



New tools for improved proteomics results


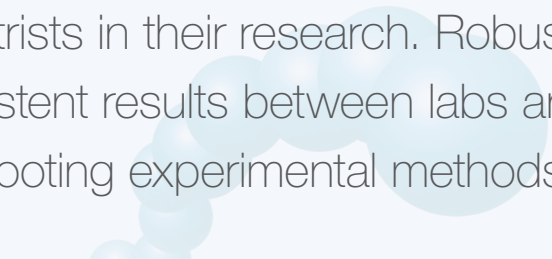
Sample preparation, protein quantitation, and instrument calibration for proteomic mass spectrometry applications



Introduction

We offer a complete portfolio of sample preparation, protein quantitation, and instrument calibration reagents designed for better mass spectrometry (MS) analysis. This portfolio has been developed 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 of the AKT pathway, RAS, and TP53. In addition, we have developed the next generation of Tandem Mass Tag™ reagents (TMTs) designed to increase the level of sample multiplexing without compromising on protein identification and quantitation.

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 provide consistent results between labs and eliminate time wasted on troubleshooting experimental methods and results.



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Sample preparation

EasyPep Mini, Maxi, and 96 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 a 96-well plate and 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).

Highlights

- **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 nonstandard 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 improves reproducibility while also saving hands-on and processing time. The number of steps and time have been reduced through the addition of Thermo Scientific™ Pierce™ Universal Nuclease to reduce viscosity from nucleic acids, a rapid “one pot” reduction/alkylation solution for cysteine modification (carbamidomethylation, +57.02), and trypsin/LysC protease mix 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 compatible with TMT and TMTpro labeling or other downstream applications, or are ready for MS analysis.

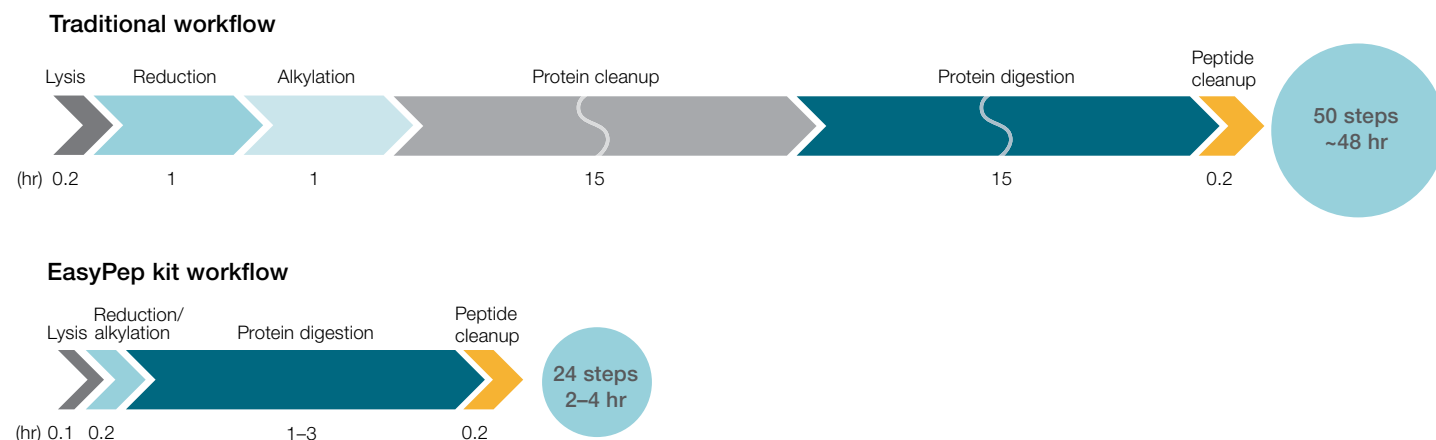


Figure 1. Comparison of sample preparation procedures for MS analysis, using a traditional workflow and EasyPep MS Sample Prep Kits. Compared to a traditional workflow, the EasyPep MS Sample Prep Kits produce high-quality peptides in less time, and with half the number of manipulation steps.



EasyPep MS Sample Prep Kit performance

Compared to other methods and commercial kits, the Thermo Scientific™ EasyPep™ MS Mini Sample Prep Kit produces MS-ready peptides with higher protein yields and more unique protein group identifications (Figure 2). This is due to the high quality of the samples, which have fewer contaminants and higher digestion efficiency compared to traditional overnight digestion with trypsin alone.

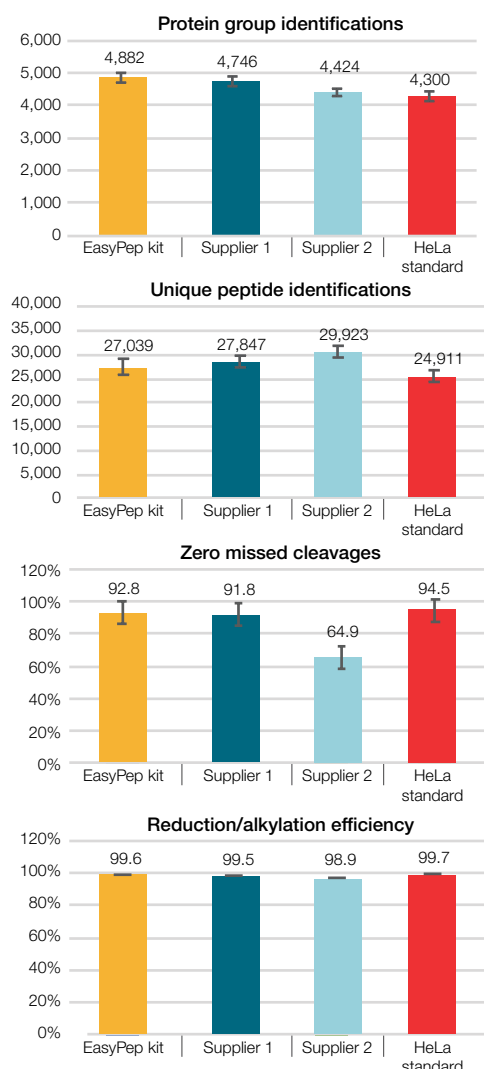


Figure 2. HeLa cell lysates prepared using the EasyPep Mini MS Sample Prep Kit compared to other commercially available kits. The EasyPep Mini MS Sample Prep Kit provides similar reduction and alkylation efficiencies with more protein identifications and better digestion efficiency (higher percentage of zero missed cleavages) than other methods per microgram of sample analyzed. Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (Cat. No. 88328) was used as a control sample.

