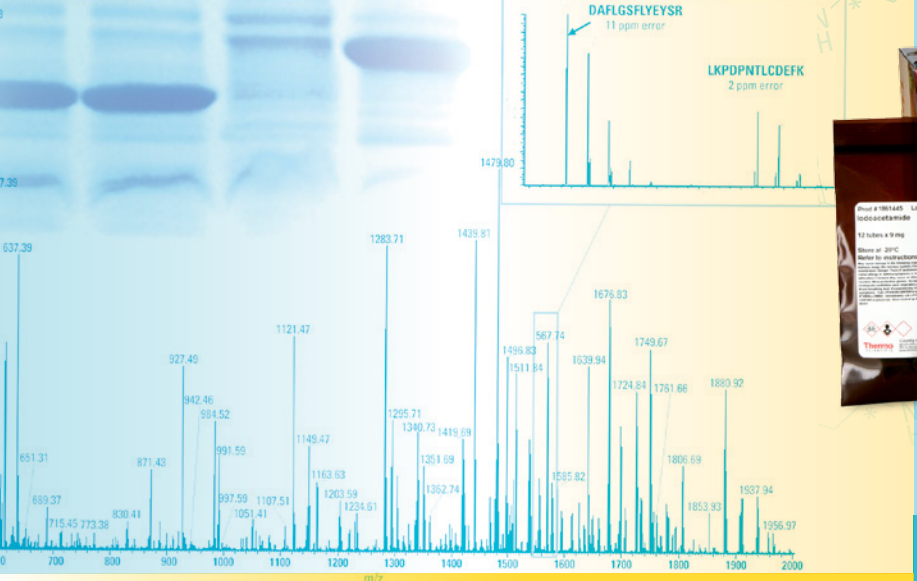


Thermo Scientific Pierce Reagents for Quantitative Proteomics

protein quantitation
using mass spectrometry

Specialized reagents for discovery and targeted analysis

Thermo SCIENTIFIC

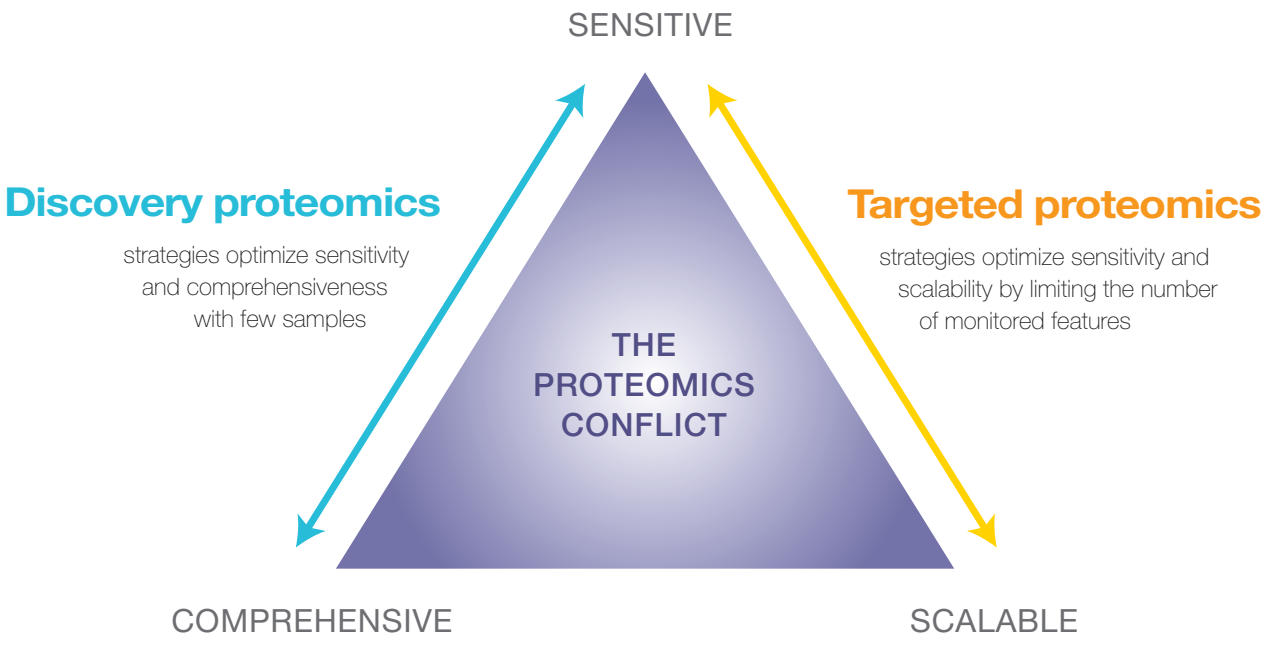
table of contents

proteomics
discovery vs. targeted analysis

an overview

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

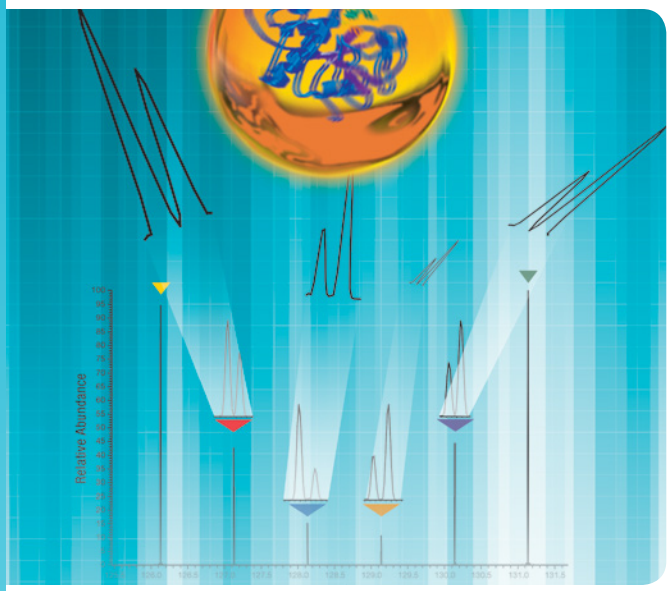
For example, if the scope of a project is increased, it is understood that it will take more time or cost more money. Similarly, proteomics researchers must recognize the conflict of scalability, sensitivity and comprehensive analysis. It is impossible to achieve all three simultaneously. Strategies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



It is impossible to optimize sensitivity, throughput and comprehensiveness simultaneously

discovery quantitation

Measuring changes in global protein expression



Discovery proteomics experiments are intended to identify as many proteins as possible across a broad dynamic range. This often requires depletion of highly abundant proteins, enrichment of relevant proteins (e.g., protein immunoprecipitation) and fractionation steps (e.g., SDS-PAGE or chromatography) to decrease sample complexity.

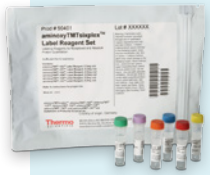
These strategies reduce the dynamic range between components in an individual sample and reduce the competition between proteins or peptides for ionization and mass spectrometry (MS) duty cycle time. Quantitative discovery proteomics experiments add a further challenge because they seek to identify *and* quantify protein levels across multiple samples. Quantitative discovery proteomics experiments utilize label-free or stable isotope labeling methods to quantify proteins. Label-free strategies require highly reproducible fractionation, increased instrumentation time and alignment of peptides across LC-MS/MS experiments to compare spectral counts or ion intensities. Stable isotope protein labeling strategies (e.g., SILAC and Thermo Scientific™ Tandem Mass Tag™ methods) incorporate ^{2}H, ^{13}C, ^{15}N or ^{18}O isotopes into proteins and peptides, resulting in distinct mass shifts but otherwise identical chemical properties. This allows two to 10 samples to be labeled and combined prior to processing and LC-MS/MS analysis. Multiplexing reduces sample processing variability, improves specificity by quantifying the proteins from each condition simultaneously and requires less LC-MS and data analysis time.

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

Specialized software including Thermo Scientific™ Proteome Discoverer™ 1.4, SIEVE™ and ProSightPC™ software ensures as much high-quality data is acquired, and as much valuable information is extracted from that data, as possible.

Discovery Quantitation Workflow by Sample Type Using Thermo Scientific™ Products

	Mammalian Cells	Tissue	Serum/Plasma Biofluids
Reagents	• Metabolic Labeling Kits **(SILAC Quantitation Reagents and Kits)**		• Abundant Protein Depletion (Albumin, Top 2 or Top 12 Protein Depletion Spin Columns and Kits)
	• Lysis & Fractionation Reagents (Mass Spec Sample Prep Kit for Cultured Cells)		
	• Protein Labeling using Active Site Probes (Kinase, GTPase and Serine Hydrolase Labeling and Enrichment Kits) • Protein Labeling of Cysteine-Reactive Sites **(iodoTMT Isobaric Labeling Reagents and Kits)**		
	• Protein Clean-Up (Desalting Spin Columns) • Protein Quantitation Reagents (BCA and Micro BCA Protein Assay Kits)		
	• Protein Digestion Verification (Digestion Indicator for Mass Spectrometry) • Protein Digestion - Proteases (Trypsin, LysC, LysN, Chymotrypsin, AspN, and/or GluC Proteases, MS Grade; In-gel Tryptic Digestion Kit; In Solution Tryptic Digestion and Guanidination Kit)		
	• Glycan Labeling of Reducing Sugars After PNGase Digestion **(aminoxyTMT Isobaric Labeling Reagents)** • Peptide Labeling of Amine-Reactive Sites **(TMT Isobaric Labeling Reagents and Kits)**		
	• Peptide Enrichment (Fe-NTA and/or TiO_2 Phosphopeptide Enrichment Columns and Kits)		
	• Peptide Clean-Up (Detergent Removal Spin Columns) • Peptide Clean-Up (C18, Graphite Spin Columns and/or Tips)		
Instruments	• Thermo Scientific™ LTQ Orbitrap™ Velos™ Mass Spectrometer • Thermo Scientific™ LTQ Orbitrap™ Elite Mass Spectrometer • Orbitrap Fusion Tribrid Mass Spectrometer • Q Exactive Mass Spectrometer		
Software	• Proteome Discoverer 1.4 Software • SIEVE Software • Prosight PC Software		



Thermo Scientific SILAC Protein Quantitation Kits and Reagents

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

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.

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** – broadest portfolio of liquid and powdered SILAC media based on classical media formulations, including DMEM, RPMI-1640, MEM, DMEM:F12, IMDM, Ham's F12 and McCoy's 5a
- **Compatible** – label proteins expressed in a wide variety of mammalian cell lines, including HeLa, 293T, COS7, U2OS, A549, NIH 3T3, Jurkat and others

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. disease states
- Identification and quantification of hundreds to thousands of proteins in a single experiment
- Simultaneous immunoprecipitation of labeled native proteins and protein complexes from multiple conditions

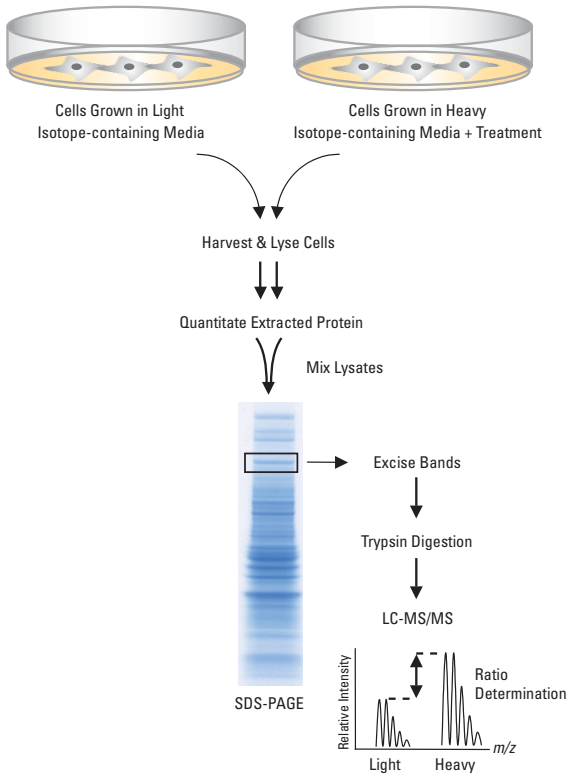


Figure 1. Schematic of SILAC workflow. A549 cells adapted to DMEM were grown for six passages (10 days) using SILAC DMEM (Product # 89983) containing 0.1mg/mL heavy $^{13}C_6$ L-lysine-2HCl or light L-lysine-HCl supplemented with 10% dialyzed FBS. After 100% label incorporation, $^{13}C_6$ L-lysine-labeled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

Three different 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. Several isotopes of lysine and arginine are available separately, enabling multiplexed experiments and analysis. In addition, dialyzed FBS and other stand-alone media are available for additional mammalian cell lines. When combined with Thermo Scientific™ Protein/Peptide Sample Enrichment Products, Thermo Scientific™ 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.

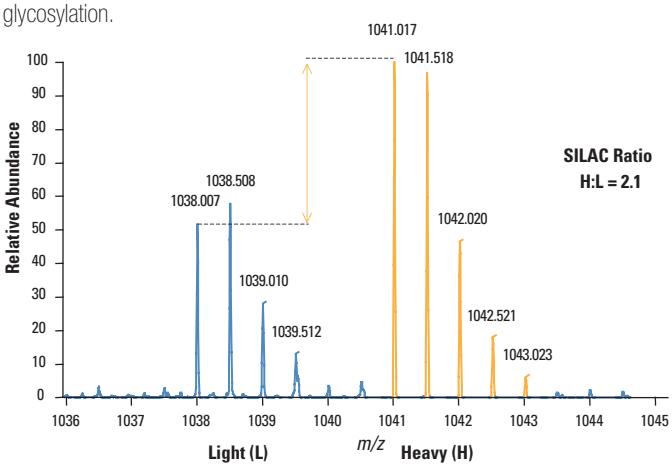


Figure 2. Representative MS spectra generated using SILAC. Light and heavy ($^{13}C_6$) L-lysine-containing peptides (AEDNADTLALVFEAPNQEK) from PCNA were analyzed by MS. Mass spectra of heavy peptides containing $^{13}C_6$ L-lysine have an increased mass of 6Da and are shifted to the right of light peptide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

Example Experiment

Using a SILAC Protein 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 and quantified using an LTQ Orbitrap Mass Spectrometer.

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 2.1-fold in response to camptothecin treatment was proliferating cell nuclear antigen (PCNA), a protein with involvement in DNA repair (Figure 2). To validate SILAC data, protein levels were separately quantitated by Western blot (Figure 3). In addition to an increase in PCNA protein levels, Capthesin-L protein abundance was decreased by 4-fold, 14-3-3σ protein was increased 3.1-fold and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein did not significantly change (Figure 4). The abundance ratios determined by Western blot were comparable to those determined by SILAC.

