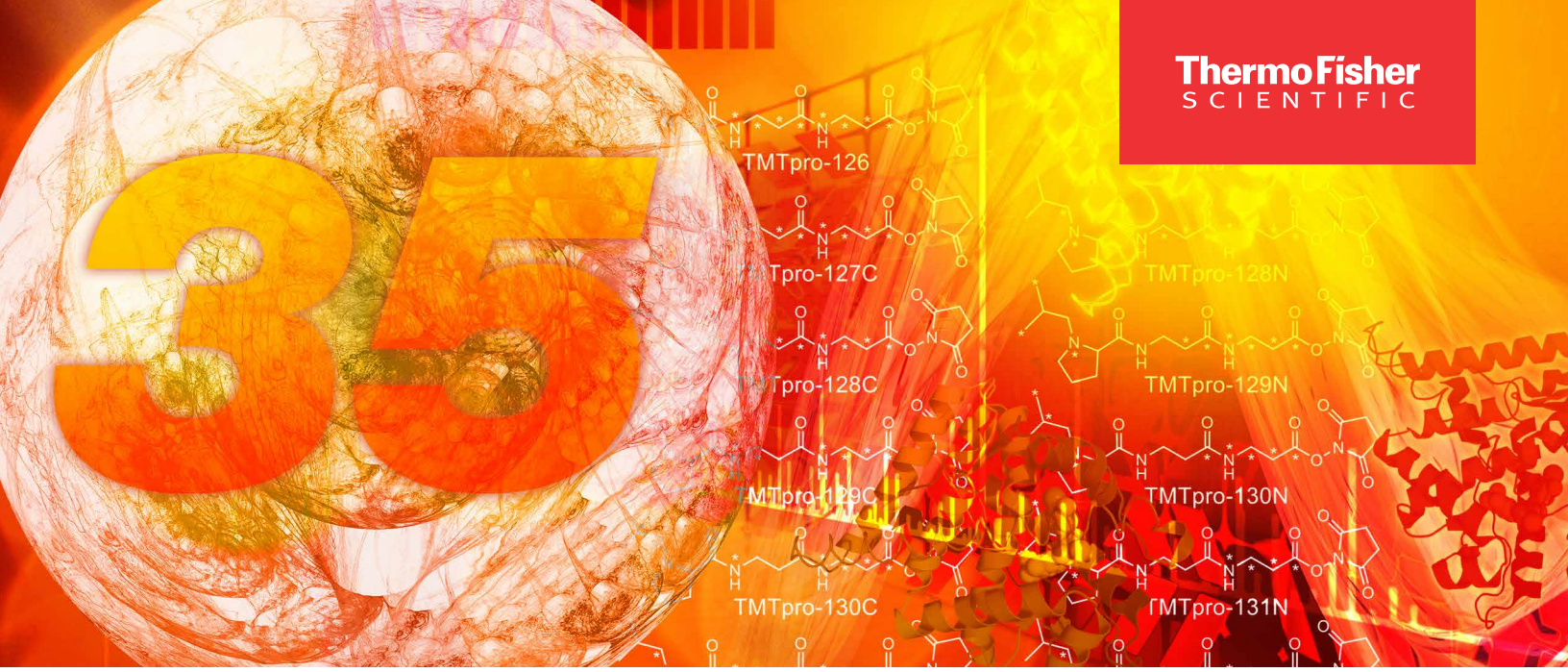


Mass spectrometry

TMTpro Label Reagents—higher multiplex quantitation for up to 35 samples

High throughput and exceptional quantitative accuracy for multiplexed proteome analysis

Thermo Scientific™ TMTpro™ 35plex label reagents are our latest evolution of tandem mass tags designed to greatly expand the level of sample multiplexing without compromising on protein identification and quantification.

Highlights

- **Multiplex**—concurrent MS analysis of up to 35 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 help 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 Ascend Tribrid™,Orbitrap Eclipse™ Tribrid™ and Orbitrap Exploris™ 480 mass spectrometers with data analysis fully supported by Thermo Scientific™ Proteome Discoverer™ 3.2 software

The original Thermo Scientific™ Tandem Mass Tags™ (TMT®) are isobaric, amine-reactive mass tagging reagents that enable

