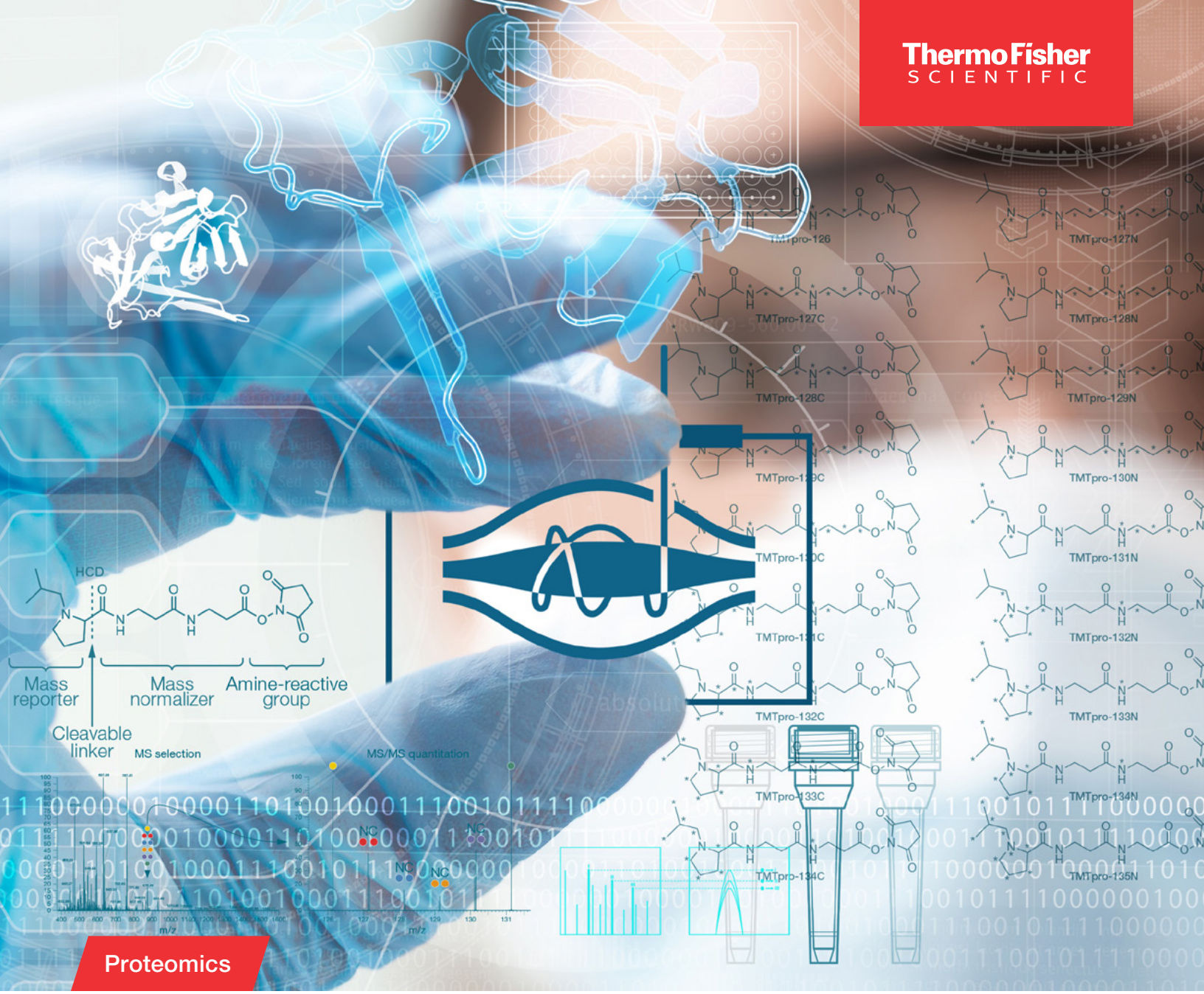


New tools for improved proteomics results

Sample preparation, quantitation, and instrument calibration reagents for proteomic mass spectrometry

thermo scientific

Introduction

We offer a complete portfolio of sample preparation, protein quantitation, and instrument calibration solutions and standards designed for better mass spectrometry (MS) analysis. This portfolio has been developed in the context of biology, and the latest products include improvements to sample preparation (Thermo Scientific™ EasyPep™ MS Sample Prep Kits) and MS-targeted multiplex pathway panel assays (Thermo Scientific™ SureQuant™ kits) for targeted protein quantitation. In addition, we have developed the next generation of Thermo Scientific™ Tandem Mass Tag™ (TMT™) reagents designed to increase the level of sample multiplexing without compromising on protein identification and quantitation.

New calibration solutions and standards enable better assessment and assurance of MS instrument performance. We recognize the need for complete solutions as well as technical support for proteomics research and analytical analysis using MS instrumentation. These reagents have been verified for use by biologists and mass spectrometrists in their research. Robust, integrated workflows help provide consistent results between labs and eliminate time wasted on troubleshooting experimental methods and results.

Contents

Sample preparation

EasyPep MS Sample Prep Kits

Optimized, rapid protein extraction and digestion of samples for MS analysis

The newly expanded EasyPep MS Sample Prep product portfolio now includes 96-well plate, mini, and maxi formats that enable efficient and reproducible processing of plasma, cultured mammalian cells, and tissues for MS analysis. These kits contain preformulated buffers, an MS-grade enzyme mix, peptide cleanup columns or plates, and an optimized protocol to generate MS-compatible peptide samples in less than 4 hours (Figure 1).

Features include:

- **Complete**—includes preformulated reagents for lysis through digestion, peptide cleanup columns, and an optimized protocol for processing up to 20 samples (mini-column), 8 samples (maxi-column), or one 96-well plate
- **Optimized**—streamlined protocol and reagents minimize the number of steps and time it takes to process samples
- **Flexible**—reagents and protocol have been verified using cells, plasma, and tissue samples for 10 µg to 2 mg samples
- **Time-saving**—sample processing has been reduced from more than 1 day to less than 4 hours
- **Compatible**—sample is ready for MS analysis and other downstream applications, including label-free quantitation, phosphopeptide enrichment, and Thermo Scientific™ TMT™ and TMTpro™ reagent labeling

Sample preparation of peptides for MS analysis is complex, with numerous steps and non-standard protocols, resulting in variable sample quality and poor reproducibility. To address these issues, the EasyPep MS Sample Prep Kits have been designed using a standardized workflow that helps improve reproducibility while also saving hands-on and processing time. The implementation of three reagents reduces the number of steps and the amount of time in the workflow. Specifically, the Thermo Scientific™ Pierce™ Universal Nuclease for Cell Lysis reduces viscosity from nucleic acids. A rapid "one pot" reduction/alkylation solution is used for cysteine modification (carbamidomethylation, +57.02). And, a trypsin/LysC protease mix has been optimized for complete digestion in 1–3 hours. In addition, these kits include peptide cleanup columns (or plates) and buffers to prepare contaminant-free peptide samples for MS analysis. The optimized reagents and protocol produce high-quality peptides that are ready for MS analysis or compatible with TMT labeling and other downstream applications such as high pH reversed phase fractionation or phosphopeptide enrichment.

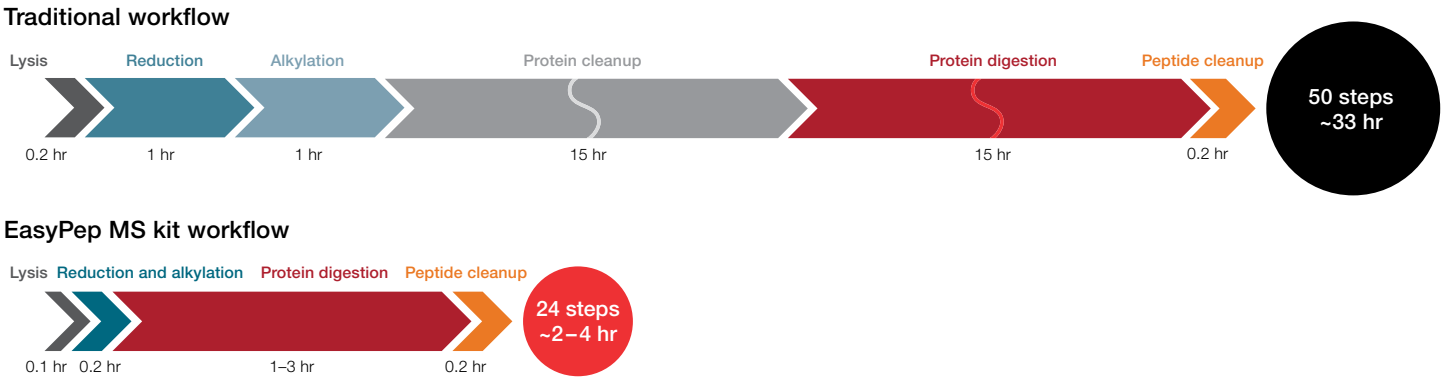


Figure 1. Comparison of sample preparation methods using a traditional workflow and a workflow using an EasyPep MS kit. Compared to a traditional workflow, the EasyPep MS Sample Prep Kits produce high-quality peptides in less time with half the number of manipulation steps.

EasyPep MS Sample Prep Kit formats

EasyPep MS Sample Prep Kits are available in three formats to support a wide range of sample numbers and input amounts (Figure 2). The Thermo Scientific™ EasyPep™ Mini MS Sample Prep Kit and the Thermo Scientific™ EasyPep™ 96 MS Sample Prep Kit are optimized to efficiently process protein samples of 10–100 µg, resulting in a high yield of MS-ready peptides. The Thermo Scientific™ EasyPep™ Maxi MS Sample Prep Kit is optimized to process 8 protein samples ranging from 0.5–2 mg per sample, which is ideal for phosphopeptide enrichment and other applications that require larger starting sample amounts. Alternatively, each column can be used to process a combined set of isobaric tag–labeled samples (10–100 µg each, ≤2 mg total) for multiplex proteomic quantitation.

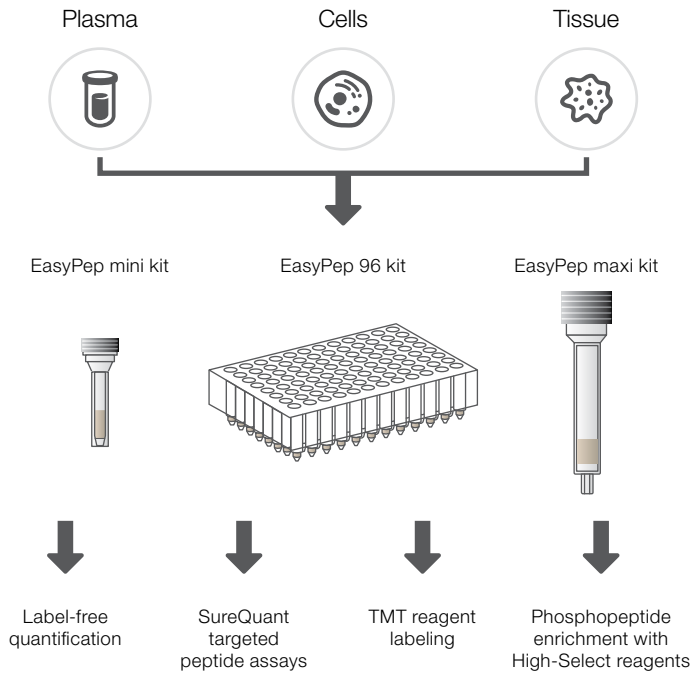


Figure 2. EasyPep MS Sample Prep Kits use the same chemistry and standardized method to process plasma, cells, and tissues for downstream MS-based proteomic applications. Mini, 96-well, and maxi formats can be used to generate samples for label-free quantification, Thermo Scientific™ SureQuant™ targeted quantitation assays, TMT reagent labeling, or phosphopeptide enrichment with Thermo Scientific™ High-Select™ reagents.

EasyPep MS Sample Prep Kits performance

Since the EasyPep kits share the same reagents, cleanup resin, and protocols, the sample preparation is highly reproducible for all formats. As shown in Figure 3, HeLa cell lysate prepared using the EasyPep Mini, Maxi, and 96-well kits have similar protein and peptide identifications. In addition, samples prepared using the different kits showed high digestion efficiency with >90% of identified peptides having zero missed cleavages with high reproducibility.

