TMT™ and TMTpro 96-well Plates

Catalog Numbers A58332, A58333, A58334, and A58335

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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 TMT™ and TMTpro 96-well Plates enable multiplex relative quantitation by mass spectrometry (MS). Each TMT™ or 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 TMT™ and TMTpro reagent sets can be used to label up to 11 or 18 different peptide samples, respectively, prepared from cells or tissues. For each sample, a unique reporter ion (126–131 Da m/z for TMT™; 126–135 Da m/z for TMTpro) generated in the low-mass region of MS/MS spectra upon peptide fragmentation is used to measure relative protein expression levels.

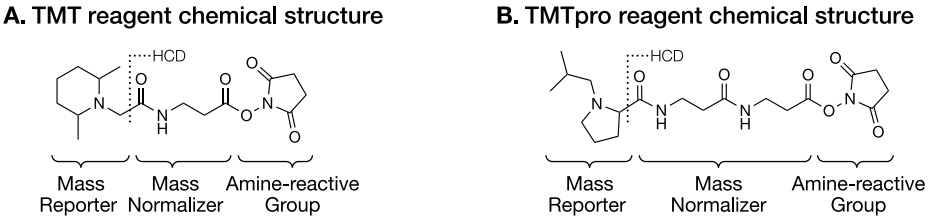

Figure 1 TMT™ and TMTpro reagent structures including functional regions and HCD fragmentation sites.

The TMT™ and TMTpro 96-well Plates enable high-throughput labeling of many samples with multiple reagent sets configured in a ready-to-use 96-well PCR microplate format. The reagents are supplied in a DMSO-based solution with stabilizing agent that enhances reagent integrity and permits reliable handling by robotic pipettors on automated liquid handling platforms. Reagents are provided in 5 µL of the stabilized DMSO solution in amounts of 40 µg TMT™ or 50 µg TMTpro reagent per well, which is appropriate for labeling protein digest samples of 1 µg to 10 µg. Each 96-well microplate contains multiple sets of reagents: 8 sets of TMT™ 10plex, 8 sets of TMT™ 11plex, 6 sets of TMTpro 16plex, and 4 sets of TMTpro 18plex reagents (see Figure 2). The plates are sealed with foil that can be peeled or pierced.

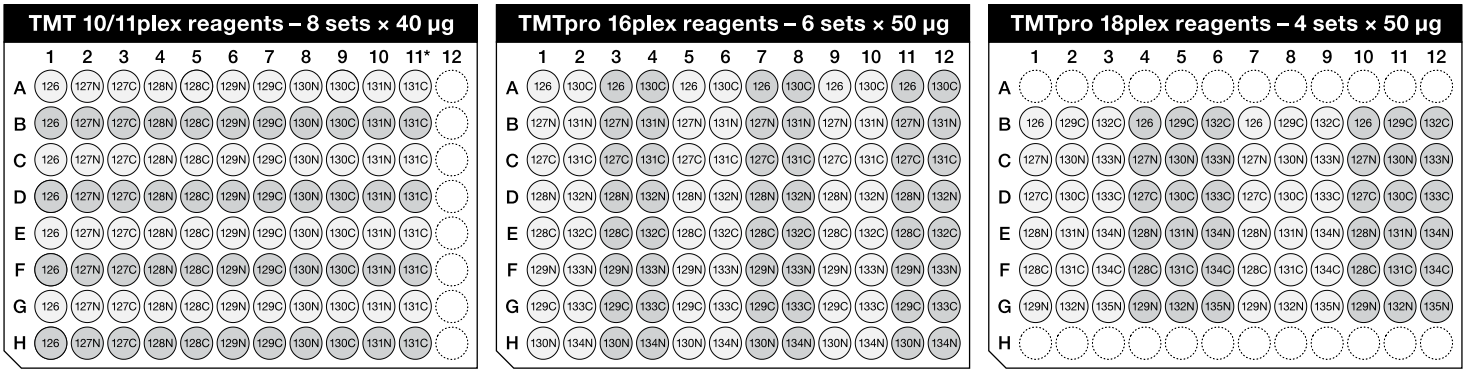

Figure 2 TMT™ and TMTpro 96-well Plate layouts. Note that the TMTpro 10plex 96-well plate does not contain TMT-131C reagents in column 11.

