TMTpro Mass Tag Labeling Reagents and Kits

Catalog Numbers A44518, A44519, A44520, A44521, A44522, A52045, A52046

Doc. Part No. 2162734 Pub. No. MAN0018773 Rev. D.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ TMTpro Mass Tag Labeling Reagents and Kits enable multiplex relative quantitation by mass spectrometry (MS). Like other isobaric mass-tagging reagents, each reagent within a set has the same nominal mass (i.e., isobaric) and chemical structure composed of an amine-reactive NHS-ester group, a spacer arm and a mass reporter (see Figure 1). The reagent set can be used to label up to 18 different peptide samples prepared from cells or tissues. For each sample, a unique reporter mass (i.e., 126-135Da) in the low mass region of the MS/MS spectrum is used to measure relative protein expression levels during peptide fragmentation (see “Data acquisition methods” on page 3).

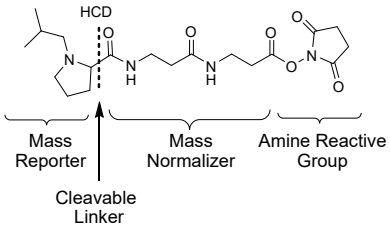


Figure 1 Functional regions of the reagent structure including MS/MS fragmentation site by higher-energy collision dissociation (HCD)

The Thermo Scientific™ TMTpro Label Reagents have a different chemical structure and are ~20% larger in mass than the Thermo Scientific™ TMT™ Label Reagents. The TMTpro Reagent structure has a longer linker region and a proline-based reporter containing different numbers and combinations of nine stable ^{13}C and ^{15}N isotopes to support higher multiplexing than TMT Reagents. Advantages of the TMTpro Label Reagents include increased sample multiplexing for relative quantitation, increased sample throughput, and fewer missing quantitative channels among samples.

Contents and storage

Table 1 TMTpro Isobaric Label Reagents

Product	Amount	Cat. No.	Storage
TMTpro 18plex Isobaric Label Reagent Set[1]	1 × 5 mg per vial (sufficient reagents for ten 18plex isobaric experiments)	A52045	–20°C
TMTpro-134C & TMTpro-135N Label Reagents	1 × 5 mg per vial (sufficient reagents for labeling ten samples)	A52046	–20°C
TMTpro 16plex Isobaric Label Reagent Set[2]	1 × 0.5 mg per vial (sufficient reagents for one 16plex isobaric experiment)	A44521	–20°C
	6 × 0.5 mg per vial (sufficient reagents for six 16plex isobaric experiments)	A44522	
	1 × 5 mg per vial (sufficient reagents for ten 16plex isobaric experiments)	A44520	

Product	Amount	Cat. No.	Storage
TMTpro Zero	5 × 0.5 mg per vial (sufficient reagents for labeling five samples)	A44519	–20°C
	1 × 5 mg per vial (sufficient reagents for labeling ten samples)	A44518	

[1] A total of 18 vials: 1 each of TMTpro-126, TMTpro-127N, TMTpro-127C, TMTpro-128N, TMTpro-128C, TMTpro-129N, TMTpro-129C, TMTpro-130N, TMTpro-130C, TMTpro-131N, TMTpro-131C, TMTpro-132N, TMTpro-132C, TMTpro-133N, TMTpro-133C, TMTpro-134N, TMTpro-134C, TMTpro-135N Label Reagent (see Table 2).

[2] A total of 16 vials: 1 each of TMTpro-126, TMTpro-127N, TMTpro-127C, TMTpro-128N, TMTpro-128C, TMTpro-129N, TMTpro-129C, TMTpro-130N, TMTpro-130C, TMTpro-131N, TMTpro-131C, TMTpro-132N, TMTpro-132C, TMTpro-133N, TMTpro-133C, TMTpro-134N Label Reagent (see Table 2).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
For TMTpro Reagent labeling:	
Low Protein Binding Microcentrifuge tubes	90410
Anhydrous acetonitrile (Acetonitrile, LC-MS Grade)	51101
50% Hydroxylamine	90115
For peptide preparation:	
EasyPep™ Mini MS Sample Prep Kit	A40006
For protein digest quantification:	
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275
For labeled peptide clean up, one of the following:	
Pierce™ Peptide Desalting Spin Columns	89852
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	84868
For LC-MS analysis:	
EASY-Spray™ HPLC Columns (2 µm particle, 50 µm x 150 mm)	ES901
EASY-nLC™ 1200 System	LC140
Orbitrap Eclipse™ Tribrid™ Mass Spectrometer	FSN04-10000

