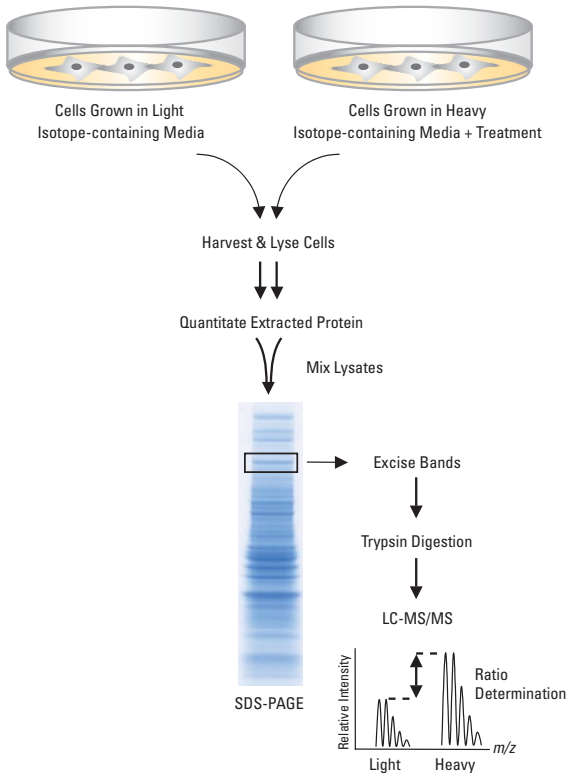


Figure 1. Schematic of SILAC workflow. A549 cells adapted to DMEM were grown for six passages (10 days) using SILAC DMEM (Product # 89983) containing 0.1mg/mL heavy $^{13}C_6$ L-lysine-2HCl or light L-lysine-HCl supplemented with 10% dialyzed FBS. After 100% label incorporation, $^{13}C_6$ L-lysine-labeled cells were treated with 5µM 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 50mg 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 a Thermo Scientific LTQ Orbitrap Hybrid Mass Spectrometer.

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 2).

Several different 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, 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.

Example Experiment

Using a 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 2). To validate SILAC data, protein levels were separately quantitated by Western blot (see Figure 3). 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.

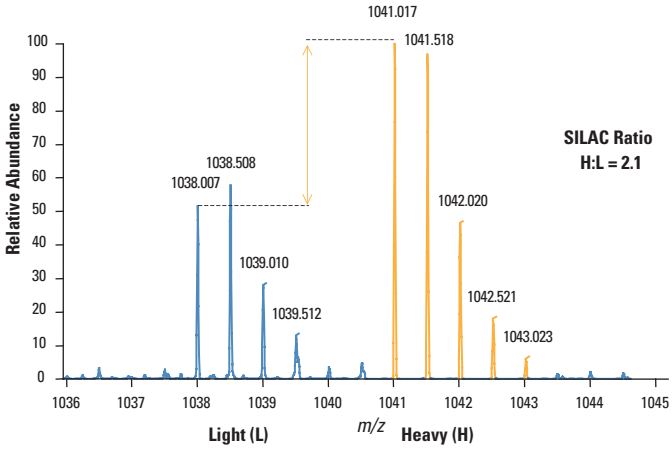


Figure 2. Representative MS spectra generated using SILAC. Light and heavy ($^{13}C_6$) L-lysine-containing peptides (AEDNADTLALVFEAPNQEK) from PCNA were analyzed by MS. Mass spectra of heavy peptides containing $^{13}C_6$ L-lysine have an increased mass of 6Da and are shifted to the right of light peptide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

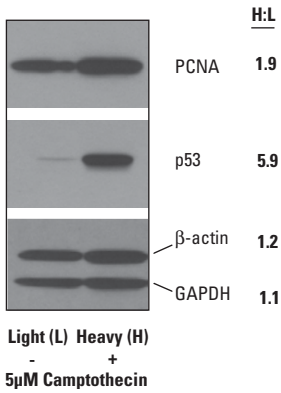


Figure 3. Comparison of A549 protein levels detected by Western blotting after camptothecin treatment. Ten micrograms of each light (L) and heavy (H) sample were analyzed by 4-20% SDS-PAGE and Western blotting using specific antibodies.