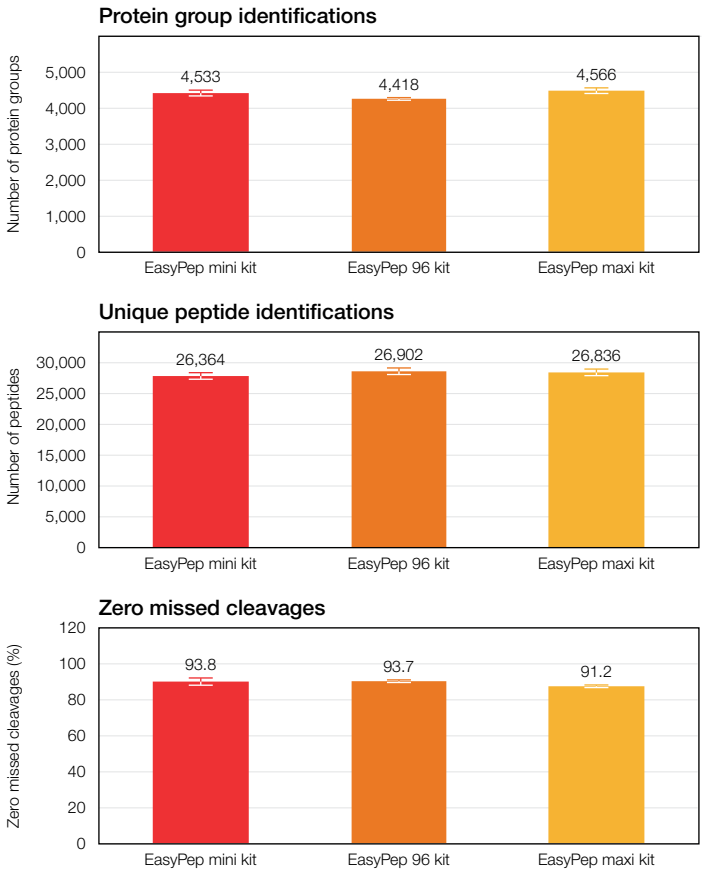


Figure 3. Comparison of the EasyPep Mini, 96, and Maxi MS Sample Prep Kits for processing HeLa cell lysates. All three formats provide comparable peptide and protein identifications with high digestion efficiency (percentage of zero missed cleavages with high reproducibility).

Sample preparation

EasyPep MS Sample Prep Kits, cont.

EasyPep MS Sample kit compatibility

EasyPep MS Sample Prep Kits are compatible with common downstream applications including peptide quantitation, peptide fractionation, and phosphopeptide enrichment. In addition, EasyPep kits are compatible with TMT reagent labeling before or after peptide cleanup. EasyPep columns (or plates) and buffers have been optimized to remove excess, unreacted, and quenched TMT reagents, resulting in more protein and peptide identifications compared to traditional sample prep workflows (Figures 4 and 5).

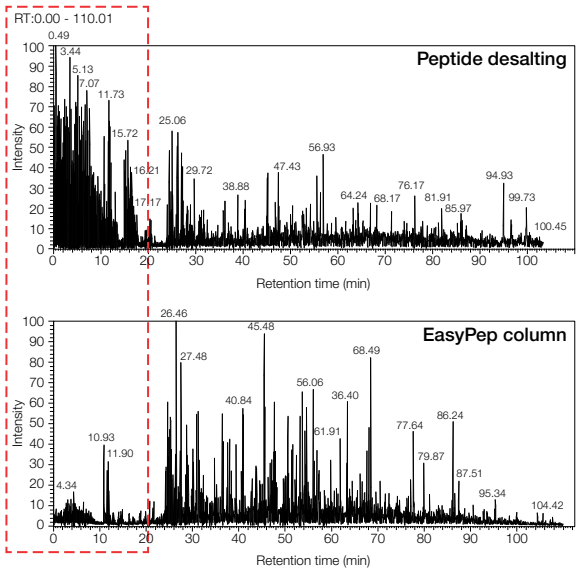


Figure 4. EasyPep cleanup removes excess TMT more efficiently than other peptide desalting methods. HeLa cells were processed using the EasyPep Mini MS Sample Prep Kit. Peptides were labeled with TMT before cleanup and processed using either the EasyPep cleanup or the conventional method.

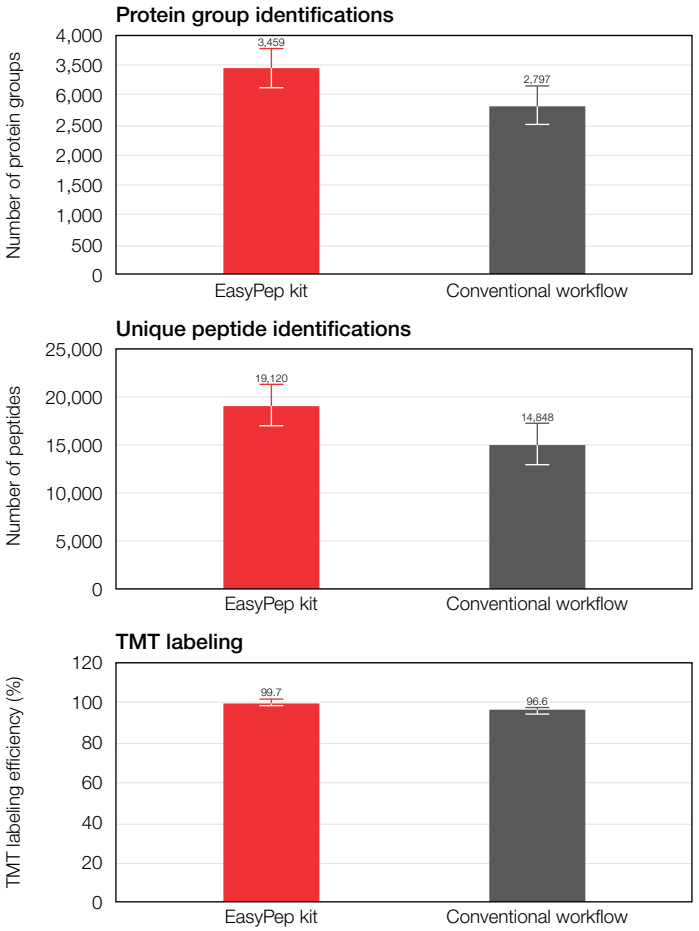


Figure 5. Comparison of the EasyPep and conventional workflows for processing TMT-labeled multiplexed samples. The EasyPep kit resulted in more peptide and protein identifications with high TMT labeling efficiency (N-terminus and lysine) compared to a conventional acetone precipitation workflow.

Ordering information

Product	Quantity	Cat. No.
EasyPep Mini MS Sample Prep Kit	20 reactions	A40006
EasyPep Maxi MS Sample Prep Kit	8 reactions	A45734
EasyPep 96 MS Sample Prep Kit	96 reactions	A45733
EasyPep Lysis Buffer	100 mL	A45735

Find out more at **thermofisher.com/easypep**

Sample preparation

High-Select Fe-NTA Magnetic Phosphopeptide Enrichment Kit

Thermo Scientific™ High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit and High-Select™ Fe-NTA Magnetic Agarose enable efficient and selective enrichment of phosphorylated peptides for MS analysis. Each reaction is sufficient to enrich 250–1,000 µg of phosphopeptides from a starting sample of 0.5–5 mg of total protein digest. The simplified procedure requires less than 45 minutes to enrich phosphopeptides from protein digests or peptide fractions, with greater than 90% selectivity. Additionally, the kit may also be used to enrich for chemically modified peptides with functional groups designed for use with immobilized metal affinity chromatography resins.

Features of the High-Select Fe-NTA Magnetic Phosphopeptide Enrichment Kit and High-Select Fe-NTA Magnetic Agarose include:

- **Convenient**—pre-formulated buffers for use with high-capacity Fe-NTA magnetic agarose beads (Figure 6) to enable parallel sample processing
- **High specificity**—phosphopeptide recovery with >90% selectivity
- **Scalable**—compatible with automation, including the use of Thermo Scientific™ KingFisher™ magnetic particle processors

Mass spectrometry is a key tool for identifying sites of protein phosphorylation and quantifying phosphorylation changes; however, MS analysis of protein phosphorylation is challenging due to the low stoichiometry, high hydrophilicity, poor ionization, and incomplete fragmentation of phosphopeptides. Because of the low relative abundance of phosphorylation modifications in complex protein samples, enrichment is essential for successful identification of phosphopeptides.

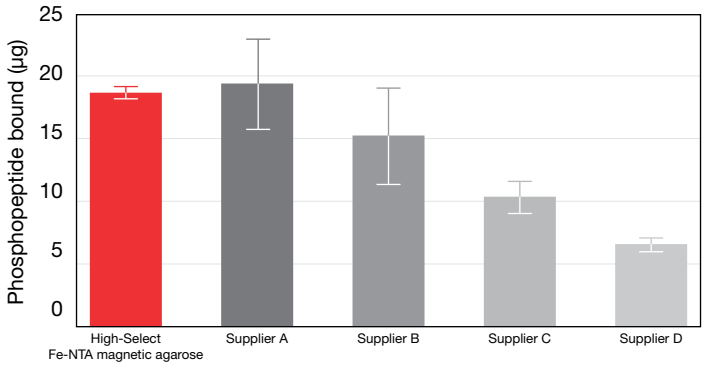


Figure 6. Fe-NTA resin binding capacity. A beta-casein monophosphopeptide standard was incubated with different magnetic and non-magnetic agarose resins. Bound peptide was measured by a subtractive method using the Thermo Scientific™ Pierce™ Phosphoprotein Phosphate Estimation Assay Kit (Cat. No. 23270).

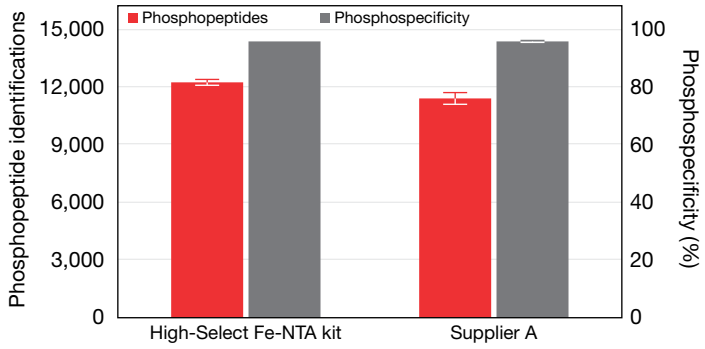


Figure 7. Phosphopeptide enrichment from nocodazole-treated HeLa cell digests. 1 mg of digest was enriched using 25 µL of High-Select Fe-NTA Magnetic Agarose Beads before LC-MS analysis (n = 3). Enrichment using High-Select Fe-NTA beads had higher phosphopeptide identifications and equivalent phosphospecificity (97%) compared to an alternative resin.

Ordering information

Product	Quantity	Cat. No.
High-Select Fe-NTA Magnetic Phosphopeptide Enrichment Kit	20 reactions	A52283
High-Select Fe-NTA Magnetic Agarose	5 mL	A52284

Protein quantitation

SureQuant IP-MS kits and standards

Validated, modular reagents for multiplexed targeted protein quantitation

SureQuant assays and standards have been designed for multiplexed, targeted quantitation of key cellular signaling proteins. The assays provide flexibility to use the immunoprecipitation (IP) sample prep kits and peptide quantitation modules together or independently based on the end application.

Features include:

- **Complete**—kits include reagents for successful sample preparation and quantitative analysis of each target peptide
- **Verified**—kits and reagents are rigorously tested for specificity and successful quantitation of each target peptide
- **Multiplex**—Thermo Scientific™ HeavyPeptide™ AQUA Custom Peptide Synthesis panels for simultaneous quantitation of target proteins and phosphorylation status from key cell signaling pathways
- **Flexible**—modular format allows for immunoenrichment only, or in combination with peptide quantitation panels

The Thermo Scientific™ SureQuant™ Protein A/G and Streptavidin IP-MS Sample Preparation Kits have been designed to support targeted quantitation using SureQuant or SRM/PRM analysis. The kits contain high-quality Thermo Scientific™ Pierce™ Protein A/G or Streptavidin Magnetic Beads together with reagents that have been optimized for MS compatibility. The streamlined procedure enables digestion immediately after the immunoprecipitation elution step, facilitating MS sample preparation in ~4 hours for same-day LC-MS/MS analysis (Figure 8). These kits have also been rigorously validated using numerous target antigens with varying expression levels, including targets previously undetected by western blotting.

The Thermo Scientific™ SureQuant™ quantitation modules contain Thermo Scientific™ HeavyPeptide™ and/or LightPeptide AQUA Ultimate panels for multiplexed quantitation of target proteins using LC and MS. These peptide panels may be used for absolute quantitation by the generation of a standard curve or as a spike-in internal standard for relative quantitation.

Together, the SureQuant IP-MS sample preparation kits and the SureQuant quantitation modules provide a complete solution for multiplexed sample preparation and analysis of select target proteins (Figures 9 and 10).

