TMTpro Mass Tag Labeling Reagents and Kits

Catalog Numbers A44518, A44519, A44520, A44521, A44522, A52045, A52046, A40000817, A40000818, A40000839, A40000853, A40000928

Doc. Part No. 2162734 Pub. No. MAN0018773 Rev. E

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). Each TMTpro reagent within the multiplex set has the same nominal mass (isobaric) and chemical structure composed of an amine-reactive NHS-ester group, a mass normalizer group, and a mass reporter (see Figure 1). The reagent set can be used to label up to 35 different peptide samples prepared from cells, biological fluids, or tissues. For each sample, a unique reporter ion (126–135 *m/z*) generated in the low mass region of the MS/MS spectra upon peptide fragmentation is used to measure relative protein expression levels (see “Data acquisition methods” on page 3).

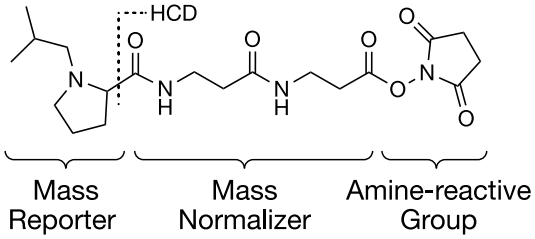

Figure 1 TMTpro reagent structure including functional regions and HCD fragmentation site

The TMTpro reagents have a different chemical structure and are about 20% larger in mass than the TMT™ reagents. The TMTpro reagent structure has a longer mass normalizer region and a proline-based reporter containing different numbers and combinations of nine stable ^{2}H, ^{13}C and ^{15}N isotopes to support higher multiplexing than TMT™ reagents. Advantages of the TMTpro reagents include increased sample multiplexing for relative quantitation, increased sample throughput, and fewer missing quantitative channels among samples.

Procedure overview

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

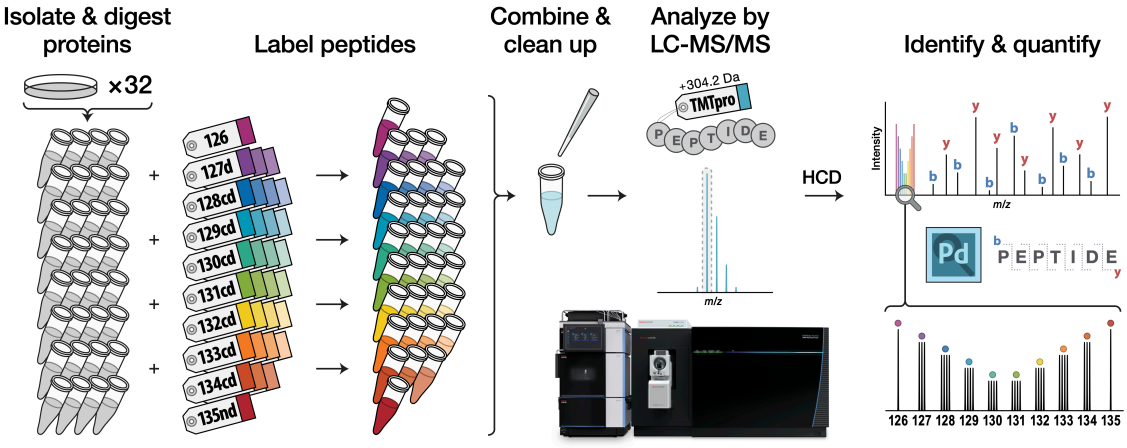

Figure 2 Procedure schematic for using TMTpro 32plex Label Reagents

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Contents and storage

Table 1 TMTpro Isobaric Label Reagents

Product	Amount	No. of Reactions	Cat. No.	Storage
TMTpro 10plex Isobaric Label Reagent Set (Unit Mass Reporter)[1]	1 × 5 mg per vial	10	A40000928	–20°C
TMTpro 16plex Isobaric Label Reagent Set[2]	1 × 5 mg per vial	10	A44520	–20°C
	1 × 0.5 mg per vial	1	A44521	
	6 × 0.5 mg per vial	6	A44522	
TMTpro 18plex Isobaric Label Reagent Set[3]	1 × 5 mg per vial	10	A52045	–20°C
TMTpro–134C and TMTpro–135N Label Reagents	1 × 5 mg per vial	10	A52046	–20°C
TMTpro 32plex Label Reagent Matched Set[4]	1 × 5 mg per vial	10	A40000839	–20°C
TMTpro 16plex Deuterated Label Reagent Set[5]	1 × 5 mg per vial	10	A40000817	–20°C
TMTpro–134C and TMTpro–135CD Label Reagents	1 × 5 mg per vial	10	A40000853	–20°C
TMTpro–135CD Label Reagent	1 × 5 mg per vial	5	A40000818	–20°C
TMTpro Zero	5 × 0.5 mg per vial	5	A44519	–20°C
	1 × 5 mg per vial	10	A44518	