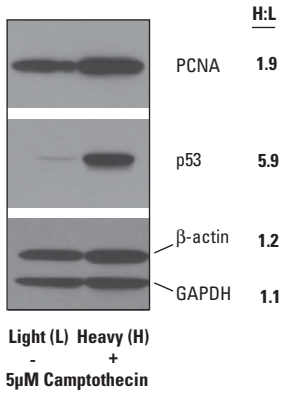


Figure 3. Comparison of A549 protein levels detected by Western blotting after camptothecin treatment. Ten micrograms of each light (L) and heavy (H) sample were analyzed by 4-20% SDS-PAGE and Western blotting using specific antibodies.

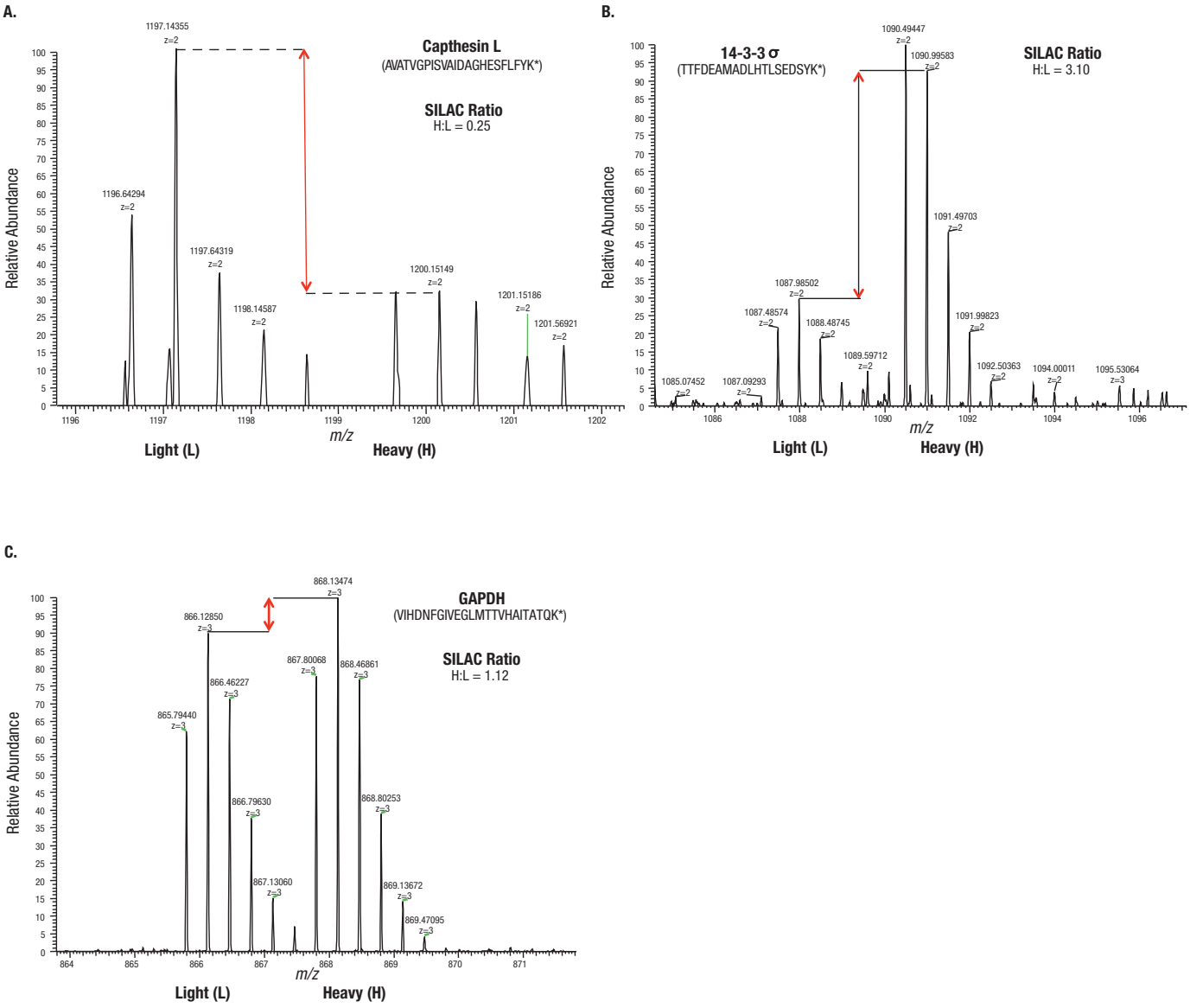


Figure 4. Identification and quantitation of Capthesin L, 14-3-3σ and GAPDH using SILAC. A. Representative MS spectra of light and heavy L-lysine containing peptide (AVATVGPISVAIDAGHESFLFYK) of Cathepsin L precursor. **B.** Representative MS spectra of light and heavy L-lysine containing peptide (TTFDEAMADLHTLSEDSYK) of 14-3-3σ. **C.** Representative MS spectra of light and heavy L-lysine containing peptide (VIHDNFGIVEGLMTTVHAITATQK) of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Mass spectra of heavy peptides containing $^{13}C_6$ L-lysine (K*) 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.

Ordering Information

Product #	Description	Pkg. Size
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
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
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
89989	**L-Arginine•HCl**	50mg
88427	**L-Arginine•HCl**	500mg
88210	**$^{13}C_6$ L-Arginine•HCl**	50mg
88433	**$^{13}C_6$ L-Arginine•HCl**	500mg
89990	**$^{13}C_6$ $^{15}N_4$ L-Arginine•HCl**	50mg
88434	**$^{13}C_6$ $^{15}N_4$ L-Arginine•HCl**	500mg
89987	**L-Lysine•2HCl**	50mg
88429	**L-Lysine•2HCl**	500mg
89988	**$^{13}C_6$ L-Lysine•2HCl**	50mg
88431	**$^{13}C_6$ L-Lysine-2HCl**	500mg
88209	**$^{13}C_6$ $^{15}N_2$ L-Lysine•2HCl**	50mg
88432	**$^{13}C_6$ $^{15}N_2$ L-Lysine•2HCl**	500mg
88437	**4,4,5,5-D_4 L-Lysine•2HCl**	50mg
88438	**4,4,5,5-D_4 L-Lysine•2HCl**	500mg
88428	**L-Leucine**	500mg
88435	**$^{13}C_6$ L-Leucine**	50mg

Product #	Description	Pkg. Size
88436	**$^{13}C_6$ L-Leucine**	500mg
88211	**L-Proline**	115mg
88430	**L-Proline**	500mg
89984	**RPMI Media for SILAC** (RPMI-1640 minus L-Lysine and L-Arginine)	500mL
88421	**RPMI Media for SILAC** (RPMI-1640 minus L-Lysine and L-Arginine)	6 x 500mL
88426	**Powdered RPMI Media for SILAC** (RPMI minus L-Leucine, L-Lysine and L-Arginine) *Sufficient for preparing 10L medium*	104g
89985	**DMEM Media for SILAC** (DMEM minus L-Lysine and L-Arginine)	500mL
88420	**DMEM Media for SILAC** (DMEM minus L-Lysine and L-Arginine)	6 x 500mL
88425	**Powdered DMEM Media for SILAC** (DMEM minus L-Leucine, L-Lysine and L-Arginine) *Sufficient for preparing 10L medium*	135g
88422	**MEM for SILAC** (MEM minus L-Lysine and L-Arginine)	500mL
88214	**Phenol Red Free MEM for SILAC** (MEM minus phenol red, L-Lysine and L-Arginine)	500mL
88215	**DMEM:F12 (1:1) Media for SILAC** DMEM:F12 (1:1) minus L-Lysine and L-Arginine for induced pluripotent cells	500mL
88424	**Ham's F12 for SILAC** Ham's F12 minus L-Lysine and L-Arginine)	500mL
88441	**McCoy's 5A Media for SILAC**	500mL
88423	**IMDM for SILAC** (IMDM minus L-Lysine and L-Arginine)	500mL
89986	**Dialyzed FBS for SILAC**	50mL
88212	**Dialyzed FBS for SILAC**	100mL
88440	**Dialyzed FBS for SILAC**	500mL

For a list of references using SILAC Reagents, please see the back cover.

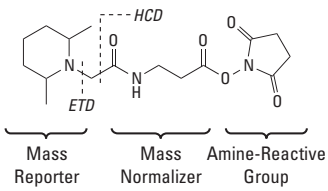
Isobaric Mass Tagging Overview

Simultaneously identify and quantify protein expression and post-translational modifications from multiple conditions in a single analysis.

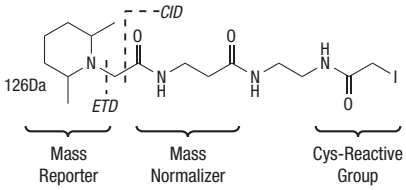
Isobaric chemical tags are powerful tools that enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry. These tags contain reactive groups that covalently label peptide amino termini, peptide cysteine amino acid side changes or glycopeptides, depending on the chemistry used. During the tandem mass spectrometry (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 tandem mass tag 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.

A. Amine-reactive



B. Sulfhydryl-reactive



C. Carbonyl-reactive

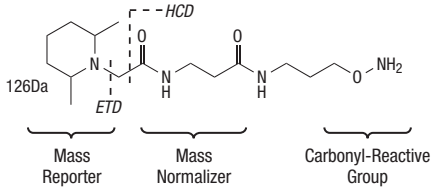


Figure 1. Structural design of Thermo Scientific Tandem Mass Tag Reagents. Mass reporter: Has a unique mass and reports sample-specific abundance of a labeled peptide during MS/MS analysis. **Cleavable linker:** Preferentially fragments under typical MS/MS conditions to release the mass reporter. **Mass normalizer:** Has a unique mass that balances the mass reporter, ensuring the same overall mass for all tags in a set. **Reactive groups: A.** Reactive NHS ester provides high-efficiency, amine-specific labeling of proteins/peptides. **B.** Reactive iodoacetyl functional group provides covalent, irreversible labeling of sulfhydryl (-SH) groups. **C.** Reactive alkoxyamine functional group provides covalent labeling of carbonyl-containing compounds.

Thermo Scientific Amine-reactive Tandem Mass Tag Reagents

Amine-reactive, sixplex and 10-plex isobaric tag reagents.

The Thermo Scientific™ Tandem Mass Tag™ Reagents are designed to enable identification and quantitation of proteins in different samples using tandem mass spectrometry (MS). Thermo Scientific™ TMT10plex™ Label Reagents share an identical structure with Thermo Scientific™ TMTzero™, TMTduplex™ and TMTsixplex™ Reagents but contain different numbers and combinations of ^{13}C and ^{15}N isotopes in the mass reporter. The different isotopes result in a 10-plex set of tags that have mass differences in the reporter that can be detected using high-resolution Thermo Scientific Orbitrap Mass Spectrometers.

Highlights:

- **Powerful** – concurrent MS analysis of multiple samples increases sample throughput and enables relative quantitation of up to 10 different samples derived from cells, tissues or biological fluids
- **Consistent** – identical reagent structure and performance among TMTzero, TMTduplex, TMTsixplex and TMT10plex Reagents allow efficient transition from method development to multiplex quantitation
- **Robust** – increased multiplex capability results in fewer missing quantitative values
- **Efficient** – amine-reactive, NHS-ester-activated reagents ensure 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 Orbitrap Fusion Tribrid, Thermo Scientific™ Orbitrap Velos™ Pro, Orbitrap Elite and Q Exactive Instruments with data analysis fully supported by Proteome Discoverer 1.4 Software

Advantages of the TMT10plex Reagents compared to the first-generation TMTsixplex Reagents include increased multiplex relative quantitation, increased sample throughput and fewer missing quantitative values among samples. TMT10plex Reagents are ideal for the analysis of multiple protein samples from inhibitor dose response experiments, time course experiments or biological replicates.

The TMT10plex Reagent Set contains 10 different isobaric compounds with the same mass and chemical structure (i.e., isotopomeric) composed of an amine-reactive NHS-ester group, a spacer arm and a mass reporter. The reagent set enables up to 10 different peptide samples prepared from cells or tissues to be labeled in parallel and then combined for analysis. For each sample, a unique reporter mass (i.e., TMT^{10} Reagent that is 126-131Da) in the low-mass region of the high-resolution MS/MS spectrum is used to measure relative protein expression levels during peptide fragmentation and tandem mass spectrometry.

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
- Multiplex up to 10 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

TMT Reagent Generic Chemical Structure

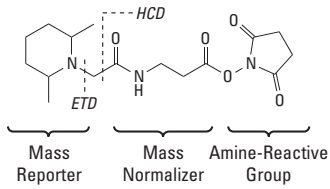


Figure 1. Functional regions of the Thermo Scientific TMT Reagent chemical structure including MS/MS fragmentation sites by higher energy collision dissociation (HCD) and electron transfer dissociation (ETD).

