Multiplexed Proteomics

Observe biological systems through space and time

Thermo SCIENTIFIC

TOP 10
Reasons to Use
TMT10plex Multiplexing

Multiplexed proteomics has allowed protein quantitation to be transformed from basic identification experiments to a multidimensional characterization of the proteome with Thermo Scientific™ Tandem Mass Tags™ Reagents and the Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer.

These solutions enable you to ask real questions of your sample and obtain a meaningful description of how protein expression changes with disease, with the progression of time, and even within different parts of the cell. Instead of a single data point, monitor the real changes at work. TMT enables more than multiplexed quantitation; it opens the door to answering a whole new set of questions. So go ahead, ask your system a tough question…

1

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

Merely identifying what proteins are present in a sample supplies only a limited understanding of the underlying biology.

Solution:

Isobaric tagging with TMT (Tandem Mass Tag Reagents) adds an important new dimension to a classic discovery experiment: quantitation enables meaningful conclusions about changes in the proteome as a result of disease, environmental factors, or time.

Isobaric tagging with Tandem Mass Tags uses an NHS ester functionality to covalently label free amines on the exposed N-terminus or lysine side chains of peptides. By shifting the location of the heavy isotopes between the linker region and the reporter region, the total mass and structure of the label can be kept exactly the same (isobaric).

2

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

Obtaining quantitative information about a sample often comes at the expense of identifications due to increased sample complexity.

Solution:

TMT labeled peptides are completely isobaric and are irresolvable by LC, SCX-fractionation, and even MS^1, meaning no added complexity.

Labeling efficiency at the peptide level is excellent and does not suffer from a significant loss in identifications, which can occur with large tags. More importantly, the technique does not increase MS^1 sample complexity and does not require metabolic labeling, making it suitable to a wide variety of sample conditions.

3

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

Quantifying on the peptide level limits the obtainable information.

Solution:

TMT labeling is amenable to labeling on the protein level, opening the door for a wide range of novel experiments.

TMT labeling can also be done on either the peptide or protein level. Intact protein identification can be expanded along a new dimension by adding quantitation. By labeling prior to digestion, a mixture of intact proteins can be quantified using TMT. This approach can be extended to study the post translational proteolysis and its role in disease by the N-terminal amino isotopic labeling of substrates (NTAILS) followed by negative enrichment to detect TMT-labeled neo N-termini and quantify the relative degree of proteolysis. On the opposite end of the spectrum, TMT-labeling of free amino acids has been used to add quantitative data to amine metabolism experiments as well as facilitate better chromatographic performance in reverse-phase HPLC.

4

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

Comparing changes in protein expression across more than one or two states at a time is necessary to obtain a complete depiction of how time, disease, and the environment affect the proteome, instead of just a snapshot.

Solution:

The multiplexing capabilities of Tandem Mass Tags reagents were recently expanded from 6 to 10 through the incorporation of both 13C and 15N.

The augmented number of reporter ions does more than just allow more simultaneous experiments; it enables a whole new class of analyses to be undertaken. One of these is hyperplexed localization of organelle proteins by isotopic labeling or hyperLOPIT. This method combines density gradient centrifugation to separate cellular organelles with TMT10plex labeling, enabling the spatial resolution of proteins within the cell. The method can be extended further to look at changes in both space and time. Indeed, TMT lends itself extremely well to time-based studies, and has been used to monitor drug dose response curves with the reporter ions acting as multiple time points.

5

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

Increasing the number of reporter ions often requires an increase in the size of the tag, which can result in a loss of identification.

Solution:

By leveraging the small mass deficit between 13C and 15N, the number of reporters was increased from six to ten without altering the size of the tag or its chemistry.

6

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

To accommodate a wide variety of labeled samples, reporter ion generation needs to be possible with a variety of fragmentation techniques.

Solution:

Generation of reporter ions can occur either by collision induced dissociation methods like CID and HCD (ten reporters) or by electron activated fragmentation techniques like ETD (six reporters) for quantitation with a variety of methods.

7

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

In order to obtain good statistical validity, multiple replicate runs need to be performed.

The same peptide may not be reproducibly identified in each run.

Solution:

With a higher number of reporter ions, channels can be used redundantly as technical replicates for statistical validation, all within a single run.

8

TOP 10 Reasons to Use TMT10plex Multiplexing

Besides their utility in providing quantitative information, Tandem Mass Tag Reagents increase the charge of labeled peptides and the resulting higher charged peptides fragment more efficiently by ETD, improving the analysis of phosphopeptides and glycopeptides.

9

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In addition to amine reactive tags, two additional reactivites are available: sulfhydryl reactive that labels cysteine residues, and carbonyl reactive used to label free glycans. The cysteine reactive TMT labels have been used to probe cysteine nitrosylation, a labile modification and important mediator of cellular activity using selective reduction, while cellular profiling of released N-linked glycans has been shown using carbonyl reactive aminoxyTMT reagents.

10

TOP 10 Reasons to Use TMT10plex Multiplexing

Problem:

Quantitation of very low abundance compounds is challenging.

Solution:

A specialized high affinity TMT-antibody is available either as free antibody or resin immobilized antibody which permits the positive enrichment of TMT labeled compounds and is compatible with any of the Tandem Mass Tag Reagents.

TOP 10 Reasons to Use TMT10plex Multiplexing

Additional resources

Go to the TMT Multiplexing Web Page

Go to the TMT10Plex Product Page

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