INSTRUCTIONS

TMT10plex Mass Tag Labeling Kits and Reagents

90110 90111 90406 90113

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Number	Description
90110	**TMT10plex™ Isobaric Label Reagent Set,** sufficient reagents for one 10plex isobaric experiment **Contents:** **TMT^{10}-126™ Label Reagent,** 1 × 0.8mg **TMT^{10}-127N™ Label Reagent,** 1 × 0.8mg **TMT^{10}-127C™ Label Reagent,** 1 × 0.8mg **TMT^{10}-128N™ Label Reagent,** 1 × 0.8mg **TMT^{10}-128C™ Label Reagent,** 1 × 0.8mg **TMT^{10}-129N™ Label Reagent,** 1 × 0.8mg **TMT^{10}-129C™ Label Reagent,** 1 × 0.8mg **TMT^{10}-130N™ Label Reagent,** 1 × 0.8mg **TMT^{10}-130C™ Label Reagent,** 1 × 0.8mg **TMT^{10}-131™ Label Reagent,** 1 × 0.8mg
90111	**TMT10plex Isobaric Label Reagent Set,** sufficient reagents for three 10plex isobaric experiments **Contents:** **TMT^{10}-126 Label Reagent,** 3 × 0.8mg **TMT^{10}-127N Label Reagent,** 3 × 0.8mg **TMT^{10}-127C Label Reagent,** 3 × 0.8mg **TMT^{10}-128N Label Reagent,** 3 × 0.8mg **TMT^{10}-128C Label Reagent,** 3 × 0.8mg **TMT^{10}-129N Label Reagent,** 3 × 0.8mg **TMT^{10}-129C Label Reagent,** 3 × 0.8mg **TMT^{10}-130N Label Reagent,** 3 × 0.8mg **TMT^{10}-130C Label Reagent,** 3 × 0.8mg **TMT^{10}-131 Label Reagent,** 3 × 0.8mg
90406	**TMT10plex Isobaric Label Reagent Set**, sufficient reagents for one 10plex isobaric experiment **Contents:** **TMT^{10}-126 Label Reagent,** 1 × 5mg **TMT^{10}-127N Label Reagent,** 1 × 5mg **TMT^{10}-127C Label Reagent,** 1 × 5mg **TMT^{10}-128N Label Reagent,** 1 × 5mg **TMT^{10}-128C Label Reagent,** 1 × 5mg **TMT^{10}-129N Label Reagent,** 1 × 5mg **TMT^{10}-129C Label Reagent,** 1 × 5mg **TMT^{10}-130N Label Reagent,** 1 × 5mg **TMT^{10}-130C Label Reagent,** 1 × 5mg **TMT^{10}-131 Label Reagent,** 1 × 5mg

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90113 **TMT10plex Isobaric Mass Tag Labeling Kit,** sufficient reagents for three 10plex isobaric experiments

Contents:

TMT^{10}-126 Label Reagent, 3 × 0.8mg
TMT^{10}-127N Label Reagent, 3 × 0.8mg
TMT^{10}-127C Label Reagent, 3 × 0.8mg
TMT^{10}-128N Label Reagent, 3 × 0.8mg
TMT^{10}-128C Label Reagent, 3 × 0.8mg
TMT^{10}-129N Label Reagent, 3 × 0.8mg
TMT^{10}-129C Label Reagent, 3 × 0.8mg
TMT^{10}-130N Label Reagent, 3 × 0.8mg
TMT^{10}-130C Label Reagent, 3 × 0.8mg
TMT^{10}-131 Label Reagent, 3 × 0.8mg
Dissolution Buffer (1M triethyl ammonium bicarbonate), 5mL
Denaturing Reagent (10% SDS), 1mL
Reducing Reagent (0.5M TCEP), 1mL
Iodoacetamide, 12 × 9mg
Quenching Reagent (50% hydroxylamine), 1mL
Pierce Trypsin Protease, MS Grade, 5 × 20µg
Trypsin Storage Solution, 250µL
Albumin, Bovine, 2.5mg

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

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

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Introduction

The Thermo Scientific™ TMT™ Mass Tag Labeling Kits and 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 (Figure 1). 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., TMT^{10} 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™ TMTzero™ and TMTsixplex™ 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 TMT10plex Label Reagents include increased sample multiplexing for relative quantitation, increased sample throughput and fewer missing quantitative channels among samples.