Workflow

Protein extracts isolated from cells or tissues are reduced, alkylated, and digested. Samples are labeled with the TMTpro Reagents and then mixed before sample fractionation and clean-up. Labeled samples are analyzed by high resolution Orbitrap LC-MS/MS before data analysis to identify peptides and quantify reporter ion relative abundances (see Figure 2).

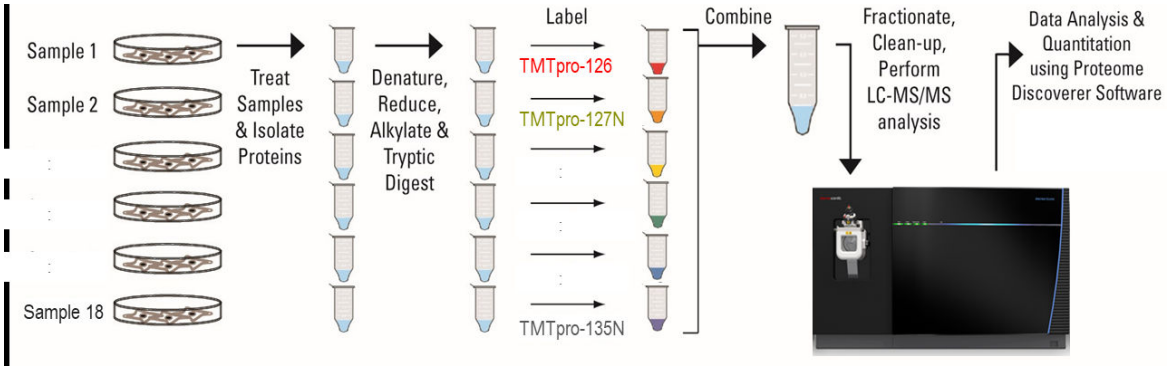


Figure 2 Procedure schematic for using TMTpro 18plex Label Reagents

Procedural guidelines

- The TMTpro reagents are highly moisture-sensitive. To avoid moisture condensation onto the product, the reagents must be equilibrated to room temperature before opening. Store unused reagent in foil pouch with desiccant at –20°C.
- The TMTpro reagents are amine-reactive and modify lysine residues and peptide N-termini. All amine-containing buffers and additives must be removed before digestion and labeling.
- The TMTpro Zero Label Reagent (Product No. A44518 or A44519) can be used to optimize methods before multiplexed analysis of samples with TMTpro 16plex or 18plex Label Reagent sets.
- All samples must be digested, labeled, and then mixed equally before desalting, fractionation, and LC-MS/MS. For optimal results, use 25–100 µg of peptide for each labeling reaction.
- To avoid contamination of MS samples, always wear gloves when handling samples and gels. Use ultrapure MS-grade reagents. Perform sample preparation in a clean work area.
- TMTpro reagents are ~20% heavier than TMT reagents, so a ratio range of 1:5-1:10, sample to tag, w:w is recommended compared to standard TMT reagent labeling ratio range of 1:4-1:8, w:w.

Guidelines for peptide labeling

- Use the EasyPep™ Mini MS Sample Prep Kit to prepare protein digests for TMTpro reagent labeling.
- Use 25–100 µg of protein digest per labeling reaction.
- For complete labeling of lysine and N-termini, use a minimum ratio of 1:5-1:10, sample to tag (w:w).