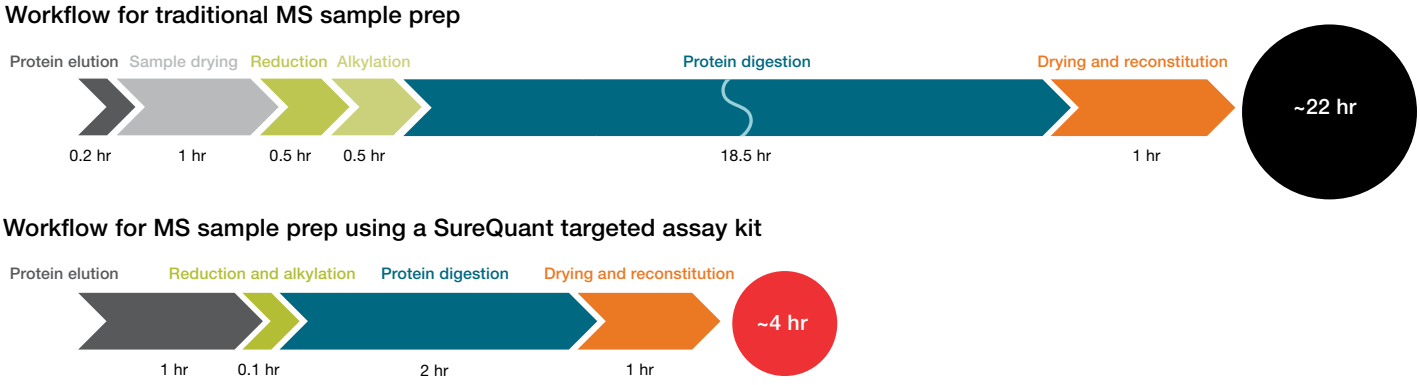


Figure 8. MS sample preparation procedure comparison between traditional methods and SureQuant targeted mass spec assay kits following immunoprecipitation

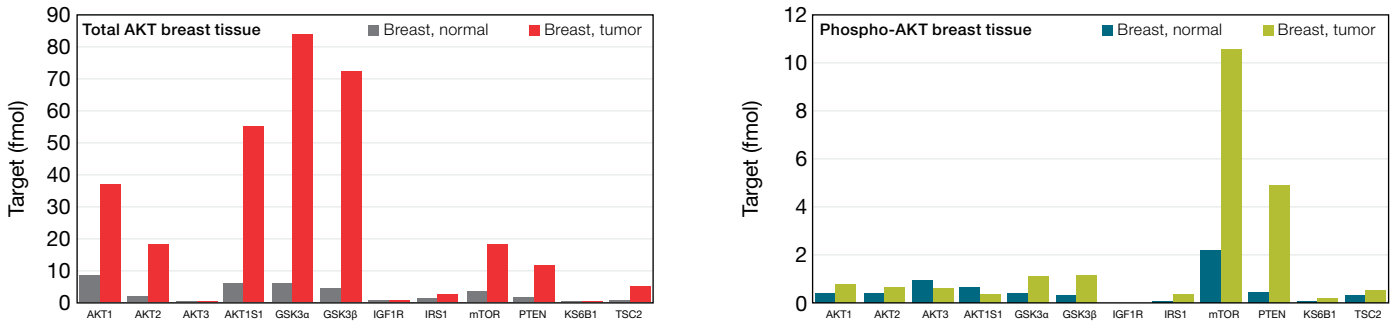


Figure 9. Multiplex IP analysis using streptavidin magnetic beads for human tissue. AKT/mTOR pathway proteins were enriched by multiplex IP using biotinylated antibodies from the Thermo Scientific™ SureQuant™ AKT Pathway kits. Parallel reaction monitoring (PRM) analysis for total AKT/mTOR pathway targets (left) and phosphorylated AKT/mTOR pathway targets (right) was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano System and Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Data were subsequently analyzed in Thermo Scientific™ Skyline software using calibration curves to determine the absolute protein amount (fmol).

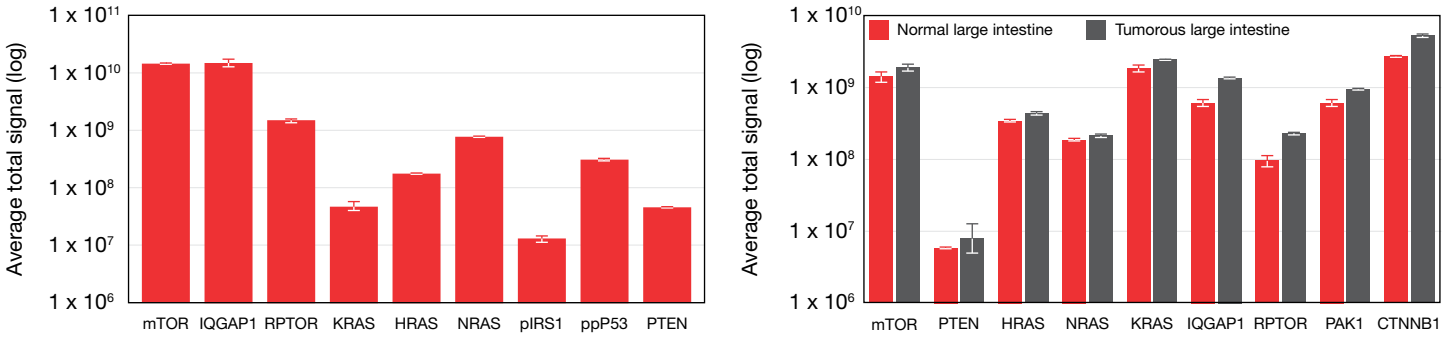


Figure 10. Multiplex IP analysis using Thermo Scientific™ Pierce™ Protein A/G Magnetic Beads for cell line and tissue lysates. Multiplex SureQuant™ Protein A/G IP-MS data using a lysate containing hIGF-1–treated MCF7 and HEK293 (left). Multiplex SureQuant™ Protein A/G IP-MS data for normal or tumorous large intestine human tissue (right). Data dependent acquisition (DDA) analysis was performed using the Dionex UltiMate 3000 RSLCnano System and Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer. Data were subsequently analyzed in Thermo Scientific™ Proteome Discoverer™ Software using the average total peptide area.

Find out more at thermofisher.com/ms-targeted-assays

Protein quantitation

SureQuant phosphopeptide standards

The Thermo Scientific™ SureQuant™ Multipathway Phosphopeptide Standard contains an optimized mixture of 131 isotopically labeled phosphopeptides to simultaneously monitor and quantitate phosphorylation signaling. The Thermo Scientific™ SureQuant™ Phosphopeptide Suitability Standard contains a mixture of 20 isotopically labeled phosphopeptides with increasing hydrophobic properties for LC optimization and monitoring of LC-MS/MS system performance. When used together, the standards help ensure optimal instrument performance and sample assessment for phosphorylation events.

Features of SureQuant standards include:

- **Optimized**—validated mixture of 131 HeavyPeptide AQUA Basic phosphorylated peptides (SureQuant Multipathway standard) or 20 HeavyPeptide AQUA Basic phosphopeptides (SureQuant Suitability standard)
- **Multiplex**—suitable for relative quantitation of phosphorylation events for 89 key proteins in seven cellular signaling pathways (SureQuant Multipathway standard)
- **Predictive**—range of peptides with increasing hydrophobic properties for method optimization and calculation of peptide retention times
- **Monitoring**—excellent QC assay tool for quality assessment of LC and MS instrument performance (SureQuant Suitability standard)
- **Convenient**—provided in a ready-to-use liquid format

The specific phosphopeptides in the SureQuant Multipathway Phosphopeptide Standard have been chosen to cover biologically relevant phosphorylation sites for 89 key proteins from seven different signaling pathways including EGFR/HER, RAS-MAPK, PI3K/AKT/mTOR, AMPK, death/apoptosis, and stress (p38/SAPK/JNK). For optimal results, MS sample preparation should include phosphopeptide enrichment, with the standard spiked in before MS sample preparation (1 pmol) or after phosphopeptide enrichment (0.8 pmol) (Figure 11). The ability to simultaneously monitor and quantitate these targets in a single sample is a powerful tool for assessment of normal and abnormal signaling pathway activity (Figure 12).

The phosphopeptides selected for the SureQuant Phosphopeptide Suitability Standard represent relevant proteins from key signaling pathways that include serine, threonine, and tyrosine phosphorylation. The 20 phosphopeptides have a wide range of retention times and have been designed for use with phosphopeptide samples to assess system performance and to better enable prediction of peptide retention times to maximize phosphopeptide identifications (Figure 13).

Applications

- Standard for data-dependent acquisition (DDA)
- Standard for targeted MS (e.g., SRM, PRM)
- Optimization of LC parameters
- Identification of total peptide elution window
- Optimization of MS parameters

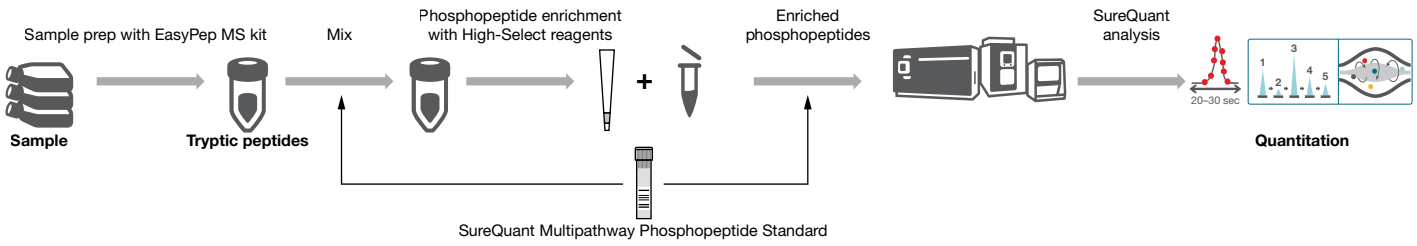


Figure 11. Workflow for sample analysis using the SureQuant Multipathway standard.

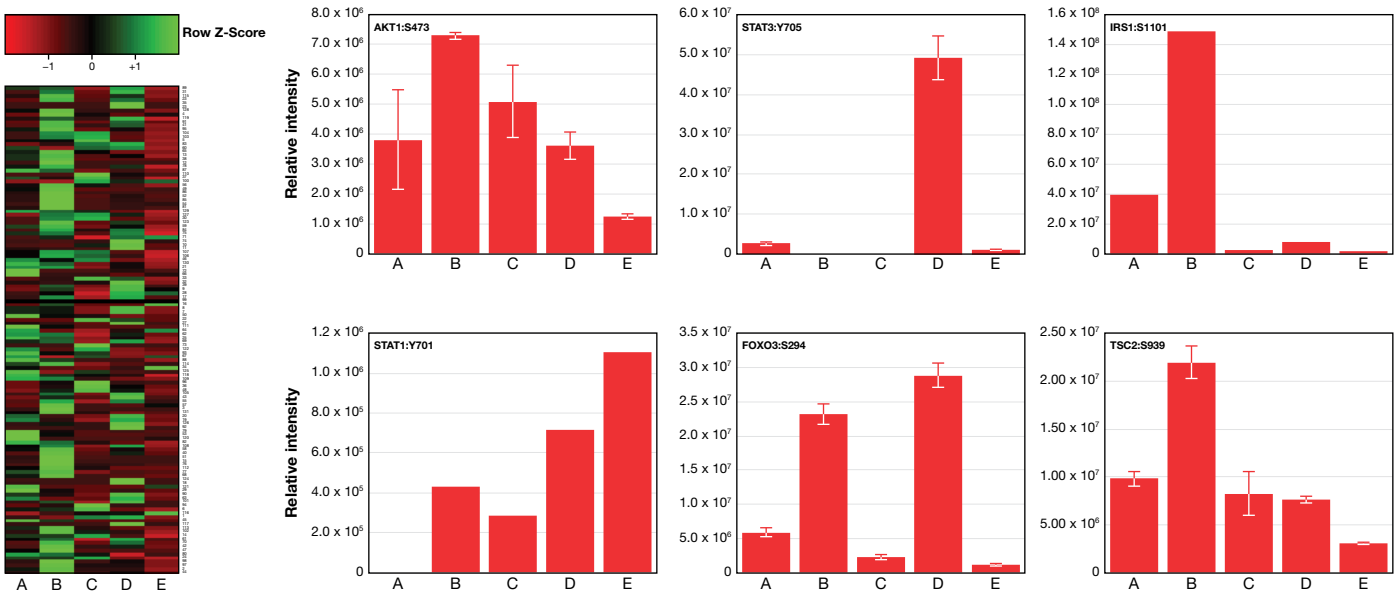


Figure 12. Use of SureQuant Multipathway Standard with phosphopeptide enrichment enables relative quantitation of more than 100 phosphoproteins. Heat map of relative levels of phosphopeptides compared between treated cell lysates (left). Changes in specific phosphopeptides (right). One µg of treated cell lines was incubated with High-Select Fe-NTA magnetic beads. Prior to enrichment, 1 pmol of the SureQuant Multipathway Phosphopeptide Standard was spiked into the lysate. PRM analysis was performed using the Dionex UltiMate 3000 RSLCnano System and Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer, with subsequent analysis in Skyline software using the average total area. A: HCT116/IGF; B: MCF7/IGF; C: LNCAP/IGF; D: A431/EGF; E: HepG2/Ins.