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	U.S. Price
89982	**SILAC Protein Quantitation Kit – RPMI 1640** Includes: RPMI Media for SILAC Dialyzed FBS $^{13}C_6$ L-Lysine•2HCl L-Lysine•2HCl L-Arginine•HCl	Kit 2 x 500mL 2 x 50mL 50mg 50mg 2 x 50mg	$ 543
89983	**SILAC Protein Quantitation Kit – DMEM** Includes: DMEM Media for SILAC Dialyzed FBS $^{13}C_6$ L-Lysine•2HCl L-Lysine•2HCl L-Arginine•HCl	Kit 2 x 500mL 2 x 50mL 50mg 50mg 2 x 50mg	$ 543
88200	**Human Mesenchymal Stem Cell SILAC Kit** Includes: Human Mesenchymal Stem Cell Universal Expansion Media for SILAC $^{13}C_6$ L-Lysine•2HCl L-Lysine•2HCl L-Arginine•HCl Stem Cell Screened Dialyzed FBS	Kit 2 x 500mL 1 x 50mg 1 x 50mg 2 x 50mg 100mL	$ 543
88206	**Mouse Embryonic Stem Cell SILAC Kit** Includes: Mouse Stem Cell Expansion DMEM for SILAC $^{13}C_6$ L-Lysine•2HCl L-Lysine•2HCl L-Arginine•HCl Stem Cell Screened Dialyzed FBS	Kit 2 x 500mL 1 x 50mg 1 x 50mg 2 x 50mg 100mL	$ 543
88439	**SILAC Protein Quantitation Kit - DMEM:F12** Includes: DMEM:F12 Media for SILAC Dialyzed FBS $^{13}C_6$ L-Lysine•2HCl L-Lysine•2HCl L-Arginine•HCl	Kit 2 x 500mL 2 x 50mL 50mg 50mg 2 x 50mg	$ 526
89989	**L-Arginine•HCl**	50mg	$ 28
88427	**L-Arginine•HCl**	500mg	$ 98
88210	**$^{13}C_6$ L-Arginine•HCl**	50mg	$ 357
88433	**$^{13}C_6$ L-Arginine•HCl**	500mg	$2,167
89990	**$^{13}C_6$ $^{15}N_4$ L-Arginine•HCl**	50mg	$ 331
88434	**$^{13}C_6$ $^{15}N_4$ L-Arginine•HCl**	500mg	$2,167
89987	**L-Lysine•2HCl**	50mg	$ 28
88429	**L-Lysine•2HCl**	500mg	$ 98
89988	**$^{13}C_6$ L-Lysine•2HCl**	50mg	$ 331
88431	**$^{13}C_6$ L-Lysine-2HCl**	500mg	$2,167
88209	**$^{13}C_6$ $^{15}N_2$ L-Lysine•2HCl**	50mg	$ 331
88432	**$^{13}C^6$ $^{15}N_2$ L-Lysine•2HCl**	500mg	$2,167
88437	**4,4,5,5-D_4 L-Lysine•2HCl**	50mg	$ 98
88438	**4,4,5,5-D_4 L-Lysine•2HCl**	500mg	$ 619
88428	**L-Leucine**	500mg	$ 98
88435	**$^{13}C_6$ L-Leucine**	50mg	$ 361
88436	**$^{13}C_6$ L-Leucine**	500mg	$2,167
88211	**L-Proline**	115mg	$ 28
88430	**L-Proline**	500mg	$ 98
89984	**RPMI Media for SILAC** (RPMI-1640 minus L-Lysine and L-Arginine)	500mL	$ 45
88421	**RPMI Media for SILAC** (RPMI-1640 minus L-Lysine and L-Arginine)	6 x 500mL	$ 180
88426	**Powdered RPMI Media for SILAC** (RPMI minus L-Leucine, L-Lysine and L-Arginine) *Sufficient for: Preparing 10L medium*	104g	$ 210
89985	**DMEM Media for SILAC** (DMEM minus L-Lysine and L-Arginine)	500mL	$ 42
88420	**DMEM Media for SILAC** (DMEM minus L-Lysine and L-Arginine)	6 x 500mL	$ 180
88425	**Powdered DMEM Media for SILAC** (DMEM minus L-Leucine, L-Lysine and L-Arginine) *Sufficient for: Preparing 10L medium*	135g	$ 210
88422	**MEM for SILAC** (MEM minus L-Lysine and L-Arginine)	500mL	$ 47
88214	**Phenol Red Free MEM for SILAC** (MEM minus phenol red, L-Lysine and L-Arginine)	500mL	$ 55
88215	**DMEM:F12 (1:1) Media for SILAC** DMEM:F12 (1:1) minus L-Lysine and L-Arginine for induced pluripotent cells	500mL	$ 49
88424	**Ham's F12 for SILAC** Ham's F12 minus L-Lysine and L-Arginine)	500mL	$ 47
88441	**McCoy's 5A Media for SILAC**	500mL	$ 47
88423	**IMDM for SILAC** (IMDM minus L-Lysine and L-Arginine)	500mL	$ 47
88207	**Mouse Stem Cell Expansion DMEM for SILAC**	500mL	$ 59
88208	**Low Osmolarity Mouse Stem Cell DMEM for SILAC**	500mL	$ 59
89986	**Dialyzed FBS for SILAC**	50mL	$ 95
88440	**Dialyzed FBS for SILAC**	550mL	$ 475
88212	**Stem Cell Screened Dialyzed FBS for SILAC**	100mL	$ 96
88213	**Serum Substitute for Mouse Embryonic Stem Cells**	50mL	$ 45

For a list of references using SILAC Reagents, please see page 17.

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. The chemical tags contain a structure that covalently attaches to the free amino termini of peptides and to lysines residues (Figure 4), thereby labeling various peptides in a given sample. During the MS/MS analysis, the 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, the 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.

The ability to generate low-m/z reporter ions and to distinguish them from isobaric interferences is essential for consistent, precise TMT quantitation. This is best accomplished using HCD fragmentation combined with the high-resolution-at-low-m/z detection that is available on Orbitrap-based systems.

