INSTRUCTIONS

Immobilized Anti-TMT™ Resin & TMT Elution Buffer

90076 90104

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Number	Description
90076	**Immobilized Anti-TMT Resin,** 6mL
90104	**TMT Elution Buffer,** 20mL

Storage: Upon receipt store all products at 4°C. Product shipped with an ice pack.

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

Introduction

Thermo Scientific Immobilized Anti-TMT Resin and TMT Elution Buffer are used for specific capture and elution of TMT Reagent-labeled peptides. Immobilized Anti-TMT Resin and TMT Elution Buffer are effective for reducing sample complexity, improving dynamic range and studying cysteine modifications such as S-nitrosylation, oxidation and disulfide bonds of protein samples labeled with iodoTMT Reagents. This approach of selective thiol labeling and affinity enrichment is similar to isotope-coded affinity tags (ICAT™ Method) but allows for higher multiplex quantitation.

The Immobilized Anti-TMT Resin uses the highly specific anti-TMT antibody (Product No. 90075) to bind peptides labeled with TMT Reagents. The antibody is specific for the mass reporter region of the TMT Reagents which allows for enrichment of TMT-labeled peptides regardless of peptide labeling chemistry (e.g., amine-reactive, sulfhydryl-reactive). The TMT Elution Buffer is a volatile neutral buffer which competitively elutes captured TMT-labeled peptides. When used in combination with the Immobilized Anti-TMT Resin, the TMT Elution Buffer provides higher specific enrichment of TMT-labeled peptides compared to acidic or basic elution buffers.

Procedure Summary

To label and prepare samples for analysis (Figure 1), protein extracts are isolated from cultured cells or tissues. After proteins are denatured and reduced, each sample is individually labeled. Excess tag is removed by acetone precipitation, SDS-PAGE or Thermo Scientific Zeba Spin Desalting Columns. For LC-MS/MS analysis, proteins are digested with a site-specific endoproteinase. After digestion, labeled peptides are enriched using the Immobilized Anti-TMT Antibody Resin and TMT Elution Buffer. Data acquisition is performed on a Thermo Scientific LTQ Orbitrap or Orbitrap Velos Mass Spectrometer and data analysis software is used for protein identification and relative quantitation of the six samples via reporter ions.

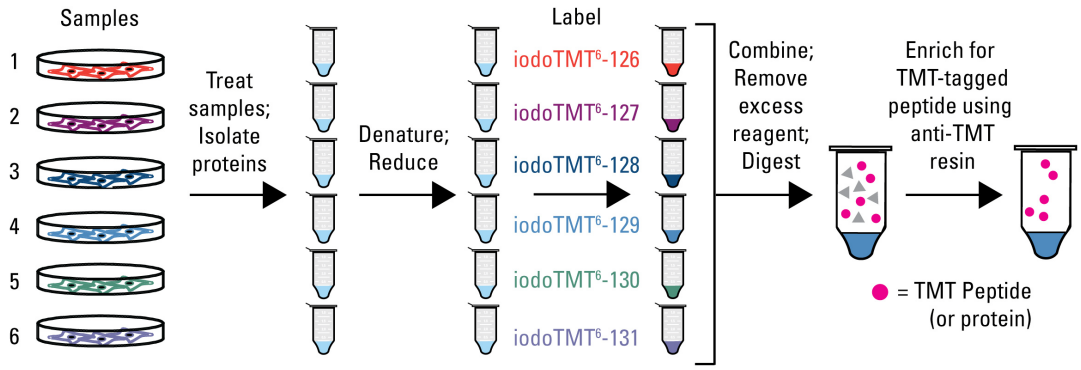

Figure 1. Workflow for the Thermo Scientific iodoTMT Reagents.

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Important Product Information

- Removal of non-reacted iodoTMT Label Reagent is required for successful enrichment of labeled peptides (or proteins) from complex samples using Anti-TMT Resin (Product No. 90076). Strategies for removal of the label reagent are found in Tech Tip #69 on our website.
- The binding capacity of the Anti-TMT Resin is ~25nmol/mL of resin. The antibody is specific for the mass reporter portion of TMT Reagents (e.g., TMT and iodoTMT™ Reagents).
- The TMT Elution Buffer is a volatile buffer at neutral pH. Acetate salts may be observed after lyophilization of eluted peptides. If necessary, eluted peptides may be cleaned up using C18 columns or tips.
- Protein labeling can be confirmed by Western blot using the Anti-TMT Antibody (Product No. 90075) or LC-MS/MS of tryptic peptide digests.