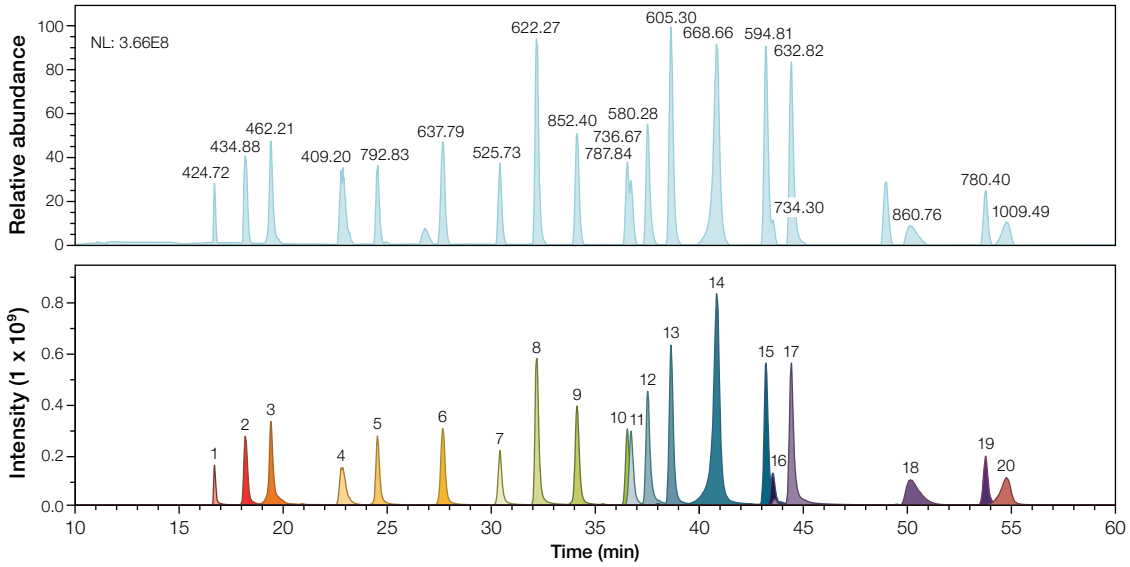


Figure 13. SureQuant Phosphopeptide Suitability Standard. Base peak chromatogram (top) and Skyline chromatogram (bottom) of 200 fmol SureQuant Phosphopeptide Suitability Standard separated using a Dionex UltiMate 3000 RSLCnano System and Thermo Scientific™ Acclaim™ PepMap RSLC C18 column (2 µm x 75 µm x 50 cm) with a 2–35% gradient (Buffer A: 0.1% FA in water, Buffer B: 0.1% FA in 100% acetonitrile) at 300 nL/min for 60 minutes and detected on a Thermo Scientific™ Q Exactive™ HF-Orbitrap™ Mass Spectrometer.

Protein quantitation

SureQuant phosphopeptide standards, cont.

Table 1. SureQuant Phosphopeptide Suitability Standard peptide information.

Peak No.	Peptide name	Peptide sequence	m/z	z	Avg RT (min)	Hydrophobicity
1	MAP2K7:S271	LVDSKAK	424.722	2	16.63	22.96
2	MAP2K4:S80	LRTHSIESSGK	434.881	3	18.08	21.82
3	TBC1D4:T642	AHTFSHPPSSTK	462.209	3	19.20	33.71
4	MAPKAPK2:T334	VPQTPLHTSR	409.204	3	22.55	24.01
5	IRS1:S1101	HSSETFSSTPSATR	792.832	2	24.39	29.12
6	SIRT1:S27	EAASSPAGEPLR	637.786	2	27.65	32.11
7	PLCB3:S537	SLGDEGLNR	525.728	2	30.37	31.22
8	TSC2:T1462	GYTISDSAPSR	622.265	2	32.16	14.25
9	ATF2:T69	NDSVIVADQTPTPTR	852.397	2	34.09	34.89
10	STAT5B:S690	YYTPVPCESATAK	787.838	2	36.49	9.36
11	AKT1:S129	SGSPSDNSGAEEMEVSLAKPK	736.659	3	36.60	31.93
12	CAV1:Y14	YVDSEGHLYTVPIR	580.274	3	37.44	5.80
13	PTK2:Y925	VYENVTGLVK	605.304	2	38.54	13.05
14	PPP1CA:T320	YGQFSGLNPGGRPITPPR	668.662	3	40.82	15.77
15	CAMKK2:S511	SLSAPGNLLTK	594.809	2	43.15	18.93
16	ERBB3:Y1328	SLEATDSAFDNPDYWHSR	734.300	3	43.42	23.66
17	CYLD:S418	FHSLPFSLTK	632.814	2	44.31	15.94
18	BCAR1:Y410	VLPPEVADGGVVDSGVYAVPPPAER	860.759	3	50.22	10.90
19	JUN:S63	NSDLLTSPDVGLLK	780.394	2	53.76	29.22
20	LRP6:S1490	GTYFPAILNPPPSPATER	1,009.487	2	54.72	13.81

Ordering information

Product	Quantity	Cat. No.
SureQuant AKT Pathway Multiplex Panel (Relative Quantitation)	10 reactions	A40080
SureQuant AKT Pathway IP and MS Sample Preparation Module	10 reactions	A40081
SureQuant AKT Pathway Relative Quantitation Module	10 reactions	A40082
SureQuant AKT Pathway (Phospho) Multiplex Panel (Absolute Quantitation)	10 reactions	A40084
SureQuant AKT Pathway (Phospho) Multiplex Panel (Relative Quantitation)	10 reactions	A40085
SureQuant AKT Pathway (Phospho) IP and MS Sample Preparation Module	10 reactions	A40086
SureQuant AKT Pathway (Phospho) Relative Quantitation Module	10 reactions	A40087
SureQuant AKT Pathway (Phospho) Absolute Quantitation Module	10 reactions	A40088
SureQuant RAS Isoform Relative Quantitation Module	10 reactions	A40097
SureQuant AKT Isoform Relative Quantitation Module	10 reactions	A40092
SureQuant TP53 Panel Relative Quantitation Module	10 reactions	A40102
SureQuant Protein A/G IP-MS Sample Preparation Kit	20 reactions	A51743
SureQuant Streptavidin IP-MS Sample Preparation Kit	20 reactions	A51744
SureQuant Multipathway Phosphopeptide Standard (100 fmol/µL)	200 µL	A51745
SureQuant Phosphopeptide Suitability Standard (500 fmol/µL)	50 µL	A51746

Protein quantitation

TMTpro 18plex Label Reagent Set

The Thermo Scientific™ TMTpro™ 18plex Label Reagent Set increases multiplexing from 16 to 18 samples, enabling even greater throughput for protein identification and quantitative analysis by tandem mass spectrometry (MS/MS).

Features include:

- **Multiplex**—concurrent MS analysis of up to 18 samples derived from cells, tissues, or biological fluids
- **Robust**—increased multiplex capability results in fewer missing quantitative values among samples and higher confidence among replicates
- **Efficient**—amine-reactive, NHS ester–activated reagents ensure efficient labeling of all peptides regardless of protein sequence or proteolytic enzyme specificity
- **Convenient**—provided in a ready-to-use, single-use format to help ensure optimal stability and performance or in bulk for custom formatting

The Thermo Scientific™ TMTpro-134C and TMTpro-135N tags are identical in composition and structure to the other TMTpro 16plex tags, but have different stable isotopes in their linker and reporter regions (Figure 14). Addition of the TMTpro-134C and TMTpro-135N tags creates a 17th and 18th channel for relative quantitation using high-resolution Thermo Scientific™ Orbitrap™ Mass Spectrometer instruments and Proteome Discoverer™ 2.4 software. These tags are available separately or as part of the TMTpro 18plex Label Reagent Set. When combined with the superior, high-resolution Orbitrap instruments and software, TMTpro reagents provide an integrated total solution for quantitative protein expression analysis.

TMTpro label reagents are the next generation of tandem mass tags, designed to increase the level of sample multiplexing without compromising protein identification and quantitation. The structures of the TMTpro and TMT tags are similar in being isobaric and amine-reactive, but the TMTpro tag has a longer spacer region and isobutyl proline mass reporter. After MS/MS fragmentation, each TMTpro tag generates a unique reporter mass (e.g., TMTpro 126–135 Da) in the low-mass region of the high-resolution MS/MS spectrum that is used for relative quantitation of protein expression levels (Figure 15).

To demonstrate the accuracy of TMTpro quantitation, yeast protein extracts from wild type and knockout strains (Met6, His4, or Ura2) were serially diluted at different fixed ratios (1:2, 1:4, 1:8, and 1:16) before being reduced, alkylated, digested, and labeled with the TMTpro reagents. Labeled samples were combined and analyzed on a Thermo Scientific™ Orbitrap Eclipse™ LC-MS/MS mass spectrometer to identify peptides and quantify the relative abundance of reporter ions. As shown in Figure 16, each protein from the knockout strain was accurately quantified relative to the abundance level in the wild-type strain.

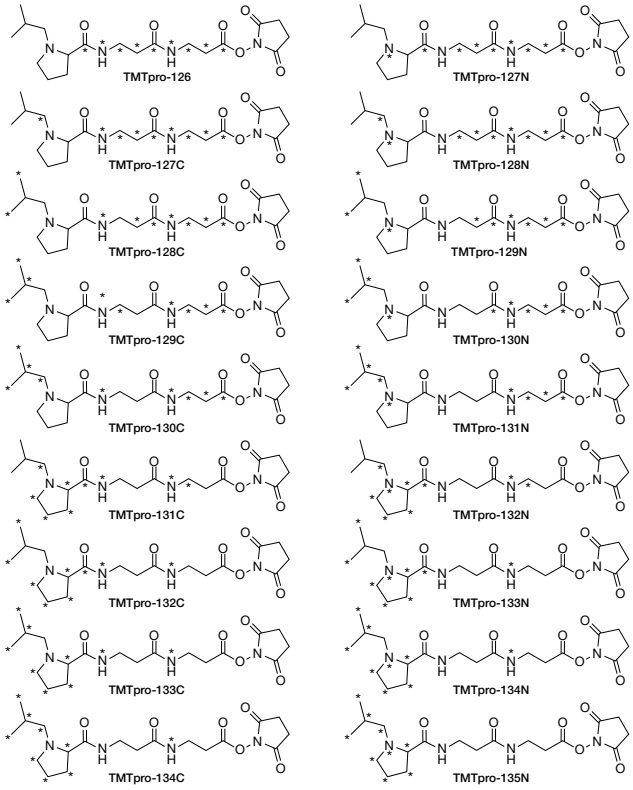


Figure 14. TMTpro 18plex reagent structures with ^{13}C and ^{15}N stable isotope positions annotated with an asterisk (*).

Protein quantitation

TMTpro 18plex Label Reagent Set, cont.

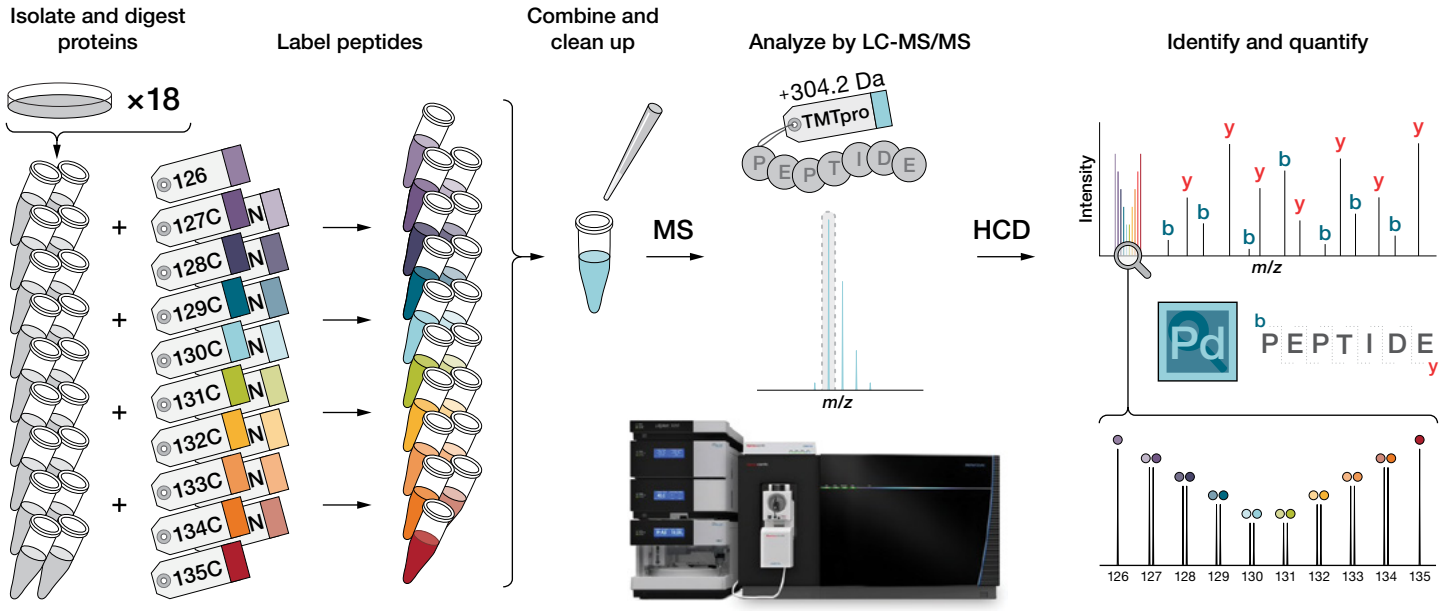


Figure 15. TMTpro reagent workflow.