Contents and storage

Item	Amount	Cat. No.	Storage
TMT™ 10plex Label Reagents in stabilized solution, 96-well plate	8 sets of TMT™ 10plex reagents x 40 µg per well (80 reactions)	A58332	–20°C
TMT™ 11plex Label Reagents in stabilized solution, 96-well plate	8 sets of TMT™ 11plex reagents x 40 µg per well (88 reactions)	A58333	
TMTpro 16plex Label Reagents in stabilized solution, 96-well plate	6 sets of TMTpro 16plex reagents x 50 µg per well (96 reactions)	A58334	
TMTpro 18plex Label Reagents in stabilized solution, 96-well plate	4 sets of TMTpro 18plex reagents x 50 µg per well (72 reactions)	A58335	

Required materials not supplied

Unless otherwise indicated, 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	51140
1M Triethylammonium Bicarbonate (TEAB)	90114
50% Hydroxylamine	90115
SpeedVac™ Vacuum Concentrator	SPD140P1
EASY-Spray™ HPLC Column (2-µm particle size, 75 µm x 750 mm) or similar	ES905
Vanquish™ Neo UHPLC System or similar	VN-S10-A-01
Orbitrap™ Ascend Tribrid™ or Eclipse™ Tribrid™ Mass Spectrometer	FSN06-10003 or FSN04-10000
Optional items	
Low Protein Binding Microcentrifuge Tubes (1.5 or 2 mL)	90410 or 88379
EasyPep™ Mini MS Sample Prep Kit	A40006 or 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 TMT™ and TMTpro reagents are highly moisture-sensitive. To avoid moisture condensation onto the product, the reagent plate must be equilibrated to room temperature before removal from the pouch or removal of the foil seal. Store unused reagents sealed in the plate in the foil pouch with desiccant at –20°C. Sealed reagents are stable for 1 month at room temperature or 2 months at 4°C and usable over multiple freeze/thaw cycles.
- The TMT™ and TMTpro reagents are amine-reactive and modify lysine residues and peptide N-termini. Remove all amine-containing buffers and additives before peptide labeling.
- 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 TMT™ or TMTpro reagents.
- Measure peptide concentration using the Pierce™ Quantitative Fluorometric Peptide Assay or the Pierce™ Quantitative Colorimetric Peptide Assay Kit.

- Use 1–10 µg of protein digest (>0.1 µg/µL) per labeling reaction with a sample to tag ratio (w:w) between 1:4–1:8 for TMT™ reagents or 1:5–1:10 for TMTpro reagents for complete labeling.
- All samples must be labeled, quenched, and then equally combined before desalting, fractionation, and LC-MS/MS analysis.

Prepare materials

- Prepare 100 mM TEAB buffer by adding 200 µL of 1 M TEAB to 1.8 mL of ultrapure water.
- Prepare 5% hydroxylamine solution by adding 10 µL of 50% hydroxylamine solution to 90 µL of 100 mM TEAB.

Label peptides with TMT™/TMTpro reagents

1. Prepare 1–10 µg protein digest samples in 10 µL of 100 mM TEAB, pH 8.5 or 100 mM HEPES, pH 8. Verify pH using pH paper.
2. Equilibrate the TMT™/TMTpro plate to room temperature in the foil pouch. Remove the plate and ensure that the reagent solution has thawed.
3. Briefly centrifuge the reagent plate at 1,000 × *g* to collect the reagent solution at the bottom of the wells.
4. Peel the foil from the reagent plate. Alternatively, puncture the foil seal for individual reagent wells.

