INSTRUCTIONS

TMT Mass Tagging Reagents

Pub. No. MAN0015866
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90308 90309

Number	Description
90308	**TMTsixplex Isobaric Label Reagent Set (96 rxn),** sufficient reagents for 16 sixplex isobaric experiments **Contents:** **TMT^{6}-126 Label Reagent,** 16 × 0.2mg **TMT^{6}-127 Label Reagent,** 16 × 0.2mg **TMT^{6}-128 Label Reagent,** 16 × 0.2mg **TMT^{6}-129 Label Reagent,** 16 × 0.2mg **TMT^{6}-130 Label Reagent,** 16 × 0.2mg **TMT^{6}-131 Label Reagent,** 16 × 0.2mg
90309	**TMT10plex Isobaric Label Reagent Set (80 rxn),** sufficient reagents for eight 10plex isobaric experiments **Contents:** **TMT^{10}-126 Label Reagent,** 8 × 0.2mg **TMT^{10}-127N Label Reagent,** 8 × 0.2mg **TMT^{10}-127C Label Reagent,** 8 × 0.2mg **TMT^{10}-128N Label Reagent,** 8 × 0.2mg **TMT^{10}-128C Label Reagent,** 8 × 0.2mg **TMT^{10}-129N Label Reagent,** 8 × 0.2mg **TMT^{10}-129C Label Reagent,** 8 × 0.2mg **TMT^{10}-130N Label Reagent,** 8 × 0.2mg **TMT^{10}-130C Label Reagent,** 8 × 0.2mg **TMT^{10}-131 Label Reagent,** 8 × 0.2mg

Storage: Upon receipt store at -20°C. Reagents are shipped with dry ice.

Note: These products are for research use only – do not use for diagnostic procedures.

Contents

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | thermofisher.com

Introduction

The Thermo Scientific™ TMT™ Mass Tagging Reagents enable multiplex relative quantitation by mass spectrometry (MS). Each mass-tagging 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. The reagent set can be used to label up to ten different peptide samples prepared from cells or tissues. For each sample, a unique reporter mass (i.e., 126-131Da) in the low mass region of the MS/MS spectrum is used to measure relative protein expression levels during peptide fragmentation.

The Thermo Scientific™ TMT10plex™ Label Reagents share an identical structure with Thermo Scientific™ TMT sixplex Reagents but contain different numbers and combinations of ^{13}C and ^{15}N isotopes in the mass reporter. The different isotopes result in a 10plex set of tags that have monoisotopic mass differences in the reporter that can be detected using high resolution Thermo Scientific™ Orbitrap™ mass spectrometry instruments. Advantages of the TMT Mass Tagging Reagents include increased sample multiplexing for relative quantitation, increased sample throughput and fewer missing quantitative channels among samples.

Procedure Summary

Protein extracts isolated from cells or tissues are reduced, alkylated and digested overnight. Samples are labeled with the TMT Reagents and then mixed before sample fractionation and clean-up. Labeled samples are analyzed by high resolution Orbitrap LC-MS/MS before data analysis to identify peptides and quantify reporter ion relative abundance (Figure 1).

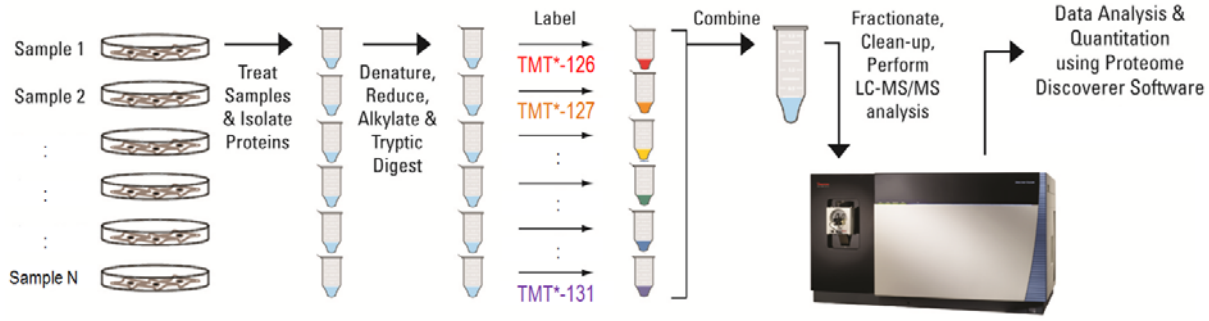

Figure 1. Schematic for using the Thermo Scientific TMT Isobaric Mass Tagging Reagents.