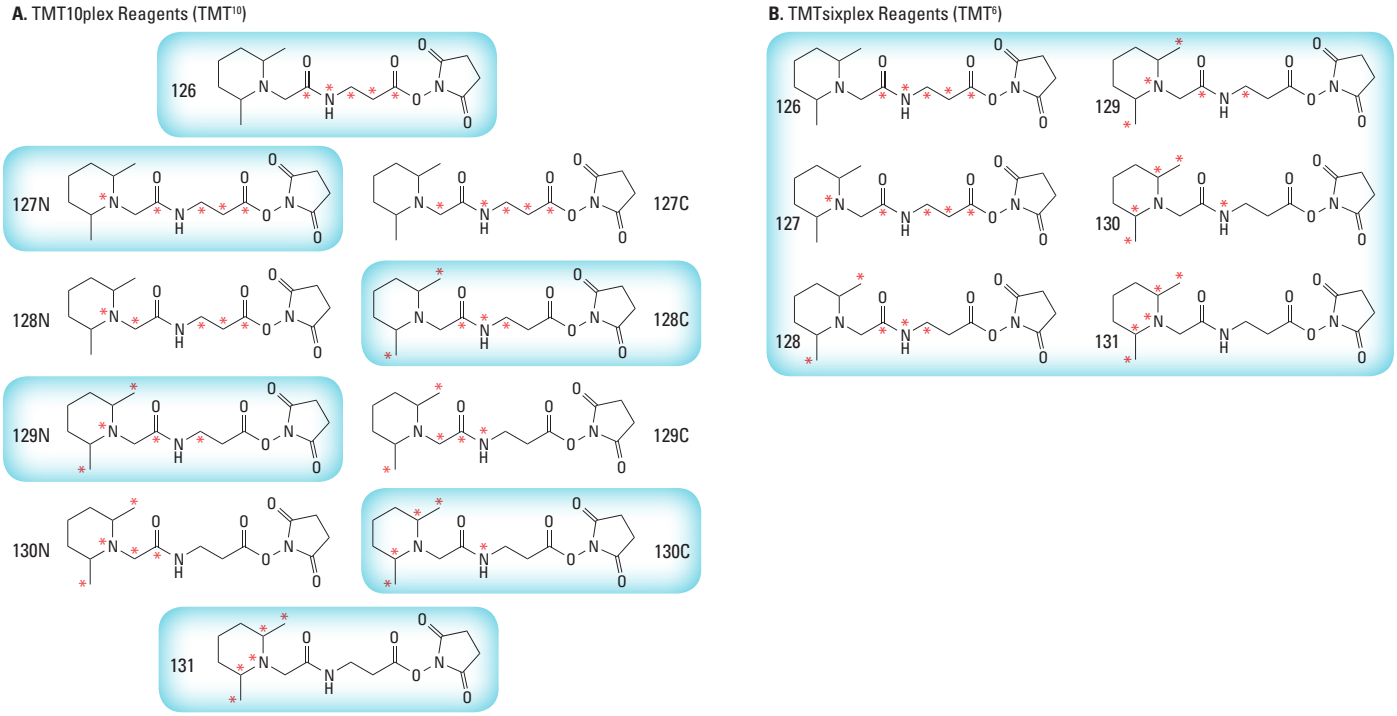
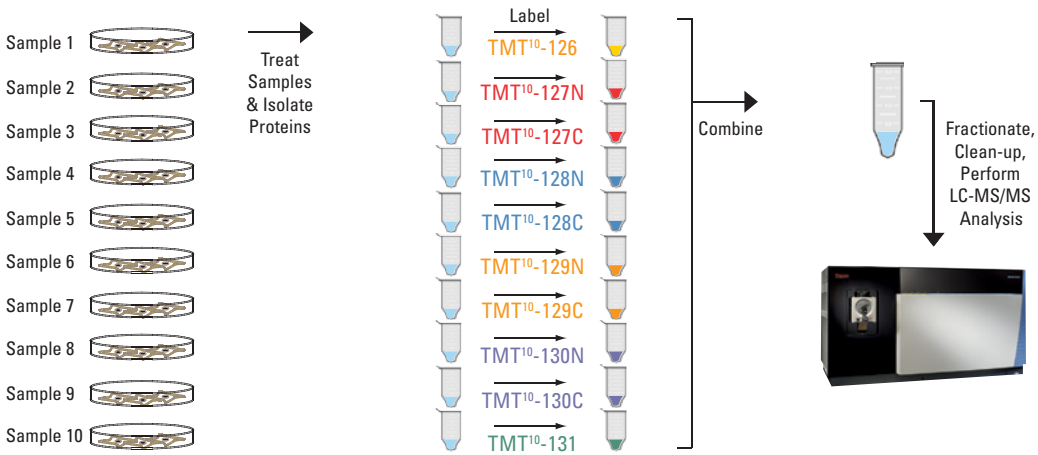


Figure 2. Thermo Scientific TMT Reagent chemical structures. A. TMT10plex Reagent structures with ^{13}C and ^{15}N heavy isotope positions (red asterisks). **B.** TMTsixplex Reagent structures with ^{13}C and ^{15}N heavy isotope positions (red asterisks).



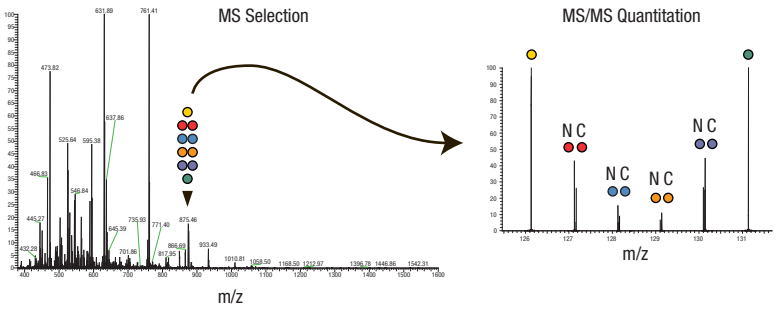


Figure 3. Procedure summary for MS experiments with Thermo Scientific TMT10plex Isobaric Mass Tagging Reagents. Protein extracts isolated from cells or tissues are reduced, alkylated and digested overnight. Samples are labeled with the TMT Reagents and then mixed before sample fractionation and clean up. Labeled samples are analyzed by high-resolution Orbitrap LC-MS/MS before data analysis to identify peptides and quantify reporter ion relative abundance.

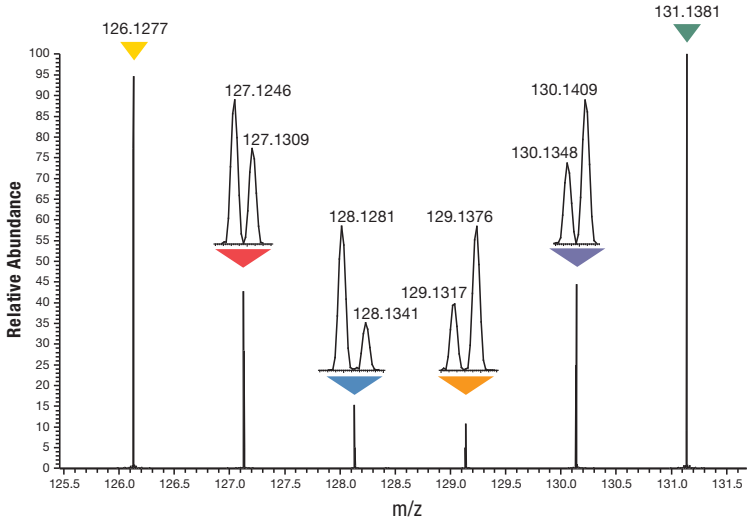


Figure 4. Example of 10-plex relative quantitation using Thermo Scientific TMT10plex Reagents. BSA tryptic digests labeled with TMT10plex Reagents (TMT[10] Reagent that is 126-131Da) were mixed 16:8:4:2:1:1:2:4:8:16 and analyzed by high-resolution Orbitrap LC-MS. The relative abundance of the target protein or peptide fragment in 10 different samples is easily measured by comparing the reporter ions generated by MS/MS fragmentation of the different mass tags.

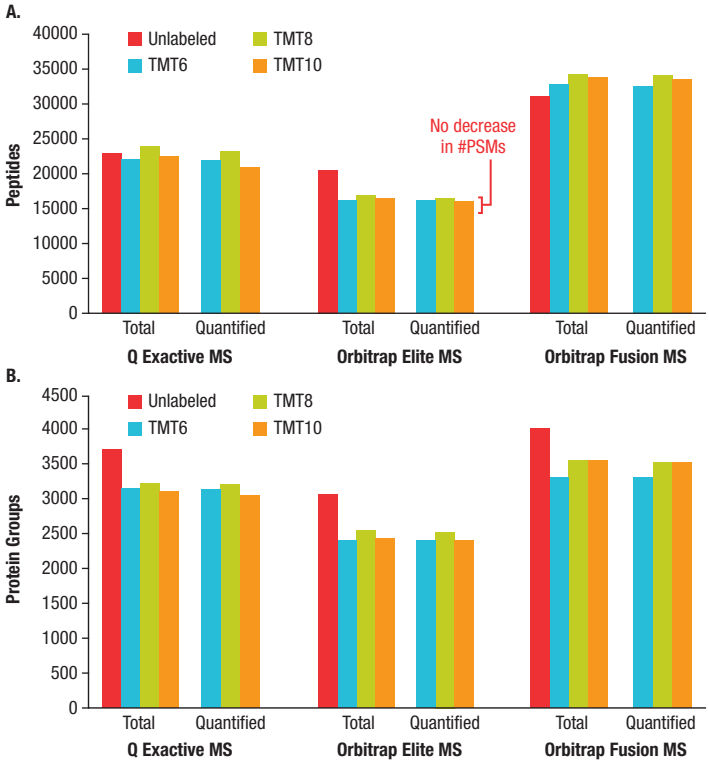


Figure 5. Benchmarking Thermo Scientific Orbitrap MS instruments using Thermo Scientific TMT Reagents with higher multiplexing. Number of total peptide identifications **(A)** and protein groups **(B)** are shown at 1% FDR for 500ng of Thermo Scientific™ Pierce™ HeLa Protein Digest. The number of quantifiable proteins and peptides is also shown. Results represent an average of two replicate runs for each sample.

Ordering Information

Product #	Description	Pkg. Size
90110	**TMT10plex Labeling Reagent Set, 1 x 0.8mg**	10-rxn set
	Sufficient for one 10plex experiment	
	TMT^{10}–126™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–127N™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–127C™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–128N™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–128C™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–129N™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–129C™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–130N™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–130C™ Label Reagent	1 x 0.8mg vial
	TMT^{10}–131™ Label Reagent	1 x 0.8mg vial
90111	**TMT10plex Labeling Reagent Set, 3 x 0.8mg**	30-rxn set
	Sufficient for three 10plex experiments	
	TMT^{10}–126 Label Reagent	3 x 0.8mg vial
	TMT^{10}–127N Label Reagent	3 x 0.8mg vial
	TMT^{10}–127C Label Reagent	3 x 0.8mg vial
	TMT^{10}–128N Label Reagent	3 x 0.8mg vial
	TMT^{10}–128C Label Reagent	3 x 0.8mg vial
	TMT^{10}–129N Label Reagent	3 x 0.8mg vial
	TMT^{10}–129C Label Reagent	3 x 0.8mg vial
	TMT^{10}–130N Label Reagent	3 x 0.8mg vial
	TMT^{10}–130C Label Reagent	3 x 0.8mg vial
	TMT^{10}–131 Label Reagent	3 x 0.8mg vial
90113	**TMT10plex Isobaric Mass Tag Kit**	30-rxn kit
	Sufficient for three 10plex experiments	
	Kit Contents:	
	TMT^{10}–126 Label Reagent	3 x 0.8mg vial
	TMT^{10}–127N Label Reagent	3 x 0.8mg vial
	TMT^{10}–127C Label Reagent	3 x 0.8mg vial
	TMT^{10}–128N Label Reagent	3 x 0.8mg vial
	TMT^{10}–128C Label Reagent	3 x 0.8mg vial
	TMT^{10}–129N Label Reagent	3 x 0.8mg vial
	TMT^{10}–129C Label Reagent	3 x 0.8mg vial
	TMT^{10}–130N Label Reagent	3 x 0.8mg vial
	TMT^{10}–130C Label Reagent	3 x 0.8mg vial
	TMT^{10}–131 Label Reagent	3 x 0.8mg vial
	Dissolution Buffer	5mL
	Denaturing Reagent	1mL
	Reducing Reagent	1mL
	Iodoacetamide	12 vials x 9mg
	Quenching Reagent	1mL
	Pierce™ Trypsin Protease MS-Grade	5 x 20µg
	Albumin, Bovine	2.5mg
	Trypsin Storage Solution	250µL
90406	**TMT10plex Isobaric Reagent Label Reagent Set**	60-rxn set
	Sufficient for 60 samples	
	TMT^{10}–126 Label Reagent	1 x 5mg vial
	TMT^{10}–127N Label Reagent	1 x 5mg vial
	TMT^{10}–127C Label Reagent	1 x 5mg vial
	TMT^{10}–128N Label Reagent	1 x 5mg vial
	TMT^{10}–128C Label Reagent	1 x 5mg vial
	TMT^{10}–129N Label Reagent	1 x 5mg vial
	TMT^{10}–129C Label Reagent	1 x 5mg vial
	TMT^{10}–130N Label Reagent	1 x 5mg vial
	TMT^{10}–130C Label Reagent	1 x 5mg vial
	TMT^{10}–131 Label Reagent	1 x 5mg vial
90061	**TMTsixplex Label Reagent Set, 1 x 0.8mg**	6-rxn set
	Sufficient for one sixplex (1 x 6-way) experiments	
	TMT^{6}-126 Label Reagent	1 x 0.8mg vial
	TMT^{6}-127 Label Reagent	1 x 0.8mg vial
	TMT^{6}-128 Label Reagent	1 x 0.8mg vial
	TMT^{6}-129 Label Reagent	1 x 0.8mg vial
	TMT^{6}-130 Label Reagent	1 x 0.8mg vial
	TMT^{6}-131 Label Reagent	1 x 0.8mg vial
90062	**TMTsixplex Label Reagent Set, 2 x 0.8mg**	12-rxn set
	Sufficient for two sixplex (2 x 6-way) experiments	
	TMT^{6}-126 Label Reagent	2 x 0.8mg vial
	TMT^{6}-127 Label Reagent	2 x 0.8mg vial
	TMT^{6}-128 Label Reagent	2 x 0.8mg vial
	TMT^{6}-129 Label Reagent	2 x 0.8mg vial
	TMT^{6}-130 Label Reagent	2 x 0.8mg vial
	TMT^{6}-131 Label Reagent	2 x 0.8mg vial

Product #	Description	Pkg. Size
90064	**TMTsixplex Isobaric Mass Tagging Kit**	35-rxn set
	Sufficient for five complete sixplex (5 x 6-way) experiments	
	Kit Contents:	
	TMT^{0} Label Reagent	5 x 0.8mg vial
	TMT^{6}-126 Label Reagent	5 x 0.8mg vial
	TMT^{6}-127 Label Reagent	5 x 0.8mg vial
	TMT^{6}-128 Label Reagent	5 x 0.8mg vial
	TMT^{6}-129 Label Reagent	5 x 0.8mg vial
	TMT^{6}-130 Label Reagent	5 x 0.8mg vial
	TMT^{6}-131 Label Reagent	5 x 0.8mg vial
	Dissolution Buffer (1M triethyl ammonium bicarbonate)	5mL
	Denaturing Reagent (10% SDS)	1mL
	Reducing Reagent (0.5 M TCEP)	1mL
	Iodoacetamide	12 x 9mg
	Quenching Reagent (50% hydroxylamine)	1mL
	Trypsin	5 x 20µg
	Trypsin Storage Solution	250µL
	Albumin, Bovine	2.5mg
90064B	**TMTsixplex Isobaric Mass Tagging Kit**	Custom
	Same formulation and kit contents as Product # 90064. Available in custom sizes.	
90066	**TMTsixplex Label Reagent Set, 5 x 0.8mg**	30-rxn set
	Sufficient for five sixplex (5 x 6-way) experiments	
	TMT^{6}-126 Label Reagent	5 x 0.8mg vial
	TMT^{6}-127 Label Reagent	5 x 0.8mg vial
	TMT^{6}-128 Label Reagent	5 x 0.8mg vial
	TMT^{6}-129 Label Reagent	5 x 0.8mg vial
	TMT^{6}-130 Label Reagent	5 x 0.8mg vial
	TMT^{6}-131 Label Reagent	5 x 0.8mg vial
90068	**TMTsixplex Label Reagent Set, 2 x 5mg**	72-rxn set
	Sufficient for 12 sixplex (12 x 6-way) experiments	
	TMT^{6}-126 Label Reagent	2 x 5mg vial
	TMT^{6}-127 Label Reagent	2 x 5mg vial
	TMT^{6}-128 Label Reagent	2 x 5mg vial
	TMT^{6}-129 Label Reagent	2 x 5mg vial
	TMT^{6}-130 Label Reagent	2 x 5mg vial
	TMT^{6}-131 Label Reagent	2 x 5mg vial
90063	**TMTduplex Isobaric Mass Tagging Kit**	15-rxn kit
	Sufficient for five complete duplex (5 x 2-way) experiments	
	Kit Contents:	
	TMT^{0} Label Reagent	5 x 0.8mg vial
	TMT^{2}-126 Label Reagent	5 x 0.8mg vial
	TMT^{2}-127 Label Reagent	5 x 0.8mg vial
	Dissolution Buffer (1M triethyl ammonium bicarbonate)	5mL
	Denaturing Reagent (10% SDS)	1mL
	Reducing Reagent (0.5M TCEP)	1mL
	Iodoacetamide	12 x 9mg
	Quenching Reagent (50% hydroxylamine)	1mL
	Trypsin	2 x 20µg
	Trypsin Storage Solution	250µL
	Albumin, Bovine	2.5mg
90065	**TMTduplex Isobaric Label Reagent Set, 5 x 0.8mg**	10-rxn set
	Sufficient for five duplex (5 x 2-way) experiments	
	TMT^{2}-126 Label Reagent	5 x 0.8mg
	TMT^{2}-127 Label Reagent	5 x 0.8mg
90060	**TMTduplex Isotopic Label Reagent Set, 5 x 0.8mg**	10-rxn set
	Sufficient for five duplex (5 x 2-way) experiments	
	TMT^{0} Label Reagent	5 x 0.8mg
	TMT^{6}-127 Label Reagent	5 x 0.8mg
90067	**TMTzero Label Reagent, 5 x 0.8mg**	5-rxn set
	Sufficient for controls in five experiments	
	TMT^{0} Label Reagent, 5 x 0.8mg	
90114	**1M Triethylammonium Bicarbonate (TEAB)**	50mL
90115	**50% Hydroxylamine**	5mL

For a list of references using amine-reactive TMT Reagents, please see the back cover.