   Note: Proceed with labeling after removing/puncturing the foil seal. TMT™ and TMTpro reagents are stable in-solution in the unsealed plate for approximately 8 hours at room temperature but may hydrolyze in humid environments (>40% relative humidity). Discard unused TMT™ or TMTpro reagent from wells with removed/punctured foil.
5. Add 10 µL of protein digest sample to each reagent well and mix briefly. Alternatively, transfer reagent solution to each sample.
6. Incubate at room temperature for 60 minutes.

   Note: TMT™ and TMTpro reagents in DMSO-based stabilized solutions can require longer incubation times compared to reagents in acetonitrile.
7. Add 1 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 low protein-binding microcentrifuge tube, then dry the pooled sample in the SpeedVac.

   Note: DMSO requires more time and higher temperatures to dry completely compared to acetonitrile. Alternatively, pooled samples can be diluted using 100 mM TEAB buffer to a DMSO concentration of ≤5% before proceeding directly to cleanup.
9. Clean up the sample using an EasyPep™ peptide clean-up column or peptide desalting column before LC-MS/MS analysis using an Orbitrap MS platform.

   For EasyPep™ SPE clean-up, no dilution of DMSO is necessary before sample binding. Reversed-phase chromatography may require dilution of DMSO and/or optimization of sample to resin ratio before sample binding to achieve high recovery. 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.

   TMT™/TMTpro-labeled peptide concentration can be measured after clean-up using the Pierce™ Quantitative Colorimetric Peptide Assay Kit. The Pierce™ Quantitative Fluorometric Peptide Assay cannot be used to measure TMT™/TMTpro-labeled peptide concentrations.

Data acquisition methods

Quantification of peptides labeled with TMT™ and TMTpro reagents requires an Orbitrap mass spectrometer. Resolving near-isobaric reporter ions in MS/MS spectra requires a resolving power of >50,000 at 150 m/z. Higher-energy collision dissociation (HCD) is recommended for TMT™/TMTpro reporter ion fragmentation. Optimal HCD fragmentation energy is instrument-dependent and can be optimized using TMTzero and TMTpro™ Zero reagents.

The peptide mass modification imparted by TMT™ and TMTpro reagents differs and is in the UNIMOD database (**https://www.unimod.org**). Proteome Discoverer™ Software (version 2.4 and above) is recommended for TMT™/TMTpro multiplex quantification.

Troubleshooting

Observation	Possible cause	Recommended action
Poor labeling	A primary amine-based buffer was used (for example Tris or glycine).	Use non-primary amine-based buffers (for example TEAB, HEPES).
	Sample buffer pH was not correct.	Ensure that the sample pH during labeling is 8.0–8.5.
	Too much sample was used.	Label 1–10 µg of sample per well of TMT™ or TMTpro reagent.
	Incubation time was too short.	Increase reaction incubation time.
	Sample concentration was too low.	Increase sample concentration.
		Increase tag to sample ratio.
	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 sample recovery after cleanup using reversed-phase SPE resin	Low peptide binding efficiency because of DMSO concentration.	Dry samples completely in SpeedVac before cleanup.
		Dilute sample volume to a DMSO concentration of ≤5%.
		Increase SPE resin amount used for cleanup.
		Use EasyPep™ peptide cleanup columns.
Poor protein quantitation	Incorrect instrument method used.	Optimize TMT™ or TMTpro reporter ion MS/MS fragmentation.
	Insufficient sample was analyzed.	Increase sample amount and optimize ion injection time.
	Chromatography was poor.	Optimize the LC gradient to maximize the MS/MS of unique peptides.
	Peptides were co-isolated during MS/MS acquisition.	Reduce sample complexity by prefractionating peptides.
		Decrease quadrupole isolation width.
		Use an SPS-MS3 acquisition method.

Related products

Item	Source
TMTpro Zero Reagent	A44518 or A44519
TMTzero Label Reagent	90067

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. MAN0029523 A.0

Revision	Date	Description
A.0	21 August 2023	New document for TMT™ and TMTpro 96-well Plates.