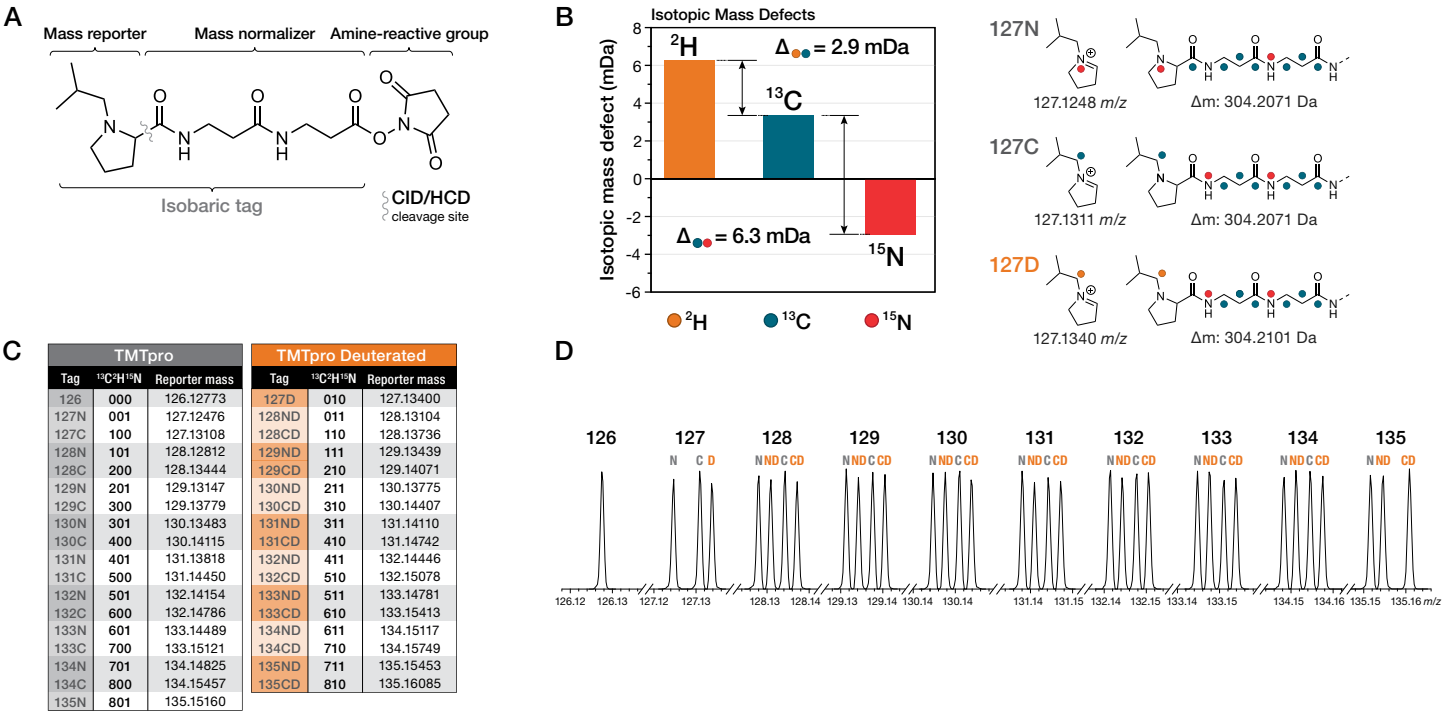
multiplexing and relative quantitative analysis of up to 11 samples in a single LC-MS/MS acquisition.

The next-generation Thermo Scientific™ TMTpro reagents increased multiplexing capability to 18 samples through a redesigned tag structure with a larger mass balancer and isobutyl proline reporter group (Figure 1A). Upon MS/MS fragmentation, each TMTpro tag generates a distinct reporter ion in the low-mass region (126–135 Da) of high-resolution MS/MS spectra that is used for relative quantification of protein expression levels.

To further increase multiplexing capability without changing the reagent structure, our latest evolution of the TMTpro reagent includes the development of an additional isobaric set of 17 isotopologues that incorporate a single ^{2}H isotope on the reporter group to yield distinct reporter ion masses that differ from the

existing set by 3 mDa (Figure 1B–C) that can be distinguished in HCD MS/MS spectra acquired in the Orbitrap mass analyzer at 90,000 resolving power (Figure 1D). In combination with the traditional reagent set, the deuterated reagents enable multiplexed quantitative analysis of up to 35 samples on Thermo Scientific™ Orbitrap platforms. Because the reagent structure of the expanded TMTpro set is unchanged, sample processing and analysis workflow (Figure 2) remains the same with the added advantage of increased multiplexed quantitation, increased sample throughput, and fewer missing quantitative values among samples.