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 3). The EasyPep mini kit and the Thermo Scientific™ EasyPep™ 96 MS Sample Prep Kit are optimized to efficiently process protein samples of 10–100 µg with 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, 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.

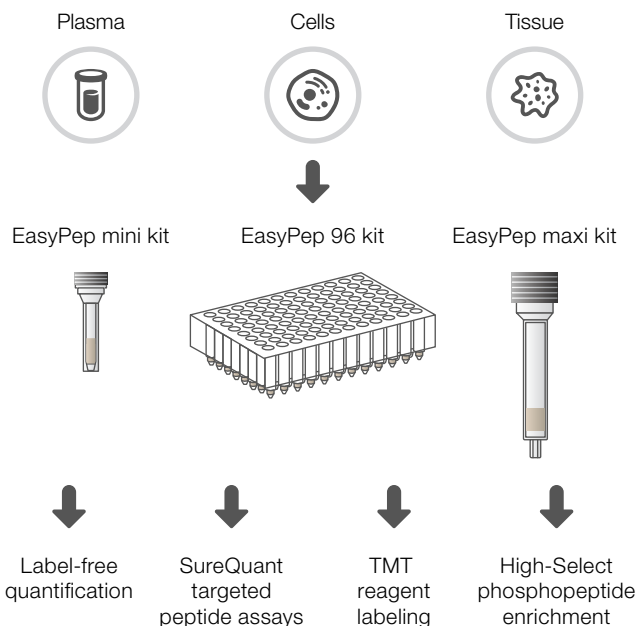


Figure 3. EasyPep MS Sample Prep Kits use the same chemistry and standardized method to process plasma, cells, and tissues for downstream MS-based proteomic applications. Mini, 96-well, and maxi formats can be used to generate samples for label-free quantification, Thermo Scientific™ SureQuant™ targeted quantitation assays, TMT reagent labeling, or phosphopeptide enrichment with Thermo Scientific™ High-Select™ reagents.

All formats of the EasyPep kits use the same chemistry and standardized method to process biological samples to have similar yields, quality, and protein and peptide identifications (Figure 4).

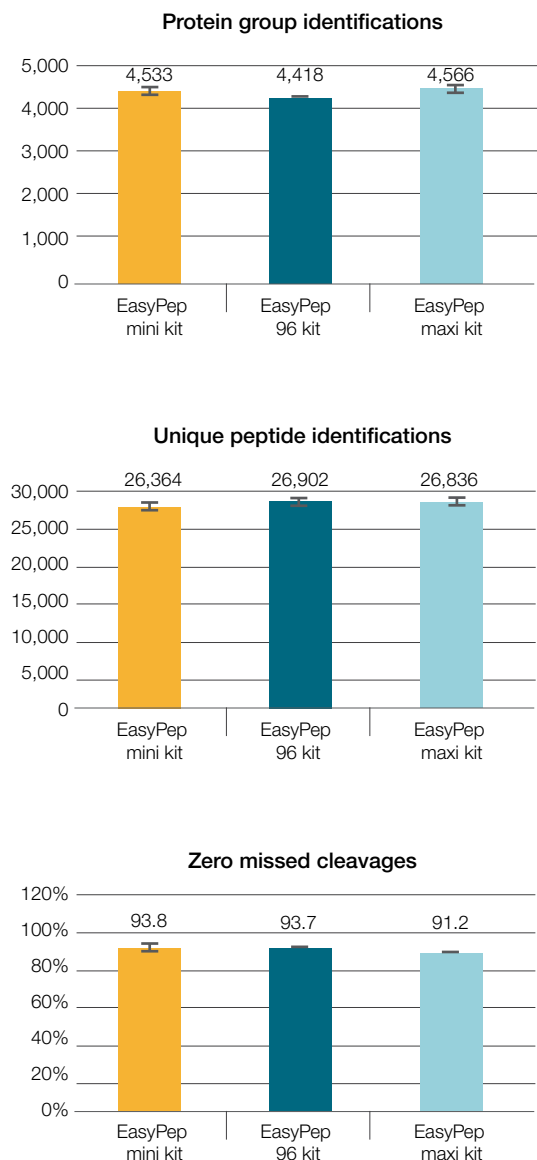


Figure 4. Comparison of the EasyPep Mini, 96, and Maxi MS Sample Prep Kits for processing HeLa cell lysates. All three formats provide comparable peptide and protein identifications with high digestion efficiency (percentage of zero missed cleavages) and good reproducibility.

Sample 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 plate) 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 (Figure 5).

Excess TMT reagents (quenched and hydrolyzed)

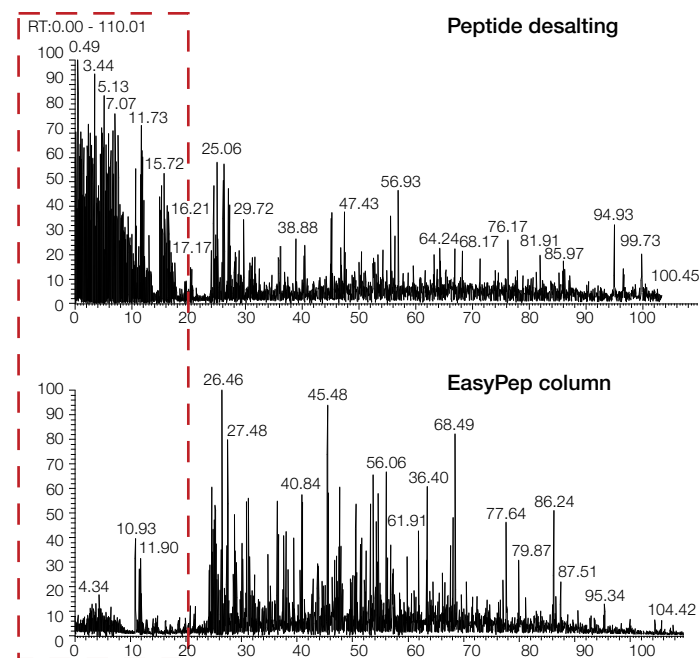


Figure 5. Comparison of LC-MS chromatograms of samples prepared using EasyPep MS Sample Prep Kits and traditional sample prep methods using reversed-phase peptide desalting. Excess, unreacted, and quenched TMT reagents are efficiently removed using EasyPep columns.

