AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits

Catalog Numbers A50949, A50950, A50951

Doc. Part No. 2162764 Pub. No. MAN0025759 Rev. D

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™ AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits enable multiplex relative quantitation by mass spectrometry (MS). The kit contains a Sample Prep Module and a TMTpro™ 16plex Labeling Reagent Module (shipped separately). Like other isobaric mass-tagging reagents, each reagent within a set has the same nominal mass (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 (126–134 Da) in the low mass region of the MS/MS spectrum is used to measure the relative protein expression levels during peptide fragmentation.

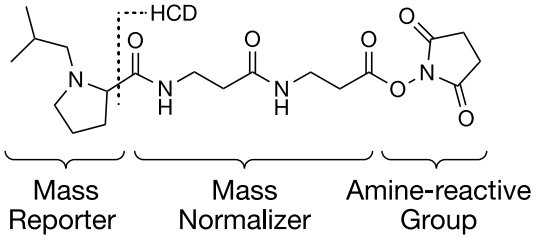


Figure 1 Functional regions of the TMTpro™ structure including MS/MS fragmentation site by higher-energy collision dissociation (HCD)

The Thermo Scientific™ TMTpro™ labeling reagents have a different chemical structure and are about 20% larger in mass than the Thermo Scientific™ TMT™ labeling reagents. The TMTpro™ reagent structure supports higher multiplexing compared to TMT™ reagents due to a longer linker region and proline-based reporter, containing different numbers of combinations of 9 stable ^{13}C and ^{15}N isotopes. Advantages of TMTpro™ labeling reagents include increased sample multiplexing for relative quantitation, increased sample throughput, and fewer missing quantitative channels among samples.

Contents and storage

Product	Kit Cat. No.	Contents	Storage
AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (10–30 µg), 2 × 16 Reactions	A50949	AccelerOme™ TMTpro™ 16plex Sample Prep Module	4°C
		AccelerOme™ TMTpro™ 16plex Module, (10–30 µg), 32 reactions, or (30–100 µg), 16 reactions[1]	-20°C
AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (30–100 µg), 1 × 16 Reactions	A50950	AccelerOme™ TMTpro™ 16plex Sample Prep Module	4°C
		AccelerOme™ TMTpro™ 16plex Module, (10–30 µg), 32 reactions, or (30–100 µg), 16 reactions[1]	-20°C
AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (30–100 µg), 2 × 16 Reactions	A50951	AccelerOme™ TMTpro™ 16plex Sample Prep Module	4°C
		AccelerOme™ TMTpro™ 16plex Module, (30–100 µg), 32 reactions[1]	-20°C

[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 “Data acquistion methods” on page 5).

Required materials not supplied

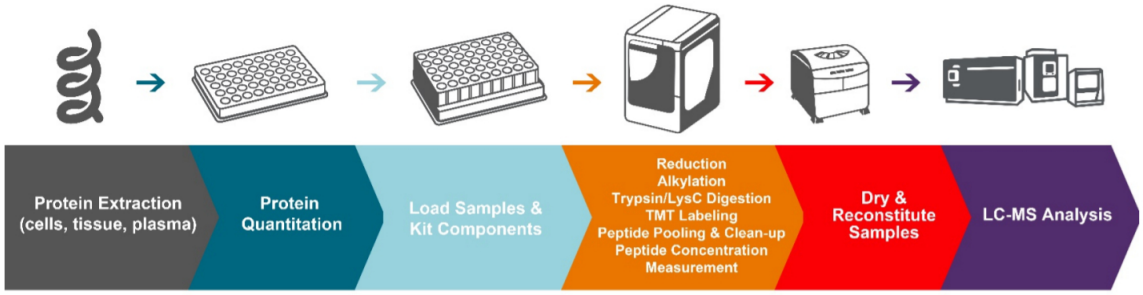
Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

