Iodoacetyl Tandem Mass Tags for Cysteine Peptide Modification, Enrichment and Quantitation

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Overview
Purpose: To develop on-chip Iodoacetyl Tandem Mass Tags (iodoTMT) reagent for multiplexed cysteine-containing peptide labeling and enrichment.

Methods: Reduced and sulfhydryl of protein cysteines were labeled with iodoTMT reagent.

Results: The iodoTMT reagent selectively labels a plethora of cysteine peptides in complex mixtures and is ideal for detection of cysteine modifications.

Introduction
Thermo Scientific Tandem Mass Tag(TM) Reagents enable concurrent identification and quantitation of up to eight differentially labeled proteomes. Here, we describe the development of an iodoacetyl Tandem Mass Tag (iodoTMT) reagent for multiplex quantitation.

Methods
Sample Preparation
Preparation of iodoTMT-labeled peptides: Proteins or cell lysates were solubilized in 0.5% TFA and incubated with 10 mM iodoTMT reagent for 1 h at room temperature. The labeling reaction was quenched by the addition of 100 mM cysteine.

Selective labeling of cysteine-containing proteins: Protein samples were mixed with 10 mM iodoTMT reagent and an equal volume of 100 mM cysteine. After 1 h at room temperature, the reaction was quenched by adding 100 mM cysteine.

Results and Discussion
Figure 1: Schematic of iodoTMT reagent labeling reaction. iodoTMT reacts with cysteine-containing peptides, resulting in a labeled peptide that can be detected by LC-MS/MS.

Figure 2: Schematic of iodoTMT reagent reaction mechanism. iodoTMT reacts with cysteine-containing peptides, resulting in a labeled peptide that can be detected by LC-MS/MS.

Figure 3: iodoTMT reagent labeling specificity and efficiency. iodoTMT reagent labeling specificity and efficiency were determined by peptide competition assays. The iodoTMT reagent labeling specificity was determined by comparing modified peptide ion signals to total peptide ion signals for each unique peptide.

Figure 4: iodoTMT reagent labeling reactivity of BAD protein. BAD protein was labeled with 10 mM iodoTMT reagent and analyzed by LC-MS/MS. The data were compared with the unlabeled BAD protein to determine the reactivity of iodoTMT reagent.

Conclusion
The iodoTMT reagent can be used as a probe for labeling S-nitrosylated cysteines in a variety of samples, including cell lysates and tissue homogenates. The reactivity of the iodoTMT reagent can be used to detect cysteine modifications in a wide range of samples, including cell lysates and tissue homogenates.

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References