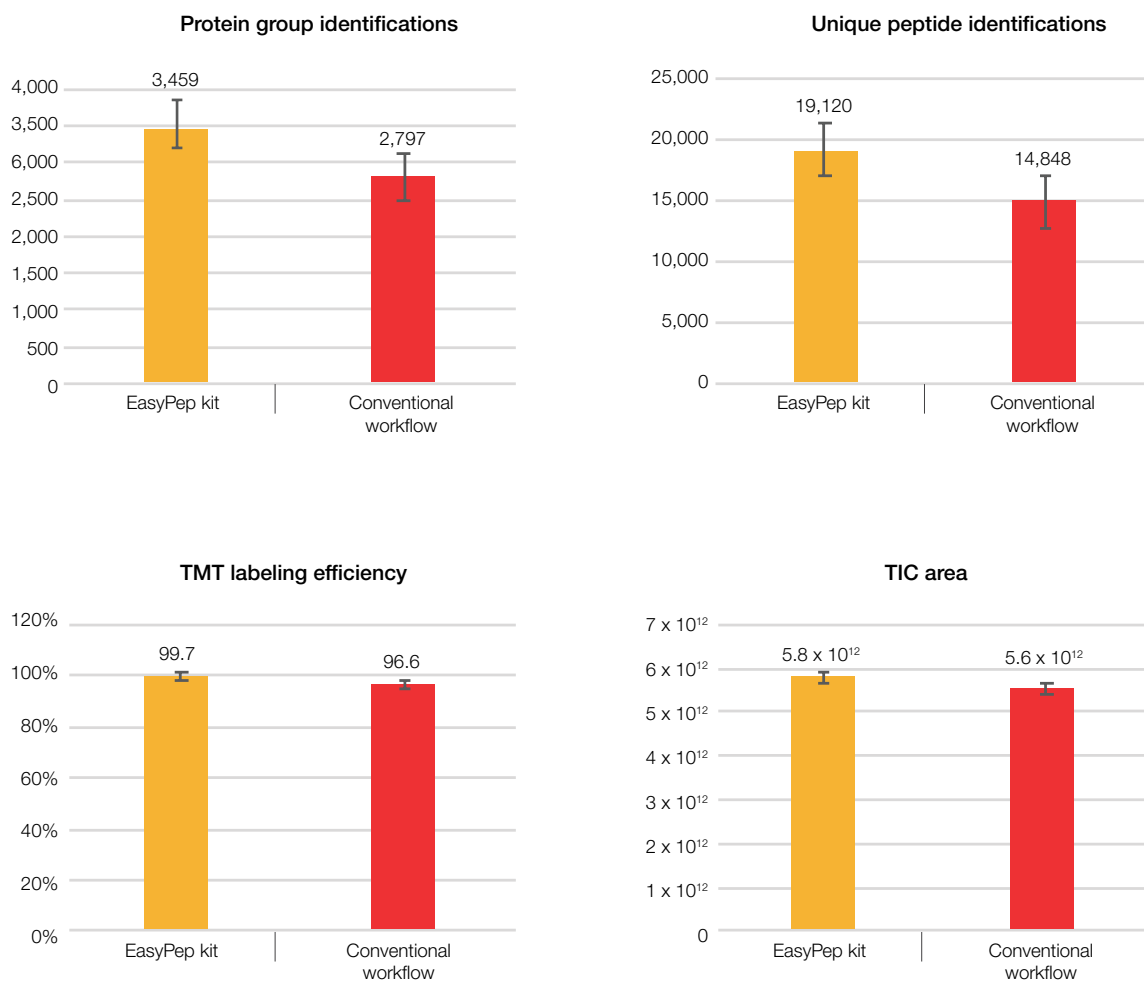


Figure 6. Comparison of the EasyPep and conventional workflows for processing TMT-labeled multiplexed samples. The EasyPep kit resulted in more peptide and protein identifications with high TMT labeling efficiency (N-terminus and lysine). Total ion current (TIC) areas are shown for equal loading of both samples.

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

Protein quantitation

SureQuant Targeted Mass Spec Assay Kits



Validated, modular reagents for multiplexed target protein quantitation

SureQuant kits utilize multiplex immunoprecipitation coupled with mass spectrometry for simultaneous enrichment and quantitation of target proteins (AKT/mTOR signaling pathways featured in Figure 7). Each multiplex panel (Table 1) contains two modules: 1) the IP and MS Sample Preparation Module, which includes all the reagents necessary to immunoenrich target proteins with subsequent in-solution MS sample preparation, and 2) an Absolute or Relative Quantitation Module, including a system suitability standard, and Thermo Scientific™ HeavyPeptide™ and LightPeptide™ AQUA Ultimate mixtures.

Highlights

- **Complete**—includes all reagents for successful monitoring of MS system performance, sample preparation, and quantitation of target proteins and peptides
- **Verified**—antibodies, peptides, and control lysate are rigorously tested for specificity and successful quantitation of each target peptide
- **Multiplex**—able to quantitate different targets from the AKT/mTOR pathway, RAS, and p53 isoforms
- **Flexible**—modular format allows for immunoenrichment only, or in combination with relative or absolute quantitation

The AKT/mTOR signaling pathway serves as a master regulatory pathway for cell growth and proliferation, and plays a vital role in the signaling cascade. Aberrant activation of this pathway promotes survival of tumor cells in many types of cancer. SureQuant kits are ideal for monitoring AKT/mTOR expression levels, isoforms, and phosphorylation status in a single assay. The ability to simultaneously monitor and quantitate in a single sample is a powerful tool for assessment of normal and abnormal cell growth.

The IP and MS Sample Preparation Module enables highly effective antigen IP and co-IP for MS analysis. Cells are first lysed and incubated with an IP-MS-verified biotinylated antibody mixture. The antibody-antigen complex is then captured on MS-compatible streptavidin magnetic beads, and nonspecific proteins are removed through washing with MS-compatible buffers. The target proteins are eluted using trypsin and are compatible with MS sample preparation. The MS sample preparation has been streamlined to be completed in ~4 hours so that the sample can be analyzed by MS on the same day (Figures 8 and 9). The Absolute Quantitation Module provides reagents for absolute quantitation of unique peptides using LC and MS. The HeavyPeptide and LightPeptide AQUA Ultimate mixtures and Thermo Scientific™ Pierce™ 6 Protein Digest are used to generate a standard curve in a matrix.