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

Item	Source
Pierce™ Rapid Gold BCA Protein Assay Kit	A53225
Needle Wash Solution W1: Water with 0.1% Formic Acid (v/v), Optima™ LC-MS Grade	LS118-212
Needle Wash Solution W2: 50% Methanol/50% Water with 0.05% Formic Acid (v/v), Optima™ LC-MS Grade	PN A456-1
EASY-Spray™ LC Columns (2–µm particle, 50 µm × 150 mm)	ES901
EASY-nLC™ 1200 System	LC140
Orbitrap Eclipse™ Tribrid™ Mass Spectrometer	FSN04-10000
Optional Materials	
Low Protein-Binding Collection Tubes (2.0 mL)	88379
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	84868
Pierce™ Peptide Retention Time Calibration Mixture	88320

Workflow

Protein extracts isolated from cells or tissues are reduced, alkylated, then digested on the instrument. Samples are labeled with the TMTpro™ labeling reagents, then mixed for sample clean-up on the instrument. Labeled samples are analyzed by a high resolution Orbitrap LC-MS/MS before data analysis to identify the peptides and quantify the reporter ion relative abundances.



Procedural guidelines

- For phosphopeptide enrichment and analysis, we recommend adding phosphatase inhibitors (for example, Halt™ Phosphatase Inhibitor Cocktail, Cat. No. 78420) to the Lysis Solution before the cell lysis.
- DO NOT add protease inhibitor cocktails containing EDTA to the Lysis Solution, as these reagents inhibit the universal nuclease and Pierce™ Trypsin/Lys-C Protease Mix, MS Grade activity.

Before you begin

- Warm the Lysis Solution to room temperature before use. Store the buffers and columns at 4°C.
- The TMTpro™ reagents are highly moisture-sensitive. To avoid condensation on the product, equilibrate the reagents to room temperature before opening the foil pouch.

Extract protein

Use either 10–30 µg or 30–100 µg of protein per sample preparation.

1. Rinse cultured cells or tissues 2–3 times with 1X PBS.

2. Resuspend the sample in Lysis Buffer without additional buffers. Use one of the following methods according to the sample type.

   - For cultured cells, add 50 µL of Lysis Buffer and 1 µL of universal nuclease to a minimum of 1×10^6 cells. Pipet up and down with a 20–200 µL tip for 10–15 cycles or until the sample viscosity is reduced.

     Note: Centrifugation of cultured cell lysates is typically not required after aspiration using a pipet.

   - For tissue samples, add 50 µL of Lysis Buffer and 1 µL of universal nuclease per 5 mg of tissue, then disrupt with a tissue homogenizer until the sample is homogenized. Centrifuge tissue lysates at 16,000 × *g* for 10 minutes, then collect the supernatant.

   - For purified proteins, serum, or plasma samples, dilute the samples directly in the Lysis Buffer to 0.2–2 mg/mL. Use 0.5–1.5 µL of undepleted plasma or serum per sample preparation.

     Note: For purified proteins or plasma samples, addition of universal nuclease is **not** required.

3. Determine the protein concentration of the prepared sample using established methods, such as the Pierce™ BCA Protein Assay Kit (Cat. No. 23227) or Pierce™ Rapid Gold BCA Protein Assay Kit (Cat. No. A53226).

   Note: If needed, dilute concentrated samples with Lysis Buffer if you are using the BCA assay.

Load samples into wells

Transfer 10–30 µg or 30–100 µg of each protein sample to the appropriate wells of the Sample Input Plate, then adjust the final volume to 50 µL with the Lysis Buffer. The sample loading wells are defined by the kit capacity and the number of samples. Use one of the following options:

Note: If you have fewer samples than the kit capacity, fill the samples sequentially in the plate within the same area, starting in the first row, going left to right.

- For the AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (16 reactions), load the samples in wells A1–A8 and B1–B8.