Important Product Information

- The TMT Reagents are moisture-sensitive. To avoid moisture condensation onto the product, tube rack containing the reagent vials must be equilibrated to room temperature before opening. Remaining unopened reagent vials should be stored at -20°C in the foil pouch with desiccant.
- Anhydrous acetonitrile is the recommended solvent to dissolve reagents. Stock solutions are stable for one week when stored at -20°C. For long term storage of unused reagent, remove all solvent by drying and store with desiccant at -20°C. Anhydrous ethanol can be used as an alternative solvent to dissolve reagents but is not recommended for stock solution storage.
- The TMT Reagents are amine-reactive and modify lysine residues and the peptide N-termini. All amine-containing buffers and additives (i.e. Tris, glycine, etc.) should be avoided or removed before digestion and labeling.
- All samples must be digested, labeled and then mixed equally before desalting, fractionation and LC-MS/MS. For optimal results, use 10-25µg of peptide for each labeling reaction.
- TMT Reagent vials can be placed in a 0.6mL microcentrifuge tube rack for easier handling. Vials can also be placed in a 2mL microcentrifuge tube if centrifugation is required.
- 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.

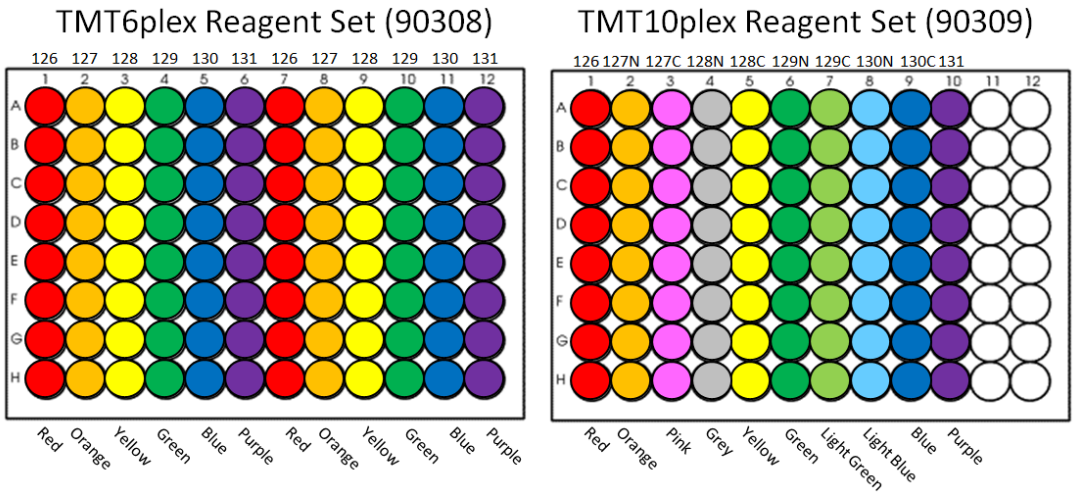

Figure 2. Layout of Thermo Scientific TMTsixplex and TMT10plex Isobaric Mass Tagging Reagents.

Additional Materials Required

- Low protein binding microcentrifuge tubes (e.g., Thermo Scientific™ Low Protein Binding Collection Tubes, Product No. 90410)
- Anhydrous acetonitrile (Thermo Scientific™ Acetonitrile HPLC grade, Product No. 51101)
- Water, LC-MS Grade (Product No. 51140)
- 1M Triethylammonium bicarbonate (TEAB) (Product No. 90114)
- 10% SDS solution (e.g. UltraPure™ SDS solution, Product No. 15553027 or Fisher Bioreagents™ SDS solution, Product No. BP2436200)
- 0.5M TCEP (e.g. Thermo Scientific™ Bond-Breaker™ TCEP Solution, Neutral pH, Product No. 77720)
- Iodoacetamide (Thermo Scientific™ Pierce™ Iodoacetamide, Single-Use, Product No. 90034)
- 50% Hydroxylamine (Product No. 90115)
- Chilled (-20°C) acetone
- 50mM acetic acid
- Protein assay (e.g., Thermo Scientific™ BCA Protein Assay Kit, Product No. 22235)
- 75-300µm capillary C_{18} reversed-phase column
- High-resolution Orbitrap Mass Spectrometer with online or offline liquid chromatography (LC) system
- Data analysis software such as Thermo Scientific™ Proteome Discoverer™ or Mascot™ Software
- Optional: Peptide assay (e.g., Thermo Scientific™ Pierce™ Quantitative Fluorescent Peptide Assay, Product No. 23290) or Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay, Product No. 23275)
- Optional: C18 spin tips or columns (e.g., Thermo Scientific™ Pierce™ C18 Spin Columns, Product No. 89870 or Pierce™ C18 Tips, Product No. 87784)