[1] A total of 10 vials: 1 each of TMTpro reagent 126, 127N, 128N, 129N, 130N, 131N, 132N, 133N, 134N, 135N (Table 2)
[2] A total of 16 vials: 1 each of TMTpro reagent 126, 127N, 127C, 128N, 128C, 129N, 129C, 130N, 130C, 131N, 131C, 132N, 132C, 133N, 133C, 134N (Table 2)
[3] A total of 18 vials: 1 each of TMTpro reagent 126, 127N, 127C, 128N, 128C, 129N, 129C, 130N, 130C, 131N, 131C, 132N, 132C, 133N, 133C, 134N, 134C, 135N (Table 2)
[4] A total of 32 vials from A44520 TMTpro 16plex Isobaric Label Reagent Set and A40000817 TMTpro 16plex Deuterated Label Reagent Set
[5] A total of 16 vials: 1 each of TMTpro reagent 127D, 128ND, 128CD, 129ND, 129CD, 130ND, 130CD, 131ND, 131CD, 132ND, 132CD, 133ND, 133CD, 134ND, 134CD, 135ND (Table 2)

Required materials not supplied

All materials are available through **thermofisher.com**.

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

Item	Source
Water, LC-MS Grade	047146.K2
Acetonitrile, Anhydrous	448391000
1M Triethylammonium Bicarbonate (TEAB)	90114
50% Hydroxylamine	90115
SpeedVac™ Vacuum Concentrator	SPD140P1-115
EASY-Spray™ HPLC Column (2–µm particle size, 75 µm x 500 mm), or similar	ES903
Vanquish™ Neo UHPLC System, or similar	VN-S10-A-01
Orbitrap Ascend™ Tribrid™ or Eclipse™ Tribrid™ Mass Spectrometer	FSN06-10000, FSN04-10000
Optional Item	
Low Protein Binding Microcentrifuge Tubes (1.5 or 2 mL)	90410, 88379
EasyPep™ Mini MS Sample Prep Kit or EasyPep™ 96 Micro MS Sample Prep Kit	A40006, A57864
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275
Pierce™ Peptide Desalting Spin Columns	89852
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	84868
Proteome Discoverer™ Software	OPTON-31099

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 removal from the pouch. Store unused reagents in the foil pouch with desiccant at –20°C.
- The TMTpro reagents actively react with amines and modify lysine residues and peptide N-termini. It is essential to eliminate all buffers and additives containing amines before proceeding with labeling.
- The TMTpro Zero Label Reagent can be used to optimize methods before multiplexed analysis of samples with TMTpro 10plex, 16plex, 18plex, or 32plex reagent sets.
- To avoid contamination of MS samples, always wear gloves when handling samples. Use ultrapure MS-grade reagents. Perform sample preparation in a clean work area.
- Use the EasyPep™ Mini MS or EasyPep™ 96 Micro MS Sample Prep Kit to prepare protein digests for labeling with TMTpro reagents.
- Use 25–100 µg of protein digest per labeling reaction with a sample to tag ratio (w:w) of 1:5–1:10 for complete labeling.
- All samples must be labeled, quenched, and then combined equally before desalting, fractionation, and LC–MS/MS analysis.

Prepare materials

- Prepare 100 mM TEAB buffer: Add 500 µL of 1M TEAB to 4.5 mL of ultrapure water.
- Prepare 5% hydroxylamine solution: Add 50 µL of 50% hydroxylamine solution to 450 µL of 100 mM TEAB.

Label peptides with TMTpro reagents

1. Prepare 25–100 µg protein digest samples in 100 µL of 100 mM TEAB, pH 8.5 or 100 mM HEPES, pH 8. Verify pH using pH paper.

   Note: Protein digest concentration may be measured using the Pierce™ Quantitative Colorimetric Peptide Assay Kit.

2. Immediately before use, equilibrate the TMTpro 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 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 reagent solution.
5. Add 20 µL of the TMTpro reagent solution to each 100 µL protein digest sample. Alternatively, transfer the sample to the reagent vial.
6. Incubate the reaction for 1 hour at room temperature.
7. Add 5 µL of 5% hydroxylamine to each sample and incubate for 15 minutes to quench the labeling reaction.
8. Combine equal amounts of each labeled sample into a new low protein binding microcentrifuge tube, then dry the pooled sample in the SpeedVac.
9. Clean up the sample using an EasyPep™ peptide clean-up column or peptide desalting column prior to LC–MS/MS analysis using an Orbitrap MS platform.

