

TMTpro Mass Tag Labeling Reagents and Kits

Catalog Numbers A44522, A44521, A44520, A44519, A44518

Doc. Part No. 2162734.0 Pub. No. MAN0018773 Rev. A.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 16 different peptide samples prepared from cells or tissues. For each sample, a unique reporter mass (i.e., 126-134Da) 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 4).

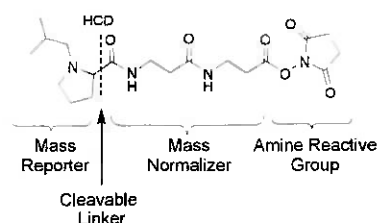


Figure 1 Functional regions of the reagent structure including MS/MS fragmentation site by higher-energy collision dissociation (HCD)

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 ¹³C and ¹⁵N 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 16plex Isobaric Label Reagent Set

Product	Amount	Cat. No.	Storage
TMTpro 16plex Isobaric Label Reagent Set ^[1]	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	
TMTpro Zero	6 × 0.5 mg per vial (sufficient reagents for labeling six samples)	A44519	
	1 × 5 mg per vial (sufficient reagents for labeling ten samples)	A44518	

^[1] 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).

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 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.

Guidelines for peptide labeling

- Use the Pierce™ Mass Spec Sample Prep Kit for Cultured Cells or 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, 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.

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 0.5 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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub No. MAN0018773

Revision	Date	Description
A.0	02 July 2019	New document.

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PRODUCT DATA SHEET
Thermo Scientific™ TMTpro 16plex Label Reagent Set, 1 x 5mg
Product Number: A44520

Lot Number: UH290428

Form: TMTpro reagents are supplied dried, 5 mg/tube. Make a stock solution by reconstituting each tube with 200 µl dry acetonitrile.

Note: ** Reporter ion isotopic distributions (+1, +2) are primarily for natural carbon isotopes with reporter ion interference for each mass tag shown in parentheses. Incomplete stable isotope incorporation (-2, -1) are also reported for carbon and nitrogen isotopes. Reporter ion isotopic distributions can be used as isotope correction factors in TMTpro 16plex method template in Proteome Discoverer software version 2.3 and above.

****Reporter Ion Isotopic Distributions:**

Mass Tag	Reporter Ion Mass	-2		-1		M+	+1		+2	
		-2x 13C	-13C -15N	-13C	-15N	-	+15N	+13C	+15N +13C	+2x 13C
TMTpro-126	126.127726	NA%	NA%	NA%	NA%	100%	0.29%	7.73%(127C)	0.02%	0.22%
TMTpro-127N	127.124761	NA%	NA%	NA%	0.68% (126)	100%	NA%	7.46%(128N)	NA%	0.22%
TMTpro-127C	127.131081	NA%	NA%	0.71% (126)	NA%	100%	0.32%	6.62% (128C)	0.01%	0.16%
TMTpro-128N	128.128116	NA%	0.00%	0.75% (127N)	1.13%	100%	NA%	6.67% (129N)	NA%	0.16%
TMTpro-128C	128.134436	0.06%	NA%	1.34% (127C)	NA%	100%	0.28%	5.31%(129C)	0.02%	0.11%
TMTpro-129N	129.131471	0.01%	0.1%	1.29% (128N)	1.12%	100%	NA%	5.48% (130N)	NA%	0.10%
TMTpro-129C	129.13779	0.26%	NA%	2.34% (128C)	NA%	100%	0.32%	4.87% (130C)	0.0%	0.08%
TMTpro-130N	130.134825	0.39%	0.02%	2.36%(129N)	1.17%	100%	NA%	4.57% (131N)	NA%	0.07%
TMTpro-130C	130.141145	0.05%	NA%	2.67% (129C)	NA%	100%	0.31%	3.85% (131C)	0.00%	0.15%
TMTpro-131N	131.13818	0.05%	0.03%	2.71% (130N)	1.21%	100%	NA%	3.73% (132N)	NA%	0.04%
TMTpro-131C	131.1445	0.09%	NA%	3.69%(130C)	NA%	100%	0.37%	2.77% (132C)	0.0%	0.01%
TMTpro-132N	132.141535	0.09%	0.01%	2.51%(131N)	0.71%	100%	NA%	2.76% (133N)	NA%	0.01%
TMTpro-132C	132.147855	0.10%	NA%	4.11%(131C)	NA%	100%	0.37%	1.63%(133C)	0.02%	0.00%
TMTpro-133N	133.14489	0.09%	0.01%	3.09% (132N)	0.76%	100%	NA%	1.58% (134N)	NA%	0.00%
TMTpro-133C	133.15121	0.36%	NA%	4.63% (132C)	NA%	100%	0.30%	0.88%(134C)	0.00%	NA%
TMTpro-134N	134.148245	0.38%	0.04%	4.82% (133N)	0.4%	100%	NA%	0.86%(135N)	NA%	NA%

Stability: Reagent is highly moisture sensitive. Product is guaranteed for one year from date of receipt in original, unopened packaging.

Storage: Store at -20°C

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