For lysine attachment

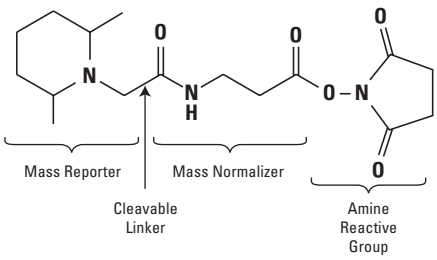
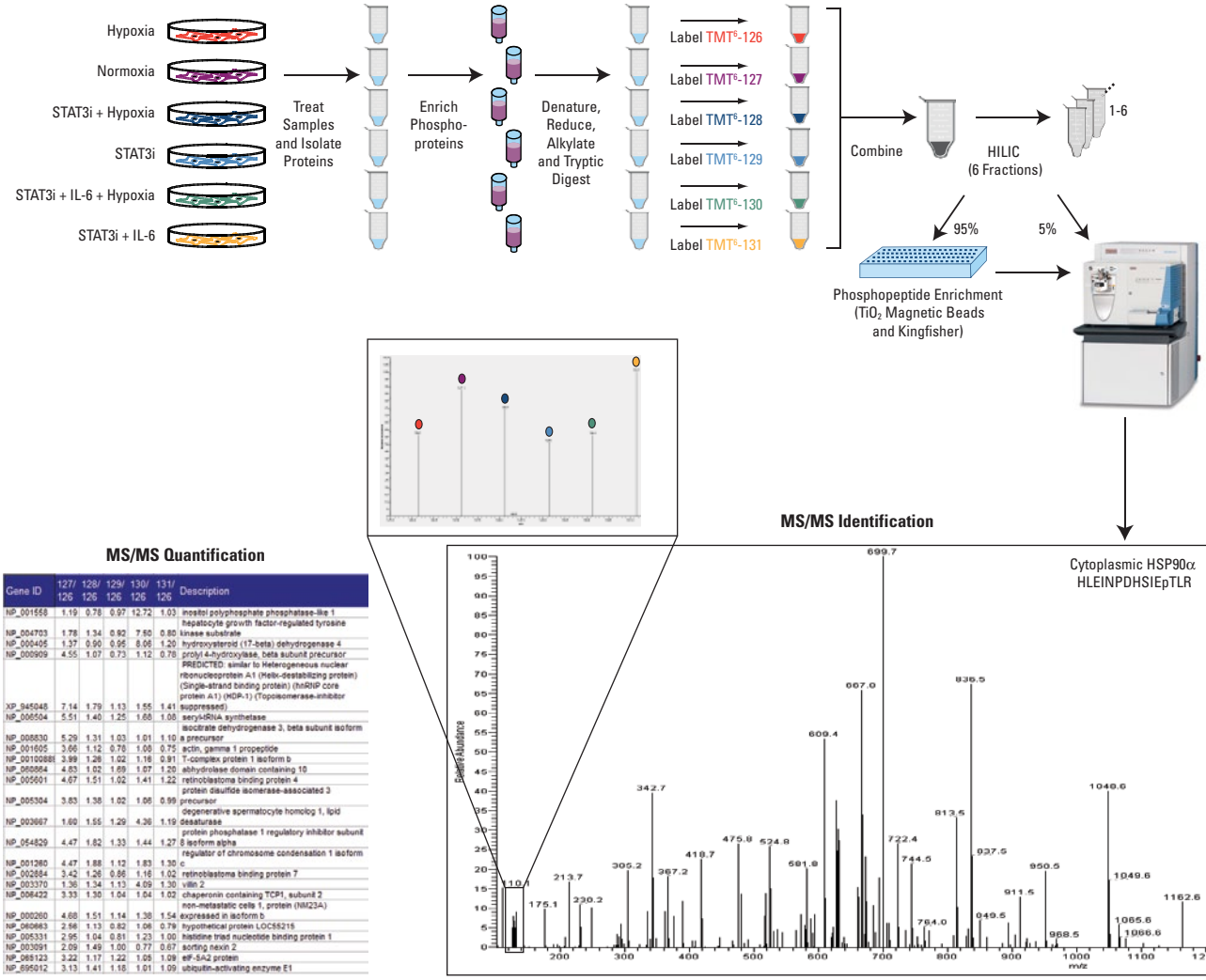


Figure 4. Structural design of tandem mass tags. Mass reporter: Has a unique mass and reports sample-specific abundance of a labeled peptide during MS/MS analysis. **Cleavable linker:** Preferentially fragments under typical MS/MS conditions to release the mass reporter. **Mass normalizer:** Has a unique mass that balances the mass reporter, ensuring the same overall mass for all tags in a set. **Reactive group:** Reactive NHS ester provides high-efficiency amine-specific labeling of proteins/peptides.



Gene ID	127/126	128/126	129/126	130/126	131/126	Description
NP_001558	1.19	0.78	0.97	12.72	1.03	inositol polyphosphate phosphatase-like 1
NP_004703	1.78	1.34	0.92	7.50	0.80	hepatocyte growth factor-regulated tyrosine kinase substrate
NP_000405	1.37	0.90	0.95	8.06	1.20	hydroxysteroid (17-beta) dehydrogenase 4
NP_000909	4.55	1.07	0.73	1.12	0.78	prolyl 4-hydroxylase, beta subunit precursor
XP_945048	7.14	1.79	1.13	1.55	1.41	PREDICTED: similar to Heterogeneous nuclear ribonucleoprotein A1 (Helix-destabilizing protein) (Single-strand binding protein) (hnRNP core protein A1) (HDP-1) (Topoisomerase-inhibitor suppressed)
NP_006504	5.51	1.40	1.25	1.68	1.08	seryl-tRNA synthetase
NP_008830	5.29	1.31	1.03	1.01	1.10	isocitrate dehydrogenase 3, beta subunit isoform a precursor
NP_001605	3.66	1.12	0.76	1.00	0.75	actin, gamma 1 propeptide
NP_00100888	3.99	1.26	1.02	1.16	0.91	T-complex protein 1 isoform b
NP_060864	4.83	1.02	1.69	1.07	1.20	abhydrolase domain containing 10
NP_005601	4.67	1.51	1.02	1.41	1.22	retinoblastoma binding protein 4
NP_005304	3.83	1.38	1.02	1.06	0.99	protein disulfide isomerase-associated 3 precursor
NP_003667	1.60	1.55	1.29	4.36	1.19	degenerative spermatocyte homolog 1, lipid desaturase
NP_054829	4.47	1.82	1.33	1.44	1.27	protein phosphatase 1 regulatory inhibitor subunit 8 isoform alpha
NP_001260	4.47	1.88	1.12	1.83	1.30	regulator of chromosome condensation 1 isoform c
NP_002884	3.42	1.26	0.86	1.16	1.02	retinoblastoma binding protein 7
NP_003370	1.36	1.34	1.13	4.09	1.30	villin 2
NP_006422	3.33	1.36	1.04	1.04	1.02	chaperonin containing TCP1, subunit 2
NP_000260	4.68	1.51	1.14	1.38	1.54	non-metastatic cells 1, protein (NM23A) expressed in isoform b
NP_060663	2.56	1.13	0.82	1.06	0.79	hypothetical protein LOC55215
NP_005331	2.95	1.04	0.81	1.23	1.00	histidine triad nucleotide binding protein 1
NP_003091	2.09	1.49	1.00	0.77	0.67	sorting nexin 2
NP_065123	3.22	1.17	1.22	1.05	1.09	eIF-5A2 protein
NP_695012	3.13	1.41	1.16	1.01	1.09	ubiquitin-activating enzyme E1

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.

discovery analysis

For quantitative analysis of differential protein expression in cultured cells, tissues and biofluids

Thermo Scientific Amine-reactive Tandem Mass Tagging Reagents

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.