Material Preparation

50mM TEAB (triethyl ammonium bicarbonate)	Add 500µL of 1M TEAB to 4.75mL of ultrapure water.
Lysis Buffer	Add 200µL of 10% SDS solution to 1.8mL of 50mM TEAB.
200mM TCEP	Add 70µL of 0.5M TCEP to 70µL of ultrapure water. Then add 35µL of 1M TEAB.
375mM iodoacetamide	Dissolve 9mg of iodoacetamide with 100µL of 50mM TEAB. Make fresh and protect from light.
5% Hydroxylamine	Add 50µL of 50% hydroxylamine to 450µL of 50mM TEAB.

Preparing and Labeling Peptides with the TMT Isobaric Mass Tags

Note: The protocol below is adapted from the Thermo Scientific™ Pierce™ Mass Spec Sample Prep Kit for Cultured Cells (Product No. 84840) to prepare 25µg of peptide digest per sample condition for TMT reagent labeling. All steps can be scaled to accommodate more or less sample. Use 10-25µg of protein per labeling reaction.

A. Preparing Whole Cell Protein Extracts

1. Culture cells to harvest at least 25µg of protein per condition. For best results, culture a minimum of 5×10^5 cells.

   Note: Rinse cells 2-3 times with 1X PBS to remove cell culture media. Pellet cells using low-speed centrifugation (i.e., < 1000 × *g*) to prevent premature cell lysis.

2. Lyse the cells by adding five cell-pellet volumes of Lysis Buffer (i.e., 50µL of Lysis Buffer for a 10µL cell pellet).

   Note: Lysis buffers such as 8M urea (Product No. 29700) in 50mM TEAB or HEPES buffer, pH 8 may be used as alternative denaturing cell lysis buffers. For urea-based lysis buffer, protein samples must be diluted to < 1M urea before digestion, and the final C18 desalting step (C.8) is not optional. Addition of protease and/or phosphatase inhibitors during lysis is optional, but addition of phosphatase inhibitors is recommended.

   Note: Depending on the Lysis Buffer used it may be necessary to reduce sample viscosity by shearing DNA using a microtip sonicator or addition of a nuclease (e.g., Thermo Scientific™ Pierce™ Universal Nuclease for Cell Lysis, Product No. 88700)

3. Centrifuge lysate at 16,000 × *g* for 10 minutes at 4°C.
4. Carefully separate the supernatant and transfer into a new tube.
5. Determine the protein concentration of the supernatant using established methods such as the BCA Protein Assay Kit (Product No. 23227).

   Note: Use samples at ≥1mg/mL. Less concentrated samples may be used; however, it might be necessary to use larger volumes of reducing/alkylating reagents.

6. Transfer 25µg per condition (i.e., six each for the TMTsixplex Label Reagents) into a new microcentrifuge tube and adjust to a final volume of 25µL with 50mM TEAB.
7. Add 1.5µL of the 200mM TCEP and incubate sample at 55°C for 1 hour.
8. Immediately before use, dissolve one tube of iodoacetamide (9mg) with 132µL of 50mM TEAB to make 375mM iodoacetamide. Protect solution from light.
9. Add 1.5µL of the 375mM iodoacetamide to the sample and incubate for 30 minutes protected from light at room temperature.
10. Add six volumes (~180µL) of pre-chilled (-20°C) acetone and freeze at -20°C. Allow the precipitation to proceed for at least 4 hours up to overnight.

    Note: Methanol/chloroform is the recommended solvent for precipitation of proteins derived from tissue extracts.

11. Centrifuge the samples at 8000 × *g* for 10 minutes at 4°C. Carefully remove the acetone without disturbing the white pellet. Allow the pellet to dry for 2-3 minutes.

B. Protein Digestion

1. Resuspend 25µg of acetone-precipitated (or lyophilized) protein pellets with 25µL of 50mM TEAB.

   Note: An acetone-precipitated pellet might not completely dissolve; however, after proteolysis at 37°C, all the protein (peptides) will be solubilized.

