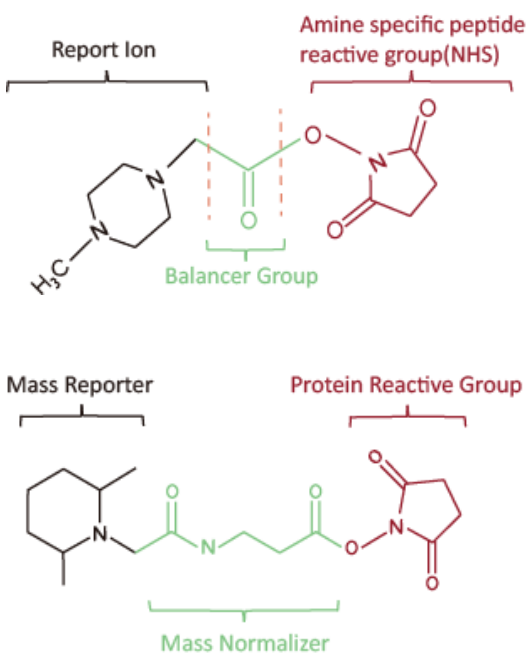
Quantitative Proteomics

Quantitative proteomics uses mass spectrometry to measure and compare protein abundance, offering insights into cellular processes and disease mechanisms.

Principles of Quantitative Proteomics

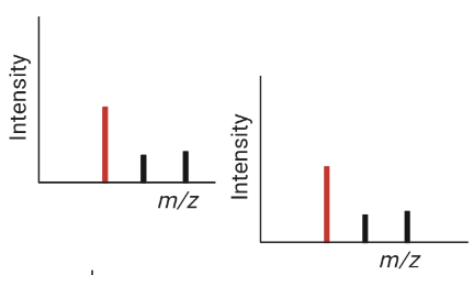
iTRAQ/TMT

iTRAQ (Isobaric Tags for Relative and Absolute Quantification) and TMT (Tandem Mass Tags) are peptide-labeling quantitative techniques developed by AB Sciex and Thermo Fisher in the United States. These methods use labels with various isotopes that can react with amines, facilitating connection. Through high-precision mass spectrometry analysis, iTRAQ/TMT enables both qualitative and quantitative analysis of protein groups from multiple samples. The reagents consist of three parts: a reporter group, a balance group, and a reactive group.



In MS/MS analysis, each isobaric tag produces a unique ion spectrum, allowing for quantitative analysis. Labeled peptides cannot be distinguished in primary mass spectrometry analysis, but in secondary mass spectrometry mode, the bonds between the groups break, generating reporter ions for quantification.

Label Free

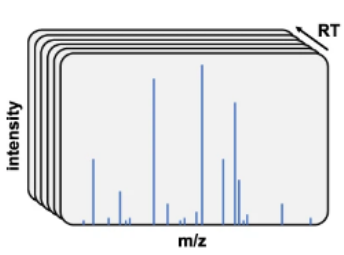


Label-free protein quantification is a technique that does not rely on isotope labeling for protein quantification. This method analyzes peptides generated from protein enzymatic digestion using liquid chromatography-mass spectrometry.

It involves analyzing mass spectrometry data generated during the large-scale identification of proteins, integrating the intensity of detected precursor ion peaks, and performing relative quantification based on the integrated area.

DIA

Data-Independent Acquisition (DIA) is a recently developed mass spectrometry data acquisition method. It is an improvement over traditional shotgun analysis using Data-Dependent Analysis (DDA). DIA allows unbiased MS analysis of all signals in complex samples, resulting in higher peptide coverage.



In the DIA scanning mode, the entire mass scan range is divided into multiple windows, and all ions in each window are rapidly and cyclically selected, fragmented, and detected. This approach ensures comprehensive and unbiased acquisition of all ion fragmentation information in the sample.

How to Choose?

1

For samples such as tissues and cells, TMT is suitable. These samples typically represent the overall protein expression of the sample type, have a diverse range of protein types, and show relatively consistent parallelism. In contrast, samples with significant individual differences and fewer protein types, like cell supernatant and exosomes, are recommended for Label-free quantification.

2

TMT can handle up to 18 samples in a single set. If there are more than 18 samples, we could perform more experiment sets. A pooled sample will be included in each set as a bridging for data nromalization.

3

Both Label-free and TMT belong to data-dependent acquisition (DDA), where the top N precursor peptides are selected for secondary fragmentation detection. DIA, on the other hand, is a data-independent mass spectrometry acquisition method, allowing all precursor ions within defined windows to undergo secondary fragmentation (thus, high reproducibility) and capturing more spectral information. The final identification of protein types in DIA depends on the reference library data. Compared to DDA, DIA is a more comprehensive data acquisition mode suitable for large-sample studies. TMT involves pooling samples for grouping before analysis, while both DIA and Label-free are performed individually, unaffected by and not limited by the number of samples.

4

When dealing with a small number of samples, label-free or iTRAQ/TMT may be preferred, whereas for a large number of samples, DIA technology is recommended.

Categories	iTRAQ/TMT	Label Free	DIA
Data acquisition mode	DDA	DDA	DDA (Library Construction) + DIA (Detection)
Reagent labeling necessity	Yes	None	None
Quantitative principles	Labeling reagent	Precursor ion	MS/MS fragment ions
Protein quantity determination	Multiple	Medium	Abundant
Quantitative precision	High	Medium	High
Experimental cost	Medium	Low	Medium to High
Validation efficacy	High	Medium	High
Project timeline	Medium	Fast	Medium
Original sample volume prerequisites	Medium	Low	Relatively Low
Sample parallelism requirements	Present	None	None
Capability to discern protein presence	Absent	Present	Present
Inter-sample impact assessment	Present	None	None
Technical constraints on sample volume	Unlimited (multiple sets of bridging)	Unlimited	Unlimited
Recommended test sample size	Several to dozens of cases	Several to dozens of cases	Dozens and above

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