Highlights:

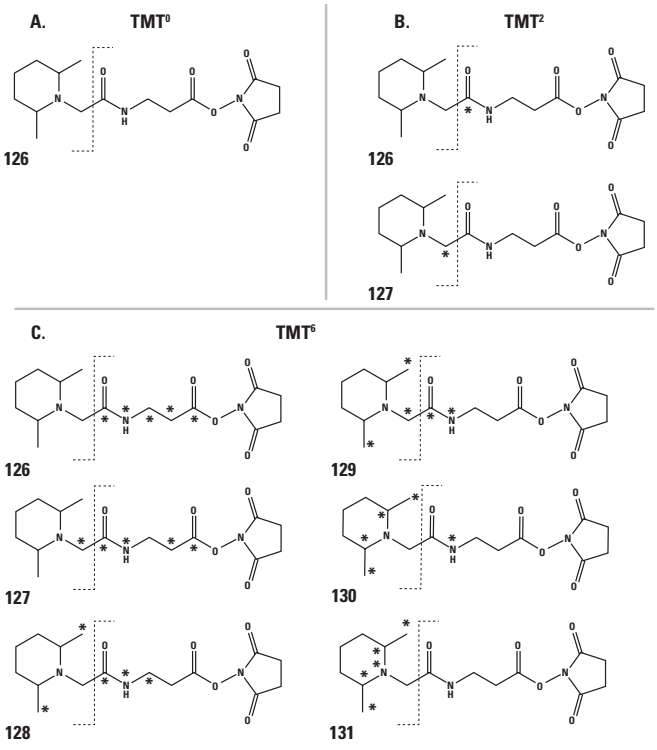
- Amine-reactive, NHS-ester activated reagents ensure efficient labeling of membrane and post-translationally modified proteins
- Expandable system allows concurrent multiplexing of up to six different samples in a single experiment
- Optimized fragmentation and fully supported quantitation with Thermo Scientific Proteome Discoverer and Pinpoint software packages on Thermo Scientific LC-MS/MS platforms

Applications:

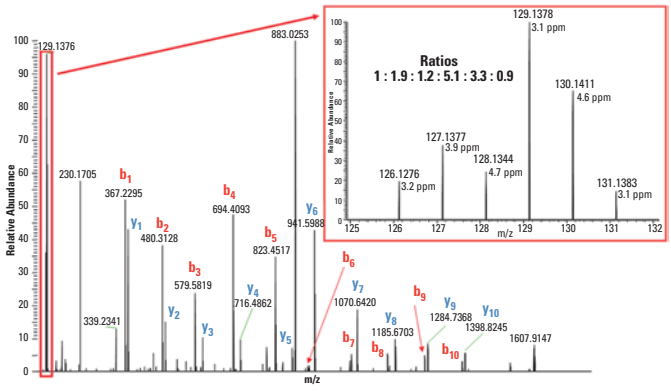
- Protein identification and quantitation from multiple samples of cells, tissue or biological fluids
- Protein expression profiling of normal vs. disease states or control vs. treated samples
- Multiplex up to six different samples concurrently in a single experiment
- Quantitative analysis of proteins 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

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 TMT^0 (zero), the TMTduplex and the TMTsixplex set. The TMT^0 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.

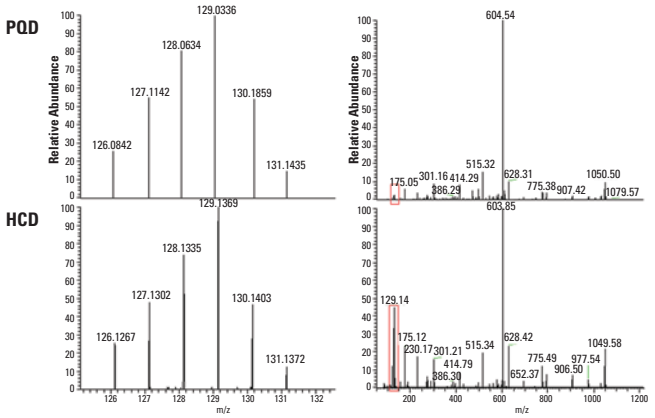
The TMT Label Reagents are provided as standalone sets or in optimized kit formats containing all necessary reagents and controls for maximum flexibility, convenience and reliability. The TMT Reagents combined with Thermo Scientific Instruments and Software provide a complete and integrated solution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



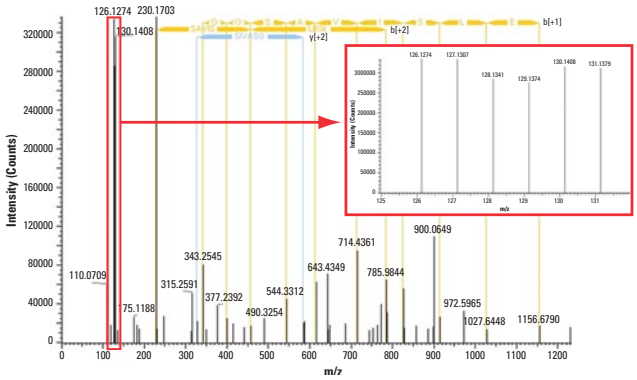
Thermo Scientific TMT Reagent chemistry. Structures of isobaric TMTsixplex Reagents with positions of ^{13}C and ^{15}N heavy isotopes identified (*) and sites of Collisionally Induced Dissociation (dashed lines). **A.** TMT^0 has no isotopic substitutions and is used for method development. **B.** A 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 TMT^6 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.