2. Immediately before use, add 40µL of the 50mM acetic acid (i.e., Trypsin Storage Solution) to 20µg lyophilized trypsin to reconstitute trypsin at 0.5µg/µL. Store any remaining trypsin in single-use aliquots at -80°C.

3. Add 1µL of trypsin (i.e., 0.5µg) per 25µg of protein (i.e., 1:50 (w:w)) and digest the sample 4-16 hours at 37°C.

4. Optional: Measure protein digest concentration using Thermo Scientific™ Pierce™ Quantitative Fluorescent Peptide Assay (Product No. 23290) or Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay (Product No. 23275).

C. Peptide Labeling

1. Immediately before use, equilibrate the TMT Label Reagents inside the foil pouch with desiccant to room temperature. Return any unused reagent to foil pouch with desiccant and store at -20°C until use.

2. For the 0.2mg vials, add 20µL of anhydrous acetonitrile to the bottom of each tube.

   Note: Reagents dissolved in anhydrous acetonitrile are stable for one week when stored at -20°C. Anhydrous ethanol can be used as an alternative solvent to dissolve reagents but is not recommended for stock solution storage.

3. Carefully add 25µl sample (10-25µg protein digest) to each 20µL of reconstituted TMT Label Reagent. Alternatively, TMT reagents can be added to the reduced and alkylated protein digest in a low protein binding microcentrifuge tube.

   Note: Labeling more than 25µg protein digest per reaction requires additional TMT Label Reagent. Labeling less than 10µg protein digest requires proportionately less TMT reagent. Labeling < 1µg protein digest is not recommended.

4. Incubate the reaction for 1 hour at room temperature.

5. Add 1µL of 5% hydroxylamine to the sample and incubate for 15 minutes to quench the reaction.

6. Combine samples at equal amounts in a new low protein binding microcentrifuge tube and speed vac to remove solvent and buffer. Store labeled peptides at -80°C or proceed with optional C18 clean up.

   Note: TMT-labeled peptide concentration can be measured using Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay. The Thermo Scientific™ Pierce™ Quantitative Fluorescent Peptide Assay cannot be used to measure TMT-labeled peptide concentrations.

7. Optional: Clean-up samples with C18 spin tips (Product No. 87784) or columns (Product No. 89870) before LC-MS analysis. Fractionation of labeled peptides using Thermo Scientific™ Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (Product No. 84868) is recommended before LC-MS analysis to increase the number of peptide identifications.

Troubleshooting

Problem	Possible Cause	Solution
Poor labeling	An amine-based buffer was used	Use a non-amine-based buffer such as TEAB or HEPES for labeling reactions
	Incorrect buffer pH	Make sure the buffer pH is 8-8.5
	Too much sample was used	Label 10-25µg per sample
	TMT reagent is hydrolyzed	Store unused reagent in foil pouch with desiccant at -20°C.
Protein precipitation	Lack of detergent present	Add detergent, such as 0.05% SDS to the preparation
	pH decreased	Make sure the pH is > 7.5

Additional Information

A. Data Acquisition Methods

Quantitation of peptides labeled with Thermo Scientific™ Tandem Mass Tag™ Reagents requires a mass spectrometer capable of MS/MS fragmentation, such as an ion trap, quadrupole time of flight, time of flight-time of flight (TOF-TOF) or triple quadrupole instrument. Higher energy collision dissociation (HCD) is recommended for TMT reporter ion fragmentation. Optimal HCD fragmentation energy is instrument-dependent and can be optimized using TMTzero Reagents. Electron transfer dissociation (ETD) may be used as an alternative fragmentation method for peptide identification and quantitation. The choice of MS/MS fragmentation method(s) depends on the instrument capabilities such as collisionally induced dissociation (CID), pulsed-Q dissociation (PQD), higher energy collisional dissociation (HCD), or electron transfer dissociation (ETD). TMT Reagent reporter ions are not visible in ion traps following traditional CID fragmentation.

Table 1. Instruments and MS/MS fragmentation options for peptide identification and quantitation with Thermo Scientific TMT Reagents.

