INSTRUCTIONS

# TMT Isobaric Mass Tagging Kits and Reagents

2073.0

| Number | Description |
|---|---|
| **90063** | **TMTduplex™ Isobaric Mass Tagging Kit**<br>**Contents:**<br>**TMT$^{0}$ Label Reagent,** 5 vials<br>**TMT$^{2}$-126 Label Reagent,** 5 vials<br>**TMT$^{2}$-127 Label Reagent,** 5 vials<br>**Dissolution Buffer** (1 M triethyl ammonium bicarbonate), 5 ml<br>**Denaturing Reagent** (10% SDS), 1 ml<br>**Reducing Reagent** (0.5 M TCEP), 1 ml<br>**Iodoacetamide,** 12 × 9 mg<br>**Quenching Reagent** (50% hydroxylamine), 1 ml<br>**Trypsin,** 2 × 20 µg<br>**Trypsin Storage Solution,** 250 µl<br>**Albumin, Bovine,** 2.5 mg |
| **90064** | **TMTsixplex™ Isobaric Mass Tagging Kit**<br>**Contents:**<br>**TMT$^{0}$ Label Reagent,** 5 vials<br>**TMT$^{6}$-126 Label Reagent,** 5 vials<br>**TMT$^{6}$-127 Label Reagent,** 5 vials<br>**TMT$^{6}$-128 Label Reagent,** 5 vials<br>**TMT$^{6}$-129 Label Reagent,** 5 vials<br>**TMT$^{6}$-130 Label Reagent,** 5 vials<br>**TMT$^{6}$-131 Label Reagent,** 5 vials<br>**Dissolution Buffer** (1 M triethyl ammonium bicarbonate), 5 ml<br>**Denaturing Reagent** (10% SDS), 1 ml<br>**Reducing Reagent** (0.5 M TCEP), 1 ml<br>**Iodoacetamide,** 12 × 9 mg<br>**Quenching Reagent** (50% hydroxylamine), 1 ml<br>**Trypsin,** 5 × 20 g<br>**Trypsin Storage Solution,** 250 µl<br>**Albumin, Bovine,** 2.5 mg |
| **90065** | **TMTduplex Label Reagent Set**<br>**Contents:**<br>**TMT$^{2}$-126 Label Reagent,** 5 vials<br>**TMT$^{2}$-127 Label Reagent,** 5 vials |

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| | |
|---|---|
| **90066** | **TMTsixplex Label Reagent Set** |
| | **Contents:** |
| | **TMT$^6$-126 Label Reagent**, 5 vials |
| | **TMT$^6$-127 Label Reagent,** 5 vials |
| | **TMT$^6$-128 Label Reagent,** 5 vials |
| | **TMT$^6$-129 Label Reagent,** 5 vials |
| | **TMT$^6$-130 Label Reagent,** 5 vials |
| | **TMT$^6$-131 Label Reagent,** 5 vials |
| **90067** | **TMTzero™ Label Reagent**, 5 vials |

**Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

**Note:** This product is for research use only. Do not use for diagnostic procedures.

## Introduction

The Thermo Scientific TMT Isobaric Mass Tagging Kits and Reagents enable quantitative labeling of proteins extracted from cells and tissues. Each isobaric tagging reagent is composed of an amine-reactive NHS-ester group, a spacer arm and an MS/MS reporter (Figure 1). The reagents label peptides prepared from distinct cell-based or tissue samples, either two samples for the duplex kit or six samples for the sixplex kit. For each sample, a unique reporter mass results in the MS/MS spectrum (i.e., 126-127 Da for TMT$^2$ and 126-131 Da for TMT$^6$ Label Reagents). These reporter ions are in the low mass region of the MS/MS spectrum and are used to report relative protein expression levels during peptide fragmentation. Each reagent in the duplex and sixplex set has the same nominal parent (precursor) mass; however, the masses differ in the balancing and reporter region. By balancing these two components, the intact molecule has the same mass. For example, for the duplex reagent a given peptide will be modified by 225 Da per amine residue. At the MS/MS level, the reporter ions will be either 126 or 127 Da (see the Additional Information Section). The mass balance is compensated by a neutral loss of the reporter ion.