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
90063	**TMTduplex Isobaric Mass Tagging Kit Labeling Reagents for Multiplexed and Absolute Protein Quantification**	Kit	**$ 694**
	Includes: TMT⁰-126 Label Reagent	5 vials	
	TMT²-126 Label Reagent	5 vials	
	TMT²-127 Label Reagent	5 vials	
	Dissolution Buffer	5mL	
	Denaturing Reagent	1mL	
	Reducing Reagent	1mL	
	Iodoacetamide	12 vials x 9mg	
	Quenching Reagent	1mL	
	Trypsin	2 x 20µg	
	Trypsin Storage Solution	250µl	
	Albumin, Bovine	2.5mg	
90064	**TMTsixplex Isobaric Mass Tagging Kit Labeling Reagents for Multiplexed and Absolute Protein Quantification**	Kit	**$2,456**
	Includes: TMT⁰-126 Label Reagent	5 vials	
	TMT⁶-126 Label Reagent	5 vials	
	TMT⁶-127 Label Reagent	5 vials	
	TMT⁶-128 Label Reagent	5 vials	
	TMT⁶-129 Label Reagent	5 vials	
	TMT⁶-130 Label Reagent	5 vials	
	TMT⁶-131 Label Reagent	5 vials	
	Dissolution Buffer	5mL	
	Denaturing Reagent	1mL	
	Reducing Reagent	1mL	
	Iodoacetamide	12 vials x 9mg	
	Quenching Reagent	1mL	
	Trypsin	5 x 20µg	
	Trypsin Storage Solution	250µl	
	Albumin, Bovine	2.5mg	
90065	**TMTduplex Label Reagent Set Labeling Reagents for Multiplexed and Absolute Protein Quantification**	Kit	**$ 537**
	Includes: TMT²-126 Label Reagent	5 vials	
	TMT²-127 Label Reagent	5 vials	
90066	**TMTsixplex Label Reagent Set Labeling Reagents for Multiplexed and Absolute Protein Quantification TMT⁶-126 Label Reagent**	Kit	**$2,240**
	Includes: TMT²-126 Label Reagent	5 vials	
	TMT⁶-127 Label Reagent	5 vials	
	TMT⁶-128 Label Reagent	5 vials	
	TMT⁶-129 Label Reagent	5 vials	
	TMT⁶-130 Label Reagent	5 vials	
	TMT⁶-131 Label Reagent	5 vials	

Product #	Description	Pkg. Size	U.S. Price
90067	**TMTzero Label Reagent Labeling Reagent for Multiplexed and Absolute Protein Quantification**	5 vials	**$ 155**
	Includes: TMT⁰-126 Label Reagent		
90060	**TMTduplex Isotopic Label Reagent Set, 5 x 0.8mg**	Kit	**$ 511**
	Sufficient reagents for 5 duplex isotopic experiments with controls.		
	Includes: TMT⁰ Label Reagent	5 x 0.8mg	
	TMT⁶-127 Label Reagent	5 x 0.8mg	
90061	**TMTsixplex Label Reagent Set, 0.8mg**	Kit	**$ 480**
	Sufficient reagents for 1 sixplex isobaric experiment.		
	Includes: TMT⁶-126 Label Reagent	1 x 0.8mg	
	TMT⁶-127 Label Reagent	1 x 0.8mg	
	TMT⁶-128 Label Reagent	1 x 0.8mg	
	TMT⁶- 129 Label Reagent	1 x 0.8mg	
	TMT⁶-130 Label Reagent	1 x 0.8mg	
	TMT⁶-131 Label Reagent	1 x 0.8mg	
90062	**TMTsixplex Label Reagent Set, 2 x 0.8mg**	Kit	**$ 903**
	Sufficient reagents for 2 sixplex isobaric experiments.		
	Includes: TMT⁶-126 Label Reagent,	2 x 0.8mg	
	TMT⁶- 127 Label Reagent	2 x 0.8mg	
	TMT⁶-128 Label Reagent	2 x 0.8mg	
	TMT⁶-129 Label Reagent	2 x 0.8mg	
	TMT⁶-130 Label Reagent	2 x 0.8mg	
	TMT⁶-131 Label Reagent	2 x 0.8mg	
90068	**TMTsixplex Label Reagent Set, 2 x 5mg**	Kit	**$4,802**
	Sufficient reagents for 12 sixplex isobaric experiments with controls.	2 x 5mg	
	Includes: TMT⁶-126 Label Reagent	2 x 5mg	
	TMT⁶-127 Label Reagent	2 x 5mg	
	TMT⁶-128 Label Reagent	2 x 5mg	
	TMT⁶-129 Label Reagent	2 x 5mg	
	TMT⁶-130 Label Reagent	2 x 5mg	
	TMT⁶-131 Label Reagent		

For a list of references using TMT Reagents, please see page 17.

targeted analysis

Reliable solutions for quantitative proteomics

Thermo Scientific HeavyPeptide AQUA Standards

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.

Based on years of experience and thousands of 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.

Proteotypic Peptide Selection and Quantitation Protocol

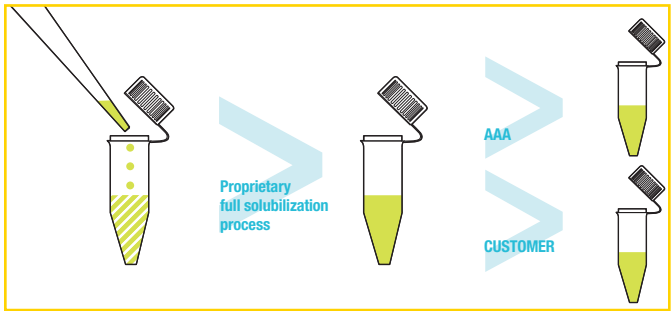
Proteotypic peptide selection

Step 1. 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: Software selected peptides can be ordered via the PEPotec SRM custom service. Crude (as synthesized) peptide libraries (starting from six proteotypic peptides) are provided for the mid- to high-throughput development of SRM/MRM assays.

Quantitation

Step 3. 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.



Thermo Scientific HeavyPeptide Standards preparation process.

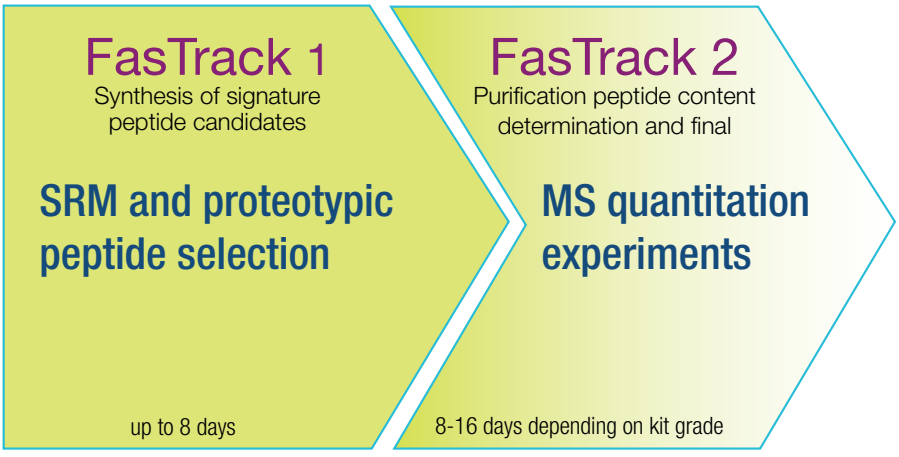
For more information on PEPotec™ SRM including optional services, please visit **www.thermoscientific.com/pepotec** (also see table on p.10)

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. 100µg 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.