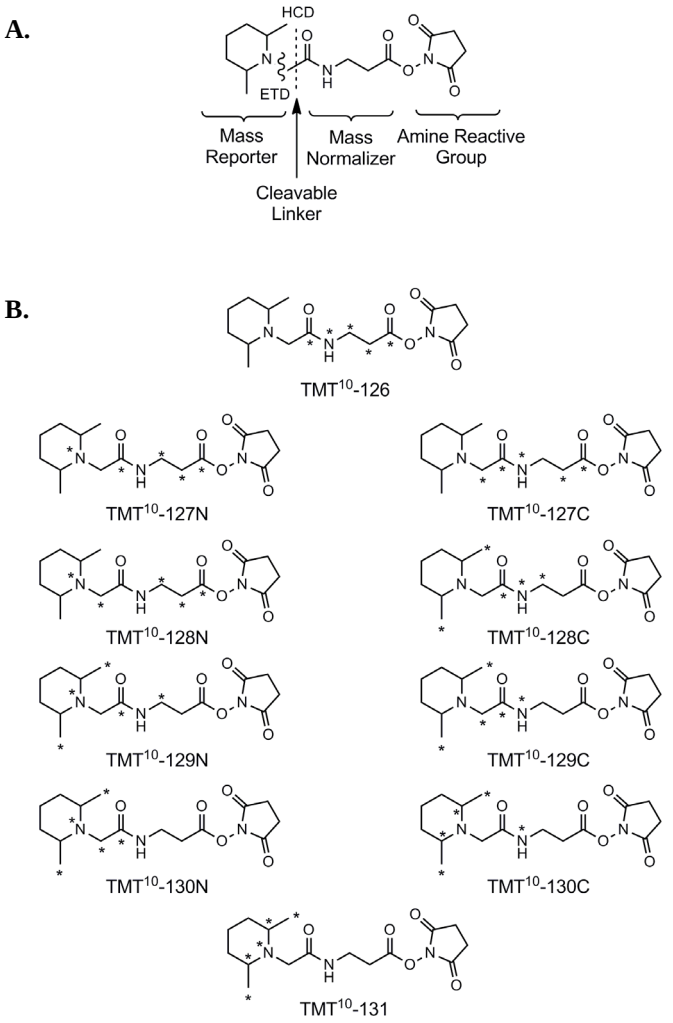

Figure 1. Chemical structures of the Thermo Scientific TMT10plex Label Reagents. A. Functional regions of the reagent structure including MS/MS fragmentation sites by higher energy collision dissociation (HCD) and electron transfer dissociation (ETD). B. TMT10plex Reagent structures and isotope positions (*).

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 2).

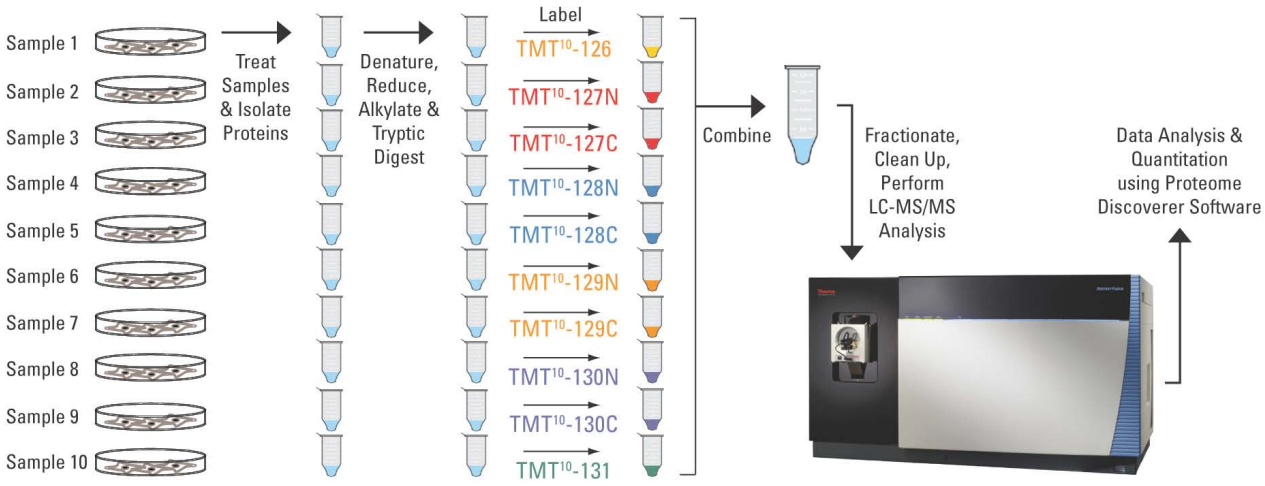

Figure 2. Procedure schematic for using the Thermo Scientific TMT10plex Label Reagents.

Important Product Information

- The TMT Reagents are moisture-sensitive. To avoid moisture condensation onto the product, the vial must be equilibrated to room temperature before opening.
- The TMT Reagents are amine-reactive and modify lysine residues and peptide N-termini. All amine-containing buffers and additives must be 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 25-100µg of peptide for each labeling reaction.
- 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.
- The TMTzero Label Reagent (Product No. 90067) can be used to optimize methods before multiplexed analysis of samples with TMT10plex Label Reagent sets.