Before you begin

- Prepare 100 mM TEAB (triethyl ammonium bicarbonate): Add 500 µL of the 1M TEAB to 4.5 mL of ultrapure water.
- Prepare 5% Hydroxylamine: Add 50 µL of the 50% hydroxylamine to 450 µL of 100 mM TEAB.

Label Peptides with TMT Isobaric Mass Tags

1. Prepare 25–100 µg of protein digest in 100 µL of 100 mM TEAB pH 8.5 or HEPES pH 8.5.
2. Immediately before use, equilibrate the TMTpro Label Reagents to room temperature in the foil pouch.
3. Add anhydrous acetonitrile to each vial according to the following table, then allow the reagent to dissolve for 5 minutes with occasional vortexing.

Vial size	Volume of anhydrous acetonitrile
0.5 mg	20 µL
5 mg	200 µL

Note: Return unused reagents to the foil pouch with a desiccant and store at –20°C. Reagents dissolved in anhydrous acetonitrile are stable for one week when stored properly at –20°C. For long term storage, store reagents dry with a desiccant.

4. Briefly centrifuge the tube to gather the solution.
5. *(Optional)* Measure protein digest concentration using Pierce™ Quantitative Colorimetric Peptide Assay Kit.
6. Add 20 µL of the TMTpro Label Reagent to each 100 µL sample.

   Alternatively, transfer the reduced and alkylated protein digest to the TMTpro Reagent vial.
7. Incubate the reaction for 1 hour at room temperature.
8. Add 5 µL of 5% hydroxylamine to the sample, then incubate for 15 minutes to quench the reaction.
9. Combine equal amounts of each sample in a new microcentrifuge tube, then speedvac to dry the labeled peptide sample.
10. Clean-up the samples using peptide desalting columns or equivalent, before high-resolution LC-MS analysis.

    Alternatively, Pierce™ High pH Reversed-Phase Peptide Fractionation Kit can be used to clean up and fractionate labeled peptides to increase the number of peptide identifications.

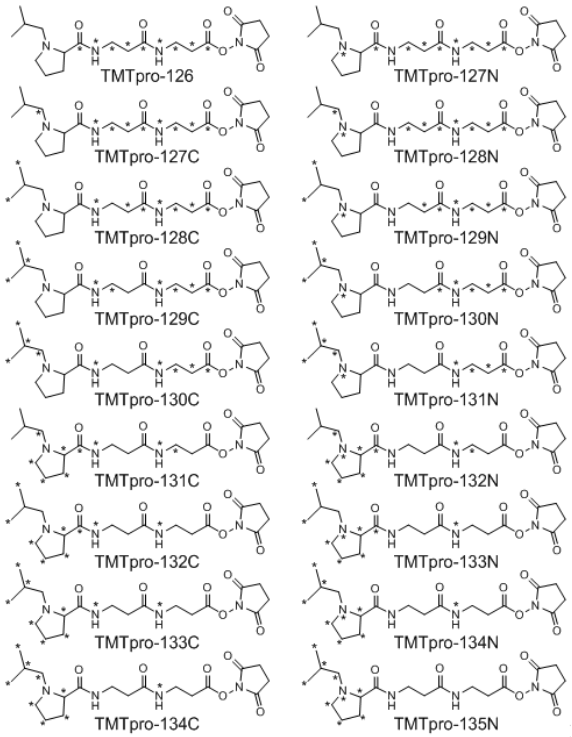
    Note: TMTpro-labeled peptides can be measured after clean up using the Pierce™ Quantitative Colorimetric Peptide Assay Kit. The Pierce Quantitative Fluorescent Peptide Assay cannot be used to measure TMTpro-labeled peptide concentrations.

Data acquisition methods

Quantitation of peptides labeled with Thermo Scientific™ Tandem Mass Tag™ Reagents requires a high-resolution Orbitrap Mass Spectrometer capable of MS/MS fragmentation. To resolve near-isobaric reporter ions, MS/MS resolution must be > 50,000 at 150 *m/z*. Higher-energy collision dissociation (HCD) is recommended for TMTpro reporter ion fragmentation. Optimal HCD fragmentation energy is instrument dependent and can be optimized using TMTpro Zero Reagents.