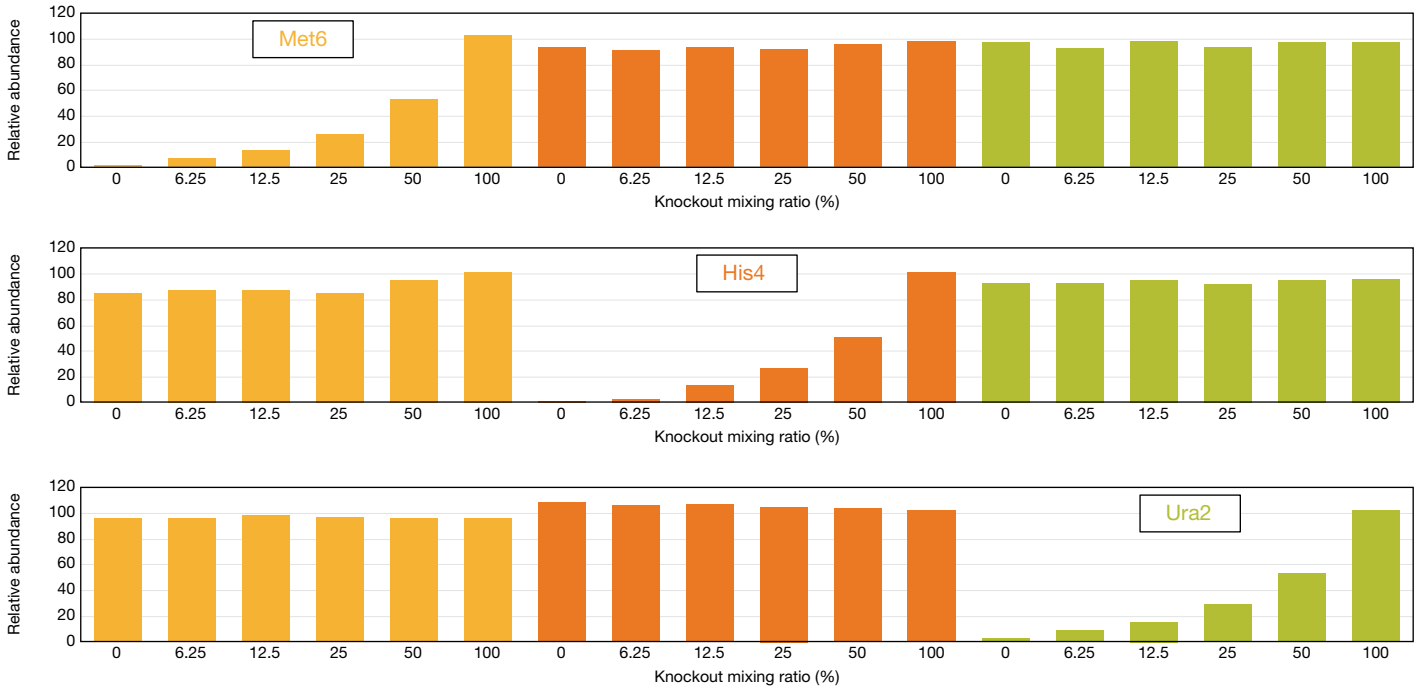


Figure 16. TMTpro 18plex standard reporter ion quantitation. Relative abundance of reporter ions from mixed protein digests of yeast wild-type strain and knockout strain (Met6, His4, or Ura2) at different fixed ratios. Data are from the Orbitrap Eclipse RTS-MS^3 close-out method with 5 peptides used per protein.

Ordering information

Product	Quantity	Cat. No.
TMTpro Zero Label Reagent (sufficient for labeling ten samples)	1 x 5 mg	A44518
TMTpro Zero Label Reagent (sufficient for labeling five samples)	5 x 0.5 mg	A44519
TMTpro 16plex Label Reagent Set (sufficient for ten 16plex isobaric experiments)	1 x 5 mg	A44520
TMTpro 16plex Label Reagent Set (sufficient for one 16plex isobaric experiment)	1 x 0.5 mg	A44521
TMTpro 16plex Label Reagent Set (sufficient for six 16plex isobaric experiments)	6 x 0.5 mg	A44522
TMTpro 18plex Label Reagent Set (sufficient for ten 18plex isobaric experiments)	1 x 5 mg	A52045
TMTpro 134C and 135N Label Reagents (sufficient for ten 18plex isobaric experiments)	1 x 5 mg	A52046
TMTpro 18plex Label Reagent Set (sufficient for six 18plex isobaric experiments)	6 x 0.5 mg	A52047
TMTpro 134C and 135N Label Reagents (sufficient for six 18plex isobaric experiments)	6 x 0.5 mg	A52048

Find out more at **thermofisher.com/tmtpro**

Protein quantitation

Super Heavy TMTpro Label Reagent

The amine-reactive Thermo Scientific™ Super Heavy TMTpro™ Label Reagent is an alternate form of TMTpro reagent that contains twice the number of heavy isotopes for labeling of peptides (Figure 17). When used with samples labeled with TMTpro reagents, Super Heavy TMTpro Label Reagent enables 2D TMTpro multiplexing applications for targeted quantitation (Figure 18).

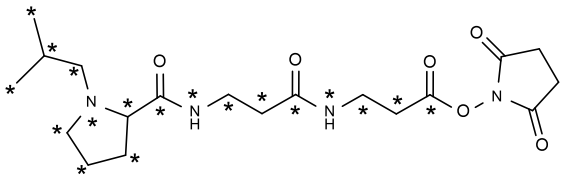


Figure 17. Structure of the Super Heavy TMTpro Label Reagent.

Super Heavy TMTpro Label Reagent is used to label synthetic peptides for use specifically with TMTpro multiplex-labeled samples. When used in combination, the mass offset from the super heavy–labeled synthetic peptide serves as a trigger for the selection of the targeted peptide in the multiplexed sample. This triggered selection reduces interference/co-isolation to enable more robust quantitation.

This new method combines the power of multiplexing from TMTpro reagents with targeted quantitation methods and is referred to as the TOMAHAQ (triggered by offset, multiplexed, accurate mass, high resolution, and absolute quantitation) method. An additional advantage of this method for targeted multiplexed quantitation is less dependence on the chromatography conditions for successful quantitation.

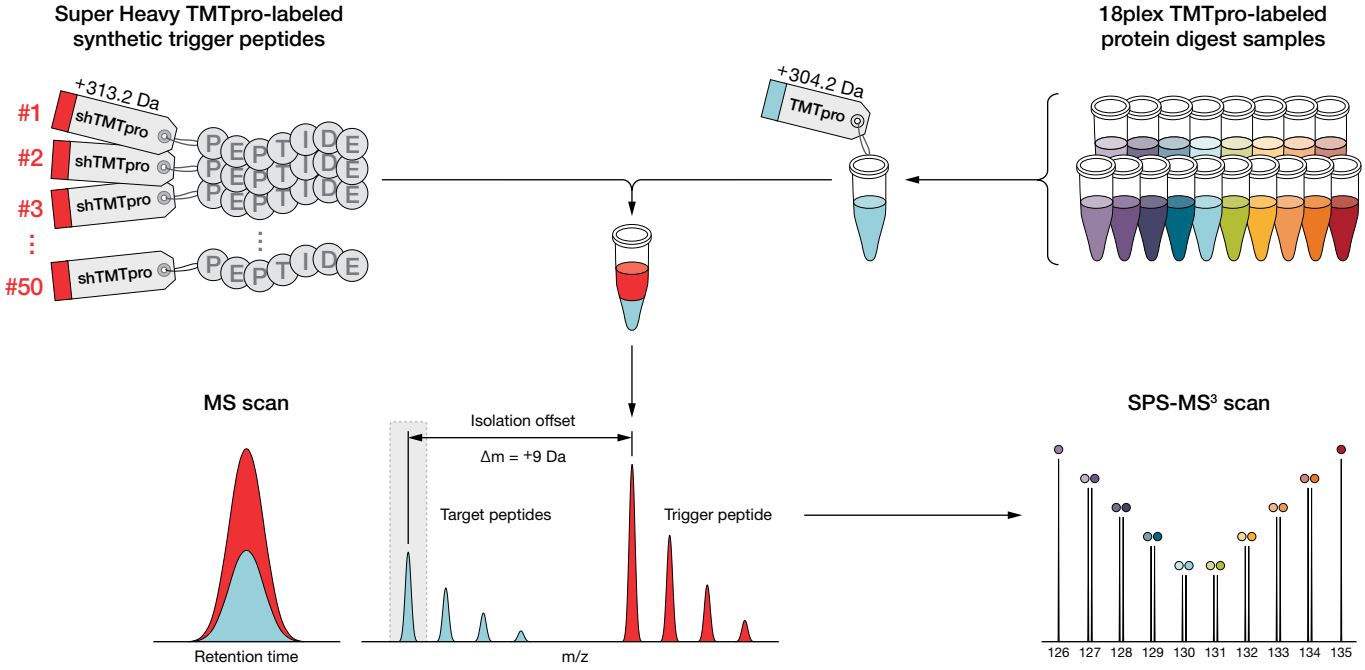


Figure 18. Super Heavy TMTpro workflow.

Ordering information

Product	Quantity	Cat. No.
Super Heavy TMTpro Label Reagent	2 mg	A52040

Find out more at **thermofisher.com/tmtpro**

Protein characterization

Protein interaction and crosslinking using mass spectrometry

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

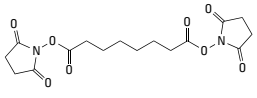
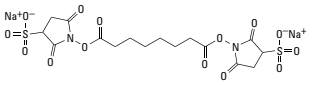
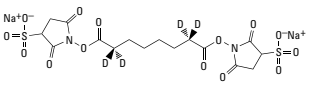
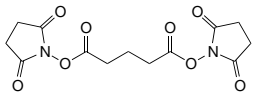
Thermo Scientific™ MS-grade crosslinkers are available with different linker lengths and as isotopically labeled sets to help elucidate protein–protein interactions (Table 2). Simplification of the analysis of crosslinked proteins is essential for successful protein characterization. In addition to traditional crosslinkers, next-generation crosslinkers have been developed to address simplifying MS analysis through crosslinker enrichment and cleavable functionality. These high-quality reagents have been validated in protein–protein interaction studies using Thermo Scientific™ mass spectrometers that use different types of fragmentation (CID, HCD, ETD, and EtHCD) and levels of tandem mass spectrometry (MS^2 and MS^3), in order to improve identification of protein–protein interaction sites.

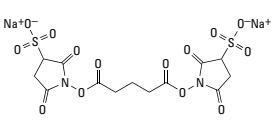
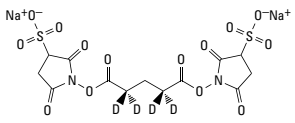
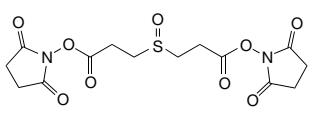
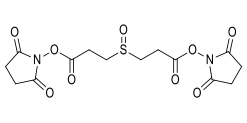
Our MS-grade crosslinkers are high-quality reagents that are available in multiple packaging options and sizes. We offer extensive technical expertise and support for various applications as well as validation of these products in workflows using Thermo Scientific™ mass spectrometers.

Features include:

- **High quality**—products manufactured in ISO 9001–certified facilities
- **Convenience**—products available in Thermo Scientific™ No-Weigh™ packaging or in multiple pack sizes
- **More choices**—available with different linker lengths, MS cleavability, and deuterium isotope labels
- **Technical support**—extensive web resources and support to help ensure successful results

Table 2. Overview of Thermo Scientific crosslinkers used for studying protein–protein interactions.