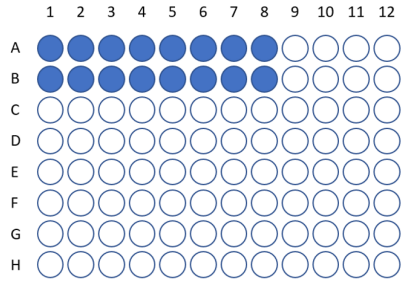


Figure 2 Sample wells: AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (16 reactions)

- For the AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (32 reactions), load the samples in wells A1–A8, B1–B8, C1–C8, and D1–D8.

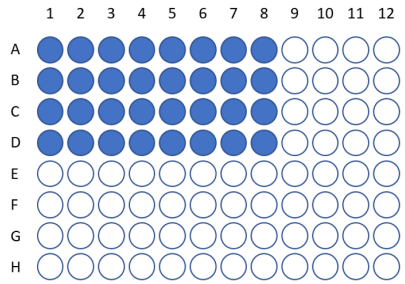


Figure 3 Sample wells: of AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (32 reactions)

Prepare and label samples with the AccelerOme™ System

1. Select the **Quick Start Setup** or **Start with Method** to start a run on the instrument. For detailed instructions about setting up a run on the AccelerOme™ system, see the AccelerOme™ User Guide.

2. Follow the on-screen guidance for loading your Sample Input Plate and the kit components into the system deck.

   Note: Remove the 6 bottle caps off of the Wash and Elution Solutions before putting the buffer tray on the instrument deck.

   IMPORTANT!
   - Ensure tray tables are front-facing on the instrument deck.
   - Ensure the labeling protein amount and the reaction numbers correspond to the kit configuration.

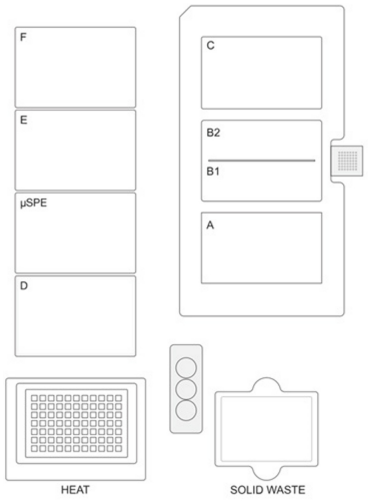


Figure 4 Instrument deck layout.

3. At the end of the run, use the **Completed Run Report** for locations of the pooled peptide samples.

Treat samples for LC-MS

1. Using a vacuum centrifuge, dry the labeled peptide samples in the Sample Output Plate.

   Note: Alternatively, transfer the peptide samples into low protein-binding collection tubes, then dry the peptide sample using a vacuum centrifuge.

2. Resuspend the sample in the Sample Reconstitution Solution (0.1% formic acid in water) for LC-MS analysis. Adjust the peptide concentration with the Sample Reconstitution Solution (see **Reconstitution for LC-MS Guidance** in the **Completed Run Report**).

3. *(Optional)* Fractionate the labeled peptides with the Pierce™ High pH Reversed-Phase Peptide Fractionation Kit to increase the number of peptide and protein identifications.

4. *(Optional)* Spike the Pierce™ Peptide Retention Time Calibration Mixture (PRTC) into the peptide sample to correct for any variability in the injection volumes for LC-MS analysis.

   Note: Additional information can be found in the instructions for the Pierce™ Peptide Retention Time Calibration Mixture.

   Table 1 Example: Calibration mixture spiked with peptide digest for LC-MS analysis

LC-MS injection amount (total 15 µL)	50 fmol/µL PRTC	0.1% Formic Acid	200 ng/µL peptide digest
PRTC: 250 fmol HeLa digest: 1 µg	5 µL	5 µL	5 µL