Instrument	Fragmentation Method	Reference(s)
Thermo Scientific Orbitrap™ Fusion™ Tribrid™ Mass Spectrometer	HCD/SPS-MS3	McAllister, G.C., *et al*. (2014), Viner, *et al*. (2013)
Thermo Scientific Orbitrap Elite™ Mass Spectrometer	HCD/MS3	McAllister, G.C., *et al*. (2012), Viner, *et al*. (2012)
Thermo Scientific Q Exactive™ Mass Spectrometer	HCD/MS2	Wühr, *et al*. (2012)
Thermo Scientific Orbitrap Velos Pro™, LTQ-Orbitrap™ XL, or MALDI-Orbitrap™ XL Mass Spectrometer	HCD/MS2	Ting, *et al*. (2011), Wenger, *et al* (2011), Schirle, *et al*. (2012), Lee, et al (2011), Xiong, *et al*. (2011), Strupat, *et al*. (2008)
Thermo Scientific™ Velos Pro™ ion trap	Trap HCD/MS2	Biringer, *et al*. (2011)
Thermo Scientific Orbitrap Elite ETD, Velos Pro ETD, LTQ-Orbitrap XL ETD	HCD/MS2 or ETD/MS2	Viner, *et al*. (2009)

B. Data Analysis and Quantitation

The masses for peptide modification by the TMTsixplex and TMT10plex reagents are the same as listed below. Several software packages directly support the modifications by TMT Reagents and the relative quantitation of reporter ions released from labeled peptides, including Thermo Scientific™ Proteome Discoverer™ 2.1 and above, Matrix Science Mascot™ 2.5 and above, MaqQuant and Proteome Software Scaffold™ Q+. For data acquired using a combination of fragmentation methods (i.e., HCD/MS3 or HCD/ETD), Proteome Discoverer may be necessary to merge spectra for identification and quantitation.

Table 2. Modification masses of the Thermo Scientific TMT Label Reagents.

Label Reagent	Label Reagent	Modification Mass (monoisotopic)	Modification Mass (average)	HCD Monoisotopic Reporter Mass*	ETD Monoisotopic Reporter Mass**
TMT^{10}-126	TMT^{6}-126	229.162932	229.2634	126.127726	114.127725
TMT^{10}-127N	TMT^{6}-127	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	TMT^{6}-128	229.162932	229.2634	128.134436	116.134433
TMT^{10}-129N	TMT^{6}-129	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	TMT^{6}-130	229.162932	229.2634	130.141145	118.141141
TMT^{10}-131	TMT^{6}-131	229.162932	229.2634	131.138180	119.138176

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

Related Thermo Scientific Products

90114	**1M Triethylammonium bicarbonate (TEAB),** 50mL
90115	**50% Hydroxylamine,** 5mL
90067	**TMTzero Label Reagent,** 5 × 0.8mg
90061	**TMTsixplex Isobaric Label Reagent Set,** 1 × 0.8mg
90064	**TMTsixplex Isobaric Mass Tagging Kit**
90110	**TMT10plex Isobaric Label Reagent Set,** 10 × 0.8mg
90113	**TMT10plex Isobaric Mass Tag Labeling Kit**
90406	**TMT10plex Isobaric Label Reagent Set,** 10 × 5mg
90114	**1M Triethylammonium bicarbonate (TEAB),** 50mL
90115	**50% Hydroxylamine,** 5mL
90100	**iodoTMTzero Label Reagent,** 5 × 0.2mg
90101	**iodoTMTsixplex Label Reagent Set,** 1 × 0.2mg
90103	**iodoTMTsixplex Isobaric Mass Tag Labeling Kit**
90076	**Immobilized Anti-TMT Antibody Resin**
90075	**Anti-TMT Antibody,** 0.1mL
90104	**TMT Elution Buffer,** 20mL
84840	**Pierce Mass Spec Sample Prep Kit for Cultured Cells**
23227	**BCA Protein Assay Kit**
23275	**Pierce Quantitative Colorimetric Peptide Assay**
23290	**Pierce Quantitative Fluorescent Peptide Assay**
90057	**Pierce Trypsin Protease, MS Grade**
90051	**Pierce™ Lys-C Protease, MS Grade**
88300	**Pierce™ Fe-NTA Phosphopeptide Enrichment Kit**
88301	**Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit**
84868	**Pierce™ High pH Reversed-Phase Peptide Fractionation Kit**
88321	**Pierce Peptide Retention Time Calibration Mixture,** 200µL
87784	**Pierce C18 Tips, 100µL bed,** 96 tips
89870	**Pierce C18 Spin Columns,** 25 columns
28904	**Trifluoroacetic Acid, Sequanal Grade**

General References

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