Crosslinker	DSS	BS^3	BS^3-d_4	DSG
Structure				
Full name	Disuccinimidyl suberate	Bis(sulfo-succinimidyl) suberate	Bis(sulfo-succinimidyl) 2,2,7,7-suberate-d_4	Disuccinimidyl glutarate
Spacer arm (Å)	11.4	11.4	11.4	7.7
Water-soluble	No	Yes	Yes	No
Isotopically labeled	No	No	Yes	No
MS-cleavable	No	No	No	No

Crosslinker	BS^2G-d_0	BS^2G-d_4	DSSO	DSBU
Structure				
Full name	Bis(sulfo-succinimidyl) glutarate-d_0	Bis(sulfo-succinimidyl) 2,2,4,4-suberate-d_4	Disuccinimidyl sulfoxide	Disuccinimidyl dibutyric urea
Spacer arm (Å)	7.7	7.7	10.1	12.5
Water-soluble	Yes	Yes	No	No
Isotopically labeled	No	Yes	No	No
MS-cleavable	No	No	Yes	Yes

Protein characterization

MS-cleavable crosslinkers (DSSO and DSBU)

Thermo Scientific™ DSSO (disuccinimidyl sulfoxide) and DSBU (disuccinimidyl dibutyric urea, also known as BuUrBu) are high-quality, MS-cleavable crosslinkers that contain an amine-reactive N-hydroxysuccinimide (NHS) ester at each end of a 10.1-angstrom and 12.5-angstrom spacer arm, respectively (Table 2, page 17). These products are offered in convenient single-use packaging (10 x 1 mg).

Features of DSSO and DSBU include:

- Amine-reactive NHS ester (at both ends) reacts rapidly with any molecule containing a primary amine
- MS-cleavable by collision-induced dissociation (CID)
- High-purity crystalline reagents for protein structure and interaction characterization
- Membrane-permeable, allowing intracellular crosslinking
- Water-insoluble (dissolve first in DMF or DMSO)

The crosslinker facilitates analysis of protein structure and complex interactions using mass spectrometry. DSSO and DSBU have similar reactivity to DSS but contain linkers that can be cleaved in the gas phase during tandem MS (MS/MS) using CID. The ability to cleave cross-linked peptides during MS/MS enables MS^3 acquisition methods, which facilitate peptide sequencing using traditional database search engines. The MS cleavage of DSSO and DSBU also generates diagnostic ion doublets during MS^2, which enables identification of cross-linked peptides from dead-end modifications and searching using novel database search engines such as MeroX or XlinkX* (Figure 19).

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

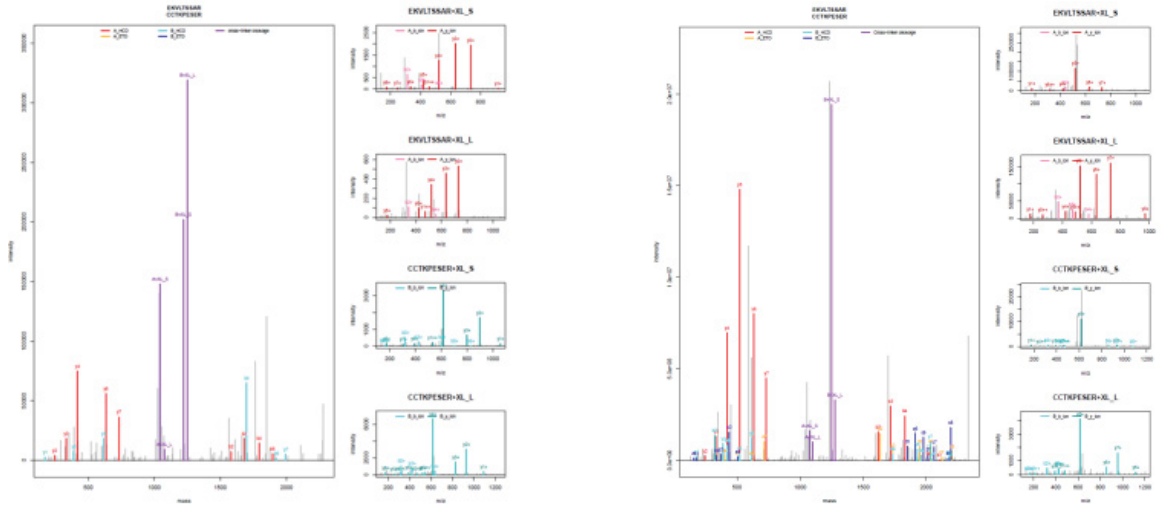


Figure 19. Spectra of BSA-crosslinked peptide identified by MS^2/MS^3 method and XlinkX software* using (left) DSSO and (right) DSBU crosslinkers. XlinkX software uses unique fragment patterns of MS-cleavable crosslinkers (purple annotation) to detect and filter crosslinked peptides for a database search.

Ordering information

Product	Quantity	Cat. No.
DSSO (Disuccinimidyl Sulfoxide)	10 x 1 mg	A33545
DSBU (Disuccinimidyl Dibutyric Urea)	10 x 1 mg	A35459

Protein characterization

Enrichable MS crosslinkers PhoX (DSPP) and TBDSPP

Thermo Scientific™ DSPP (Disuccinimidyl Phenyl Phosphonic Acid, PhoX) and TBDSPP (*tert*-Butyl Disuccinimidyl Phenyl Phosphonate, tBu-PhoX) are amine-reactive, enrichable crosslinkers designed for mass spectrometry analysis. Both crosslinkers have amine-reactive N-hydroxysuccinimide (NHS) esters at the ends of a 7-atom spacer arm containing either a phosphonic acid group (DSPP) or phosphonate ester (TBDSPP) for enrichment (Figure 20). These phospho groups are used for enrichment of crosslinked peptides using immobilized metal affinity chromatography (IMAC) or metal oxide affinity chromatography (MOAC). Additionally, TBDSPP is cell-permeable, allowing for intracellular crosslinking applications.

Features of DSPP and TBDSPP include:

- Tri-functional crosslinker–reactive groups: NHS ester (both ends), phosphonic acid (DSPP) or phosphonate ester (TBDSPP) in the spacer for enrichment
- High-purity crystalline reagents for protein structure and interaction characterization
- Membrane-permeable version (TBDSPP) for intracellular crosslinking
- Enrichable using Fe-NTA IMAC or TiO_2 MOAC

Of the two enrichable MS crosslinkers, DSPP is more water soluble than TBDSPP and is better for *in vitro* crosslinking of simple purified proteins and protein complexes. TBDSPP is better for intracelluar crosslinking due to increased membrane permeability. Although both crosslinkers can be enriched using traditional phosphopeptide methods, TBDSPP crosslinked peptides must first be incubated with TFA to remove the *tert*-butyl protection groups (Figure 21).

Chemical crosslinking in combination with MS is a powerful method to determine protein–protein interactions. This method has been applied to recombinant and native protein complexes and to whole cell lysates or intact unicellular organisms in efforts to identify protein–protein interactions on a global scale. Both traditional, non-cleavable, and MS-cleavable crosslinkers can be used for identification of protein–protein interaction sites, but phospho-enrichable crosslinkers are advantageous because they can be used to enrich low-abundance crosslinked peptides and thereby improve MS identification rates (Figures 22 and 23).

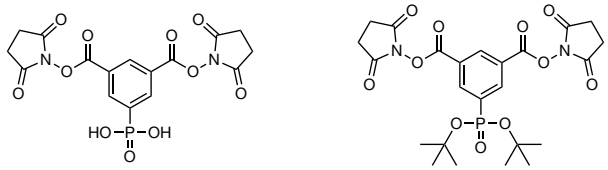


Figure 20. Chemical structure of enrichable crosslinkers DSPP (PhoX) and TBDSPP.

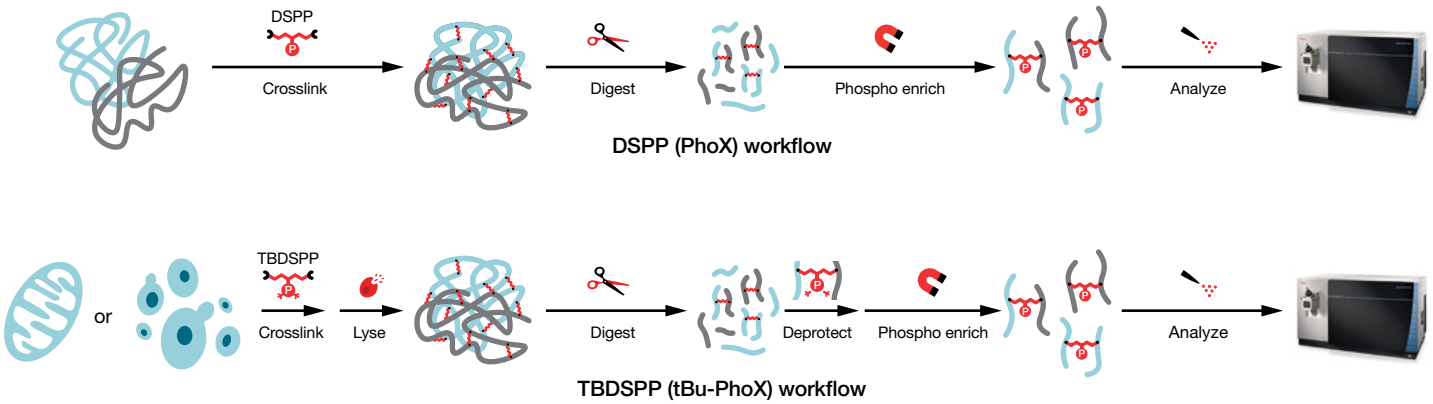


Figure 21. Workflows of DSPP or TBDSPP crosslinking for LC-MS analysis. Simple proteins and complexes are crosslinked using DSPP before digestion, phospho enrichment, and LC-MS analysis. Proteins, organelles, or cells can be crosslinked using TBDSPP, but they require deprotection using TFA before phospho enrichment and LC-MS analysis.

Protein characterization

Enrichable MS crosslinkers PhoX (DSSP) and TBDSPP, cont.

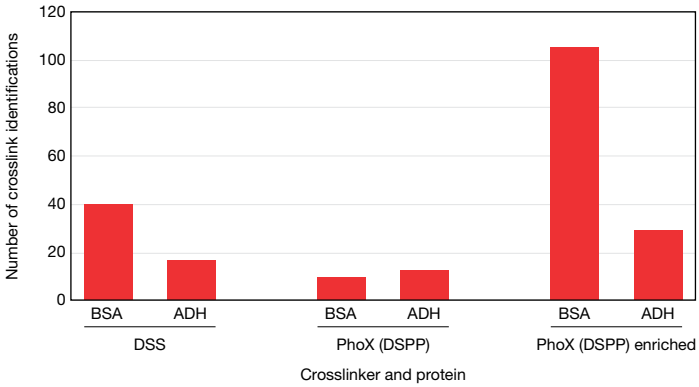


Figure 22. BSA and ADH crosslinking using DSS and PhoX (DSPP). Purified proteins were crosslinked using 20-fold molar excess of crosslinkers and acetone-precipitated before digestion and LC-MS analysis. DSPP samples enriched using the High-Select Fe-NTA Magnetic Agarose Kit had over 2-fold more crosslinks identified than DSS samples.

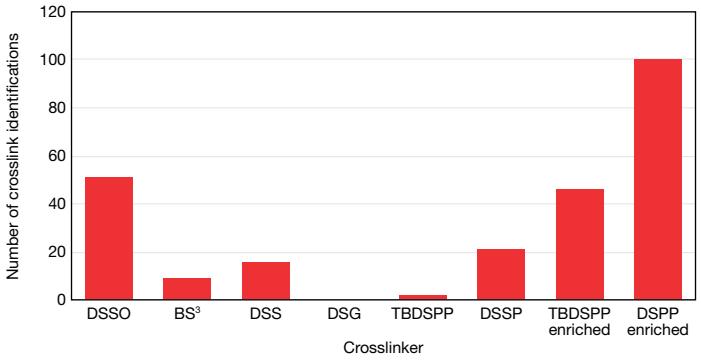


Figure 23. Crosslinking of *E. coli* ribosomes. Ribosomes were crosslinked with 40-fold molar excess of different crosslinkers for 1 hour at room temperature. Samples were reduced and alkylated before acetone precipitation to remove excess crosslinker. Samples crosslinked with TBDSPP were deprotected for 30 minutes at 37°C with 2.5% TFA. All samples were digested overnight before C18 cleanup and LC-MS analysis.