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
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.
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)
Formulation	5pmol/µl in 5% v/v Acetonitrile/H_2O	5pmol/µl in 5% v/v Acetonitrile/H_2O	lyophilized
Actual concentration	measured by AAA	measured by AAA	measured by AAA
Concentration precision	±5%	±25%	
Peptide purity	>97%	>97%	>95%
Isotopic enrichment	>99%	>99%	>99%
Length	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
Production time*	~15 working days	~15 working days	~15 working days
Shipment	in solution on wet ice	in solution on wet ice	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.*

PEPotec SRM Peptide Libraries, Standard Service (Product # 500116)

Quantity	>0.1mg
Length	6 to 25 amino acids; L-isoforms only
Purity	Crude (as synthesized)
QC	MS, 5% of samples plus 1 control peptide
Formulation	Suspension in 0.1% TFA in 50% (v/v) acetonitrile/water
Vessel	Thermo Scientific Matrix 96-tube plates **(Product # 3712MTX)**
Minimum Order	24 peptides
C-terminal amino acid restrictions	R or K
Counter-ion	TFA

PEPotec SRM Peptide Libraries, Optional Services

Product #	Service	Unit Size
500503	Phosphorylation at 1 site	Peptide
500504	Phosphorylation at 2 sites	
500505	All cysteines protected by carbamidomethylation (CAM)	
500507	Choice of C-terminal amino acid (with the exception of cysteine)	
500506	Isotopic labeling of the C-terminus with heavy R	
500509	Isotopic labeling of the C-terminus with heavy K	
500508	Isotopic labeling of single internal site with a heavy amino acid	
500510	Peptides >3 and <6 amino acids in length	
500517	Peptides >25 and <31 amino acids in length	
500512	QC: MS of 100% of samples; includes certificate with MS spectra of the peptides	96-well tube array
500511	QC: Simplified HPLC followed by a separate MALDI-TOF MS of 100% of samples; includes certificate with MS spectra and estimated purity of the peptides	
500513	QC: LC/MS of 100% of samples; includes certificate with HPLC and MS spectra of the peptides (on request only)	

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

For a list of references using HeavyPeptide Reagents, **please see page 17.**

Optional Heavy Amino Acids

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

Other amino acids on request.

For more information on HeavyPeptides AQUA Standards grades and services, please visit **www.thermoscientific.com/heavypeptides**

calibration tools

Easy prediction of peptide retention time

Thermo Scientific Pierce Peptide Retention Time Calibration Mixture

The prediction of peptide retention time is a tool to assess chromatographic performance and to assist in the development of multiplexed, high throughput mass spectrometric assays. The Pierce® Peptide Retention Time Calibration Mixture and Thermo Scientific Pinpoint Software can be used to predict peptide retention time from sequence alone or to streamline the transition from qualitative protein discovery results to the development of targeted MS assays on Thermo Scientific triple quadrupole, Orbitrap, Exactive and ion trap mass spectrometers.

The Pierce Peptide Retention Time Calibration Mixture can be used for optimization and regular assessment of chromatographic performance and for rapid development of multiplexed, scheduled targeted MS assays for the quantification of dozens to hundreds of peptide targets per run.

Thermo Scientific Pierce Peptide Retention Time Calibration Mixture Components and Properties

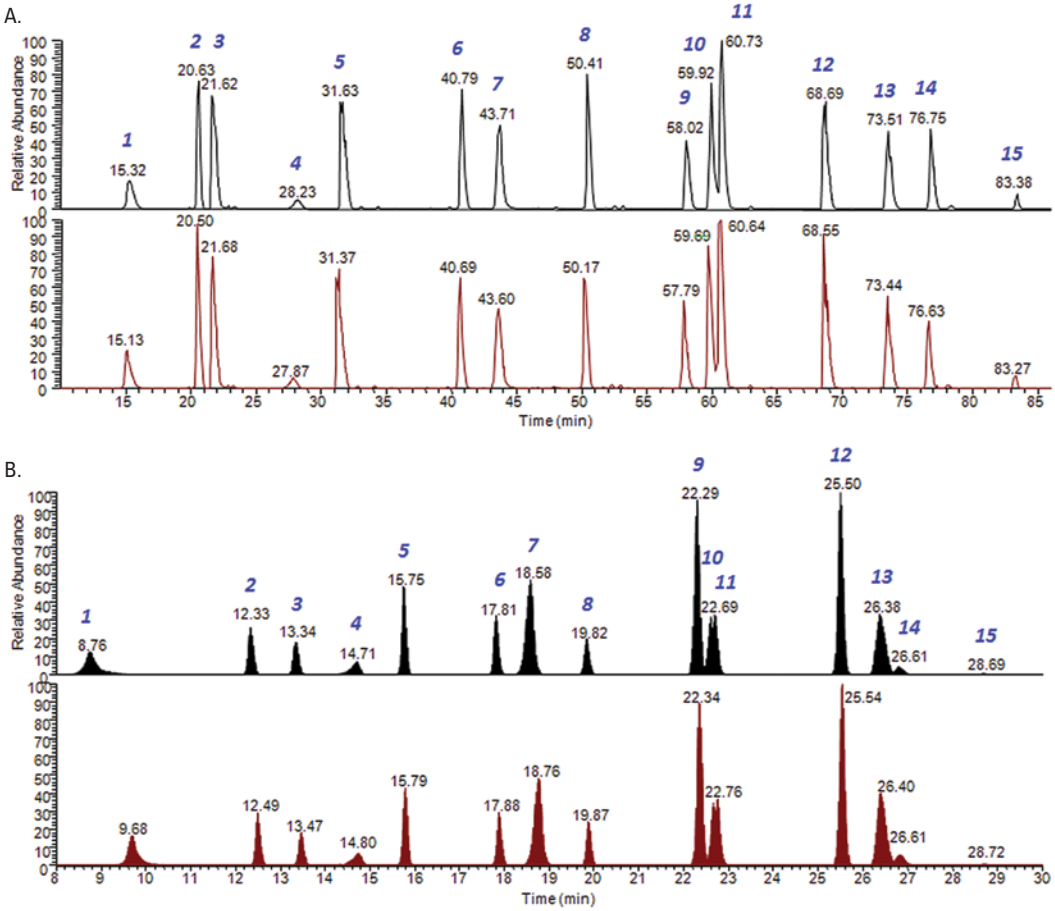
The peptide sequences, peptide masses and chromatographic behavior of each component of the Pierce Peptide Retention Time Calibration Mixture are given below. The position and identity of the heavy isotope-labeled amino acid in each sequence is indicated in bold.

