AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kits

Catalog Numbers A50946, A50947, A50948

Doc. Part No. 2162747 Pub. No. MAN0025760 Rev. B.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™ AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kits enable multiplex relative quantitation by mass spectrometry (MS). The kit contains a Sample Prep Module and a TMT11plex™ 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 11 different peptide samples prepared from cells or tissues. For each sample, a unique reporter mass (i.e., 126–131 Da) in the low mass region of the MS/MS spectrum is used to measure the relative protein expression levels during peptide fragmentation.

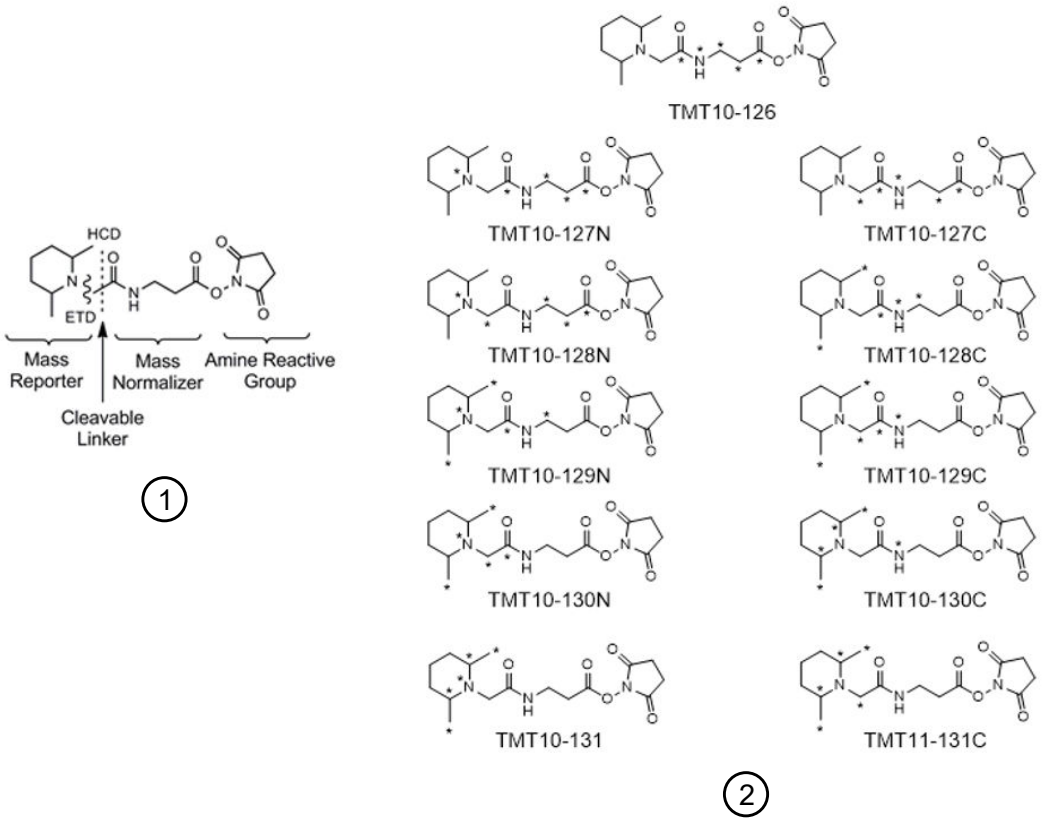


Figure 1 Chemical structures of the TMT™ label ing reagents

① Functional regions of the reagent structure including MS/MS fragmentation sites by Higher-energy Collision Dissociation (HCD) and Electron Transfer Dissociation (ETD)

② TMT11plex™ reagent structures and isotope positions (*)

The Thermo Scientific™ TMT11plex™ labeling reagents share an identical structure with TMTzero™, TMTsixplex™, and TMT10plex™ reagents, but contain different numbers and combinations of ^{13}C and ^{15}N isotopes in the mass reporter. The different isotopes result in a set of tags that have 6 mDa monoisotopic mass differences in the reporter that can be detected using high resolution Thermo

Scientific™ Orbitrap™ mass spectrometry instruments. Advantages of TMT™ labeling reagents include increased sample multiplexing for relative quantitation, increased sample throughput, and fewer missing quantitative channels among the samples.