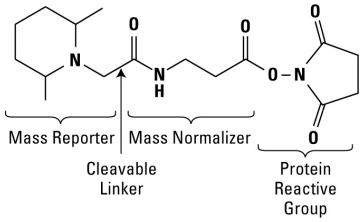


**Figure 1. Chemical structure of the TMT Label Reagents.**

## Procedure Summary

Protein extracts are isolated from cells grown in culture or from tissue samples. After removing amine- and thiol-based buffers, samples are reduced, alkylated and digested overnight. Samples are labeled with the TMT reagents and then mixed at the duplex or the sixplex level. Strong-cation exchange (SCX) fractionation simplifies complex samples before LC-MS/MS analysis. Data analysis software is used to analyze the reporter ions in the low mass region (Figure 2).

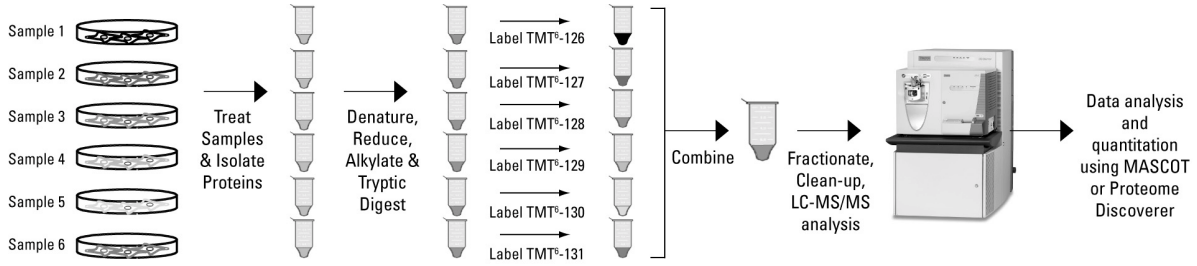


**Figure 2. Schematic for using the Thermo Scientific TMTsixplex Isobaric Mass Tagging Reagents.**

## Important Product Information

- The TMT reagents are amine-reactive and modify lysine residues and the 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 25-100 µg 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 cleaned work area cleaned with 70% methanol (Fisher Product No. A454-1).
- The TMTzero Label Reagent can be used to optimize methods before multiplexed analysis of samples with the TMTduplex or TMTsixplex Reagent Set.

## Additional Materials Required

- Acetonitrile (Thermo Scientific Acetonitrile HPLC grade, Product No. 51101)
- Glass syringe (100 µl)
- HPLC grade water (Fisher, Product No. W6-4)
- Cell lysis reagent such as Thermo Scientific M-PER® Mammalian Protein Extraction Reagent (Product No. 78501), RIPA Lysis and Extraction Buffer (Product No. 89901) or 8 M Urea (Product No. 29700)
- Protease inhibitors (Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail, EDTA-free, Product No. 78425)
- Phosphatase inhibitors (Thermo Scientific Halt Phosphatase Inhibitor Cocktail, Product No. 78420)
- Thermo Scientific Coomassie Plus (Bradford) Protein Assay (Product No. 23236)
- 75-300 µm capillary $C_{18}$ reversed phase column
- Ion trap or time-of-flight (TOF) mass spectrometer with online or offline liquid chromatography (LC) system
- Data analysis software such as Thermo Scientific Proteome Discoverer or Mascot Software (Matrix Science, Ltd.)