Ordering information

Product	Quantity	Cat. No.
DSPP (Disuccinimidyl Phenyl Phosphonic Acid, PhoX)	50 mg	A52286
TBDSPP (*tert*-Butyl Disuccinimidyl Phenyl Phosphonate, tBu-PhoX)	50 mg	A52287

Learn more at **thermofisher.com/ms-crosslinking**

Protein characterization

Enrichable chemical alkylating reagent (6C-CysPAT)

Thermo Scientific™ Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) is a sulfhydryl-reactive alkylating reagent that has been designed to chemically modify and enrich cysteine-containing peptides for MS analysis. In contrast to phosphate groups, the phosphonic acid group of 6C-CysPAT (Figure 24) is not a substrate for phosphatases, which can be used to remove endogenous phosphate-modified peptides before or after enrichment. Alkylation of reduced cysteine-containing proteins/peptides with 6C-CysPAT results in a covalent thioester bond with a modification mass of 221.082 Da. After protein alkylation and enzymatic digestion, labeled peptides can be enriched using immobilized metal affinity chromatography (IMAC) or metal oxide affinity chromatography (MOAC) (Figures 25 and 26).

Features of 6C-CysPAT include:

- Hetero-bifunctional sulfhydryl alkylating reagent with phosphonic acid reactive group for enrichment of cysteine-containing peptides
- High-purity, crystalline reagent provided in convenient single-use, No-Weigh format
- Compatible with EasyPep MS Sample Prep and High-Select™ Phosphopeptide Enrichment kits

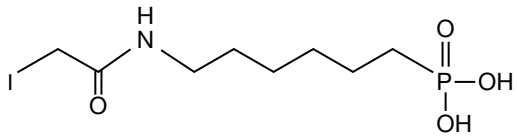


Figure 24. Structure of 6C-CysPAT.

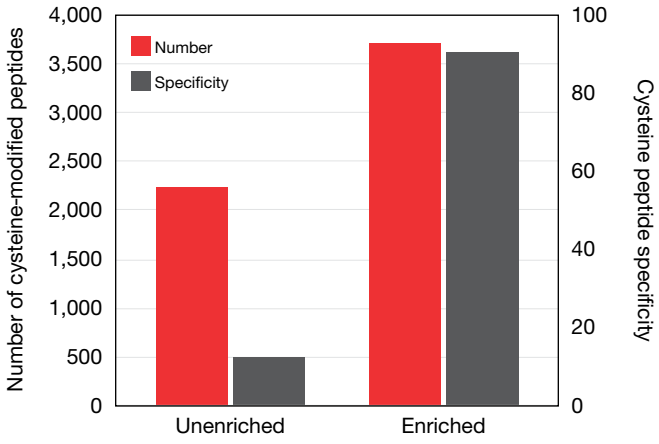


Figure 26. 6C-CysPAT labeling and enrichment specificity. A HeLa lysate was reduced and alkylated using 5 mM TCEP and 5 mM 6C-CysPAT, respectively. Digests were cleaned up using Thermo Scientific™ EasyPep™ Peptide Cleanup Columns before enrichment using High-Select™ Fe-NTA Magnetic Beads and LC-MS analysis.

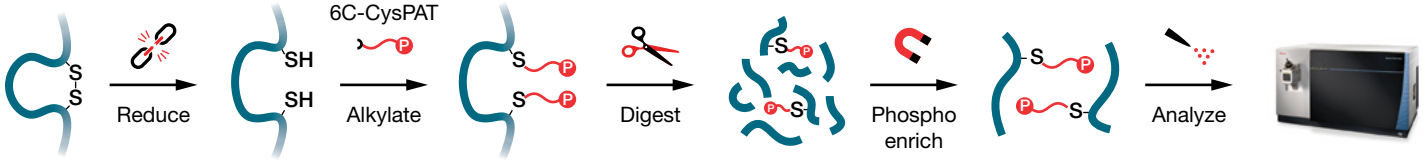


Figure 25. Workflow for cysteine peptide labeling and enrichment.

Protein characterization

Enrichable chemical alkylating reagent (6C-CysPAT), cont.

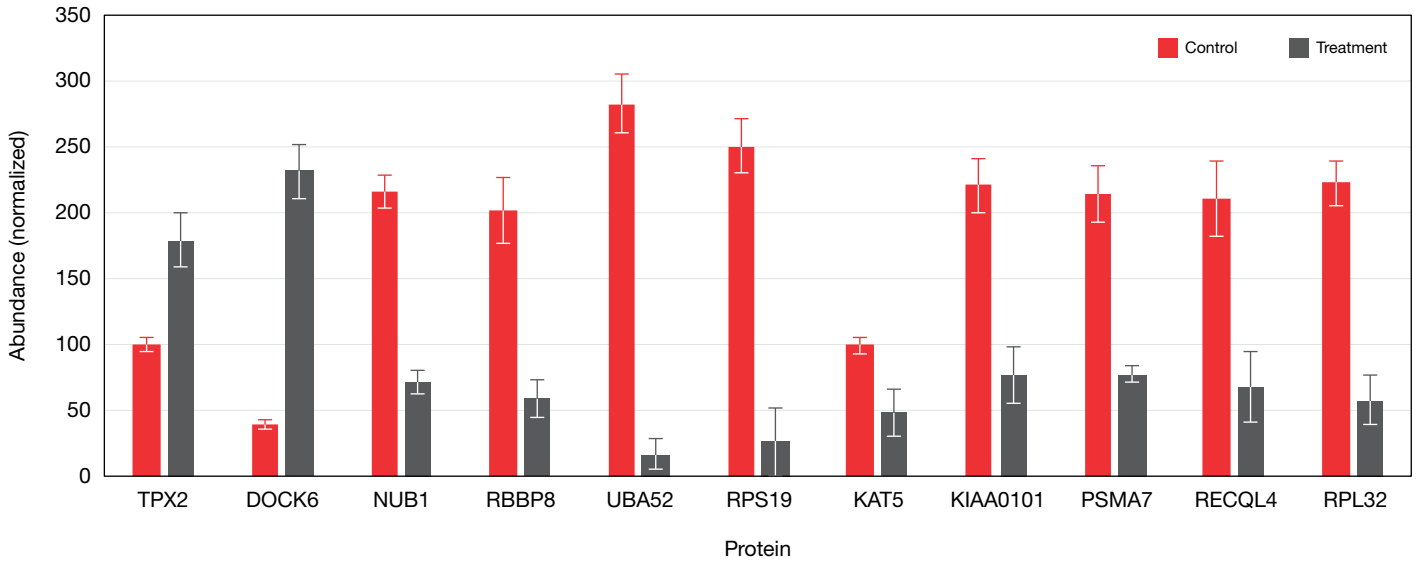


Figure 27. 6C-CysPat labeling enables identification of protein expression changes after oxidative stress. HeLa cells were treated with 1% H_2O_2 for 5 minutes before 6C-CysPAT labeling, digestion, and enrichment. Samples were analyzed by LC-MS using label-free, precursor quantitation that identified differentially regulated proteins from DNA replication/repair, apoptosis, RNA processing, and proteosome regulation pathways.

Ordering information

Product	Quantity	Cat. No.
Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT)	10 x 3.5 mg	A52285

Instrument calibration and QC

Pierce LC-MS/MS System Suitability Standard (7 x 5 mix)

Convenient standard for scheduling of MS acquisition windows

The Thermo Scientific™ Pierce™ LC-MS/MS System Suitability Standard (7 x 5 mix) contains seven HeavyPeptide AQUA Ultimate peptides (provided in a mixture at five different concentrations) distinguished by differential isotopic labeling to assess sensitivity and dynamic range of LC-MS/MS systems (Figure 28).

The Pierce LC-MS/MS System Suitability Standard (7 x 5 mix) contains 7 of 15 peptides from the Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (Cat. No. 88320). Each of the seven peptides has five versions (amino acids labeled with 0, 1, 2, 3, or 4 heavy isotopes), at five distinct concentrations (Figure 29). The 7 x 5 mix can be used with Skyline software to assess dynamic range, linearity, and lower limit of quantitation (LLOQ).

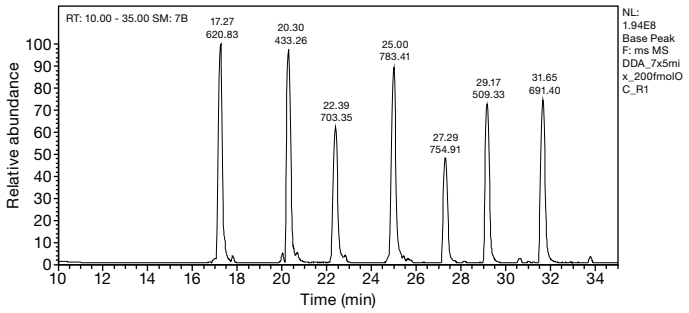


Figure 28. MS analysis of the Pierce LC-MS/MS Suitability Standard (7 x 5 mix). The standards were analyzed on a Thermo Scientific™ UltiMate™ 3000 RSLCnano System and Q Exactive HF system using a Thermo Scientific™ EASY-Spray™ HPLC Column (Cat. No. ES900) with a 3–30% gradient of Buffer B (0.1% formic acid, 99.9% acetonitrile) and Buffer A (0.1% formic acid) at 0.3 µL/min.

Features include:

- **High quality**—contains five sets of seven HeavyPeptide AQUA Ultimate peptides, where each peptide in each set is distinguishable by unique isotopolog labeling
- **Optimized**—enables scheduling of MS acquisition windows for improved quantification and increased multiplexing
- **Quantitative**—enables assessment of dynamic range of instrument and instrument sensitivity
- **Predictive**—enables assessment of chromatography and MS instrument performance

Applications

- Standard for data-dependent acquisition (DDA)
- Standard for targeted MS (e.g., parallel-reaction monitoring (PRM))
- Optimization of LC and/or MS parameters
- Identification of total peptide elution window
- Assessment of dynamic range of nano or capillary-flow LC-MS/MS systems

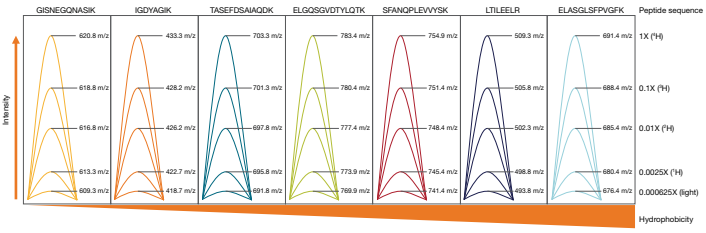


Figure 29. Composition of Pierce LC-MS/MS System Suitability Standard (7 x 5 mix).

Reference

Krokhin OV and Spicer V (2009) Peptide retention standards and hydrophobicity indexes in reversed-phase high-performance liquid chromatography of peptides. *Anal Chem* 81(22):9522–9530.

Ordering information

Product	Quantity	Cat. No.
Pierce LC-MS/MS System Suitability Standard (7 x 5 Mix) (0.5 pmol/µL)	25 µL	A40010
Pierce LC-MS/MS System Suitability Standard (7 x 5 Mix) (5 pmol/µL)	100 µL	A51747

Find out more at **thermofisher.com/ms-standards**

Instrument calibration and QC

Pierce Yeast Digest Standard

The Thermo Scientific™ Pierce™ Yeast Digest Standard is a lyophilized yeast peptide mixture ideal for monitoring LC-MS system performance.

Features include:

- **Positive control sample**—well-characterized, eukaryotic protein digest (>3,000 proteins; Figure 30) for the LC-MS method of standardization, development, and troubleshooting
- **Verified peptide quality**—digestion conditions optimized for minimal missed cleavages and peptide modifications during processing
- **Rigorously tested**—high-quality, consistent protein digest documented via lot-specific certificates of analysis
- **Convenient**—ready-to-use, lyophilized format

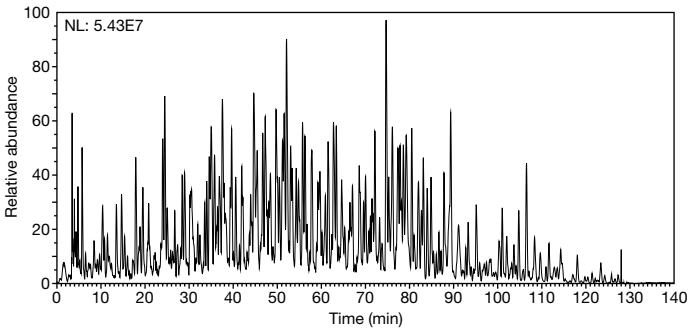


Figure 30. Base peak chromatogram of 300 ng Pierce Yeast Digest Standard. The standard was separated using a Thermo Scientific™ Acclaim PepMap™ 100 C18 3 µm x 75 µm x 15 cm column (Cat. No. 164568) with 2–35% gradient (Buffer A: 0.1% formic acid in water, Buffer B: 0.1% formic acid in 100% acetonitrile) at 300 nL/min for 120 minutes and detected on either a Thermo Scientific™ LTQ Orbitrap™ XL or a Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometer platform.