	Peptide Sequence	Mass	Hydrophobicity Factor (HF)
1	SSAAPPPPP**R**	985.5220	7.56
2	GISNEGQNASI**K**	1224.6189	15.50
3	HVLTSIGE**K**	990.5589	15.52
4	DIPVPKP**K**	900.5524	17.65
5	IGDYAGI**K**	843.4582	19.15
6	TASEFDSAIAQD**K**	1389.6503	25.88
7	SAAGAFGPELS**R**	1171.5861	25.24
8	ELGQSGVDTYLQT**K**	1545.7766	28.37
9	GLILVGGYGT**R**	1114.6374	32.18
10	GILFVGSGVSGGEEGA**R**	1600.8084	34.50
11	SFANQPLEVVYS**K**	1488.7704	34.96
12	LTILEEL**R**	995.5890	37.30
13	NGFILDGFP**R**	1144.5905	40.42
14	ELASGLSFPVGF**K**	1358.7326	41.18
15	LSSEAPALFQFDL**K**	1572.8279	46.66

Highlights:

- Assessment of chromatography and MS instrument performance
- Prediction of peptide retention across multiple instrument platforms
- Prediction of peptide retention time from sequence using calculated hydrophobicity factor
- Optimization of scheduled MS acquisition windows for improved quantification and increased multiplexing
- Internal standard to normalize for variation in retention times and peak intensities between runs

The Pierce Peptide Retention Time Calibration Mixture contains 15 synthetic heavy peptides mixed at an equimolar ratio that elute across the chromatographic gradient. The peptide sequences and chromatographic results are used to assess LC performance. In addition, the observed retention times and hydrophobicity factors (HF) for these calibrants are fit to a linear equation to determine the slope of the retention time/HF relationship. This equation and the HF of uncharacterized peptides are then used to predict retention time.



Chromatographic Analysis of the Thermo Scientific Pierce Peptide Retention Time Calibration Mixture. A. The Peptide Retention Time Calibration Solution (250fmoles) was analyzed in duplicate on a Thermo Scientific LTQ XL Orbitrap Mass Spectrometer using a self-packed column (75µm x 20cm) containing Magic™ C18 (Michrom Bioresources) using a 0.25% per minute gradient of Buffer A (0.1% formic acid) and Buffer B (0.1% formic acid/99.9 % acetonitrile) at 300nL per minute. **B.** The Retention Time Calibration Solution was also analyzed on a Thermo Scientific TSQ Vantage Mass Spectrometer using a Thermo Scientific Hypersil GOLD C18 column (1.0 x 150mm, Part No. 25005-150165) with a 1.0% per minute gradient at 120µL per minute. Numbered peaks correspond to the calibrant peptides described above.

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
88320	Pierce Peptide Retention Time Calibration Mixture, 0.5pmol/µL	50µL	$ 62
88321	Pierce Peptide Retention Time Calibration Mixture, 5pmol/µL	200µL	$408

calibration tools

Convenient solutions to calibrate Thermo Scientific Mass Spectrometers

Thermo Scientific Pierce Calibration Solutions

The optimal performance of mass spectrometers relies on the routine calibration of the instrument.

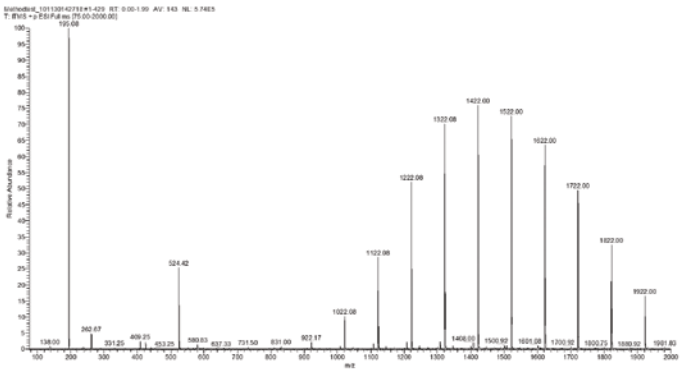
The Pierce Calibration Solutions for mass spectrometers are ready-to-use liquid formulations that can quickly calibrate your Thermo Scientific Mass Spectrometers. Four different Pierce Calibration Solutions are available and each solution consists of highly purified ionizable molecules that produce strong peaks.

The calibration solutions are manufactured in an ISO 9001 environment with strict specifications in quality-controlled lots. The solutions are provided in leak-proof, high-purity PTFE bottles.

Pierce LTQ ESI Positive Ion Calibration Solution

Use to calibrate the LTQ series, the LTQ Orbitrap® series, the LXQ, LCQ FLEET® and the Exactive™ Mass Spectrometer instruments.

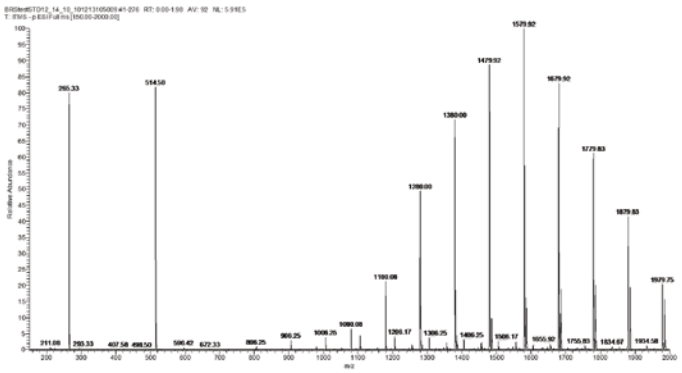
Formulation	Stability
20µg/mL caffeine, 1µg/mL MRFA and 0.001% Ultramark® 1621 in an acetonitrile/methanol/acetic solution	Store at 2–8°C for up to one year



Pierce ESI Negative Ion Calibration Solution

Use to calibrate the LTQ series, LTQ Velos series, the LTQ Orbitrap series, and Exactive Mass Spectrometer instruments.