Contents and storage

Product	Cat. No.	Contents	Storage
AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (10–30 µg), 3 × 11 Reactions	A50946	AccelerOme™ TMT11plex™ Sample Prep Module	4°C
		AccelerOme™ TMT11plex™ Module, (10–30 µg), 33 reactions, or (30–100 µg),11 reactions [1]	-20°C
		Pierce™ Peptide Retention Time Calibration Mixture, 0.5 pmol/µL[2]	-80°C
AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg), 1 × 11 Reactions	A50947	AccelerOme™ TMT11plex™ Sample Prep Module	4°C
		AccelerOme™ TMT11plex™ Module, (10–30 µg), 33 reactions, or (30–100 µg),11 reactions[1]	-20°C
		Pierce™ Peptide Retention Time Calibration Mixture, 0.5 pmol/µL	-80°C
AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg), 3 × 11 Reactions	A50948	AccelerOme™ TMT11plex™ Sample Prep Module	4°C
		AccelerOme™ TMT11plex™ Module, (30–100 µg), 33 reactions[1]	-20°C
		Pierce™ Peptide Retention Time Calibration Mixture, 0.5 pmol/µL	-80°C

[1] A total of 11 vials: 1 each of TMT–126, TMT–127N, TMT–127C, TMT–128N, TMT–128C, TMT–129N, TMT–129C, TMT–130N, TMT–130C, TMT–131N, TMT–131C label reagent.
[2] Pierce™ Peptide Retention Time Calibration Mixture (Cat. No. 88320) also available as a separate product.

Required materials not supplied

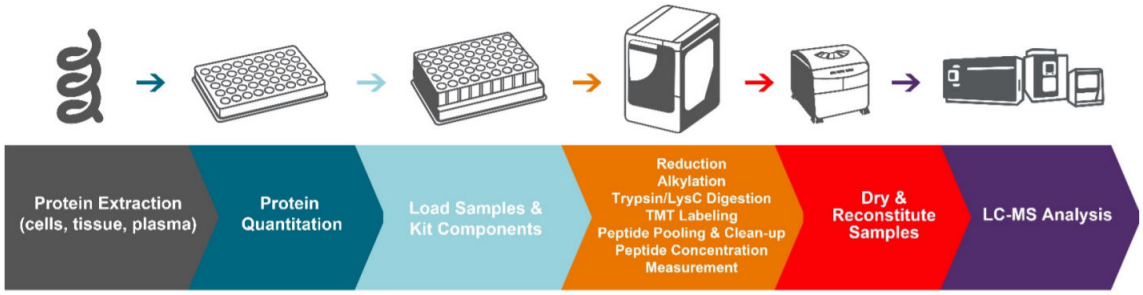
Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

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

Workflow

Protein extracts isolated from cells or tissues are reduced, alkylated, then digested on the instrument. Samples are labeled with the TMT™ the TMT 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™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg) (11 reactions), load the samples in wells A1–A11.

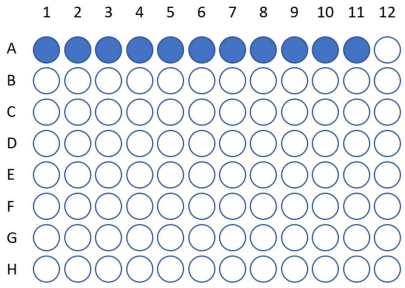


Figure 2 Sample wells: AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg) (11 reactions)

- For the AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (10–30 µg) (33 reactions) and AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg) (33 reactions) , load the samples in wells A1–A11, B1–B11, and C1–C11.

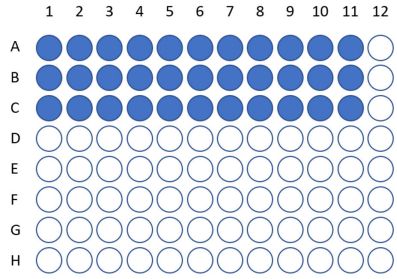


Figure 3 Sample wells:AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (10–30 µg) (33 reactions) and AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kit (30–100 µg) (33 reactions)

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.