Thermo Scientific Cysteine-reactive Tandem Mass Tag Reagents

For protein expression analysis of sulfhydryl groups by mass spectrometry.

Thermo Scientific Tandem Mass Tag (TMT) Reagents enable concurrent identification and multiplexed quantitation of proteins in different samples using tandem mass spectrometry. The Thermo Scientific™ iodoTMT™ Reagents are sets of isobaric isomers (i.e., same mass and structure) that contain iodoacetyl function groups for covalent, irreversible labeling of sulfhydryl (–SH) groups. Similar to iodoacetamide, iodoTMT Reagents react specifically with reduced cysteines in peptides and proteins. iodoTMT Reagents can be differentiated by tandem mass spectrometry (MS/MS), enabling identification and relative quantitation of cysteine modifications, such as S-nitrosylation, oxidation and disulfide bonds, across different samples or experimental conditions. IodoTMT Reagents replace our previously offered Thermo Scientific™ CysTMT™ Reagents, which utilized a dithiopyridine reactive group to reversibly label cysteine sulfhydryls.

Highlights:

- **Specific** – only reacts with reduced sulfhydryl groups
- **Irreversible** – labeled proteins and peptides are not susceptible to reducing agents
- **Flexible** – options for duplex isotopic (MS) or sixplex isobaric (MS/MS) quantitation
- **Complete** – workflow combines efficient labeling, enrichment and elution for identification and quantitation of cysteine-containing peptides

Applications:

- Identify and quantify low abundant cysteine-containing peptide subproteome
- Combine up to six different samples or experimental conditions in a single LC-MS analysis
- Determine sites of cysteine post-translational modifications (e.g., S-nitrosylation, oxidation and disulfide bonds)
- Measure cysteine post-translational modification occupancy across different samples or experimental conditions

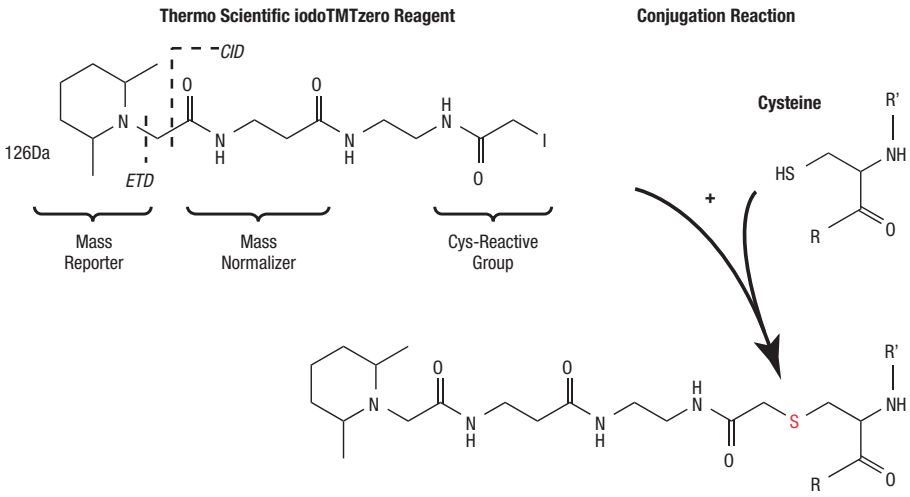


Figure 1. Mechanism of Thermo Scientific iodoTMT Reagent reaction with cysteine-containing proteins or peptides.

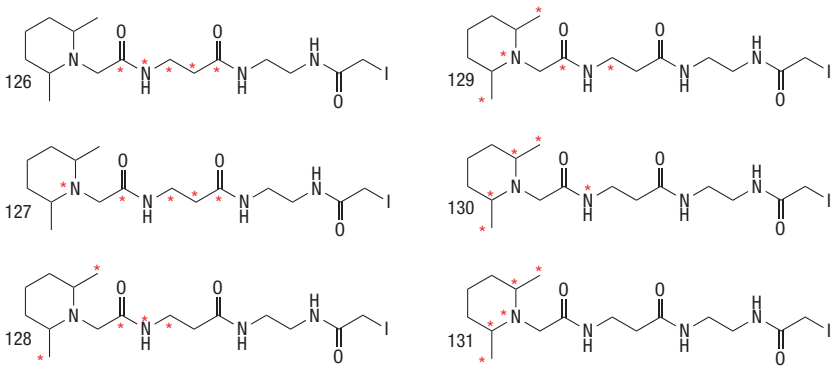
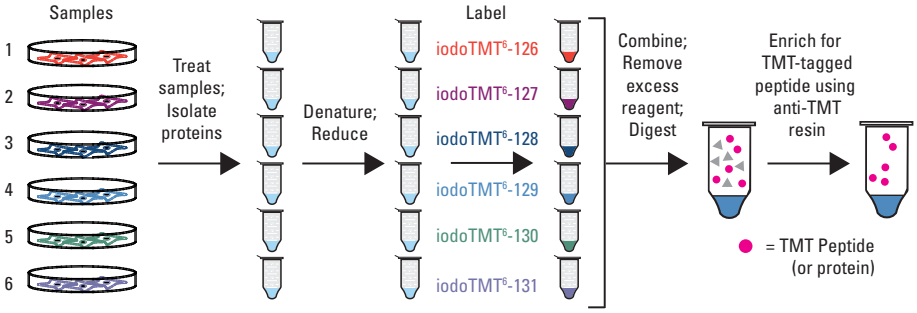


Figure 2. Structure of Thermo Scientific iodoTMTsixplex Reagents for cysteine labeling, enrichment and isobaric MS quantitation.



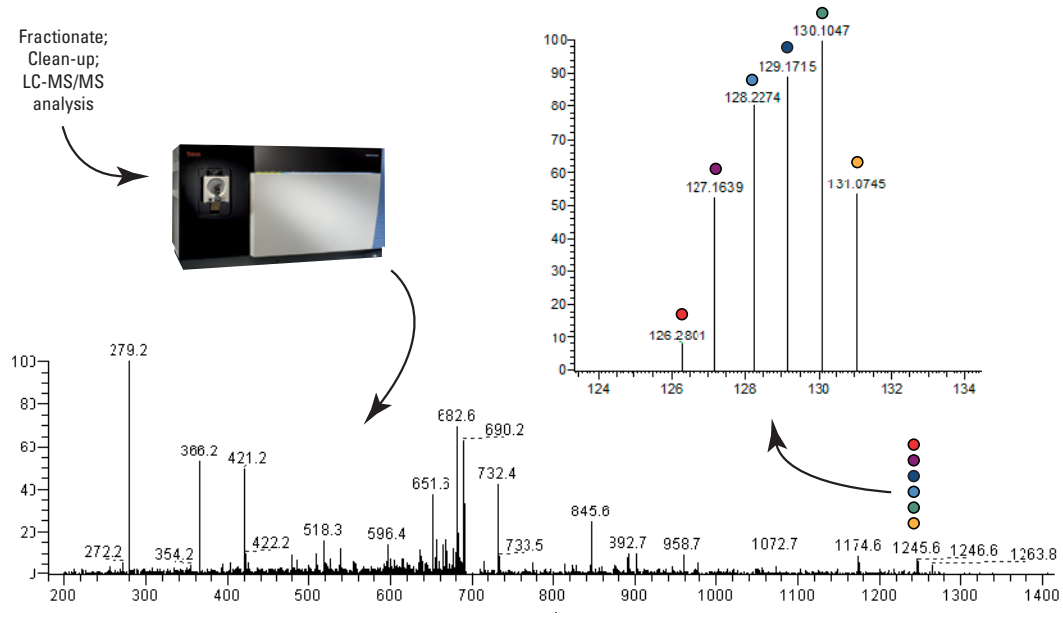


Figure 3. Schematic of Thermo Scientific iodoTMTsixplex Reagent workflow. Six different sample conditions can be prepared for iodoTMT Reagent labeling. Labeled proteins are combined before iodoTMT-labeled peptide enrichment using immobilized anti-TMT antibody resin and subsequent LC-MS/MS analysis of isobaric reporter ions.

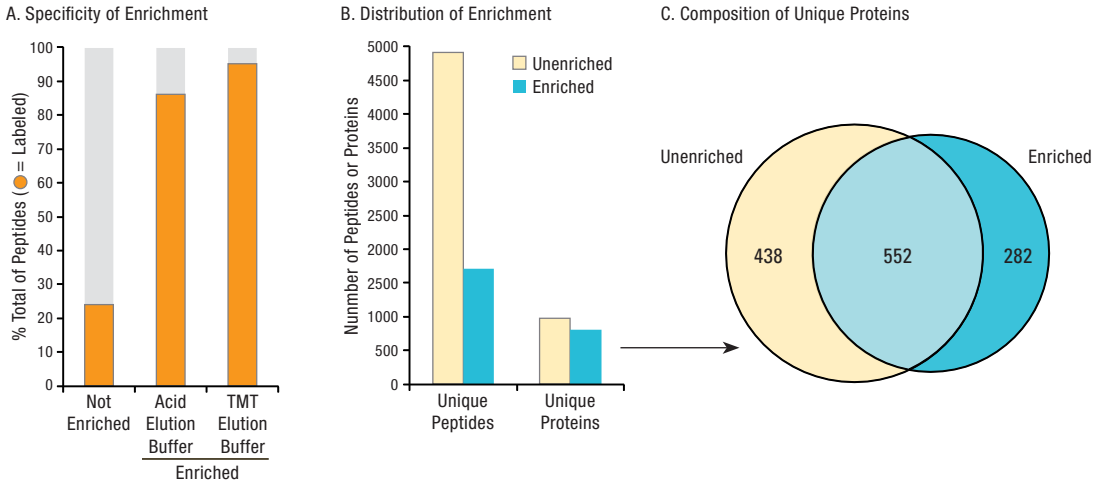


Figure 4. Anti-TMT enrichment of iodoTMT-labeled peptides. Panel A: Percent of iodoTMT-labeled peptide modifications from A549 cell lysate proteins identified before and after enrichment using acidic and TMT elution buffers. **Panel B:** Comparison of total number of unique peptides and proteins identified before and after anti-TMT antibody resin enrichment. **Panel C:** Venn diagram of unique proteins identified before and after anti-TMT antibody resin enrichment.

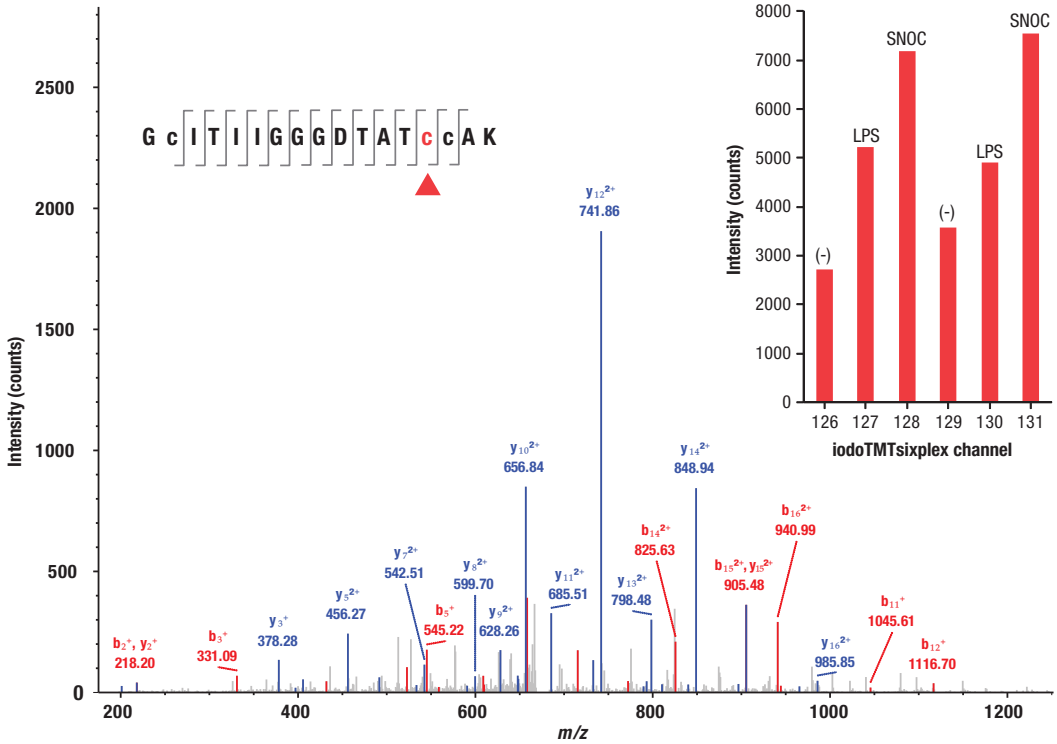


Figure 5. Measured induction of S-nitrosylation in phosphoglycerate kinase 1 peptide. BV-2 glioma cells were either untreated or treated with lipopolysaccharide (LPS) or S-nitrosocysteine (SNOC) for 20 hours to induce S-nitrosylation and selectively labeled with iodoTMTsixplex Reagents using the S-nitrosylation switch assay. MS spectrum includes phosphoglycerate kinase 1 peptide (GcITIIGGGDTATccAK, inset) showing localization of the iodoTMT-modified cysteine (red). Inserted graph shows an increase in S-nitrosylation of phosphoglycerate kinase 1 peptide in response to S-nitrosylation inducing agents lipopolysaccharide (LPS, 127 & 130) and S-nitrosocysteine (SNOC, 129 &131) determined by relative quantitation of TMT reporter ions in duplicate samples.