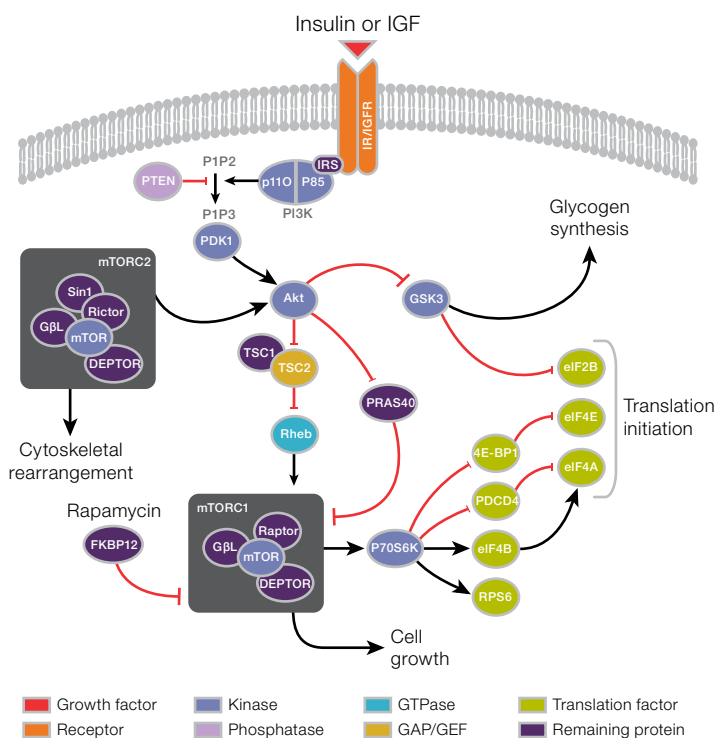


Figure 7. AKT/mTOR signaling pathway.

The HeavyPeptide AQUA Ultimate mixture is provided for use as a spike-in internal standard for relative quantitation in the multiplexed sample.

The Thermo Scientific™ Pierce™ LC-MS/MS System Suitability Standard (7 x 5 mix) is included in this module to assess the dynamic range of LC-MS/MS systems before running the calibration curves and samples (see p. 14).

Together, the SureQuant IP and MS Sample Preparation Module and the SureQuant Absolute or Relative Quantitation Modules provide a complete solution for sample preparation and analysis of the target proteins in the AKT/mTOR pathway (Figure 10).

Table 1. SureQuant Targeted Mass Spec Assay Kit panels.

AKT pathway	AKT1, AKT2, PTEN, IRS1, IGF1R, GSK3a, GSK3b, PRAS40, mTOR, P70S6K, TSC2
AKT pathway (phospho)	pAKT1(pSer473), pAKT2(pSer474), pPTEN(pSer380), pIRS1(pSer312), pIGF1R(pTyr1162, 1163), pGSK3a(pSer21), pGSK2b(pSer9), pPRAS40(pThr246), pmTOR(pSer2448), pP70S6K(pThr389), pTSC2(pSer939)
RAS	KRAS, HRAS, NRAS
TP53	TP53, pTP53(pSer15)
AKT isoform	AKT1, AKT2, pAKT1(pSer473), pAKT2(pSer474)

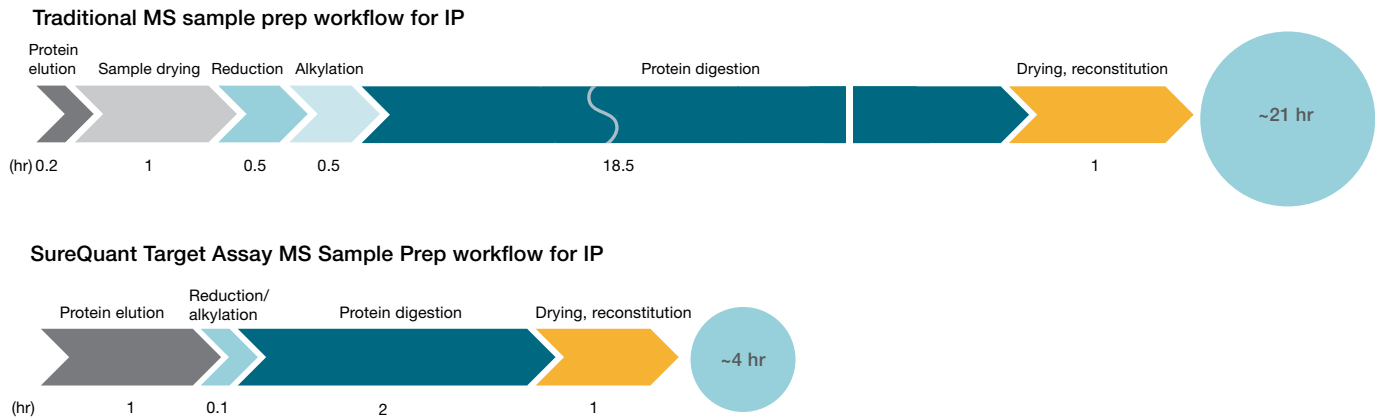


Figure 8. MS sample preparation procedure comparison between SureQuant Targeted Mass Spec Assay Kits and traditional methods following immunoprecipitation.

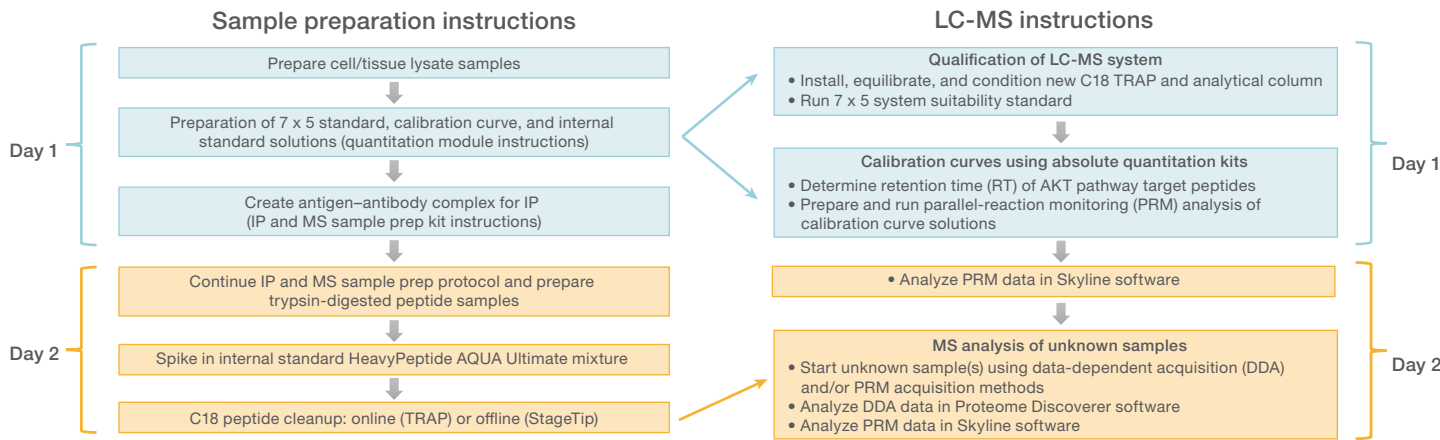


Figure 9. Procedure overview for SureQuant Targeted Mass Spec Assay Kits.