With our latest development in the TMTpro label reagents, different combinations of products can yield multiplexing of 32, 34, or 35 samples.



C

TMTpro			TMTpro Deuterated		
Tag	$^{13}C^{2}H^{15}N$	Reporter mass	Tag	$^{13}C^{2}H^{15}N$	Reporter mass
126	000	126.12773	127D	010	127.13400
127N	001	127.12476	128ND	011	128.13104
127C	100	127.13108	128CD	110	128.13736
128N	101	128.12812	129ND	111	129.13439
128C	200	128.13444	129CD	210	129.14071
129N	201	129.13147	130ND	211	130.13775
129C	300	129.13779	130CD	310	130.14407
130N	301	130.13483	131ND	311	131.14110
130C	400	130.14115	131CD	410	131.14742
131N	401	131.13818	132ND	411	132.14446
131C	500	131.14450	132CD	510	132.15078
132N	501	132.14154	133ND	511	133.14781
132C	600	132.14786	133CD	610	133.15413
133N	601	133.14489	134ND	611	134.15117
133C	700	133.15121	134CD	710	134.15749
134N	701	134.14825	135ND	711	135.15453
134C	800	134.15457	135CD	810	135.16085
135N	801	135.15160			

Figure 1. Chemical structure of the TMTpro reagent. (A) Functional regions of the reagent structure, including MS/MS fragmentation site by higher-energy collision dissociation (HCD). (B) Exchanging ^{2}H, ^{13}C, and ^{15}N isotopes imparts milliDalton mass differences between isotopologues. Incorporation of a single deuterium (^{2}H) isotope onto the TMTpro reporter group yields a mass difference of 3 mDa. (C) The TMTpro 35plex reagent set consists of the traditional 18plex set of TMTpro reagents combined with the 17plex set of TMTpro deuterated reagents. (D) Upon MS/MS fragmentation, 35 distinct reporter ions are generated in HCD MS/MS spectra acquired in the Orbitrap mass analyzer at 90,000 resolving power. The color-coded peak annotations designate the deuterated reporters interleaved between the nondeuterated reporters.

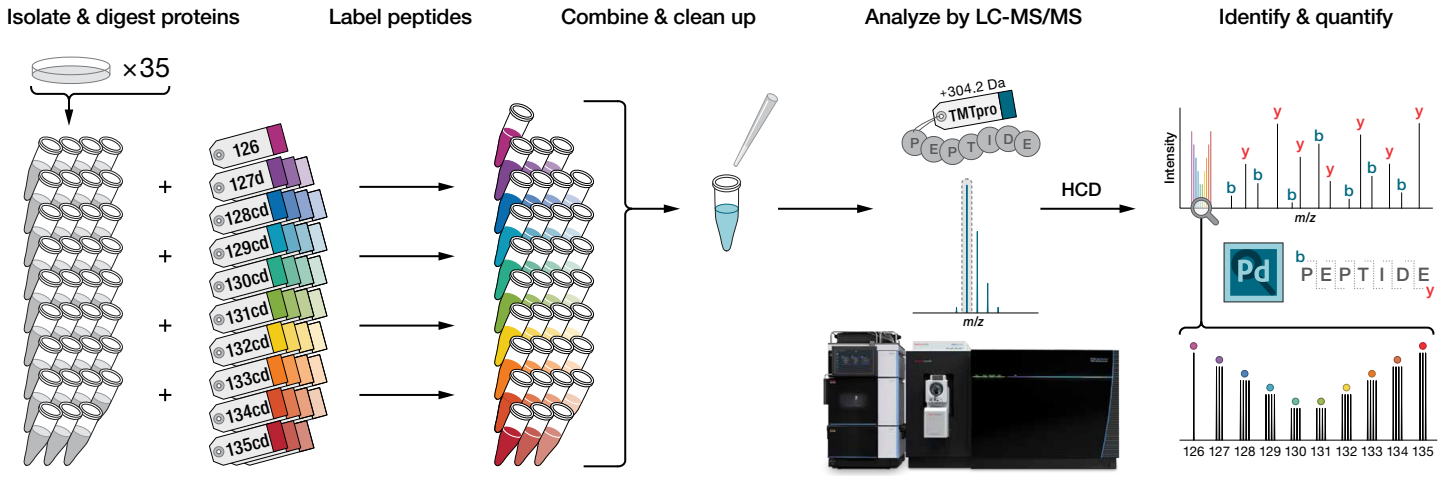


Figure 2. Procedure summary for LC-MS experiments using TMTpro 35plex reagents. Protein extracts from cells or tissues are reduced, alkylated, and digested using the EasyPep™ Mini MS Sample Prep Kit or an equivalent method. Samples are then labeled with TMTpro reagents before combining and cleaning up. Labeled samples are analyzed using a high-resolution Orbitrap LC-MS/MS system and data is processed to identify peptides and quantify relative sample abundances using reporter ion signal intensities.