Ordering Information

Product #	Description	Pkg. Size
90100	**iodoTMTzero Label Reagent Set, 5 x 0.2mg** *Sufficient for controls in five experiments* iodoTMT^0-126 Label Reagent	5-rxn set 5 x 0.2mg
90101	**iodoTMTsixplex Label Reagent Set, 1 x 0.2mg** *Sufficient for one sixplex (1 x 6-way) experiment* iodoTMT^6-126 Label Reagent iodoTMT^6-127 Label Reagent iodoTMT^6-128 Label Reagent iodoTMT^6-129 Label Reagent iodoTMT^6-130 Label Reagent iodoTMT^6-131 Label Reagent	6-rxn set 1 x 0.2mg 1 x 0.2mg 1 x 0.2mg 1 x 0.2mg 1 x 0.2mg 1 x 0.2mg
90102	**iodoTMTsixplex Label Reagent Set, 5 x 0.2mg** *Sufficient for five sixplex (5 x 6-way) experiments* iodoTMT^6-126 Label Reagent iodoTMT^6-127 Label Reagent iodoTMT^6-128 Label Reagent iodoTMT^6-129 Label Reagent iodoTMT^6-130 Label Reagent iodoTMT^6-131 Label Reagent	30-rxn set 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg
90103	**iodoTMTsixplex Isobaric Mass Tag Labeling Kit** *Sufficient for five complete sixplex (5 x 6-way) experiments* Kit Contents: iodoTMT^6-126 Label Reagent iodoTMT^6-127 Label Reagent iodoTMT^6-128 Label Reagent iodoTMT^6-129 Label Reagent iodoTMT^6-130 Label Reagent iodoTMT^6-131 Label Reagent HES Buffer Bond-Breaker™ TCEP Solution DTT, No-Weigh™ Format Trypsin Trypsin Storage Solution Albumin (bovine)	30-rxn kit 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 5 x 0.2mg 15mL 0.5mL 8 x 7.7mg 5 x 20µg 250µL 2.5mg
90075	**Anti-TMT Antibody** *Sufficient for detection of the TMT label in Western blots*	0.1mL
90076	**Immobilized Anti-TMT Antibody Resin** *Sufficient for binding 250µg TMT-BSA/mL resin*	6mL
90104	**TMT Elution Buffer** *Sufficient for elution of TMT-labeled peptides from immobilized anti-TMT antibody resin*	20mL
90106	**HENS Buffer** *Sufficient for detection of the TMT label in Western blots*	100mL

Thermo Scientific Carbonyl-reactive Tandem Mass Tag Reagents

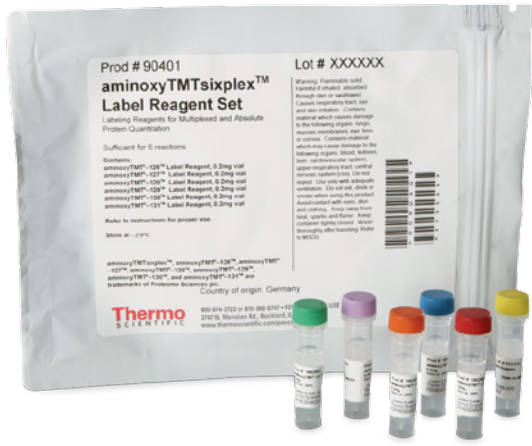
Enable characterization and multiplex quantitation of carbonyl-containing biomolecules.

The Thermo Scientific™ aminoxyTMT™ Mass Tag Labeling Reagents enable multiplexed relative quantitation of carbonyl-containing compounds by mass spectrometry (MS). The six compounds of the Thermo Scientific™ aminoxyTMTsixplex™ Reagent Set have the same nominal mass (i.e., isobaric) and chemical structure (carbonyl-reactive aminoxy group, spacer arm and mass reporter). However, the specific distribution of ^{13}C and ^{15}N isotopes on either side of the high energy collision dissociation (HCD) or electron transfer dissociation (ETD) MS/MS fragmentation site in each reagent results in a unique reporter mass (126-131Da) in the low mass region. This distribution of reporter masses is used to measure the relative abundance of labeled molecules in a combined (multiplexed) MS sample representing six different treatment conditions. The aminoxyTMT Reagents may be used to quantify a broad range of biologically important molecules including carbohydrates, steroids or oxidized proteins.

For glycobiology MS applications, native glycans are difficult to study by mass spectrometry because of their poor ionization efficiency. Quantitation of glycans is particularly challenging due to the lack of standards for all naturally occurring glycans and difficulties reproducibly quantifying multiple samples. The aminoxy group has better reactivity with carbonyls and better stability of the labeled product compared to hydrazide groups. Labeling with the aminoxyTMT Reagents improves ionization of glycans, thus improving sensitivity, and enables relative quantitation of glycans for up to six samples concurrently.

Highlights:

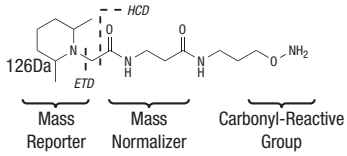
- **Quantitative** – enables relative quantitation of glycans or other carbonyl-reactive proteins from multiple samples of cells, tissues or biological fluids
- **Stable** – stable product formed after labeling reaction
- **Efficient** – achieve labeling efficiency greater than 90% in one hour
- **Sensitive** – labeled glycan signal-to-noise ratio improved greater than 20-fold when compared to native glycan
- **Multiplex** – identify and characterize up to six samples concurrently
- **Optimized** – procedure and reagents optimized for excellent labeling efficiency and recovery of glycans



Applications:

- Relative quantitation of glycans
- Study of structural diversity of protein glycosylation
- Study of glycosylation in cell signaling and regulation
- Study of cancer progression, biomarker discovery and analysis of biotherapeutics
- Study of protein oxidation

A. aminoxyTMTzero Reagent



B. Labeling the reducing end carbonyl of a glycan with aminoxyTMT Reagent

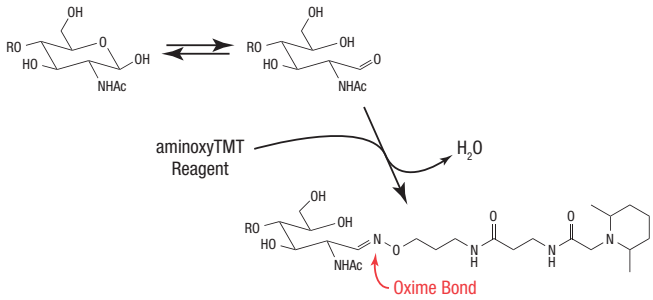


Figure 1. Thermo Scientific aminoxyTMT Reagent chemical structures. A. Functional regions of the aminoxyTMTzero Reagent structure including MS/MS fragmentation sites by HCD and ETD. **B.** Reaction scheme for labeling of reducing-end sugars with aminoxyTMT Reagent.

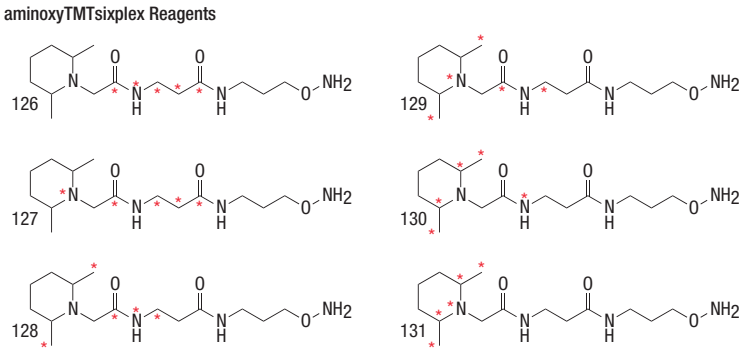


Figure 2. Thermo Scientific aminoxyTMTsixplex Reagent structures and isotope positions (*).

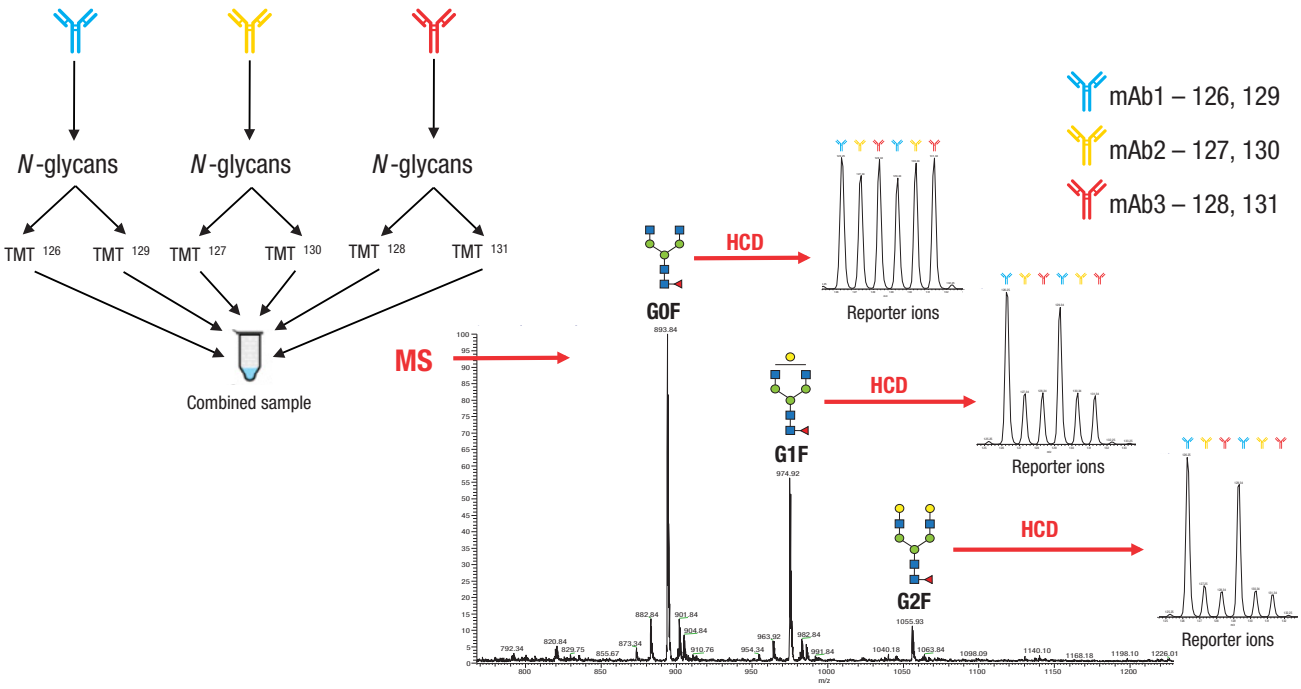


Figure 3. Relative quantitation of glycans from monoclonal antibodies. *N*-glycans were released from three different mouse monoclonal antibodies (approximately 100µg each) using PNGase F glycosidase. Each of the three glycan samples was split into two equal parts and labeled with a different mass tag, indicated in the figure. After labeling, quenching and clean-up, the samples were mixed and analyzed by direct infusion ESI-MS in the positive ion mode on a Velos Pro Mass Spectrometer. Glycoforms of interest were identified in the MS spectra and were subjected to HCD MS/MS. Reporter ion relative peak intensities provide information on relative glycoform abundance in the three samples (insets).