The Pierce Yeast Digest Standard is specifically formulated for LC-MS experiments and does not contain salts or detergents. The protein digest is derived from *Saccharomyces cerevisiae* yeast strain BY4741 (genetic background: MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0). The yeast proteome is well-documented and characterized, and the digest is an ideal standard for more complex proteome mass spectrometry applications or when a non-mammalian digest is required for analysis. Using the standard routinely before analysis of complex samples makes it possible to monitor and normalize LC-MS performance, between samples and over time.

Ordering information

Product	Quantity	Cat. No.
Pierce Yeast Digest Standard	20 µg	A47951

Instrument calibration and QC

Pierce HeLa Digest/PRTC Standard

The Thermo Scientific™ Pierce™ HeLa Digest/PRTC Standard is a validated complex mammalian protein digest containing additional PRTC heavy isotope-labeled peptides designed for LC-MS performance testing.

Features include:

- **Convenient**—premixed formulation of HeLa digest and PRTC standard in a ready-to-use format
- **System performance standard**—enables robust performance assessment of LC-MS instruments
- **Rigorously tested**—high-quality, consistent mixture documented via lot-specific certificates of analysis
- **Stable**—provided in a lyophilized format

The Pierce HeLa Digest/PRTC Standard is a lyophilized mixture of 10 µg of HeLa protein digest with 5 pmol PRTC peptides for the assessment of LC-MS instrument performance. The protein digest is derived from a well-established adenocarcinoma reference HeLa S3 cell line that expresses over 15,000 proteins with relevant post-translational modifications (Figure 31, top). Pierce Peptide Retention Time Calibration (PRTC) Mixture contains 15 synthetic heavy isotope-labeled peptides mixed at an equimolar ratio that elute across the chromatographic gradient (Figure 31, bottom). In combination, the mixture is a powerful tool to optimize and assess LC parameters, identify the total peptide elution window, and optimize and monitor MS instrumentation.

Applications

- Qualitative LC assessment
- QC assay tool for assessment of LC-MS system performance
- LC-MS standardization
- LC-MS method development and optimization
- Normalization of results for variation in retention times and peak intensities between runs

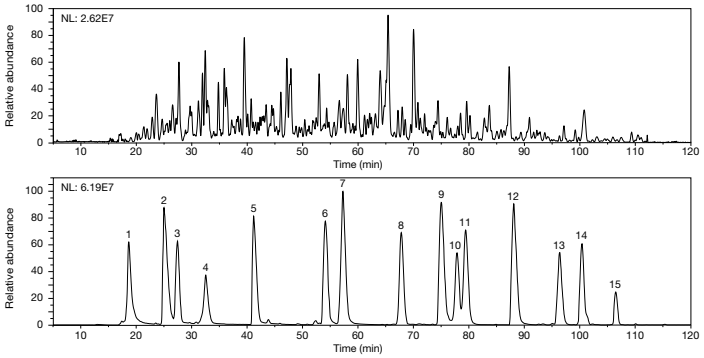


Figure 31. Base peak chromatogram of 200 ng of Pierce HeLa Protein Digest Standard. The standard was separated using a Thermo Scientific™ Acclaim™ PepMap™ 100 C18 3 µm x 75 µm x 15 cm column (Cat. No. 164568) with a 2–35% gradient (Buffer A: 0.1% formic acid in LC-MS–grade water, Buffer B: 0.1% formic acid in 100% LC-MS–grade acetonitrile) at 300 nL/min for 120 minutes and detected on a Thermo Scientific™ LTQ Orbitrap™ XL Mass Spectrometer (top). Pierce Peptide Retention Time Calibration Mixture (10 µL at 5 pmol/µL, before dilution to 0.5 pmol/µL) was analyzed on a Thermo Scientific™ EASY-nLC 1000 nano system and Thermo Scientific™ Velos Pro mass spectrometer system using an Acclaim PepMap 100 C18 3 µm x 75 µm x 15 cm column with a 2–30% gradient of Buffer B using Buffer A (0.1% formic acid) and Buffer B (0.1% formic acid/99.9% acetonitrile) at 0.3 µL/min (bottom).

Ordering information

Product	Quantity	Cat. No.
Pierce HeLa Digest/PRTC Standard	20 µg	A47996
	5 x 20 µg	A47997

Instrument calibration and QC

Pierce Small Molecule System Suitability Standard

The Thermo Scientific™ Pierce™ Small Molecule System Suitability Standard provides a preformulated mixture of nine small-molecule standards to assess system performance in both positive and negative ionization modes for Thermo Scientific™ TSQ™ Triple Quadrupole and Orbitrap Exploris™ mass spectrometers.

Features include:

- **Comprehensive**—mixture of nine small-molecule compounds (mass range: 76–1,279 Da) designed to assess instrument performance in both positive and negative ionization modes
- **Validated**—high-quality, rigorously tested formulation with lot-specific certificates of analysis
- **Convenient**—preformulated, ready-to-use liquid format
- **Monitoring**—excellent QC assay tool for quality assessment of LC and MS instrument status

The Pierce Small Molecule Suitability Standard is a ready-to-use formulation optimized for the assessment of MS instrument performance for metabolomics applications. The standard contains a mixture of nine compounds with a mass range of 76–1,279 Da, appropriate for small molecule analysis (Figure 32). The standard has been designed for use with TSQ Triple Quadrupole and Orbitrap Exploris MS series instruments to verify individual system and multiple system performance. Additionally, the Pierce Small Molecule Suitability Standard enables quicker identification of suboptimal system performance, enhances troubleshooting, and provides standardization to help obtain higher quality data. The standard is manufactured at an ISO 9001 facility, and each lot is quality controlled with strict specifications.

Compound	Ion(s)	Exact mass(es) (Da)
Glycine	$[M+H]^+$	76.0393
Atenolol	$[M+H]^+$	267.1703
Flumetsulam	$[M-H]^+$ / $[M-H]^-$	326.0518
Atrazine	$[M+H]^+$	216.10105
Terfenadine	$[M+H]^+$	472.32101
Warfarin	$[M-H]^+$ / $[M-H]^-$	307.9650 / 309.1121
Ultramark 1621	$[M+formate]^-$ / $[M+H]^+$	1,279.99722 / 1,221.99064
Methylmalonic acid	$[M-H]^-$	117.01933
Rafoxanide	$[M-H]^-$	623.81326

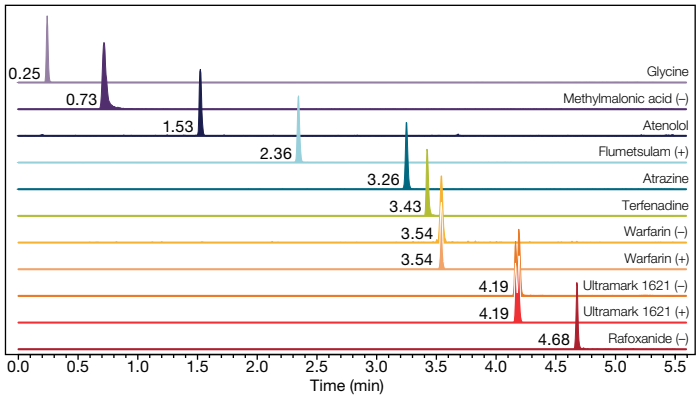


Figure 32. Chromatogram of the target compounds. A 1 µL sample of 10-fold diluted Pierce Small Molecule System Suitability Standard was separated using a Thermo Scientific™ Hypersil GOLD™ C18 Selectivity HPLC Column (Cat. No. 25002-052130) with 1–98% gradient (Buffer A: 0.1% formic acid in LC-MS–grade water, Buffer B: 100% LC-MS–grade methanol) at 0.5 mL/min on a Thermo Scientific™ Vanquish™ Binary Pump UHPLC and Thermo Scientific™ TSQ Quantis™ Triple Quadrupole Mass Spectrometer.

Ordering information

Product	Quantity	Cat. No.
Pierce Small Molecule System Suitability Standard	0.25 mL	A51740

Instrument calibration and QC

Pierce FlexMix Calibration Solution for Auto-Ready Mass Spectrometers

The Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution for Auto-Ready Mass Spectrometers is a room-temperature stable, ready-to-use liquid formulation for the calibration of Thermo Scientific mass spectrometers with the integrated Auto-Ready ion source.

Features include:

- **Improved mass accuracy**—extended mass range helps improve mass accuracy
- **Validated**—high-quality, rigorously tested formulation with lot-specific certificates of analysis
- **Ready-to-use**—bottle may be directly loaded into the instrument for scheduled automatic calibration
- **Stable**—room temperature for 18 months and within the Auto-Ready mass spectrometer for up to 4 months

The Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution is a ready-to-use formulation that has been optimized for both positive and negative ionization calibration of instruments. The extended mass range (50 to 3,000 m/z) helps improve the sensitivity and mass accuracy of Orbitrap mass spectrometers. The solution is now available in an Auto-Ready format for the Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ Mass Spectrometer using Thermo Scientific™ Orbitrap Tribrid Series 3.5 Instrument Control Software.

Component	m/z	Purity
*LC-MS–grade acetonitrile (87% v/v)	—	Purity ≥99.9%
*LC-MS–grade water (13% v/v)	—	LC-MS grade
LC-MS–grade acetic acid	59.0128[b](–)	Purity ≥99.7%
Imidazole	69.0447[a](+)	Purity ≥99.0%
Triethylamine	102.1277[a](+)	Purity ≥99.5%
Trifluoroacetic acid (TFA)	112.9845[b](–)	Purity ≥99.5%
Tetramethylpiperidine	142.1590[a](+)	Purity ≥97.5%
Pentafluoropropionic acid	162.9824[b](–)	Purity ≥97.0%
Caffeine	195.0876[a](+)	Purity ≥97.0%
Hexamethoxyphosphazine	322.0481[a](+)	Purity ≥99.0%
Perfluoroheptanoic acid	362.9696[b](–)	Purity ≥96.0%
MRFA	524.2649[a](+)	Purity ≥98.0%
2,4,6-Tris(heptafluoropropyl)-1,3,5-triazine	601.9779[c](–)	Purity ≥95.0%
Hexakis(2,2-difluoroethoxy) phosphazene	622.0290[a](+)	Purity ≥97.0%
Hexakis(2,2,3,3-tetrafluoropropoxy) phosphazene	922.0098[a](+), 1,033.9881[d](–)	Purity ≥95.0%
Ultramark 1621	**See note below	Purity ≥95.0%
Hexakis(1h,1h,7h-perfluoroheptoxy) phosphazene	2,121.9331[a](+), 2,233.9115[d](–)	Purity ≥95.0%
Hexakis(1h,1h,9h-perfluorononyloxy) phosphazene	2,721.8948[a](+), 2,833.8731[d](–)	Purity ≥95.0%

a: $[M+H]^+$; b: $[M-H]^-$; c: $[M+OH]^-$; d: $[M+TFA]^-$.

* Combined concentration of all other components listed is less than 1% w/v.

** Note: Ultramark 1621 is a well-defined mixture of compounds that produces a well-defined peak envelope in the range of 900–2,200.

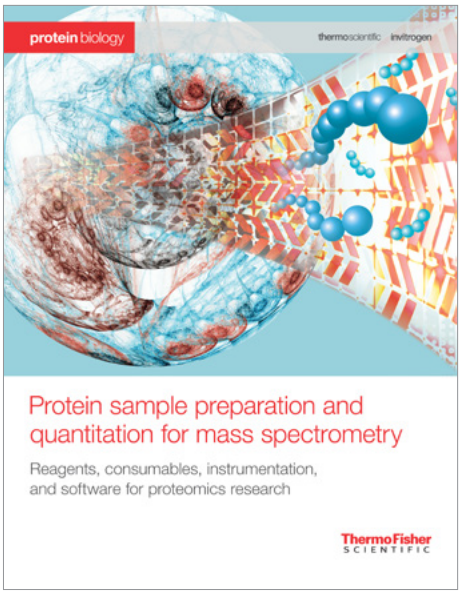
Ordering information

Product	Quantity	Cat. No.
Pierce FlexMix Calibration Solution for Auto-Ready Mass Spectrometers	2 x 4 mL	A51739

Find hardware related support at **unitylabservices.com**

ThermoFisher SCIENTIFIC

Mass spectrometry digital resources



Download this free handbook on protein sample preparation and quantitation for mass spectrometry, and browse other relevant literature, to learn more about tools and techniques for more robust and reproducible outcomes. Access helpful white papers and late-breaking posters for specific applications like subcellular fractionation, peptide fractionation, isobaric labeling, and more. Increase your knowledge of sample preparation and protein quantitation through our free, on-demand webinars.

Explore content at **thermofisher.com/msresources**

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

thermo scientific