Additional Materials Required

- Microcentrifuge tubes
- Anhydrous acetonitrile (Acetonitrile, LC-MS Grade, Product No. 51101)
- Water, LC-MS Grade (Product No. 51140)
- Chilled (-20°C) acetone
- 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 liquid chromatography system (see Table 1 for recommended instruments)
- Data analysis software (e.g., Thermo Scientific™ Proteome Discoverer™ Software)
- 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

Note: The 50% hydroxylamine and 10% SDS stock solutions provided with the kit may precipitate during storage. Warm both solutions to room temperature and vortex before use. The amounts listed below are sufficient for preparing and labeling 10 samples.

100mM TEAB (triethyl ammonium bicarbonate)	Add 500µL of the Dissolution Buffer (1M TEAB) to 4.5mL of ultrapure water.
Lysis Buffer	Add 200µL of the Denaturing Reagent (10% SDS) to 1.8mL of 100mM TEAB.
200mM TCEP	Add 70µL of the Reducing Reagent (0.5M TCEP) to 70µL of ultrapure water. Then add 35µL of the Dissolution Buffer (1M TEAB).
5% Hydroxylamine	Add 50µL of the Quenching Reagent (50% hydroxylamine) to 450µL of 100mM TEAB.

Preparing and Labeling Peptides with the TMT Isobaric Mass Tags

Note: BSA can be used as a control sample for method optimization. Dissolve BSA to 1mg/mL using 100mM TEAB. Use 25-100µg of protein per labeling reaction. The Thermo Scientific™ Pierce™ Mass Spec Sample Prep Kit for Cultured Cells can also be used to prepare peptide digests for TMT reagent labeling.

A. Preparing Whole Cell Protein Extracts

1. Culture cells to harvest at least 100µg of protein per condition. For best results, culture a minimum of 2×10^6 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., 100µL of Lysis Buffer for a 20µL cell pellet).

   Note: Lysis buffers such as Thermo Scientific™ RIPA Lysis and Extraction Buffer (Product No. 89901) or 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.6) is not optional. Addition of protease and/or phosphatase inhibitors during lysis is optional and may interfere with MS analysis.

   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 ≥ 2mg/mL. Less concentrated samples may be used; however, it might be necessary to use larger volumes of reducing/alkylating reagents.

6. Transfer 100µg per condition into a new tube and adjust to a final volume of 100µL with 100mM TEAB.
7. Add 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 100mM TEAB to make 375mM iodoacetamide. Protect solution from light.
9. Add 5µL of the 375mM iodoacetamide to the sample and incubate for 30 minutes protected from light at room temperature.
10. Add six volumes (~600µL) of pre-chilled (-20°C) acetone. Allow the precipitation to proceed overnight.
11. Centrifuge the samples at 8000 × *g* for 10 minutes at 4°C. Carefully invert the tubes to decant the acetone without disturbing the white pellet. Allow the pellet to dry for 2-3 minutes.

B. Protein Digestion

1. Resuspend 100µg of acetone-precipitated (or lyophilized) protein pellets with 100µL of 100mM TEAB.

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

2. Immediately before use, add 20µL of the Trypsin Storage Solution to the bottom of the trypsin glass vial and incubate for 5 minutes. Store any remaining reagent in single-use volumes at -80°C (e.g., 2.5µg of trypsin per 100µg of protein).

3. Add 2.5µL of trypsin (i.e., 2.5µg) per 100µg of protein. Digest the sample overnight at 37°C.

C. Peptide Labeling

1. Immediately before use, equilibrate the TMT Label Reagents to room temperature. For the 0.8mg vials, add 41µL of anhydrous acetonitrile to each tube. For the 5mg vials, add 256µL of solvent to each tube. Allow the reagent to dissolve for 5 minutes with occasional vortexing. Briefly centrifuge the tube to gather the solution.

   Note: Reagents dissolved in anhydrous acetonitrile are stable for one week when stored at -20°C and warmed to room temperature before opening. Anhydrous ethanol can be used as an alternative solvent to dissolve reagents.

2. Carefully add 41µL of the TMT Label Reagent to each 25-100µg sample. Alternatively, transfer the reduced and alkylated protein to the TMT Reagent vial.

   Note: A 100µL glass syringe or positive displacement pipette may be necessary to accurately measure and dispense TMT Reagents in volatile acetonitrile solvent.

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

4. Add 8µL of 5% hydroxylamine to the sample and incubate for 15 minutes to quench the reaction.

5. Combine samples in a new microcentrifuge tube at equal amounts and store at -80°C.

6. Optional: Clean-up samples with C18 spin tips (Product No. 87784) or columns (Product No. 89870) before high-resolution LC-MS analysis. Peptide clean up is recommended before LC-MS analysis but is not required.