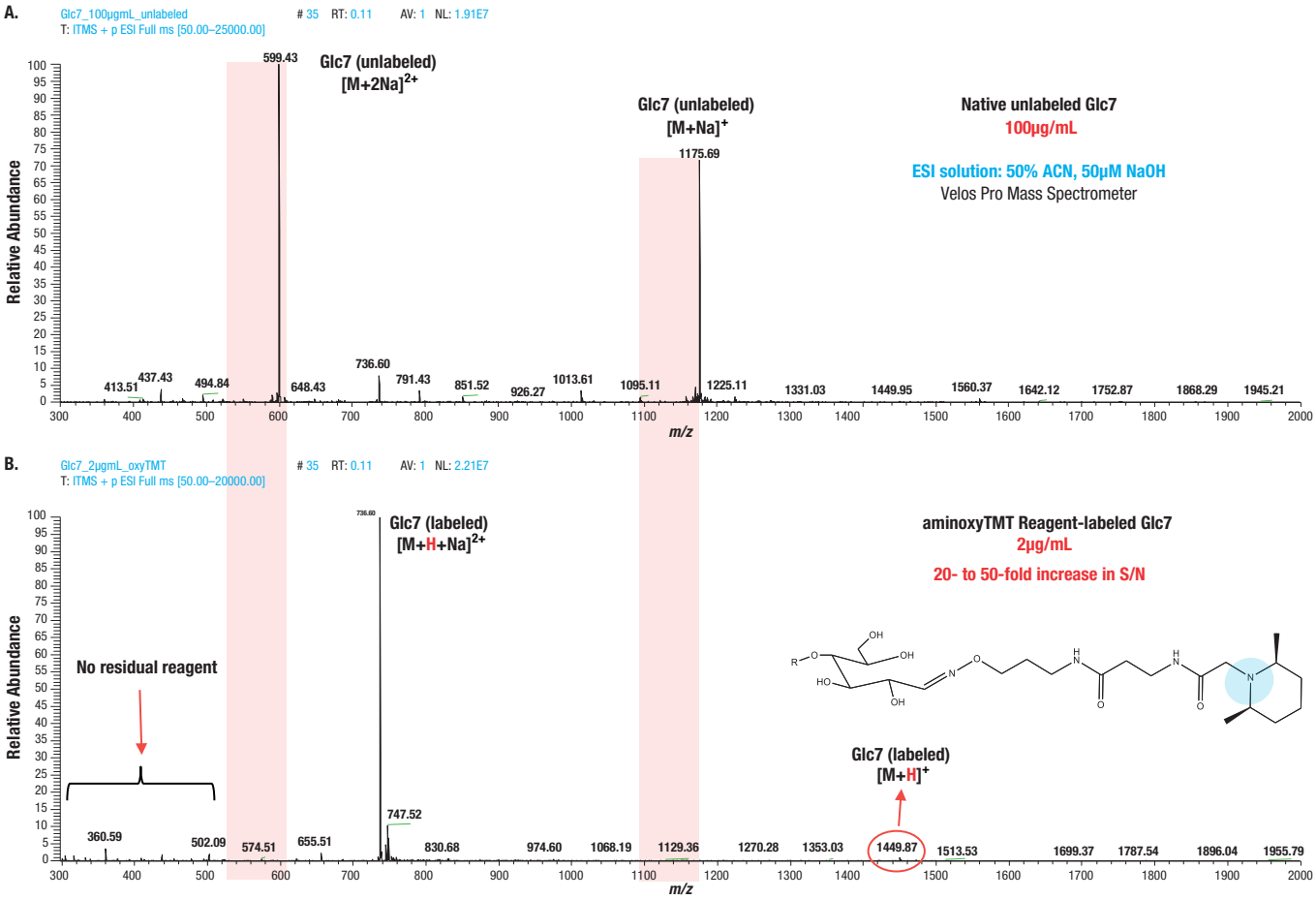


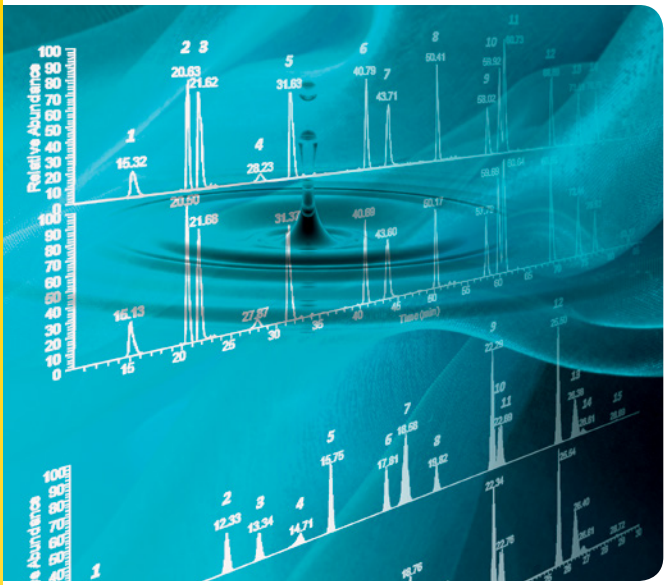
Figure 4. Positive ion mode electrospray ionization (ESI) of native unlabeled and aminoxyTMT-labeled maltoheptaose (Glc7) standard. Panel A: MS spectrum of native unlabeled Glc7; **Panel B:** MS spectrum of aminoxyTMT-labeled Glc7 standard. Fifty-fold lower concentration of the aminoxyTMT-labeled sample solution produced similar base peak intensity as the unlabeled sample. Residual aminoxyTMT reagent or unlabeled Glc7 were not detected after labeling and sample clean-up (Panel B).

Ordering Information

Product #	Description	Pkg. Size
90400	**aminoxyTMTzero Label Reagent Set, 6 x 0.2mg**	6-rxn set
	Sufficient for controls in six experiments	
	aminoxyTMT^0 Label Reagent	6 x 0.2mg vial
90401	**aminoxyTMTsixplex Label Reagent Set, 1 x 0.2mg**	6-rxn set
	Sufficient for six reactions	
	aminoxyTMT^6–126 Label Reagent	1 x 0.2mg vial
	aminoxyTMT^6–127 Label Reagent	1 x 0.2mg vial
	aminoxyTMT^6–128 Label Reagent	1 x 0.2mg vial
	aminoxyTMT^6–129 Label Reagent	1 x 0.2mg vial
	aminoxyTMT^6–130 Label Reagent	1 x 0.2mg vial
	aminoxyTMT^6–131 Label Reagent	1 x 0.2mg vial
90402	**aminoxyTMTsixplex Label Reagent Set, 5 x 0.2mg**	30-rxn set
	Sufficient for five sixplex reactions	
	aminoxyTMT^6–126 Label Reagent	5 x 0.2mg vial
	aminoxyTMT^6–127 Label Reagent	5 x 0.2mg vial
	aminoxyTMT^6–128 Label Reagent	5 x 0.2mg vial
	aminoxyTMT^6–129 Label Reagent	5 x 0.2mg vial
	aminoxyTMT^6–130 Label Reagent	5 x 0.2mg vial
	aminoxyTMT^6–131 Label Reagent	5 x 0.2mg vial

targeted quantitation

Measuring expression of selected proteins



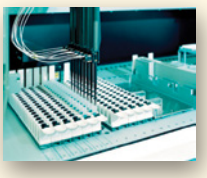
Targeted proteomics experiments are typically designed to quantify less than 100 proteins with very high precision, sensitivity, specificity and throughput. Targeted MS quantitation strategies use specialized workflows and instruments to improve the specificity and quantification of a limited number of features across hundreds or thousands of samples.

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

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

Specialized software such as Thermo Scientific™ Pinpoint 1.4 Software ensures as much high-quality data is acquired, and as much valuable information is extracted from that data, as possible.

Targeted Quantitation Workflow by Sample Type Using Thermo Scientific Products

	Mammalian Cells	Tissue	Serum/Plasma Biofluids
Reagents	• Lysis & Fractionation Reagents (Mass Spec Sample Prep Kit for Cultured Cells)		• Abundant Protein Depletion (Albumin, Top 2 or Top 12 Protein Depletion Spin Columns and Kits)
	• Targeted Protein Digestion Standard **(1-Step Heavy Protein *In Vitro* Protein Expression Kit)**		
	• Protein Immunoprecipitation (Pierce IP/co-IP Kits)		
	• Protein Quantitation Reagents (BCA and Micro BCA Protein Assay Kits)		
	• Protein Digestion Verification **(Digestion Indicator for Mass Spectrometry)** • Protein Digestion - Proteases (Trypsin, LysC, LysN, Chymotrypsin, AspN, and/or GluC Proteases, MS Grade)		
	• Peptide Enrichment (Fe-NTA and/or TiO_2 Phosphopeptide Enrichment Columns and Kits)		
	• Protein Quantitation Standards **(HeavyPeptide AQUA Standards, PEPotech SRM Libraries)** • **Peptide Retention Time Calibration Mixture**		
	• Peptide Clean-Up (Detergent Removal Spin Columns) • Peptide Clean-Up (C18, Graphite Spin Columns and Tips)		
Instruments			
	• TSQ Vantage Triple Quadrapole Mass Spectrometer • TSQ Endura Triple Quadrapole Mass Spectrometer • TSQ Quantiva Triple Quadrapole Mass Spectrometer • Q Exactive Mass Spectrometer		
Software	• Pinpoint 1.4 Software		

Thermo Scientific HeavyPeptide AQUA Standards

High-quality isotopically labelled peptides for absolute quantitation.

The Thermo Scientific™ HeavyPeptide™ AQUA Custom Synthesis Service provides isotopically labelled, AQUA-grade peptides for the relative and absolute quantitation of proteins at very low concentrations in complex protein mixtures.

HeavyPeptide sequences up to 15 amino acids in length are synthesized using the latest Fmoc solid-phase technology and purified by HPLC. All HeavyPeptide sequences are analyzed by mass spectrometry, and AQUA- (Absolute QUAntitation), Ultimate- and QuantPro-grade peptides are validated using stringent analytical HPLC to determine the final purity and assure that you are receiving only the highest quality peptides for absolute quantitation. We offer advanced heavy peptide synthesis capabilities with a wide range of labels, modifications, scales and purities to meet your research needs. HeavyPeptide AQUA-grade peptides are also part of our Thermo Scientific™ HeavyPeptide™ FasTrack Service to accelerate targeted assay development.

These peptides are packaged using our Thermo Scientific™ ArgonGuard service, in which peptides are packaged in argon gas to minimize amino acid oxidation during shipping and storage. This standard service helps maintain biological activity of custom peptides and reduce experimental variation.

Highlights:

- **Accurate** – concentration precision to meet your application needs
- **Consistent results** – ArgonGuard service helps maintain biological activity of peptides
- **Sensitive** – enables the absolute quantification of low-abundant proteins
- **Specific** – 100% peptide sequence specificity
- **Flexible** – extensive list of available modifications, solvents, concentrations, aliquots and formats

Includes:

- One isotopically labelled peptide or non-labelled control peptide sequence

Applications:

- Biomarker discovery, verification and validation
- Functional quantitative proteomics
- Quantitation of post-translational modifications
- Confirmation of RNA interference (RNAi)
- Pharmacokinetics
- Metabolomics
- Clinical biochemistry for drug and metabolite monitoring
- Anti-doping testing
- Protein expression monitoring
- Cell signal profiling and pathway validation

Table 1. Thermo Scientific HeavyPeptide AQUA Grades.

Grade	Description
AQUA Ultimate	Fully solubilized peptides with a concentration precision ±5%. The best choice for biomarker validation and experiments demanding ultimate quantitative precision and batch-to-batch reproducibility.
AQUA QuantPro	Fully solubilized peptides with a concentration precision ±25% that are ideal for biomarker verification.
AQUA Basic	Lyophilized peptides that are more adequate for relative quantitation. The batch-to-batch consistency is not quantitated.

Table 2. Specifications of Thermo Scientific HeavyPeptide AQUA-Grade Standards.

Grade	AQUA Ultimate	AQUA QuantPro	AQUA Basic
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%	NA
Peptide purity	>97%	>97%	>95%
Isotopic enrichment	>99%	>99%	>99%
Peptide length	Up to 30 amino acids	Up to 30 amino acids	Up to 30 amino acids
Amount/No. of aliquots	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots	15 to 30nmol†† (0.05 to 0.1mg)/aliquot
Quality control	MS & analytical HPLC, AAA (+/-5-10%)	MS & analytical HPLC, AAA (+/-25%)	MS & analytical HPLC
Delivery time§	6-8 weeks	6-8 weeks	6-8 weeks
Shipment	In solution on wet ice	In solution on wet ice	Lyophilized at room temp.
Product options	• Additional light amino acids to extend the peptide length • Additional heavy amino acid on each peptide • Multiple solvents, concentrations and aliquot sizes available • Peptides delivered in various formats (i.e., 96-well plate with or without detachable tubes in glass or plastic, 2D barcodes, etc.)		
Peptide modifications	• Single or double phosphorylation (pY, pT or pS) • Cysteine carbamidomethylation (CAM)‡ • Chloro-L-Tyrosine • Pryoglutamic acid • Methionine oxidation (Met[O]) • Other modifications available on request		

† *Amino acid analysis.*
§ *These production times are estimates that vary based on the number of peptides ordered.*
†† *30nmol is valid for peptides 6-15 amino acids in length. For shorter or longer peptides, the amount might decrease to as little as 15nmol.*
‡ *CAM tends to cause cyclisation at the N-terminus. Fully cyclized form can be provided upon request.*

Table 3. Heavy amino acids offered with Thermo Scientific HeavyPeptide Custom Synthesis.†

Amino acid	Code	Mass difference	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-$^{13}C_5$, $^{15}N_2$	>99%
Valine	V	+6Da	U-$^{13}C_5$, $^{15}N_2$	>99%

† *Other amino acids on request.*

For a list of references using HeavyPeptide Reagents, please see the back cover.

targeted quantitation

Measuring expression of selected proteins

Thermo Scientific HeavyPeptide AQUA FasTrack Service

Maximize your budget using two-step targeted assay development for SRM.

The HeavyPeptide AQUA FasTrack Service is a two-step approach to accelerate targeted assay development and absolute quantitation by selected reaction monitoring (SRM) within a controlled-budget environment.

Quantitative proteomics workflows usually begin with the software-assisted selection of proteotypic peptide candidates. After synthesis, these crude peptides are used to identify the best peptide candidates and optimize the quantitative liquid chromatography-mass spectrometry (LC-MS) assay. Highly pure HeavyPeptide sequences are then synthesized and purified for target quantitation. Traditionally, these steps required two independent syntheses, which are time-consuming and expensive. The HeavyPeptide FasTrack Service combines targeted assay development and quantitation for a rapid, precise workflow that saves you time and money.

The HeavyPeptide FasTrack Service provides highly pure AQUA-, Ultimate- or QuantPro-grade peptides through two phases:

FasTrack 1:

Crude HeavyPeptide sequences (up to 96 per order) are synthesized within two weeks, and a 100µg aliquot of each peptide is shipped for proteotypic peptide selection and assay development. The remainder of each crude peptide is stored until optimization is completed. The customer then indicates the specific peptides and number of aliquots required for target quantitation.

FasTrack 2 (optional):

Because the crude peptides selected in FasTrack phase 1 have already been synthesized, there is no need to resynthesize the peptides for target quantitation by SRM. The selected crude peptides are purified to a minimum purity of 97% and fully solubilized using a proprietary process. The HeavyPeptide concentrations are measured by amino acid analysis, and quality control is performed using MALDI MS and analytical HPLC. AQUA-grade peptides are then shipped on wet ice for target quantitation in three weeks or less.

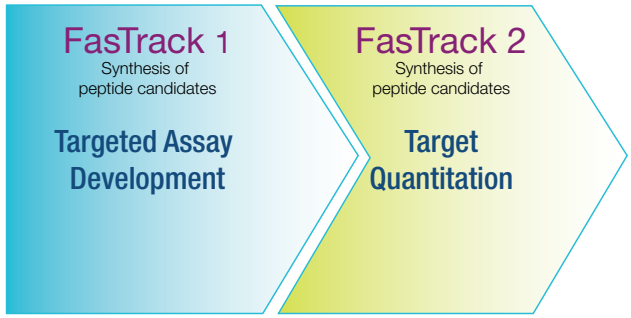
Another cost-effective alternative for the medium- to high-throughput development of SRM assays as part of the targeted assay development are Thermo Scientific™ PEPotec™ SRM Custom Peptide Libraries (see pgs. 26-27), which can be supplied in library sizes from 24 to >1000 peptides.