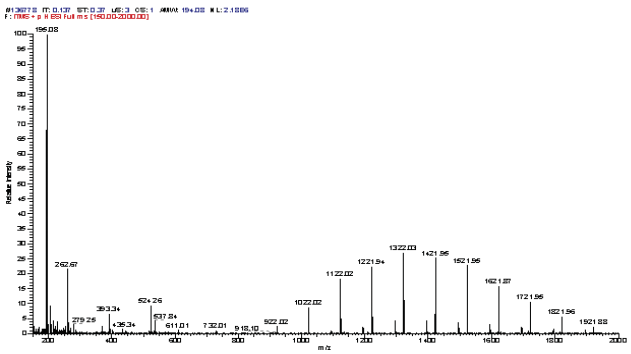
Formulation	Stability
2.9µg/mL sodium dodecyl sulfate, 5.4µg/mL sodium taurocholate and 0.001% Ultramark 1621 in an acetonitrile/methanol/acetic solution	Store at 2–8°C for up to one year



Pierce LTQ Velos ESI Positive Ion Calibration Solution

Use to calibrate the LTQ Velos series and LTQ Orbitrap Velos series and Exactive mass spectrometer instruments.

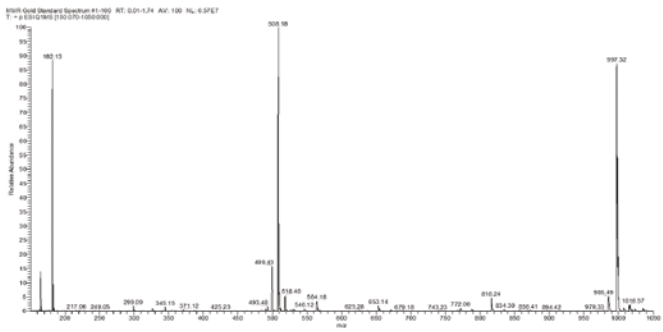
Formulation	Stability
2µg/mL caffeine, 1µg/mL MRFA, 0.001% Ultramark 1621 and 0.0005% n-butylamine in an acetonitrile/methanol/acetic solution	Store at 2–8°C for up to one year



Pierce Triple Quadrupole Calibration Solution

Use to calibrate the TSQ Quantum® series, TSQ Discovery™ series, TSQ Quantum Ultra™ series, TSQ Quantum Access™ series and TSQ Vantage™ series mass spectrometer instruments.

Formulation	Stability
25µM Tyr_1, 25µM Tyr_3, 25µM Tyr_6 in methanol/formic acid solution	Store at 2–8°C for up to one year



Ordering Information

Product #	Description	Pkg. Size	U.S. Price
88322	Pierce LTQ ESI Positive Ion Calibration Solution	10mL	$155
88324	Pierce ESI Negative Ion Calibration Solution	10mL	$155
88323	Pierce LTQ Velos ESI Positive Ion Calibration Solution	10mL	$155
88325	Pierce Triple Quadrupole Calibration Solution	10mL	$155

instruments and software

Detection and analysis for quantitative proteomics studies

Mass Spectrometry Instrument and Software Solutions for Quantitative Proteomics

Successful proteomic analyses require optimum technology in all phases of the workflow, including effective sample preparation; robust, reproducible separations; accurate, 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 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, and triple quadrupole mass spectrometers 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 high-quality data is acquired, and as much valuable information is extracted from that data, as possible.

Thermo Scientific Orbitrap and Orbitrap Hybrid Mass Spectrometers

- Exactive MS
- Q Exactive Hybrid Quadrupole-Orbitrap MS
- LTQ Orbitrap XL Hybrid Ion Trap-Orbitrap MS
- Orbitrap Velos Pro Hybrid Ion Trap-Orbitrap MS
- Orbitrap Elite Hybrid Ion Trap-Orbitrap MS

Orbitrap Velos Pro Hybrid Ion Trap-Orbitrap Mass Spectrometer

Thermo Scientific Ion Trap Mass Spectrometers

- LTQ XL Linear Ion Trap MS
- Velos Pro Dual-pressure Linear Ion Trap MS

LTQ XL Linear Ion Trap-Orbitrap Mass Spectrometer

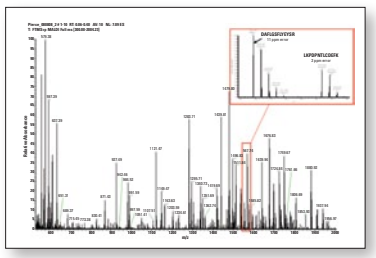
Thermo Scientific Triple Stage Quadrupole Mass Spectrometers

- TSQ Quantum Access MAX Triple Stage Quadrupole MS
- TSQ Quantum Ultra Triple Stage Quadrupole MS
- TSQ Vantage Triple Stage Quadrupole MS

TSQ Quantum Access MAX Triple Stage Quadrupole Mass Spectrometer

Thermo Scientific Software

- Proteome Discoverer Software for proteomic data analysis
- SIEVE Software for differential expression analysis
- ProSightPC Software for top-down protein analysis
- Pinpoint Software for quantitative proteomics
- Xcaliber Instrument control software



For more information on these Thermo Scientific mass spectrometers, please visit **www.thermoscientific.com/ms**

Mass Spec Sample Preparation

Get the most from your mass spectrometry experiments. This handbook breaks the mass spectrometry process into logical steps and includes helpful hints and troubleshooting information for cell lysis, detection, sample prep and downstream applications.

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.

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.

To download or request a free copy of these or other handbooks visit **www.thermoscientific.com/pierce** or call 800-874-3723 or 815-968-0747. Outside the United States, contact your local branch office or distributor.

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