## Material Preparation

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|---|---|
| Albumin, Bovine (BSA) | Reconstitute BSA (2.5 mg) with 2.5 ml of ultrapure water. Divide solution into 100 µl aliquots and lyophilize to dryness. |
| 200 mM TEAB (triethyl ammonium bicarbonate) | Add 100 µl of the Dissolution Buffer (1 M TEAB) to 400 µl of ultrapure water. |
| 2% SDS | Add 50 µl of the Denaturing Reagent (10% SDS) to 200 µl of ultrapure water. |
| 200 mM TCEP | Add 70 µl of the Reducing Reagent (0.5 M TCEP) to 70 µl of ultrapure water. Then add 35 µl of the Dissolution Buffer (1 M TEAB). |
| 5% Hydroxylamine | Dilute the Quenching Reagent (50% hydroxylamine) 1:10 with 200 mM TEAB. |

## Procedure for Labeling with the TMT Isobaric Mass Tags

### A. Preparing Whole Cell Protein Extracts

1. Culture cells to harvest at least 100 µg per condition. For best results, culture a minimum of $5 \times 10^6$ cells.
2. Lyse cells in either RIPA buffer, M-PER Reagent or 8 M urea. Add protease and phosphatase inhibitors to the lysis reagent. Use 4 ml of lysis reagent for each milliliter of cells.
3. Determine the protein concentration using the Coomassie Plus (Bradford) Protein Assay.
4. Place 100 µg per condition (two for the TMTduplex or six for the TMTsixplex Label Reagents) in a polypropylene, 15 ml centrifuge tube and add six volumes of pre-chilled (-20°C) acetone. Allow the precipitation to proceed overnight.
5. Centrifuge the samples at $8{,}000 \times g$ 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 10 minutes.

### B. Protein Digestion

**Note:** Use 25-100 µg of purified or lyophilized protein per sample. If the protein is in solution, make sure it is free of amine-containing buffers. Use the BSA (100 µg) as a control sample for method optimization.

1. Add 5 µl of 2% SDS, 45 µl of 200 mM TEAB to the sample and adjust to a final volume of 100 µl with ultrapure water.

   **Note:** An acetone-precipitated pellet might not completely dissolve; however, after proteolysis at 37°C (Step B.6), all the protein will be solubilized.

2. Add 5 µl of the 200 mM TCEP and incubate sample at 55°C for 1 hour.
3. Immediately before use, dissolve one tube of Iodoacetamide (9 mg) with 132 µl of 200 mM TEAB to make 375 mM iodoacetamide. Protect solution from light.
4. Add 5 µl of the 375 mM iodoacetamide (with TEAB) to the sample and incubate for 30 minutes protected from light.
5. Immediately before use, add 20 µl 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 aliquots at -80°C (e.g., 2.5 µg of Trypsin per 100 µg of protein).
6. Add 2.5 µg of Trypsin (i.e., 2.5 µl) per 100 µg of protein. Digest the sample overnight at 30-37°C.

### C. Protein Labeling

1. After sample digestion and immediately before use, equilibrate the TMT Label Reagents to room temperature. Add 41 µl of anhydrous acetonitrile to each tube containing 0.8 mg of reagent. Allow the reagent to dissolve for 5 minutes with occasional vortexing. Briefly centrifuge the tube to gather the solution.
2. Using a 100 µl glass syringe add exactly 41 µl of the TMT Label Reagent to each sample. For example, if comparing two samples using the duplex set, add the TMT$^2$-126 Label Reagent to the test sample and TMT$^2$-127 Label Reagent to the control sample or vice versa.
3. Incubate the reaction for 1 hour at room temperature.
4. Add 8 µl of 5% hydroxylamine to the sample and incubate for 15 minutes.
5. Combine samples at equal amounts or store isolated at -80°C.

## Sample Cleanup and Fractionation

For best results, use an HPLC system to perform strong cation exchange fractionation to remove the anionic SDS and fractionate complex proteomic extracts. Perform the separation with a Strong-Cation Exchange Column (PolyLC, Inc., Table 1). Alternatively, cleanup samples using the TopTip™ Strong Cation-Exchange Tips (PolyLC, Product No. TT1000SEA-2015), according to the manufacturer's instructions.