Highlights:

- **Efficient** – no need to resynthesize selected peptides for target quantitation
- **Accurate** – concentration precision to meet your application needs
- **Sensitive** – enables the absolute quantification of low-abundant proteins
- **Consistency** – peptides from the same synthesis reaction are used for both assay development and quantitation

Applications:

- Biomarker discovery, verification and validation
- Functional quantitative proteomics
- Quantitation of post-translationally modified proteins
- Confirmation of results from RNA interference (RNAi)
- Pharmacokinetics
- Metabolomics
- Clinical biochemistry for drug and metabolite monitoring
- Anti-doping testing
- Protein expression monitoring
- Cell signal profiling and pathway validation



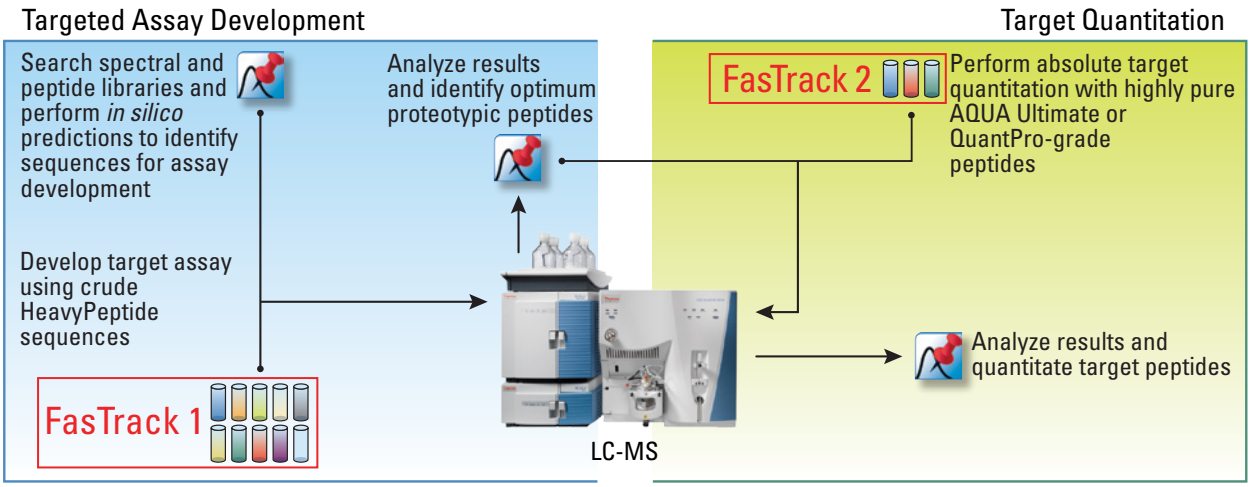


Figure 2. The Thermo Scientific HeavyPeptide FasTrack Service accelerates target assay development and quantitation.
A key step for targeted quantitation is identifying sequence-specific or unique target peptides with sufficient signal intensity for the absolute quantitation of the target peptide(s). This step begins with a combination of peptide and spectral database searches and computational (in silico) predictions using programs such as Pinpoint Software to identify an initial list of potential peptides that corresponds to multiple proteins of interest. Crude HeavyPeptide sequences are synthesized in FasTrack phase 1 and screened by LC-MS. Results are analyzed to optimize LC and MS workflows and identify the best proteotypic sequences for target quantitation. The selected crude peptides are purified in FasTrack phase 2 and used for absolute target quantitation by SRM via LC-MS.

Table 1. Thermo Scientific HeavyPeptide AQUA grades.

Grade	Description
AQUA Ultimate	Fully solubilized peptides with a concentration precision +/-5% that are the best choice for biomarker validation and experiments demanding ultimate quantitative precision and batch-to-batch reproducibility.
AQUA QuantPro	Fully solubilized peptides with a concentration precision ±25% that are ideal for biomarker verification.

Table 2. Specifications of Thermo Scientific HeavyPeptide† FasTrack phases.

Phase	FasTrack 1	FasTrack 2	
Grade	**Crude**	**AQUA Ultimate**	**AQUA QuantPro**
Formulation	Lyophilized	5pmol/µL in 5% (v/v) acetonitrile/H_2O	5pmol/µL in 5% (v/v) acetonitrile/H_2O
Peptide purity	Crude	>97%	>97%
Amount/No. of aliquots	0.1mg/1 aliquot	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots
Quality control	Mass spectrometry	MALDI MS and analytical HPLC	MALDI MS and analytical HPLC
Delivery time§	2 weeks	3 weeks	3 weeks
Shipment	Lyophilized at room temp.	In solution on wet ice	In solution on wet ice

† See HeavyPeptide Synthesis Service for all information on HeavyPeptide custom synthesis.
§ These production times are estimates that vary based on the number of kits ordered.

Thermo Scientific PEPotec SRM Peptide Libraries

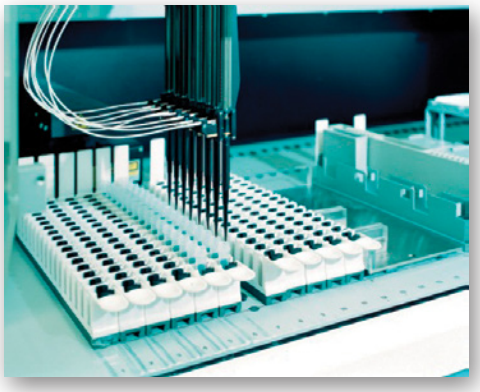
Fully synthetic, crude peptides customized for the development of mid- to high-throughput SRM and MRM assays.

The study of proteomes, sub-proteomes and protein pathways often requires quantitative MS analysis that depends on the identification and validation of SRM and MRM assays. PEPotec SRM Peptide Libraries offer great convenience and flexibility in the development of quantitative MS approaches while accelerating biological assays by reducing the setup time of MS experiments. These peptide libraries were developed as a result of our involvement in the SRMAtlas project, which seeks to map the entire human proteome.

The standard service supplies a suspension of at least 0.1mg of each crude peptide housed in individual tubes in a 96-well plate format with either arginine (R) or lysine (K) as the C-terminal amino acid. Three quality control grades are available, and optional services and peptide modifications are offered to give you the peptide libraries that fit your experimental needs.

Highlights:

- **Convenient** – peptides are provided in individual 2-D barcoded tubes in 96-tube plates
- **Application-specific** – grades and optional services help you get the right peptides for your assay
- **Easy to use** – standard libraries are delivered suspended in 0.1% trifluoroacetic acid (TFA) in 50% (v/v) acetonitrile/water
- **Flexible** – extensive list of available modifications



Applications:

- Mid- to high-throughput development of SRM and MRM assays
- MS workflows with relative and absolute quantitation strategies

Includes:

- Fully synthetic crude (as synthesized) peptides
- Multiple grades of QC analysis and optional services and modifications
- Provided in individual Thermo Scientific™ Matrix™ 96-Tube plates

Table 1. Thermo Scientific PEPotec SRM Peptide Libraries – Three grades to fit your experimental needs.

	Grade 1 Fast and Easy	Grade 2 Greater Analysis	Grade 3 Maximum Assurance
Quantity	>0.1mg		
Length[1]	6 to 25 amino acids. Up to 35 amino acids are available for an additional fee		
Purity	Crude (as synthesized)		
Formulation[1]	Suspended in 0.1% TFA in 50% (v/v) acetonitrile/water		
Delivery format	**Matrix 96-Tube plates (Product # 3712MTX)**		
C-terminal residue[1]	R or K		
Counter ion	TFA		
Quality control (QC)	MS check of 5% of peptides	MS check of 100% of peptides	MS analysis of 100% of peptides
Peptide resynthesis[2]	Not provided	Not provided	One resynthesis provided
Failed synthesis policy	You pay for entire set of peptides ordered	You only pay for peptides synthesized	You only pay for peptides synthesized
Included documentation	Peptide amount	Peptide amount	Peptide amount and MS spectra
Minimum order[3]	24 peptides	4 peptides	4 peptides

[1] *Changes to the standard length restrictions, formulation and C-terminal residues are available as optional services.*
[2] *Peptides not detected during MS analysis will be resynthesized (depending on the grade selected).*
[3] *Orders for fewer than 48 peptides incur a plate fee.*

Table 2. Thermo Scientific PEPotec SRM Peptide Library optional services.

QC: Analytical HPLC & MALDI-MS of 100% of samples
QC: LC -MS of 100% of samples
Lyophilized
Individually labeled tubes
Peptides that are 3-5 or 26-35 amino acids in length

Table 3. Thermo Scientific PEPotec SRM Peptide optional modifications – Available with all grades on a per-peptide basis.

C-terminal heavy labeling at R or K	Acetylation at side chain of Lysine [Lys(Ac)]
Internal heavy labeling at A,R,I,L,K,F,P or V	Methylation at side chain of Lysine [Lys(Me)] and Arginine [Arg(Me)]
Alternative heavy amino acid at C-term	Dimethylation at side chain of Lysine [Lys$(Me)_2$]
Alternative light amino acid at C-term	3-Chloro-tyrosine
Phosphorylation at 1–3 site	Hydroxyprolinew (Hyp)
All cysteines protected by carbamidomethylation (CAM)	Isoaspartic acid
Diglycine ubiquitination motif on Lysine [Lys(GG)]	Formylation at C-term
Methionine sulfoxide [Met(O)]	

Thermo Scientific Pierce Peptide Retention Time Calibration Mixture

Easy prediction of peptide retention time.

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 Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture and 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.

Highlights:

- Assess chromatography and MS instrument performance
- Predict peptide retention across multiple instrument platforms
- Predict peptide retention time from sequence using calculated hydrophobicity factor
- Optimize scheduled MS acquisition windows for improved quantification and increased multiplexing
- Normalize results for variation in retention times and peak intensities between runs
- Normalize collision energies between instruments and platforms

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.

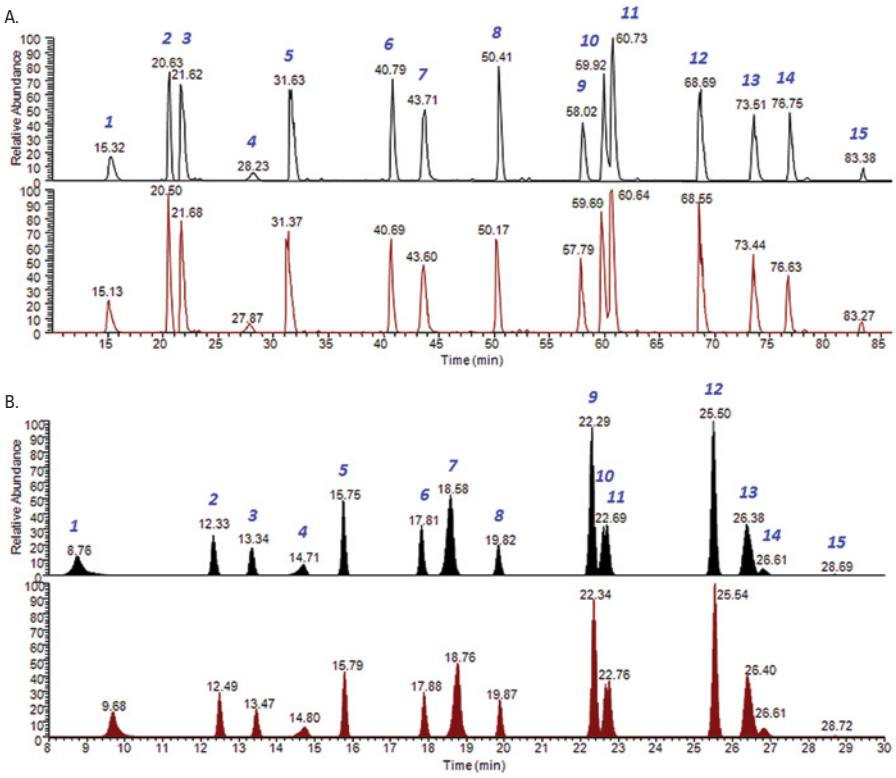


Figure 1. Chromatographic analysis of the Thermo Scientific Pierce Peptide Retention Time Calibration Mixture. A. The Pierce Peptide Retention Time Calibration Mixture (250fmol) was analyzed in duplicate on an LTQ XL Orbitrap Mass Spectrometer using a self-packed column (75µm x 20cm) containing Magic™ 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 Pierce Peptide Retention Time Calibration Mixture was also analyzed on a Thermo Scientific™ TSQ Vantage Mass Spectrometer using a Thermo Scientific™ Hypersil™ GOLD C18 column (1.0 x 150mm, Product # 25005-150165) with a 1.0% per minute gradient at 120µL per minute. Numbered peaks correspond to the calibrant peptides described above.

Table 1. 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

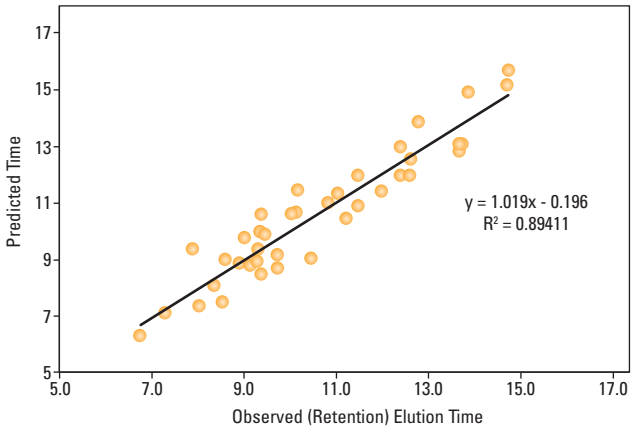


Figure 2. Retention time prediction with hydrophobicity. The Pierce Peptide Retention Time Calibration Mixture sequences and chromatographic data from Figure 1B were used to calculate the retention time-to-hydrophobicity relationship in Pinpoint 1.1 Software. The observed retention times of 29 tryptic peptides from bovine serum albumin on a TSQ Vantage Mass Spectrometer were plotted against the predicted retention times.

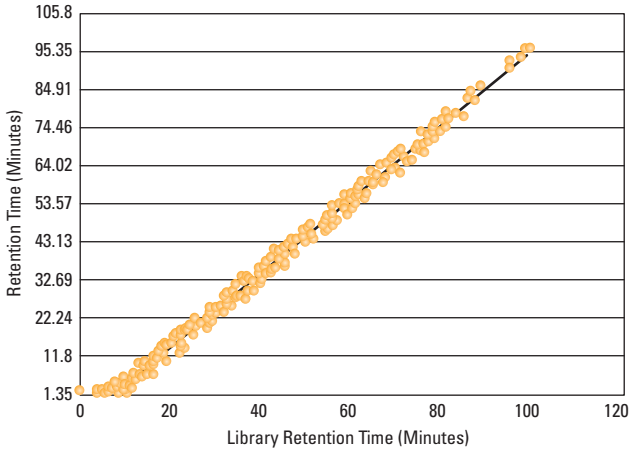


Figure 3. Prediction of relative peptide retention times between LC-MS systems. The Pierce Peptide Retention Time Calibration Mixture was analyzed using LTQ Orbitrap XL and TSQ Vantage Mass Spectrometers. Enzymatic digests from lysed 293T cells treated with 5µg/mL insulin for 30 minutes were analyzed on an LTQ Orbitrap XL Mass Spectrometer. Search results from Proteome Discoverer 1.1 Software and the calibrant results from both platforms were imported into Pinpoint 1.1 Software to develop scheduled selective reaction monitoring assays for 478 peptides in 35 proteins. Retention times observed on the TSQ Vantage Instrument were plotted against the retention times observed on the Orbitrap XL Instrument.

Ordering Information

Product #	Description	Pkg. Size
88320	Pierce Peptide Retention Time Calibration Mixture, 0.5pmol/µL	50µL
88321	Pierce Peptide Retention Time Calibration Mixture, 5pmol/µL	200µL

Thermo Scientific 1-Step Heavy Protein *In Vitro* Protein Expression Kits

Rapid, cell-free expression of recombinant proteins containing stable isotope-labeled (i.e., heavy) amino acids.