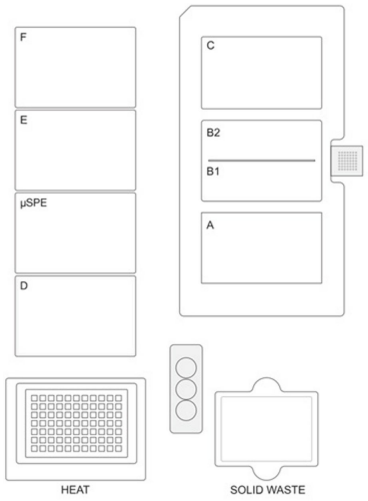


Figure 4 Instrument deck layout.

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 (see Table 2). 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 TMT11plex™ reporter ion fragmentation. Optimal HCD fragmentation energy is instrument-dependent and can be optimized using TMTpro™ Zero reagents. Electron Transfer Dissociation (ETD) is used as an alternative fragmentation method for peptide identification and quantitation; however, ETD is not recommended for TMT11plex™ reagents because of reporter ion overlap (See Table 3).

Table 2 Instruments and MS/MS fragmentation options for peptide identification and quantitation with TMT™ reagents

Instrument	Fragmentation Method	Minimum Resolution Setting
Thermo Scientific™ Orbitrap™ Fusion™ Mass Spectrometer	HCD/SPS-MS3	60,000
Thermo Scientific™ Orbitrap™ Elite™ Mass Spectrometer	HCD/MS3	30,000
Thermo Scientific™ Q Exactive™ Mass Spectrometer	HCD/MS2	35,000
Thermo Scientific™ Orbitrap™ Velos Pro Mass Spectrometer	HCD/MS2	30,000

Data analysis and quantification

The peptide mass modification by the TMT10plex™ reagents is identical to TMTsixplex™ reagents present in the UNIMOD database (*www.unimod.org*) and are listed below. Proteome Discoverer Software (2.1 and later) is recommended for TMT11plex™ relative quantitation. Additional software programs that are used for TMT™ quantitation include Matrix Science™ Mascot™ Software (2.5 and later) and Proteome Software™ Scaffold™ Q+ Software. For data acquired using a combination of fragmentation methods (i.e., HCD/MS3 or HCD/ETD), Proteome Discoverer Software may be necessary to merge the search results.

Table 3 Modification Masses of the Thermo Scientific TMT™ Label Reagents.

Label Reagent	Modification Mass (monoisotopic)	Modification Mass (average)	HCD Monoisotopic Reporter Mass[1]	ETD Monoisotopic Reporter Mass[2]
TMT^{10}–126	229.162932	229.2634	126.127726	114.127725
TMT^{10}–127N	229.162932	229.2634	127.124761	115.124760
TMT^{10}–127C	229.162932	229.2634	127.131081	114.127725
TMT^{10}–128N	229.162932	229.2634	128.128116	115.124760
TMT^{10}–128C	229.162932	229.2634	128.134436	116.134433
TMT^{10}–129N	229.162932	229.2634	129.131471	117.131468
TMT^{10}–129C	229.162932	229.2634	129.137790	116.134433
TMT^{10}–130N	229.162932	229.2634	130.134825	117.131468
TMT^{10}–130C	229.162932	229.2634	130.141145	118.141141
TMT^{10}–131	229.162932	229.2634	131.138180	119.138176
TMT^{11}–131C	229.169252	229.2634	131.144499	118.141141

[1] HCD is a collisional fragmentation method that generates ten unique reporter ions from 126 to 131 Da.
[2] ETD is a non-ergodic fragmentation method that generates six unique reporter ions from 114 to 119 Da.

Troubleshooting

Observation	Possible cause	Recommended action
Poor labeling	Sample contained primary amine-based compounds.	Remove the primary amine-based compounds in 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.

Observation	Possible cause	Recommended action
Poor labeling *(continued)*	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, 16 Reaction	A50944
AccelerOme™ Label-Free MS Sample Preparation Kits, 32 Reaction	A50945
AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (10–30 µg), 2 × 1 Reactions	A50949
AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (30–100 µg), 1 × 16 Reactions	A50950
AccelerOme™ TMTpro™ 16plex MS Sample Preparation and Labeling Kits (30–100 µg), 2 × 16 Reactions	A50951
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/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**.

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

Revision History: Pub. No. MAN0025760

Revision	Date	Description
B.0	7 June 2022	The instructions for using the Experiment Designer software and system-generated bridge samples were removed from the manual.
A.0	11 January 2022	New manual for introduction of AccelerOme™ TMT11plex™ MS Sample Preparation and Labeling Kits.