**Table 1. Strong-cation exchange column information.**

| PolyLC Part # | Column | Particle | Pore | Load Range | Flow Range |
|---|---|---|---|---|---|
| 102SE0503 | 2.1 × 100 | 5 µm | 300 Å | 0.1-1.0 mg | 0.14-0.2 ml/min |
| 104SE0503 | 4.2 × 100 | 5 µm | 300 Å | 0.4-4.0 mg | 0.7-1.0 ml/min |

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Poor labeling | An amine-based buffer was used | Use a non-amine-based buffer |
| | Incorrect buffer pH | Make sure the buffer pH is ~8.0 |
| 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. Mass Modification

Each reagent in the duplex and sixplex sets has the same nominal parent (precursor) mass; however, the masses differ in the balancing and reporter region. By balancing these two components, the intact molecule has the same mass. For example, for the duplex reagent a given peptide will be modified by 225 Da per amine residue. At the MS/MS level, the reporter ions will be either 126 or 127 Da (Table 2). The mass balance is compensated by a neutral loss of the reporter ion.

**Table 2. Modification masses of the TMT Label Reagents.**

| Label Reagent | Modification Mass (monoisotopic) | Modification Mass (average) | Monoisotopic Reporter Mass | Average Reporter Mass |
|---|---|---|---|---|
| $TMT^{0}$-126 | 224.1525 | 224.2994 | 126.1283 | 126.2193 |
| $TMT^{2}$-126 | 225.1558 | 225.2921 | 126.1283 | 126.2193 |
| $TMT^{2}$-127 | 225.1558 | 225.2921 | 127.1316 | 127.2120 |
| $TMT^{6}$-126 | 229.1629 | 229.2634 | 126.1283 | 126.2193 |
| $TMT^{6}$-127 | 229.1629 | 229.2634 | 127.1316 | 127.2120 |
| $TMT^{6}$-128 | 229.1629 | 229.2634 | 128.1350 | 128.2046 |
| $TMT^{6}$-129 | 229.1629 | 229.2634 | 129.1383 | 129.1973 |
| $TMT^{6}$-130 | 229.1629 | 229.2634 | 130.1417 | 130.1900 |
| $TMT^{6}$-131 | 229.1629 | 229.2634 | 131.1387 | 131.1834 |

### B. Please visit the website for additional information related to this product including the following:

- Tech Tip Protocol #49: Acetone Precipitation of Proteins
- Tech Tip Protocol #19: Remove Detergents from Protein Samples

## Related Products

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| **90008** | **Pierce Strong Cation Exchange Spin Column, Mini**, 24 spin columns and 48 collection tubes |
| **90009** | **Pierce Strong Cation Exchange Spin Column, Maxi,** 8 spin columns and 16 collection tubes |
| **89983** | **Pierce SILAC Protein Quantitation Kit – DMEM** |
| **89982** | **Pierce SILAC Protein Quantitation Kit – RPMI 1640** |
| **89870** | **PepClean C-18 Spin Columns,** 25 columns |
| **28904** | **Trifluoroacetic Acid, Sequanal Grade** |
| **23227** | **Pierce BCA Protein Assay** |
| **23208** | **Pre-Diluted Protein Assay Standards** |
| **89853** | **Phosphopeptide Isolation Kit** |
| **90003** | **Pierce Phosphoprotein Isolation Kit** |
| **89885** | **iCON Concentrator, 20 ml/9K MWCO,** 25 units |
| **89893** | **Zeba Desalt Spin Columns, 10 ml,** 5 columns |

### General References

Dayon, L., *et al.* (2008). Relative quantification of proteins in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. *Anal.Chem.* **80(8):**2921-31.

Ross, P.L., *et al.* (2004). Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics* **3 (12):**1154-69.

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