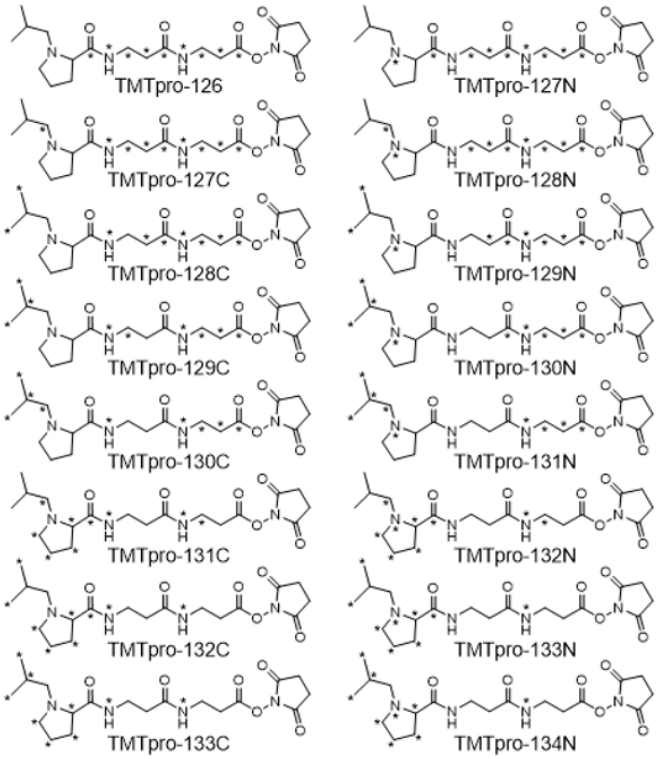
5. *(Optional)* Inject between 250 fmol and 1.5 pmol of the calibration mixture with the peptide digest per run.

Data acquistion methods

Quantitation of peptides labeled with Thermo Scientific™ TMT™ 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 (v2.4 and later) 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	

[1] HCD is a collisional fragmentation method that generates 16 unique reporter ions from 126–134 Da

[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 (monoisotopic) = 295.1896.

[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 (monoisotopic) = 304.2071.

Troubleshooting

Observation	Possible cause	Recommended action
Poor labeling	Sample contained primary amine-based compounds.	Remove the primary amine-based compounds in the samples.
	Wrong method setting was used for the kit (for example, 30–100 µg samples were used in the the kit targeted for 10–30 µg samples).	Measure the protein amount to ensure it is in the correct sample range. Ensure the labeling protein amount and the reaction numbers correspond to the kit configuration.
	Reagents were hydrolyzed.	Avoid exposing tags to moisture. Equilibrate TMTpro™ label reagents to room temperature before opening the foil pouch.
Poor protein quantification	Incorrect instrument method was used.	Optimize the TMTpro™ reporter ion MS/MS fragmentation.
	Too little sample was analyzed.	Increase the sample amount and optimize the ion injection.
	Chromatography was poor.	Optimize the LC gradient to maximize the MS/MS of unique peptides.
	Peptides were co-isolated during MS.	Reduce the sample complexity by pre-fractionating the peptides.
		Decrease quadrupole isolation width if applicable.
		Use MS3 methods (SPS-MS3).

Related products

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.

Product	Cat. No.
Pierce™ Peptide Retention Time Calibration Mixture	88320
AccelerOme™ Label-Free MS Sample Preparation Kits, 1 × 16 Reactions	A50944
AccelerOme™ Label-Free MS Sample Preparation Kits, 1 × 36 Reactions	A50945
AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (10–30 µg), 3 × 11 Reactions	A50946
AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg), 1 × 11 Reactions	A50947
AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg), 3 × 11 Reactions	A50948
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™ Trifluoroacetic Acid (TFA), Sequencing Grade	28904
Pierce™ Formic Acid, LC-MS Grade	28905

Limited product warranty

Life Technologies Corporation and its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.

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

Revision history: Pub. No. MAN0025759 D

Revision	Date	Description
D	22 April 2025	SKU 88320 was removed from the contents and storage table, then added to the required materials not supplied table.
C.0	7 June 2022	The instructions for using the Experiment Designer software and system-generated bridge samples were removed from the manual.
B.0	15 April 2022	The Doc. Part No. was corrected to 2162764.
A.0	12 January 2022	New document for AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits.

The information in this guide is subject to change without notice.

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