The 1-Step Heavy Protein IVT Kit uses a unique HeLa cell lysate supplemented with heavy amino acids for *in vitro* translation (IVT) of proteins with 90 to 95% isotope incorporation efficiency in less than 8 hours. Heavy proteins expressed using this system can be used as mass spectrometry controls for sample preparation loss, digestion efficiency determination or as quantification standards.

Highlights:

- **Efficient** – express heavy proteins with 90 to 95% stable isotope incorporation
- **Functional** – uses the human translational machinery to express more biologically active proteins than other IVT systems
- **Flexible** – express light proteins or heavy proteins containing $^{13}C_6{}^{15}N_2$ L-lysine and/or $^{13}C_6{}^{15}N_4$ L-arginine
- **Convenient** – perform transcription and translation in a single step
- **High performance** – greater yields compared to rabbit reticulocyte *in vitro* translation

Applications:

- Identify ideal peptides for targeted quantitation
- Verify protein digestion efficiency
- Control for protein sample preparation variability and affinity enrichment loss

The 1-Step Heavy Protein IVT Kit contains all of the necessary components to express a heavy protein including HeLa cell lysate, proprietary accessory proteins, reaction mix, heavy amino acids, positive-control GFP DNA and the pT7CFE1-CGST-HA-His cloning vector. The benefits of *in vitro* expression of heavy proteins over traditional *in vivo* systems include expression of toxic or insoluble proteins, a more rapid protein synthesis, and a more economical use of heavy amino acids compared to stable-isotope labeled cell lines. This small-scale expression method makes it easy to express numerous heavy proteins simultaneously or to express large quantities of a single heavy protein up to 100µg/mL. Tandem affinity purification of heavy proteins is aided using an expression vector containing multiple affinity tags including GST, HA and 6xHis.

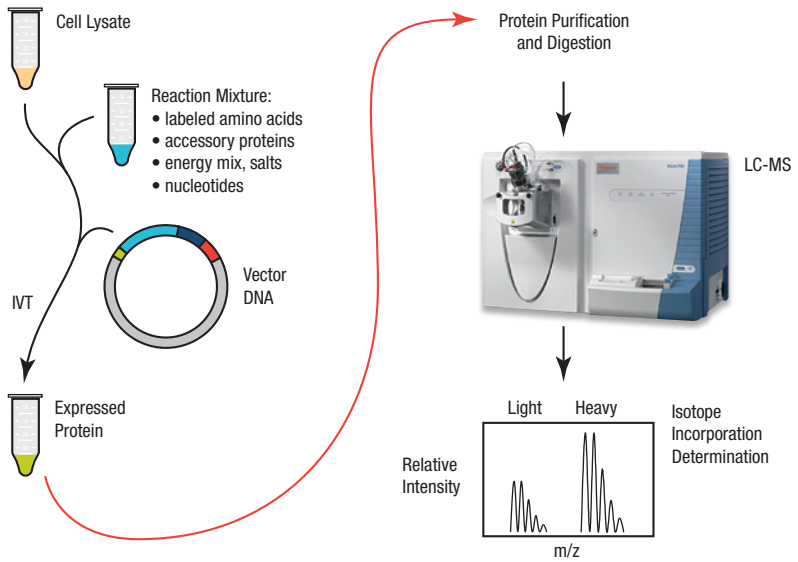


Figure 1. Workflow for heavy recombinant protein expression, purification and mass spectrometry analysis. Cell lysates are combined with the reaction mixture, vector DNA and stable isotope-labeled amino acids to express recombinant proteins. Expressed proteins are then purified and digested into peptides for LC-MS analysis.

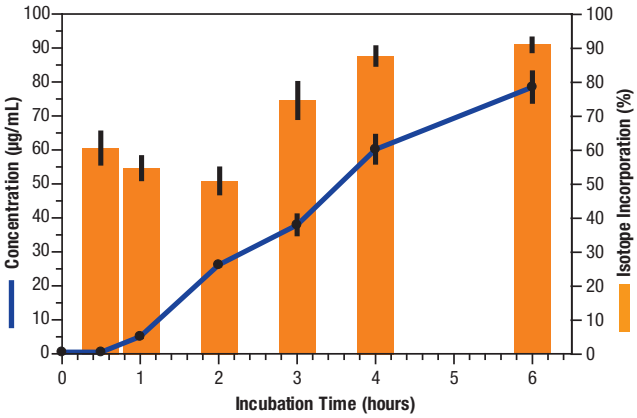
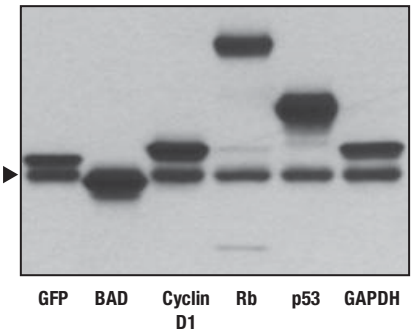


Figure 2. Heavy green-fluorescent protein (GFP) expression.
GFP expression with time, showing corresponding heavy-isotope incorporation.

A.



B.

Protein Sample	Mass (kDa)	Isotope Incorp.
GFP	27	95%
BAD	19	92%
Cyclin D1	36	97%
Rb	110	96%
p53	53	91%
GAPDH	37	94%

Figure 3. Expression of heavy mammalian proteins. Panel A: Western blot of GST-fusion proteins GFP, BAD, cyclinD1, retinoblastoma (RB), p53 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expressed using human IVT extract (the arrow indicates an anti-GST cross-reacting band in the lysate). **Panel B:** Isotope incorporation from peptides derived from the proteins in Panel A.

Ordering Information

Product #	Description	Pkg. Size
88330	**1-Step Heavy Protein IVT Kit**	8-rxn set
	Sufficient for eight reactions of 25µL each	
	Kit Contents:	
	HeLa Lysate	110µL
	Accessory Proteins	25µL
	Reaction Mix	40µL
	$^{13}C_6$ $^{15}N_4$ L-Arginine	25µL
	$^{13}C_6$ $^{15}N_2$ L-Lysine	25µL
	Positive Control DNA: pCFE-GFP	10µg
	pT7CFE1-CGST-HA-His	10µg
	Nuclease-Free Water	1.5mL
88331	**1-Step Heavy Protein IVT Kit**	40-rxn set
	Sufficient for 40 reactions of 25µL each	
	Kit Contents:	
	HeLa Lysate	500µL
	Accessory Proteins	100µL
	Reaction Mix	200µL
	$^{13}C_6$ $^{15}N_4$ L-Arginine	25µL
	$^{13}C_6$ $^{15}N_2$ L-Lysine	25µL
	Positive Control DNA: pCFE-GFP	10µg
	pT7CFE1-CGST-HA-His	10µg
	Nuclease-Free Water	1.5mL

For a list of references using the 1-Step Heavy Protein IVT Kit, please see the back cover.

Table 1. Thermo Scientific Mass Spectrometry systems and capabilities.

Instrument	SILAC/iCAT™/Mass Tags	Isobaric Mass Tags (iTRAQ 4™, TMT6plex, iTRAQ8™)	Isobaric Mass Tags (TMT10plex)	Targeted Quantitation	Discovery Proteomics (Data Dependent Mass Spectrometry - Protein Indentification)
LTQ Velos	No	Yes (PQD)	No	Yes (tSIM, SRM)	Good
LTQ Velos Pro	No	Yes (PQD	No	Yes (tSIM, SRM)	Good
LTQ XL ETD	No	Yes (PQD)	No	Yes (tSIM, SRM)	Good
MALDI LTQ XL	No	Yes (PQD)	No	Yes (tSIM, SRM)	Good
LTQ Orbitrap Velos	Yes	Yes (PQD and HCD)	Yes†	Yes (HR/AM MS1 & MS^n)	Excellent
LTQ Orbitrap XL ETD	Yes	Yes (PQD and HCD)	Yes†	Yes (HR/AM MS1 & MS^n)	Excellent
LTQ Orbitrap XL	Yes	Yes (PQD and HCD)	Yes†	Yes (HR/AM MS1 & MS^n)	Excellent
MALDI LTQ Orbitrap XL	Yes	Yes (PQD and HCD)	Yes†	Yes (HR/AM MS1 & MS^n)	Good
LTQ Orbitrap Discovery	Yes	Yes (PQD only)	No	Yes (HR/AM MS1 & MS^n)	Good
LTQ Orbitrap Velos Pro	Yes	Yes (PQD and HCD)	Yes	Yes (HR/AM MS1 & MS^n)	Excellent
Orbitrap ELITE	Yes	Yes (PQD and HCD)	Yes	Yes (HR/AM MS1 & MS^n)	Excellent
Orbitrap Fusion Tribrid	Yes	Yes (HCD)	Yes	Yes (HR/AM MS1 & MS^n)	Excellent
Exactive	Yes	No	No	Yes (HR/AM MS1)	N/A
Exactive plus	Yes	No	No	Yes (HR/AM MS1)	N/A
Q Exactive	Yes	Yes (HCD only)	Yes	Yes (HR/AM MS1 & MS^n)	Excellent
Q Exactive plus	Yes	Yes (HCD only)	Yes	Yes (HR/AM MS1 & MS^n)	Excellent
LTQ FT Ultra	Yes	No	No	Yes (HR/AM MS1 & MS^n)	Good
TSQ Access Max	No	No	No	Yes (SRM)	Not recommended
TSQ Quantam Ultra	No	No	No	Yes (SRM)	Not recommended
TSQ Vantage	No	No	No	Yes (SRM)	Not recommended
TSQ Endura	No	No	No	Yes (SRM)	Not recommended
TSQ Quantiva	No	No	No	Yes (SRM)	Not recommended

Please note:
† Can be done but with very slow duty cycle.
PQD option included in all instruments via the Xcalibur software
tSIM = Targeted Selective Reaction Monitoring
HR/AM = High Resolution/Accurate Mass
SRM = Selective Reaction Monitoring

Ordering Information

Abundant Protein Depletion

Product #	Description	Pkg. Size
85160	Albumin Depletion Kit	24-rxn kit
85161	Top 2 Abundant Protein Depletion Spin Columns†	6 columns
85162	Top 2 Abundant Protein Depletion Spin Columns†	24 columns
85164	Top 12 Abundant Protein Depletion Spin Columns†	6 columns
85165	Top 12 Abundant Protein Depletion Spin Columns†	24 columns

Protein Desalting

89877	Zeba™ Micro Spin Desalting Columns, 7K MWCO, 75µL	25 columns
89882	Zeba Spin Desalting Columns, 7K MWCO, 0.5mL	25 columns
89890	Zeba Spin Desalting Columns, 7K MWCO, 2mL	25 columns
89892	Zeba Spin Desalting Columns, 7K MWCO, 5mL	25 columns
89894	Zeba Spin Desalting Columns, 7K MWCO, 10mL	25 columns
89807	Zeba 96-well Spin Desalting Plates, 7K MWCO	2 plates
87764	Zeba Micro Spin Desalting Columns, 40K MWCO, 75µL	25 columns
87766	Zeba Spin Desalting Columns, 40K MWCO, 0.5mL	25 columns
87769	Zeba Spin Desalting Columns, 40K MWCO, 2mL	25 columns
87771	Zeba Spin Desalting Columns, 40K MWCO, 5mL	25 columns
87773	Zeba Spin Desalting Columns, 40K MWCO, 10mL	25 columns
87774	Zeba 96-well Spin Desalting Plates, 40K MWCO	2 plates

Active Site Protein Labeling and Enrichment

88310	Pierce™ Kinase Enrichment Kit with ATP Probe	16-rxn kit
88311	ActivX™ Desthiobiotin-ATP Probe	16 x 12.6µg
88312	Pierce™ Kinase Enrichment Kit with ADP Probe	16-rxn kit
88313	ActivX™ Desthiobiotin-ADP Probe	16 x 9.9µg
88314	Pierce™ GTPase Enrichment Kit with GTP Probe	16-rxn kit
88315	ActivX Desthiobiotin-GTP Probe	16 x 12.9µg
88316	ActivX Azido-FP Serine Hydrolase Probe	3.5µg
88317	ActivX Desthiobiotin-FP Serine Hydrolase Probe	4.6µg
88318	ActivX TAMRA-FP Serine Hydrolase Probe	6.8µg

† *Limit one pack per order* †† *Limit five packs per order*

Protein Digestion – Mass Spec Grade Proteases, Reagents and Kits

Product #	Description	Pkg. Size
90057	Trypsin Protease, MS Grade††	5 x 20µg
90058	Trypsin Protease, MS Grade††	5 x 100µg
90059	Trypsin Protease, MS Grade††	1mg
90305	Trypsin Protease, MS Grade, Frozen Liquid	100µg
90300	LysN Protease, MS Grade	20µg
90301	LysN Protease, MS Grade	5 x 20µg
90051	LysC Protease, MS Grade	20µg
90053	AspN Protease, MS Grade	2µg
90054	GluC Protease, MS Grade	5 x 10µg
90056	Chymotrypsin (TLCK treated), MS Grade	4 x 25µg
89871	In-Gel Tryptic Digestion Kit	Kit
89895	In-Solution Tryptic Digestion and Guanidination Kit	Kit
84840	Mass Spec Sample Prep Kit for Cultured Cells	20-rxn kit
84841	Digestion Indicator for Mass Spectrometry	10µg

Phosphopeptide Enrichment and Clean-Up

88300	Pierce™ Fe-NTA Phosphopeptide Enrichment Kit	30-rxn kit
88301	Pierce™ TiO_2 Phosphopeptide Enrichment and Clean-Up Kit	24-rxn kit
88811	Pierce™ Magnetic TiO_2 Phosphopeptide Enrichment Kit	96-rxn kit
88812	Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit	24-rxn kit
88302	Pierce™ Graphite Spin Columns, 0.5mL	30 columns

Peptide Clean-Up

87776	Pierce™ Detergent Removal Spin Column, 125µL	25 columns
87777	Pierce Detergent Removal Spin Column, 0.5mL	25 columns
87778	Pierce Detergent Removal Spin Column, 2mL	5 columns
87779	Pierce Detergent Removal Spin Column, 4mL	5 columns
87780	Pierce™ Detergent Removal Resin	10mL
88304	Pierce™ Detergent Removal Spin Plates	2 plates
88305	HiPPR Detergent Removal Spin Column Kit (Resin + Columns)	5mL kit
88306	HiPPR Detergent Removal Spin Columns, 0.1mL	24 columns
88307	HiPPR Detergent Removal 96-well Spin Plates	2 plates
89870	Pierce™ C18 Spin Columns	25 columns
87782	Pierce C18 Tips, 10µL bed	96 tips
87784	Pierce C18 Tips, 100µL bed	96 tips
84850	Pierce C18 Spin Tips	96 tips

† *Limit one pack per order.* †† *Limit five packs per order.*

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