Troubleshooting

Problem	Possible Cause	Solution
Poor labeling	A primary amine-based buffer was used (e.g., Tris, glycine)	Use non-primary amine-based buffers (e.g., TEAB, HEPES)
	Incorrect buffer pH	Make sure the buffer pH is ~8.0
	Too much sample was used	Label 25-100µg per sample
Protein precipitation	Lack of detergent present	Add detergent (e.g., 0.05-0.1% SDS) to the preparation
	pH decreased	Make sure pH is > 7.5

Additional Information

A. Data Acquisition Methods

Quantitation of peptides labeled with Thermo Scientific™ Tandem Mass Tag™ Reagents requires a high-resolution Orbitrap Mass Spectrometer capable of MS/MS fragmentation (Table 1). 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 TMT10plex 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; however, ETD is not recommended for TMT10plex Reagents because of reporter ion overlap (Table 2).

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

Instrument	Fragmentation Method	Minimum Resolution Setting	Reference(s)
Thermo Scientific Orbitrap Fusion™ Tribrid™ Mass Spectrometer	HCD/SPS-MS3	60,000	Viner, *et al.* (2013)
Thermo Scientific Orbitrap Elite™ Mass Spectrometer	HCD/MS3	30,000	Viner, *et al.* (2012)
Thermo Scientific Q Exactive™ Mass Spectrometer	HCD/MS2	35,000	Wühr, *et al.* (2012)
Thermo Scientific Orbitrap Velos Pro Mass Spectrometer	HCD/MS2	30,000	Ting, *et al.* (2011), Wenger, *et al.* (2011)

B. Data Analysis and Quantitation

The peptide mass modification by the TMT10plex Reagents is identical to TMTsixplex Reagents and present in the UNIMOD database (*www.unimod.org*) and are listed below. Proteome Discoverer Software (1.4 and above) is recommended for TMT10plex relative quantitation. Additional software programs that may be used for TMT quantitation include Matrix Science™ Mascot™ Software (2.2 and above) 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 search results.

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	126.127725	114.127725
TMT^{10}-127N	TMT^{6}-127	229.162932	229.2634	127.124760	115.124760
TMT^{10}-127C	-	229.162932	229.2634	127.131079	114.127725
TMT^{10}-128N	-	229.162932	229.2634	128.128114	115.124760
TMT^{10}-128C	TMT^{6}-128	229.162932	229.2634	128.134433	116.134433
TMT^{10}-129N	TMT^{6}-129	229.162932	229.2634	129.131468	117.131468
TMT^{10}-129C	-	229.162932	229.2634	129.137787	116.134433
TMT^{10}-130N	-	229.162932	229.2634	130.134822	117.131468
TMT^{10}-130C	TMT^{6}-130	229.162932	229.2634	130.141141	118.141141
TMT^{10}-131	TMT^{6}-131	229.162932	229.2634	131.138176	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.

C. Information Available from Our Website

- Tech Tip #49: Acetone precipitation of proteins
- Tech Tip #19: Remove detergent from protein samples

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**
90100 **iodoTMTzero™ Label Reagent,** 5 × 0.2mg

90101	**iodoTMTsixplex™ Label Reagent Set,** 1 × 0.2mg
90103	**iodoTMTsixplex Isobaric Mass Tag Labeling Kit**
84840	**Pierce™ Mass Spec Sample Prep Kit for Cultured Cells**
23227	**BCA Protein Assay Kit**
90057	**Pierce Trypsin Protease, MS Grade**
90051	**Lys-C Protease, MS Grade**
88300	**Fe-NTA Phosphopeptide Enrichment Kit**
88301	**Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit**
89870	**Pierce C18 Spin Columns,** 25 columns
28904	**Trifluoroacetic Acid, Sequanal Grade**

General References

Altelaar A.F., *et al*. (2012). Benchmarking stable isotope labeling based quantitative proteomics. *J Proteomics* Oct 22. pii: S1874-3919(12)00704-X. doi: 10.1016/j.jprot.2012.10.009.

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McAllister, G.C., *et al*. (2012). Increasing the multiplexing capacity of TMTs using reporter ion isotopologues with isobaric masses. *Anal Chem* **84(17):**7469-78.

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Wenger, C.D., *et al*. (2011). Gas-phase purification enables accurate, multiplexed proteome quantification with isobaric tagging. *Nat Methods* **8(11):**933-5.

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Viner, R.I., *et al*. (2012). Relative quantitation of TMT-labeled proteomes – Focus on sensitivity and precision. Application note #566.

Viner, R.I., *et al*. (2009). Quantification of post-translationally modified peptides of bovine α-crystallin using tandem mass tags and electron transfer dissociation. J Proteomics 72(5):874-85.

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Zhang, T., *et al*. (2010). Improving quantitation of TMT-labeled peptides using stepped higher-energy collisional dissociation. Application note #483.