The peptide mass modification by the TMTpro Reagents (see Table 2) is different from the TMT Reagents, and can be found in the UNIMOD database (*www.unimod.org*). Proteome Discoverer™ Software (2.4 and above) is recommended for TMTpro multiplex quantitation.

Table 2 Mass information and chemical structure for TMTpro Label Reagents

Label reagent	HCD Monoisotopic Reporter Mass[1]	Chemical structures and ^{13}C and ^{15}N stable isotope positions (*)
TMTpro-zero[2]	126.127726	—
TMTpro-126[3]	126.127726	
TMTpro-127N[3]	127.124761	
TMTpro-127C[3]	127.131081	
TMTpro-128N[3]	128.128116	
TMTpro-128C[3]	128.134436	
TMTpro-129N[3]	129.131471	
TMTpro-129C[3]	129.137790	
TMTpro-130N[3]	130.134825	
TMTpro-130C[3]	130.141145	
TMTpro-131N[3]	131.138180	
TMTpro-131C[3]	131.144500	
TMTpro-132N[3]	132.141535	
TMTpro-132C[3]	132.147855	
TMTpro-133N[3]	133.144890	
TMTpro-133C[3]	133.151210	
TMTpro-134N[3]	134.148245	
TMTpro-134C[4]	134.154565	
TMTpro-135N[4]	135.151600	

[1] HCD is a collisional fragmentation method that generates eighteen unique reporter ions from 126 to 135 Da

[2] Molecular formula = C19H30N4O6, molecular weight = 410.46 Da, modification formula = C15H25N3O3, modification mass (monoisotopic) = 295.1896.

[3] Molecular formula = C12[13C]7H30N2[15N]2O6, molecular weight = 419.4 Da, modification formula = C8[13C]7H25N[15N]2O3, modification mass (monoisotopic) = 304.2071.

[4] Molecular formula = C12[13C]8H30N3[15N]O6, molecular weight = 419.4 Da, modification formula = C7[13C]8H25N2[15N]O3, modification mass (monoisotopic) = 304.2135.

For TMTpro 18plex analysis, use the TMTpro 16plex monoisotopic modification mass (304.2071) for database searching.

Troubleshooting

Observation	Possible cause	Recommended action
Poor labeling	A primary amine-based buffer was used (e.g., Tris, glycine)	Use non-primary amine-based buffers (e.g., TEAB, HEPES).
	Incorrect buffer pH	Ensure the buffer pH is ~8.0–8.5
	Too much sample was used	Label 25–100 µg sample per 0.25–1 mg of TMTpro reagent.
	Incorrect solvent was used	Use dry acetonitrile or ethanol to reconstitute tags.
	Reagents hydrolyzed	Avoid exposing tags to moisture.
		Store unused reagents dry with a desiccant at –20°C.
Poor protein quantitation	Incorrect instrument method used	Optimize TMTpro reporter ion MS/MS fragmentation.
	Too little sample analyzed	Increase sample amount and optimize ion injection.
	Poor chromatography	Optimize LC gradient to maximize MS/MS of unique peptides.
	Co-isolation of peptides during MS	Reduce sample complexity by pre-fractionating peptides.
		Decrease quadrupole isolation width if applicable.
		Use MS3 methods (i.e. SPS-MS3).

Related products

Product	Source
Pierce™ Trypsin Protease, MS Grade	90057
Pierce™ Lys-C Endoproteinase, MS Grade	90051
Pierce™ Trypsin/Lys-C Protease Mix, MS Grade	A40009
High-Select™ Fe-NTA Phosphopeptide Enrichment Kit	A32992
High-Select™ TiO_2 Phosphopeptide Enrichment Kit	A32993
Pierce™ C18 Spin Columns	89870
Pierce™ C18 Tips	87784
Pierce™ Trifluoroacetic Acid (TFA), Sequencing Grade	28904
Pierce™ Formic Acid, LC-MS Grade	28905

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.