Additional Materials Required

- LC/MS-grade acetonitrile (Product No. 51101)
- LC/MS-grade water (Product No. 51140)
- Trifluoroacetic acid (TFA) (Product No. 28904)
- Tris-buffered saline (TBS) (Product No. 28358)
- 75-300µm capillary C_{18} reversed-phase column (10-25cm long) (Thermo Scientific Hypersil Product No. 25005-150065)
- 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™ (Matrix Science, Ltd.)
- Optional: Thermo Scientific Pierce Spin Columns, 2mL (Product No. 89896)
- Optional: Pierce C18 clean-up columns or tips (Product No. 89870, 87783 or equivalent)

Enrichment of TMT Reagent-labeled Peptides

Note: Use at least 100µL of settled resin (200µL of 50% slurry) for every 100µg peptide digest of samples labeled with iodoTMT Reagent. For S-nitrosylated protein samples labeled using iodoTMT Reagents, use 100µL of settled resin (200µL of 50% slurry) for every 1mg of sample. Pierce Spin Columns – Screw Cap (Product No. 69705) may be used to facilitate resin washes.

1. Wash the Anti-TMT Resin three times with one column volume of 1X TBS.
2. Resuspend lyophilized peptides with 100µL of 1X TBS. Save a small portion of this unfractionated sample for direct analysis of the non-enriched samples.
3. Add peptides to the Anti-TMT Resin and incubate for 2 hours at room temperature or overnight with end-over-end mixing at 4°C.
4. Remove the supernatant and wash the resin 5 times (5 minutes/wash) with 1 column volume of TBS.

   Note: Addition of 2M urea or MS-compatible detergents (0.05-0.2%) to TBS wash buffers may be used to decrease nonspecific peptide binding.
5. Wash the resin 3 times with 1 column volume of 1X TBS.
6. Wash the resin 3 times with 1 column volume of water.
7. Elute the sample with 4 column volumes of TMT Elution Buffer.
8. Pool the eluate, freeze peptides and lyophilize using a vacuum concentrator.
9. Optional: Clean up peptides using C18 clean-up columns or tips.
10. Resuspend the samples in 25µL of 5% acetonitrile/0.1% formic acid and inject 1-5µL directly onto an LC-MS/MS system (e.g., Orbitrap™ Velos Mass Spectrometer).

Troubleshooting

Problem	Possible Cause	Solution
Poor enrichment of TMT-labeled peptides	Nonspecific, unlabeled peptides bound antibody resin	Increase incubation time during wash steps
		Add urea or MS-cleavable detergent to wash buffers
	Nonspecific labeling of non-cysteine peptides	Quench non-reacted iodoTMT Reagent with DTT
		Maintain pH 8-8.5 to minimize iodoTMT Reagent labeling of non-cysteine amino acids
	Excess non-reacted tag was present	Perform labeling using a lower excess of reagent
		Desalt labeled samples using Zeba™ Spin Desalting Columns before enrichment
	Sample amount was insufficient	Increase sample load for anti-TMT enrichment
		Load more sample onto the C18 LC-MS/MS column

Additional Information

A. Information Available from our Website

- Tech Tip #69: Strategies for removal of non-reacted TMT tag
- Tech Tip #70: TMT data acquisition on the LTQ Orbitrap XL Mass Spectrometer
- Tech Tip #49: Acetone precipitation of proteins
- Tech Tip #19: Remove detergent from protein samples

Related Thermo Scientific Products

90075 **Anti-TMT Antibody,** 100µL
90100 **iodoTMTzero™ Label Reagent Set**
90102 **iodoTMTsixplex™ Isobaric Label Reagent Set**
90103 **iodoTMTsixplex Isobaric Labeling Kit**
90105 **Pierce S-Nitrosylation Western Blot Kit**
90064 **TMTsixplex™ Isobaric Mass Tagging Kit**
90066 **TMTsixplex Label Reagent Set**
90067 **TMTzero™ Label Reagent Set**
69705 **Pierce Spin Columns – Screw Cap,** 25/pkg
28358 **20X TBS Buffer**, 500mL
29700 **Urea,** 1kg
89890 **Zeba Spin Desalting Columns, 7K MWCO, 2mL,** 5/pkg
88305 **HiPPR™ Detergent Removal Spin Column Kit**
89870 **Pierce C18 Spin Columns,** 25/pkg
28904 **Trifluoroacetic Acid, Sequanal Grade**

General References

Gygi, S.P., *et al*. (1999). Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotech* **17:**994-9.

Murray, C.I. (2012). Identification and quantification of S-nitrosylation by cysteine-reactive tandem mass tag switch assay. *Mol Cell Proteomics* **11(2):**M111.013441.

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