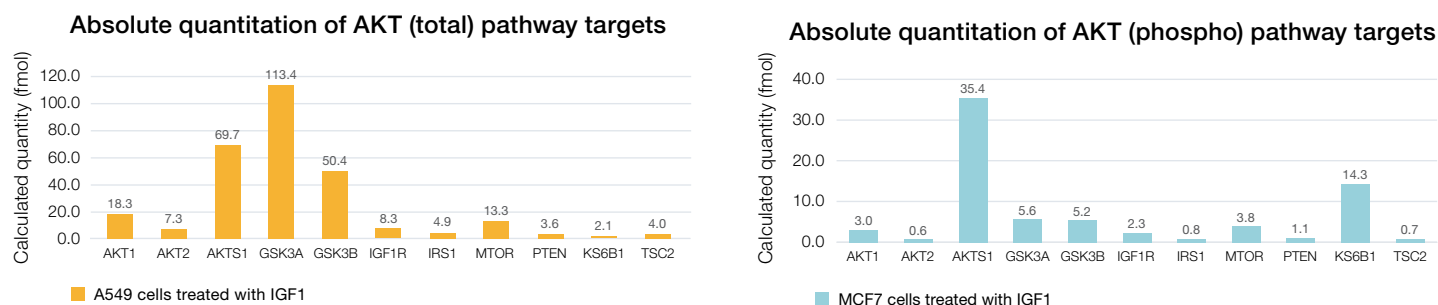


Figure 10. Absolute quantitation of AKT/mTOR signaling pathway proteins using the SureQuant AKT Pathway Multiplex Panel (Absolute Quantitation) and SureQuant AKT Pathway (Phospho) Multiplex Panel (Absolute Quantitation). The panels were run on MCF7 and A549 cells treated with IGF1. AKT/mTOR pathway proteins were enriched through multiplex immunoprecipitation, then analyzed by nanoLC-PRM/MS on a Thermo Scientific™ Q Exactive™ HF-Orbitrap™ mass spectrometer. All targets were quantified using a standard curve generated for each target peptide included in the Quantitation Modules.

Ordering information

Product	Quantity	Cat. No.
SureQuant AKT Pathway Multiplex Panel (Absolute Quantitation)	10 reactions	A40011
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 Absolute Quantitation Module	10 reactions	A40083
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 Panel (Absolute Quantitation)	10 reactions	A40094
SureQuant RAS Isoform Panel (Relative Quantitation)	10 reactions	A40095
SureQuant RAS Isoform IP and MS Sample Preparation Module	10 reactions	A40096
SureQuant RAS Isoform Relative Quantitation Module	10 reactions	A40097
SureQuant RAS Isoform Absolute Quantitation Module	10 reactions	A40098
SureQuant AKT Isoform Panel (Absolute Quantitation)	10 reactions	A40089
SureQuant AKT Isoform Panel (Relative Quantitation)	10 reactions	A40090
SureQuant AKT Isoform IP and MS Sample Preparation Module	10 reactions	A40091
SureQuant AKT Isoform Relative Quantitation Module	10 reactions	A40092
SureQuant AKT Isoform Absolute Quantitation Module	10 reactions	A40093
SureQuant TP53 Panel (Absolute Quantitation)	10 reactions	A40099
SureQuant TP53 Panel (Relative Quantitation)	10 reactions	A40100
SureQuant TP53 Panel IP and MS Sample Preparation Module	10 reactions	A40101
SureQuant TP53 Panel Relative Quantitation Module	10 reactions	A40102
SureQuant TP53 Panel Absolute Quantitation Module	10 reactions	A40103

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

TMTpro 16plex label reagents

Thermo Scientific™ TMTpro™ 16plex label reagents are the next generation of tandem mass tags designed to increase the level of sample multiplexing without compromising on protein identification and quantitation.



Highlights

- **Multiplex**—concurrent MS analysis of up to 16 samples derived from cells, tissues, or biological fluids
- **Robust**—increased multiplex capability results in fewer missing quantitative values among samples and within replicates for better quantitative accuracy and precision
- **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 Thermo Scientific™ Orbitrap™ platforms, including the Orbitrap Eclipse™ Tribrid™ and Orbitrap Exploris™ 480 mass spectrometers with data analysis fully supported by Thermo Scientific™ Proteome Discoverer™ 2.4 software

The original Thermo Scientific™ TMT label reagents were designed to enable multiplexing of up to 11 samples in a single LC-MS analysis without any changes in reagent structure or molecular weight.

The new TMTpro label reagents are similar in design to TMT label reagents in that they are both isobaric and amine-reactive, but TMTpro reagents differ in structure, having a longer spacer region and isobutyl proline mass reporter region (Figure 11). After MS/MS fragmentation, each TMTpro tag generates a unique reporter mass (126–134 Da) in the low-mass region of the high-resolution MS/MS spectrum that is used for relative quantitation of protein expression levels.