Getting the most from TMTpro reagents with Real Time Search SPS-MS3 acquisition on the Orbitrap Tribrid platform

Due to the additional overhead of the SPS-MS3 scan cycle, the higher resolving power required for TMTpro 35plex reagents can be used without reducing acquisition speed. A 32plex experiment acquired using a 90K MS3 scan with real time search yields the same number of quantified proteins as an 18plex experiment acquired using a TurboTMT 30K MS3 scan, but with twice the sample throughput. Analyzing 32 samples across two 18plex experiments yields fewer fully quantified proteins due to missing values between runs—the single 32plex experiment yields 15% more identified proteins, and the greater number of identified peptides can support higher confidence of protein identification and quantification (Figure 3). When using control channels to normalize quantitative data or by quantifying nondeuterated and deuterated sets as separate sub-plexes, quantitative metrics are equivalent between 16plex and 32plex reagents (Figure 4).

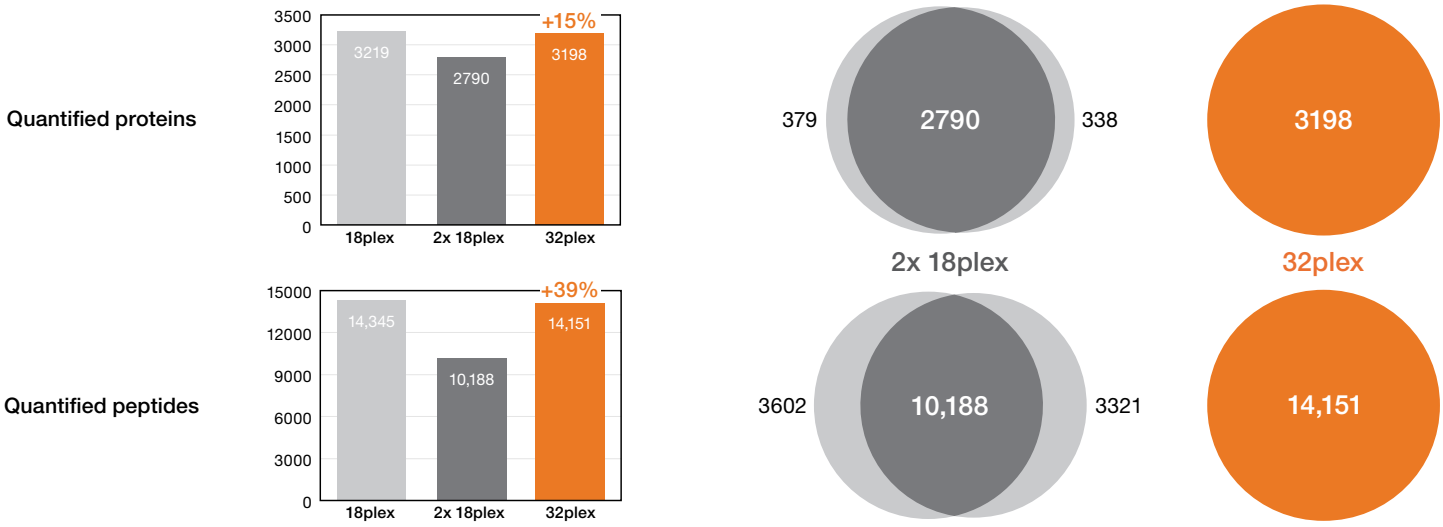


Figure 3. TMTpro 32plex vs. two 18plex bridged LC-MS experiments. HeLa digest samples labeled with TMTpro 32plex & 18plex reagents were analyzed using RTS SPS-MS3 acquisition methods at RP 90K and TurboTMT 30K, respectively. The 32plex experiment yields greater protein & peptide identifications than two 18plex experiments due to missing values between two LC-MS runs.

