

## TMTpro Label Reagents—higher multiplex quantitation for up to 16 samples

Next generation of TMT labeling reagents for higher throughput and greater quantitative accuracy for multiplexed proteome analysis

# TMTpro 16plex Label Reagents

## Next-generation TMT reagent set enabling 16plex sample multiplexing

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.



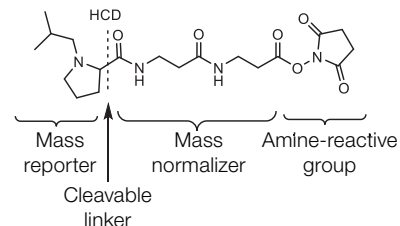
### Highlights

- **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 new Orbitrap Eclipse™ Tribrid™ and Orbitrap Exploris™ 480 mass spectrometers with data analysis fully supported by Thermo Scientific™ Proteome Discoverer™ 2.4 software

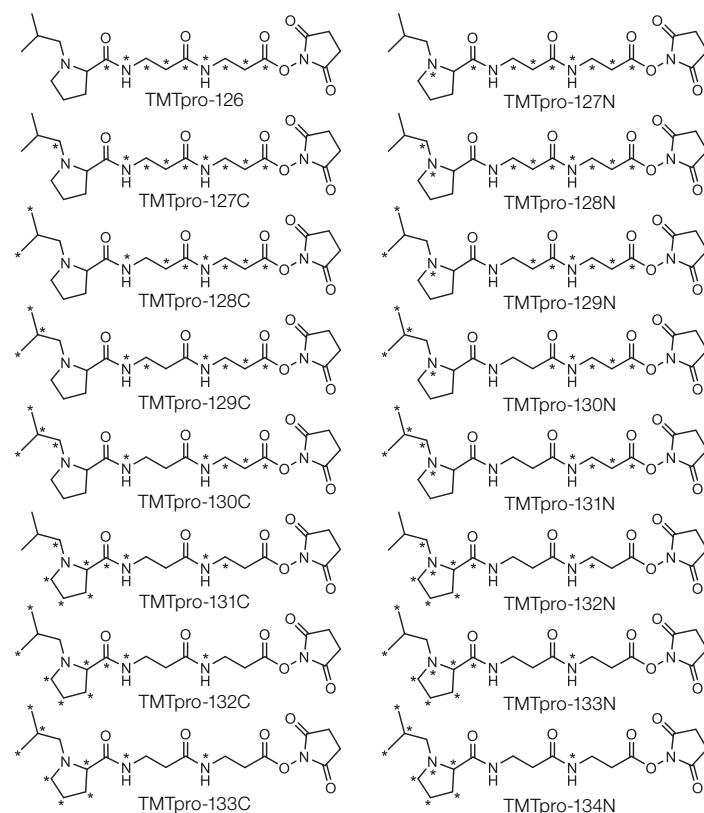
The original Thermo Scientific™ Tandem Mass Tags (TMT) were designed to enable multiplexing of up to 11 samples in a single LC-MS analysis without any changes in reagent structure or molecular weight.

The new TMTpro tag is similar in design to TMT as it is isobaric and amine reactive, but differs in structure with a longer spacer region and isobutyl proline mass reporter region (Figure 1). 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.

**A**

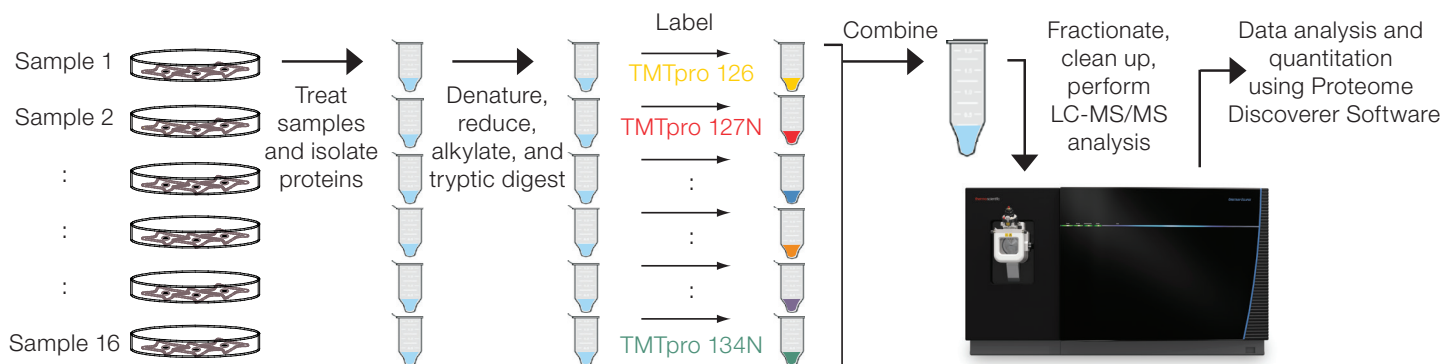


**B**



**Figure 1. Chemical structure of the Thermo Scientific 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 <sup>13</sup>C and <sup>15</sup>N stable isotope positions (\*).