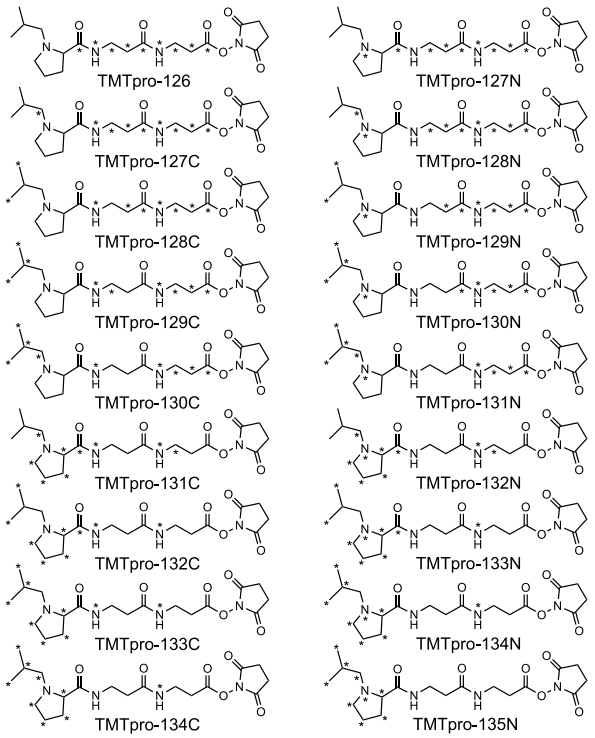
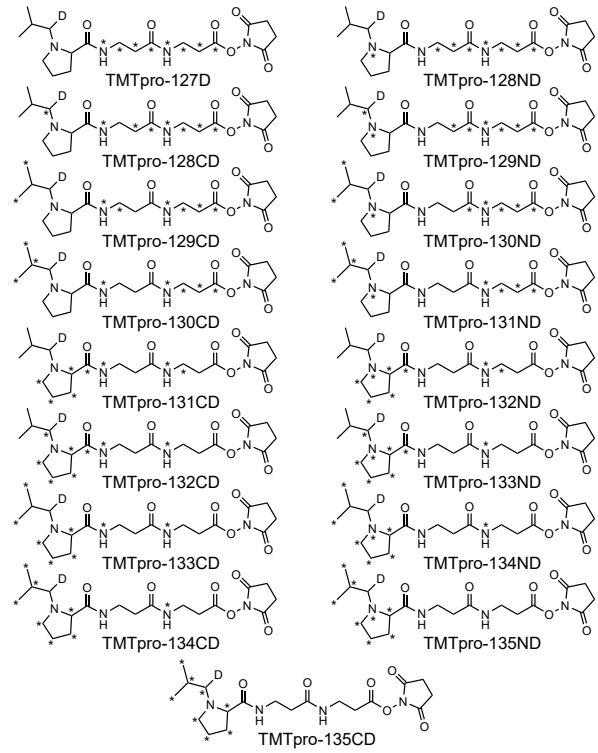
   Alternatively, the Pierce™ High pH Reversed-Phase Peptide Fractionation Kit can be used to clean up and fractionate labeled peptides to increase the number of peptide identifications.

   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

- Quantification of peptides labeled with TMTpro reagents requires an Orbitrap mass spectrometer. Resolving TMTpro reporter ions in MS/MS spectra requires a resolving power of ≥7,500 at 200 *m/z* for TMTpro 10plex reagents, ≥50,000 for TMTpro 16plex and 18plex reagents, and ≥75,000 for TMTpro 32plex and 35plex reagents.
- 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.
- Maximum injection time and automatic gain control (AGC) target parameters should be optimized for different levels of multiplexing and amount of sample loaded on–column.
- The peptide mass modification of TMTpro multiplex reagents is 304.2071 Da.
- For Real–Time Search MS^3 acquisition methods, specify TMTpro 16plex as static modification on sites Kn (lysine and N–termini).
- Proteome Discoverer™ Software (3.2 and above) is recommended for TMTpro multiplex quantification. For processing of TMTpro multiplex data, specify TMTpro 16plex as static modification on K and N–termini.