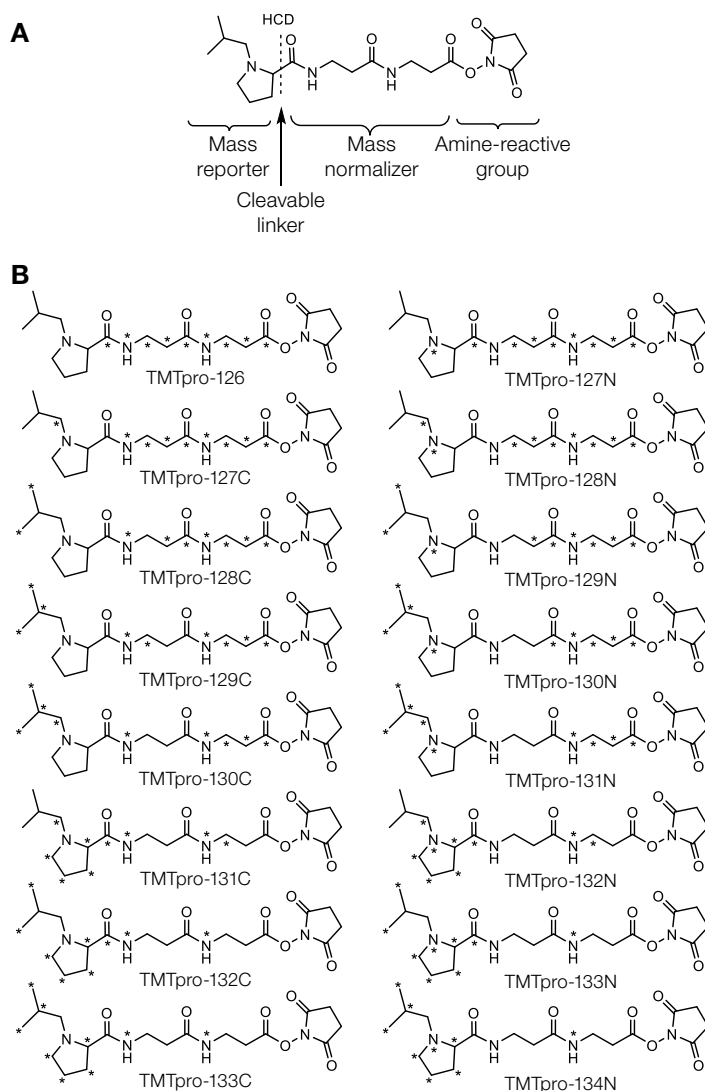


Figure 11. Chemical structure of TMTpro label reagents. (A) Functional regions of the reagent structure, including MS/MS fragmentation site by higher-energy collision dissociation (HCD). **(B)** TMTpro 16plex reagent structures and ¹³C and ¹⁵N stable isotope positions (*).

While TMTpro label reagents have a different chemical structure and are ~20% larger in mass than the TMT reagents, the sample processing and analysis workflow (Figure 12) remains the same, with the added advantage

of increased multiplexed quantitation, increased sample throughput, and fewer missing quantitative values among samples.

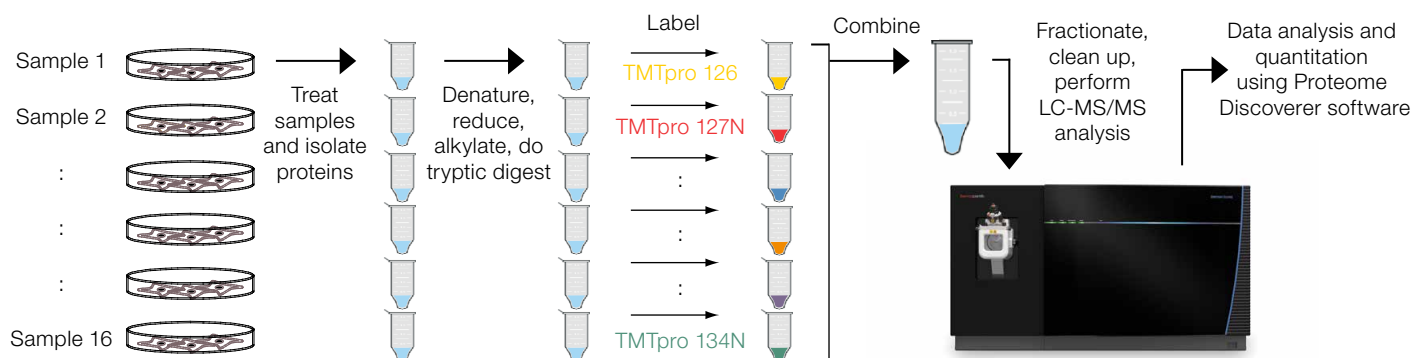


Figure 12. Procedure summary for MS experiments using TMTpro isobaric mass tagging reagents. Protein extracts from cells or tissues are reduced, alkylated, and then digested using the EasyPep Mini MS Sample Prep Kit or an equivalent method. Samples are then labeled with the TMTpro reagents before combining, fractionating, and cleaning up. Labeled samples are analyzed on a high-resolution Orbitrap LC-MS/MS mass spectrometer before data analysis to identify peptides and quantify relative abundance of reporter ions.

New TMTpro 16plex label reagents have the same labeling efficiency and peptide/protein ID rates as TMT reagents (Figures 13 and 14). The TMTpro reagents provide the same level of quantitative precision as the original TMT reagents,

with the benefit of improved quantitative accuracy with larger sample sets. Higher-multiplex tags also have fewer missing values among replicates, resulting in up to 20% more quantifiable proteins in 50% less time (Figure 15).

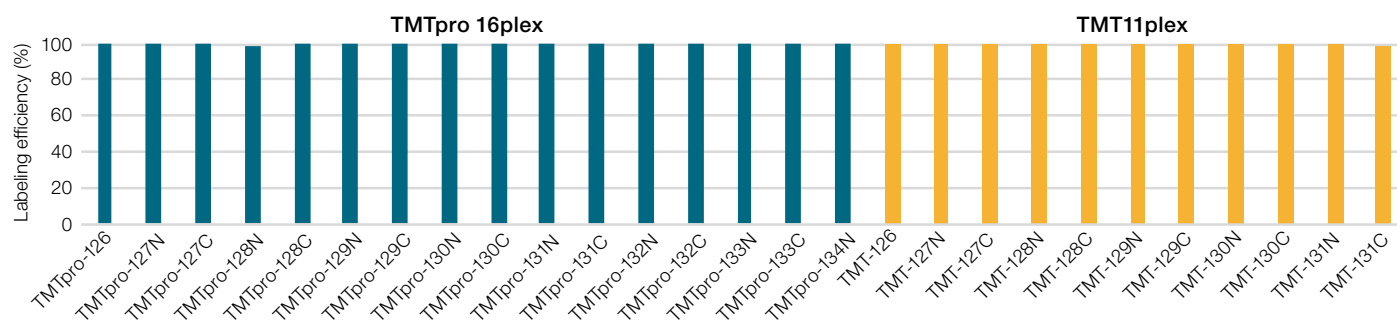


Figure 13. HeLa cell digests were individually labeled with Thermo Scientific™ TMTpro or TMT11plex™ reagents and analyzed by LC-MS to determine labeling efficiency of peptide amino groups (lysine and N termini).