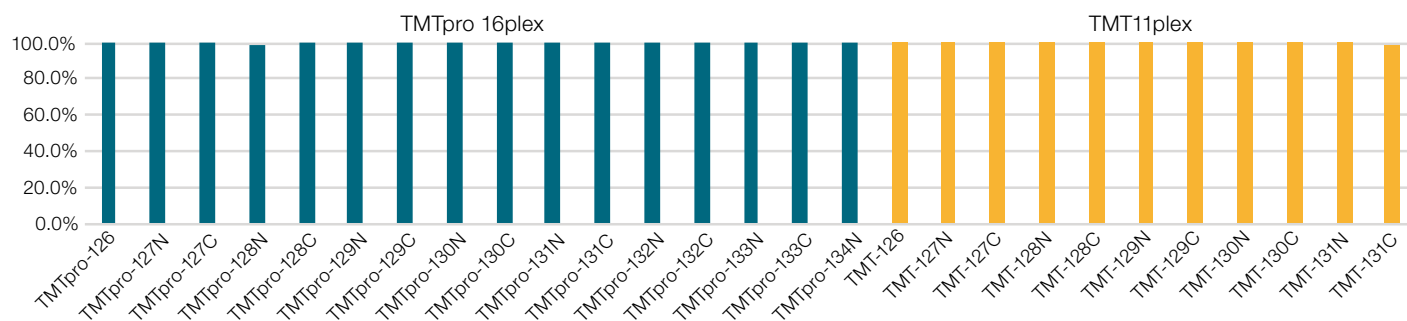
While Thermo Scientific TMTpro label reagents have a different chemical structure and are ~20% larger in mass than Thermo Scientific TMT reagents, the sample processing and analysis workflow (Figure 2) remains the same, with the added advantage of increased multiplexed quantitation, increased sample throughput, and fewer missing quantitative values among samples.



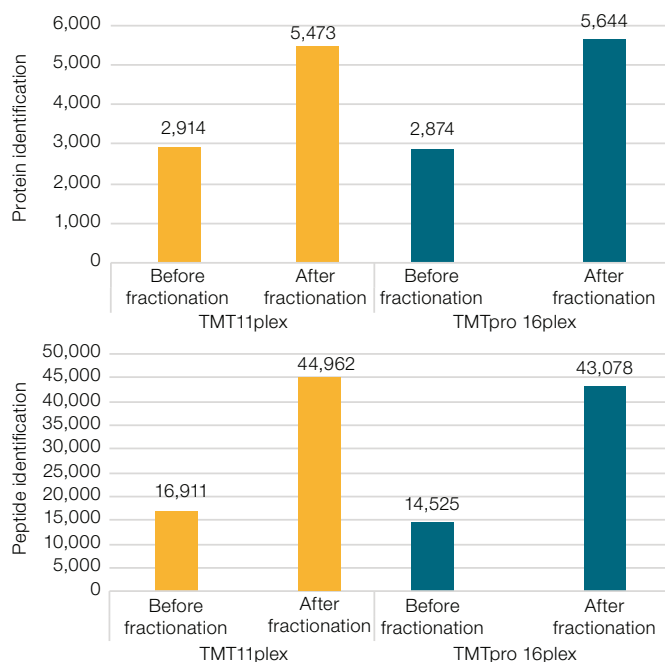
**Figure 2. Procedure summary for MS experiments using TMTpro isobaric mass tagging reagents.** Protein extracts from cells or tissues are reduced, alkylated, and then digested using the EasyPep™ Mini MS Sample Prep Kit or an equivalent method. Samples are then labeled with the TMTpro reagents before 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 3 and 4). The TMTpro reagents provide the same level of quantitative precision as the original TMT reagents,

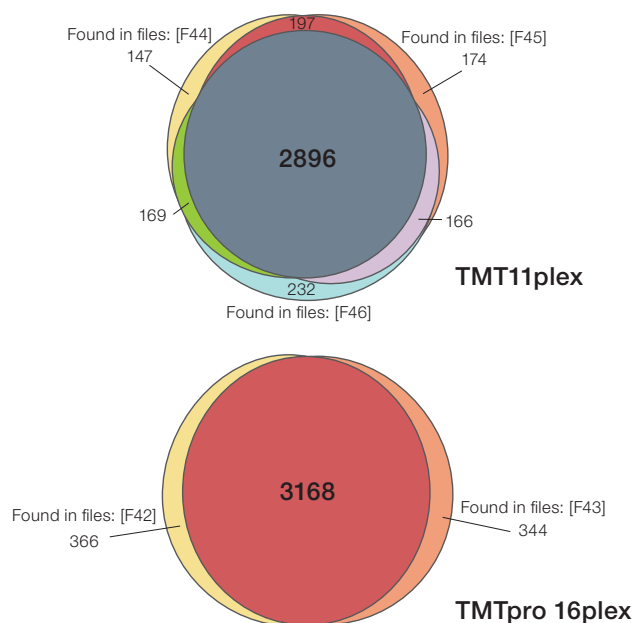
with the benefit of improved quantitative accuracy with larger sample sets. Higher-multiplex tags also have fewer missing values among replicates, resulting in up to 20% more quantifiable proteins in 50% less time (Figure 5).



**Figure 3. 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 4. Unique protein and 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 5. Venn diagrams of overlapping proteins quantified among 30 replicate HeLa cell digests labeled with three TMT11plex reagent sets or two TMTpro 16plex reagent sets.**

## 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.
<b>Sample preparation</b>		
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
Pierce Trypsin/Lys-C Mix, MS grade	20 µg	A40007
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 <sub>2</sub> Phosphoenrichment Kit	1 kit	A32993
High-Select HSA/Immunoglobulin Depletion Mini Spin Columns	6 columns	A36366
High-Select Top14 Abundant Protein Depletion Mini Spin Columns	6 columns	A36370
<b>Protein quantitation</b>		
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
<b>Standards</b>		
Pierce TMT11plex Yeast Digest Standard	20 µg	A40938
Pierce TMT11plex Yeast Digest Standard	5 x 20 µg	A40939

Find out more at [thermofisher.com/tmtpro](http://thermofisher.com/tmtpro)