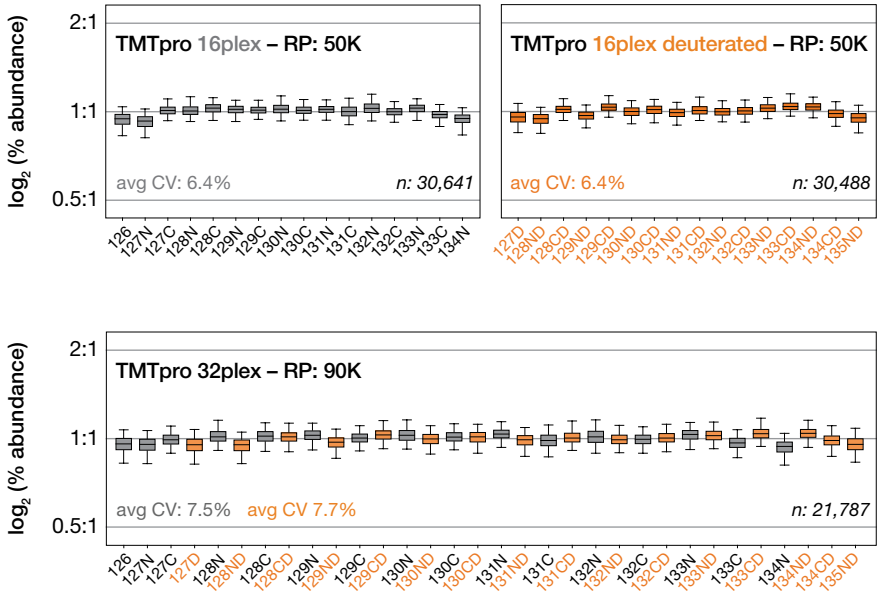


Figure 4. TMTpro 32plex vs. 16plex quantification. HeLa digest samples labeled with TMTpro 16plex, 16plex deuterated, and 32plex reagents show that the three multiplex sets achieve equivalent quantitative performance for MS2 acquisition at RP 50K & 90K. The % abundance was calculated separately for the deuterated and non-deuterated sets by dividing the abundance of each channel by the sum of abundances for that set. Isotopic interference correction not applied.

Applications

- Protein identification and quantification from multiple samples of cells, tissues, or biological fluids
- Protein expression profiling of normal vs. abnormal states, control vs. treated cells
- Quantitative analysis of proteins for which no antibodies are available
- Identification and quantification of membrane and post-translationally modified proteins
- Identification and quantification of hundreds to thousands of proteins in a single experimental workflow
- Activity-based protein profiling (ABPP), single-cell barcoding, and thermal proteome profiling (TPP)

Ordering information

Product	Quantity	Cat. No
Sample preparation		
EasyPep 96 MS Sample Prep Kit	96 reactions	A45733
EasyPep 96 Micro MS Sample Prep Kit	96 reactions	A57864
EasyPep Magnetic MS Sample Prep Kit	20 reactions	A57866
EasyPep Magnetic MS Sample Prep Kit	96 reactions	A57867
Pierce Trypsin/Lys-C Mix, MS grade	5 x 20 µg	A41007
Pierce Trypsin/Lys-C Mix, MS grade	100 µg	A40009
Pierce High pH Reversed-Phase Fractionation Kit	1 kit	84868
High-Select Fe-NTA Phosphoenrichment Kit	1 kit	A32992
High-Select TiO_2 Phosphoenrichment Kit	1 kit	A32993
High-Select HSA/Immunoglobulin Depletion Mini Spin Columns	24 columns	A36366
High-Select Top14 Abundant Protein Depletion Mini Spin Columns	24 columns	A36370
Protein quantification		
TMTpro 32plex Label Reagent Matched Set	1 x 5 mg (per tag)	A40000839
TMTpro 16plex Deuterated Label Reagent Set	1 x 5 mg (per tag)	A40000817
TMTpro-134C and TMTpro-135CD Label Reagents	1 x 5 mg (per tag)	A40000853
TMTpro-135CD Label Reagent	1 x 5 mg (per tag)	A40000818
TMTpro 16plex Label Reagents in stabilized solution, 96-well plate	6 x 50 µg (per tag)	A58334
TMTpro 18plex Label Reagents in stabilized solution, 96-well plate	4 x 50 µg (per tag)	A58335
TMTpro 16plex Isobaric Label Reagent Set	1 x 5 mg (per tag)	A44520
TMTpro 18plex Isobaric Label Reagent Set	1 x 5 mg (per tag)	A52045
Standards		
Pierce TMT11plex Yeast Digest Standard	20 µg	A40938
Pierce TMT11plex Yeast Digest Standard	5 x 20 µg	A40939

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