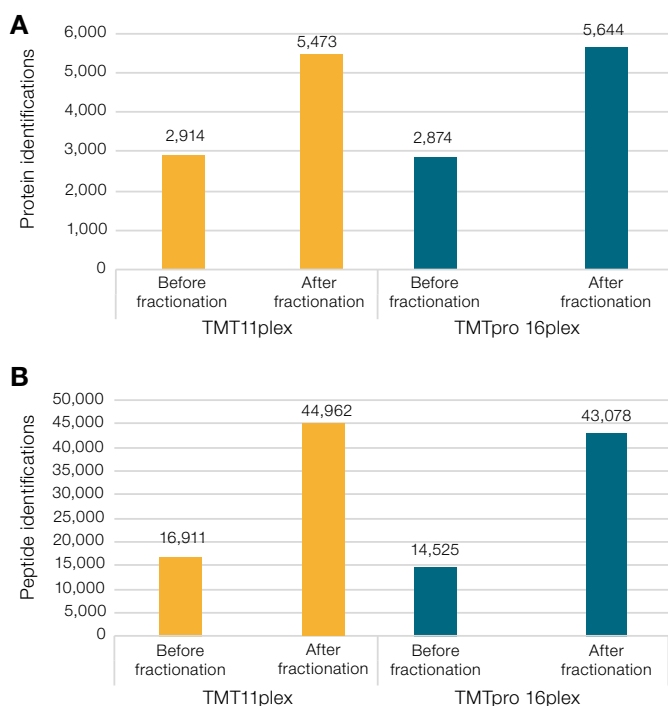


Figure 14. Unique (A) protein and (B) peptide identifications of HeLa cell digest labeled with TMT11plex or TMTpro reagents analyzed by LC-MS before or after high-pH reversed-phase fractionation.

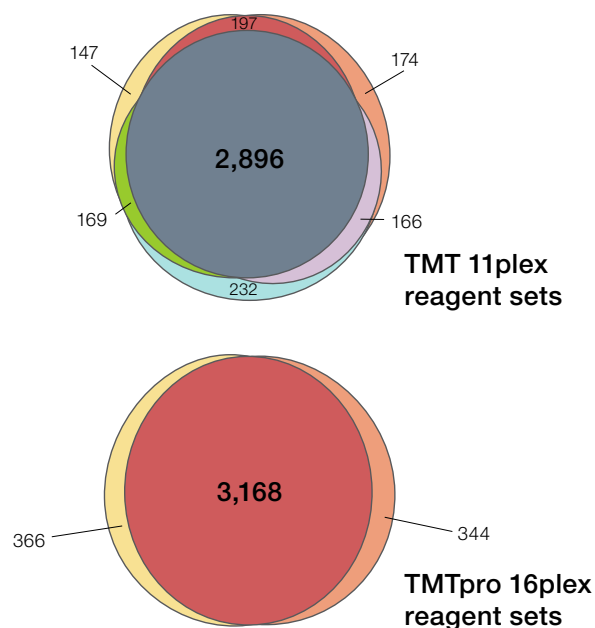


Figure 15. Venn diagrams of overlapping proteins quantified among 30 replicate HeLa cell digests labeled with three TMT11plex reagent sets (top) or two TMTpro 16plex reagent sets (bottom).

Applications

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

Ordering information

Product	Quantity	Cat. No.
TMTpro 16plex Isobaric Label Reagent Set (sufficient for one 16plex isobaric experiment)	1 x 0.5 mg	A44521
TMTpro 16plex Isobaric Label Reagent Set (sufficient for six 16plex isobaric experiments)	6 x 0.5 mg	A44522
TMTpro 16plex Isobaric Label Reagent Set (sufficient for ten 16plex isobaric experiments)	1 x 5 mg	A44520
TMTpro-Zero Isobaric Label Reagent (sufficient for labeling five samples)	5 x 0.5 mg	A44519
TMTpro-Zero Isobaric Label Reagent (sufficient for labeling ten samples)	1 x 5 mg	A44518

Find out more at thermofisher.com/tmtpro

Instrument calibration and QC

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

Convenient standard for scheduling of MS acquisition windows

The Pierce LC-MS/MS System Suitability Standard (7 x 5 mix) contains seven HeavyPeptide AQUA Ultimate peptides (provided at five dilutions) distinguished by differential isotopic labeling to assess sensitivity and dynamic range of LC-MS/MS systems (Figure 16).

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) (Figure 17). Each of the seven peptides has five versions (amino acids labeled with 0, 1, 2, 3, or 4 heavy isotopes), and are provided at five distinct concentrations (Figure 18). The 7 x 5 mix can be used with Thermo Scientific™ Pinpoint™ software (thermofisher.com/pinpoint) to assess dynamic range, linearity, and lower limit of quantitation (LLOQ).

Highlights

- **High quality**—contains five sets of seven HeavyPeptide AQUA Ultimate peptides, where each peptide in each set is distinguishable through 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