Table 2 Reporter ion masses and chemical structures for TMTpro reagents

Reagent	HCD Reporter Ion Mass[1]	Chemical structures and positions of ^{13}C and ^{15}N stable isotopes (*)
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]	129.137791	
TMTpro–130N[3]	130.134826	
TMTpro–130C[3]	130.141146	
TMTpro–131N[3]	131.138181	
TMTpro–131C[3]	131.144501	
TMTpro–132N[3]	132.141536	
TMTpro–132C[3]	132.147856	
TMTpro–133N[3]	133.144891	
TMTpro–133C[3]	133.151211	
TMTpro–134N[3]	134.148246	
TMTpro–134C[4]	134.154566	
TMTpro–135N[4]	135.151601	
TMTpro–127D[5]	127.134003	
TMTpro–128ND[5]	128.131038	
TMTpro–128CD[5]	128.137358	
TMTpro–129ND[5]	129.134393	
TMTpro–129CD[5]	129.140713	
TMTpro–130ND[5]	130.137748	
TMTpro–130CD[5]	130.144068	
TMTpro–131ND[5]	131.141103	
TMTpro–131CD[5]	131.147423	
TMTpro–132ND[5]	132.144458	
TMTpro–132CD[5]	132.150778	
TMTpro–133ND[5]	133.147813	
TMTpro–133CD[5]	133.154133	
TMTpro–134ND[5]	134.151171	
TMTpro–134CD[6]	134.157491	
TMTpro–135ND[6]	135.154526	
TMTpro–135CD[7]	135.160846	

[1] HCD is a collisional fragmentation method that generates unique reporter ions from 126–135 *m/z*

[2] Molecular formula = $C_{19}H_{30}N_4O_6$; molecular weight = 410.46 Da; modification formula = $C_{15}H_{25}N_3O_3$; modification mass = 295.1896 Da

[3] Molecular formula = $C_{12}{}^{13}C_7H_{30}N_2{}^{15}N_2O_6$; molecular weight = 419.4 Da; modification formula = $C_8{}^{13}C_7H_{25}N^{15}N_2O_3$; modification mass = 304.2071 Da

[4] Molecular formula = $C_{11}{}^{13}C_8H_{30}N_3{}^{15}NO_6$; molecular weight = 419.4 Da; modification formula = $C_7{}^{13}C_8H_{25}N_2{}^{15}NO_3$; modification mass = 304.2135 Da
[5] Molecular formula = $C_{13}{}^{13}C_6H_{29}{}^2HN_2{}^{15}N_2O_6$; molecular weight = 419.4 Da; modification formula = $C_9{}^{13}C_6H_{24}{}^2HN^{15}N_2O_3$; modification mass = 304.2101 Da
[6] Molecular formula = $C_{12}{}^{13}C_7H_{29}{}^2HN_3{}^{15}NO_6$; molecular weight = 419.4 Da; modification formula = $C_8{}^{13}C_7H_{24}{}^2HN_2{}^{15}NO_3$; modification mass = 304.2164 Da
[7] Molecular formula = $C_{11}{}^{13}C_8H_{29}{}^2HN_4O_6$; molecular weight = 419.4 Da; modification formula = $C_7{}^{13}C_8H_{24}{}^2HN_3O_3$; modification mass = 304.2227 Da

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).
	Sample buffer pH was incorrect	Ensure that the sample pH during labeling is ~8.0–8.5.
	Too much sample was used	Label 25–100 µg sample per 0.25–1 mg of TMTpro reagent.
	Incubation was too short	Increase reaction incubation time.
	Sample concentration was too low	Increase sample concentration.
		Increase tag to sample ratio.
	Incorrect solvent was used	Use dry acetonitrile or ethanol to reconstitute reagents.
	Reagents were hydrolyzed	Avoid exposing tags to moisture or high-humidity environments.
		Equilibrate reagents to room temperature before use.
		Store unused reagents sealed in foil pouch with desiccant at –20°C.
Poor protein quantitation	Incorrect instrument method was used	Optimize TMTpro reporter ion MS/MS fragmentation.
	Too little sample was analyzed	Increase sample amount and optimize ion injection time.
	Chromatography was poor	Optimize LC gradient to maximize MS/MS of unique peptides.
	Peptides were co-isolated during MS	Decrease quadrupole isolation width.
		Use a SPS–MS3 acquisition method.

Related products

Product	Source
Pierce™ HeLa Protein Digest Standard	88329
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
High-Select™ Top14 Abundant Protein Depletion Mini Spin Columns	A36370

Limited product warranty

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

Revision history: Pub. No. MAN0018773 E

Revision	Date	Description
E	12 July 2024	Updated to include expanded TMTpro reagent set
D00	7 July 2021	Created Rev. D00
C00	11 November 2019	Correcting branding bar from Invitrogen to Thermo Scientific
B00	2 October 2019	Corrections - Removing Revision history
A00	18 July 2019	New document for TMTpro Mass Tag Labeling Reagents and Kits