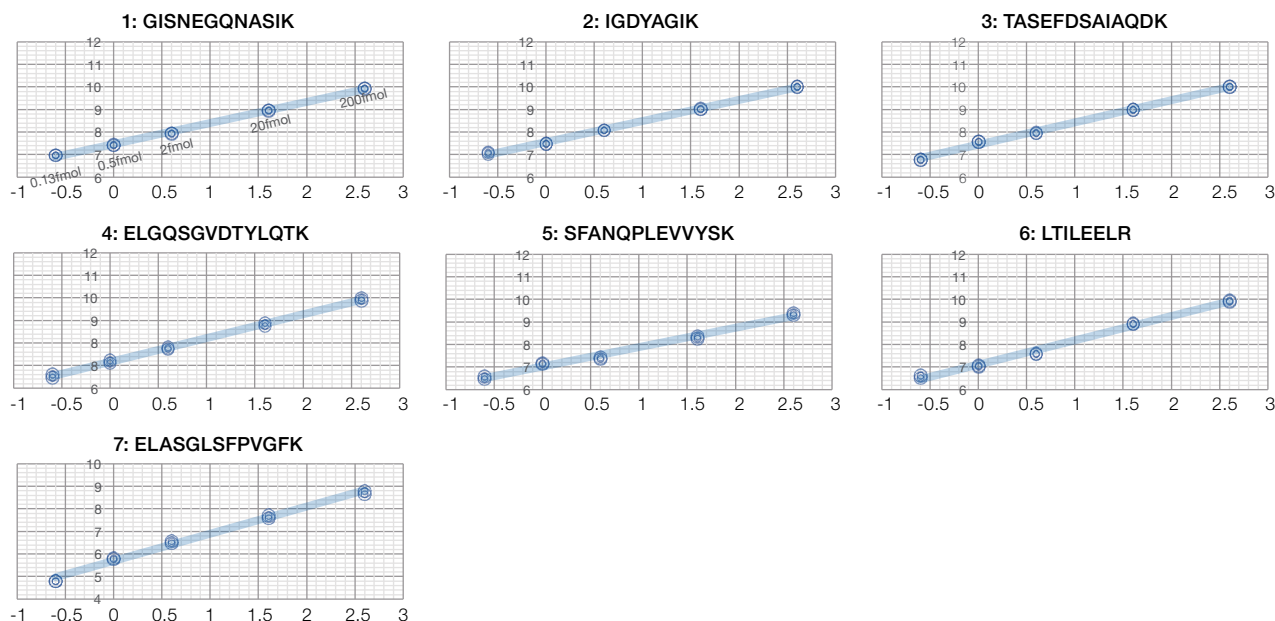
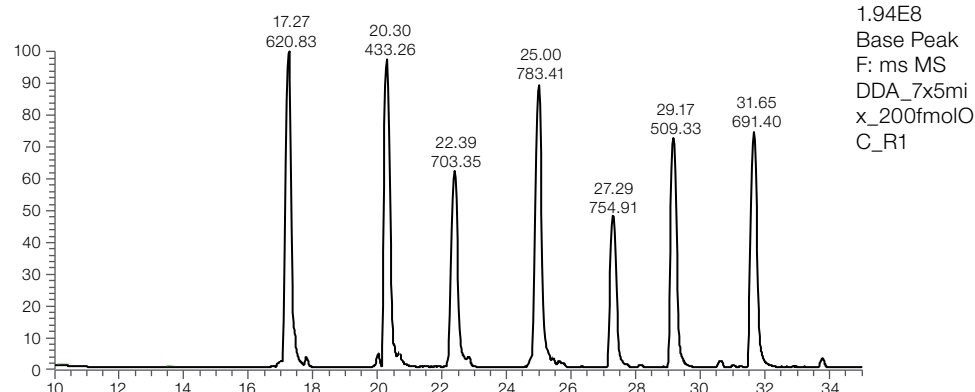


Figure 16. Calibration curves generated for the Pierce LC-MS/MS Suitability Standard (7 x 5 mix) demonstrate >3 orders of magnitude of dynamic range and linearity ($R^2 > 0.99$).

RT: 10.00 - 35.00 SM: 7B



NL:
1.94E8
Base Peak
F: ms MS
DDA_7x5mi
x_200fmolO
C_R1

Figure 17. 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™ Column (Cat. No. ES800) using 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.

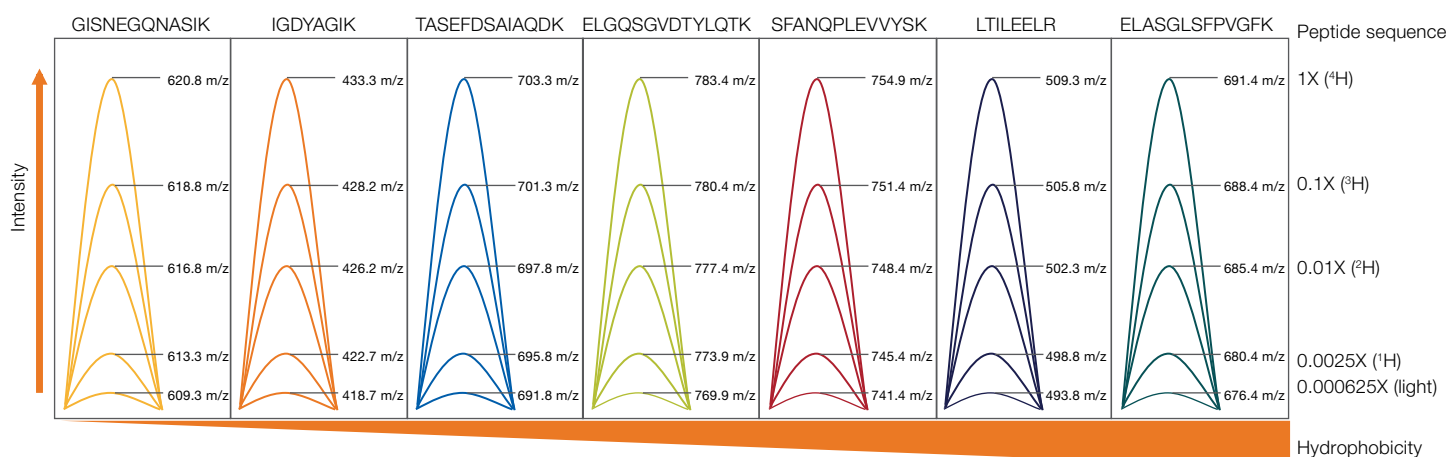


Figure 18. Composition of Pierce LC-MS/MS System Suitability Standard (7 x 5 mix).

Reference

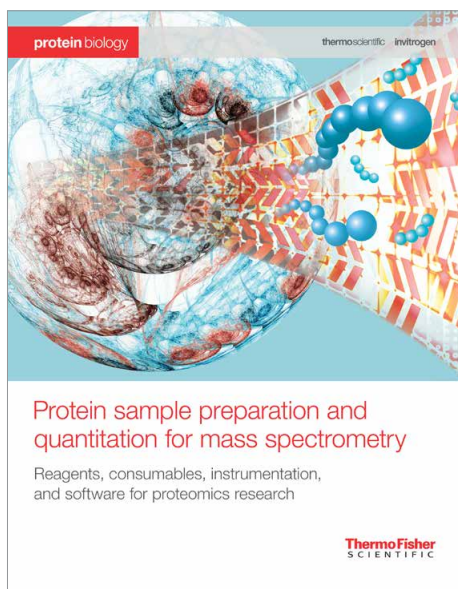
Krokhin OV, Spicer V (2009) Peptide retention standards and hydrophobicity indexes in reversed-phase high-performance liquid chromatography of peptides. *Anal Chem* 81(22):9522-9530.

Ordering information

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

Find out more at thermofisher.com/ms-standards

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.

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