iST-NHS Sample Preparation Kit

Objective

The PreOmics iST-NHS kit provides a streamlined solution for robust and reproducible sample preparation compatible with chemical labeling such as iTRAQ or TMT.

Background

Mass spectrometry (MS)-based proteomics typically employs multiple sample processing steps, representing a crucial part of routine MS analyses. Complex workflows, extensive sample fractionation and proteolytic digestion are highly time-consuming and restrict the overall technical reproducibility. The accuracy and robustness of the MS platform is also strongly influenced by the quality of sample. The PreOmics' iST technology is gaining global credibility for its ability to address the aforementioned sample preparation challenges. Isobaric mass tags have become popular for multiplexing approaches enabling precise quantification of peptides and proteins in multiple samples (1). Whilst commercially available isobaric mass tags reduce MS measurement time through multiplexing of up to 11 samples per MS run, the upfront sample preparation workflow typically still takes between 1.5 and 2 days of time. In this application note, we present the iST-NHS sample preparation kit that allows streamlined chemical labeling of peptides directly followed by an efficient peptide cleanup step (Figure 1), thus minimizing sample loss, overall hands-on time and the amount of required chemical labels. The PreOmics' NHS-compatible sample preparation kit is designed to assist you to achieve best results with few sample preparation steps and little hands-on time for multiplexing applications.

Figure 1 | iST-NHS workflow for plant tissues

Material and methods

Baker's yeast (Saccharomyces cerevisiae) derived from a yeast cube was resuspended in PBS. Aliquots of OD600 = 1 were harvested and centrifuged. Pellets were frozen at -20°C until use. Each cell pellet was then resuspended in 50 µl LYSE-NHS, boiled at 95°C for 10 min and then sonicated using the Diagenode Bioruptor® Pico (10 cycles, 30 sec ON/OFF). After the heat and sonication treatment, each sample was transferred to the CARTRIDGES, before continuing with the protocol according to the iST-NHS Sample Preparation Kit instructions.

Material: S.cerevisiae cells
Quantity: 100 µg protein starting material

For the chemical labeling step, TMTzero™ Label Reagent (Thermo Fisher Scientific) was incubated with the peptides for one hour at room temperature with different TMT label:peptide ratios and a min. 30% (v/v) acetonitrile concentration during the labeling reaction:

TMT:peptide ratio	TMT reagent [µg]	peptides [µg]
2:1	200	100
4:1	400	100
8:1	800	100

Quenching was achieved by adding 5% hydroxylamine. MS analysis was performed on a LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) with standard settings, except for a higher normalized collision energy of 42. Data analysis was performed using MaxQuant (2) and setting a specific cysteine modification (C6H11NO, +113.084 Da) as fixed modification in the database search. For the calculation of the TMT labeling efficiency, the TMTzero™ mass tag was searched as variable modification on peptide N-termini and lysines. Biological duplicates were used throughout the experiment.

Results

While most chemical labeling protocols are laborious and time-consuming, our iST-NHS workflow takes less than 4 hours from cell lysis to ready-to-measure chemically labeled peptides, providing significant time saving. Optimized and patented peptide washing procedures eliminate both hydrophobic and hydrophilic contaminants after the TMT labeling reaction resulting in clean peptides, decreasing MS downtime and guaranteeing reproducible and reliable results.
High labeling efficiency is essential to guarantee optimal quantification results. Thus, the amount of labeling reagent used per peptide input material is critical. However, a high labeling efficiency inversely correlates with peptide identification. Here, we employed the iST-NHS method to yeast cells and compared different ratios of TMT reagent per µg of peptides input material. While the manufacturer recommends to utilize a TMT to peptide ratio of 8:1, we found that a ratio of 4:1 results in the highest number of identified peptides while still achieving labeling efficiencies of more than 98% using the iST-NHS kit (Figure 2).

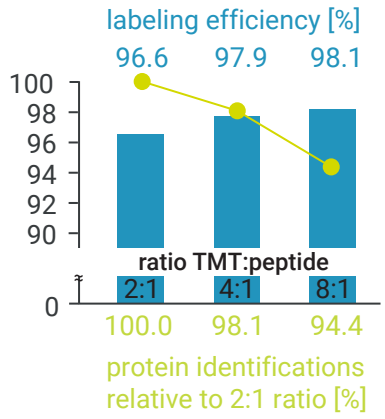

Figure 2 | Higher TMT:peptide ratios result in slightly higher labeling efficiencies but also in reduced protein identifications. In combination with the iST-NHS kit, PreOmics recommends to use a TMT:peptide ratio of 4:1 as the best tradeoff between labeling efficiency and protein identifications.

Finally, we assessed the reproducibility of the replicate measurements. Both the reproducibility across different biological experiments (Figure 3A-C), as well between different technical replicates (Figure 3D-F) was excellent (average R2 = 0.92). These results indicate high reproducibility of sample preparation with our iST-NHS kit compatible with chemical labeling.

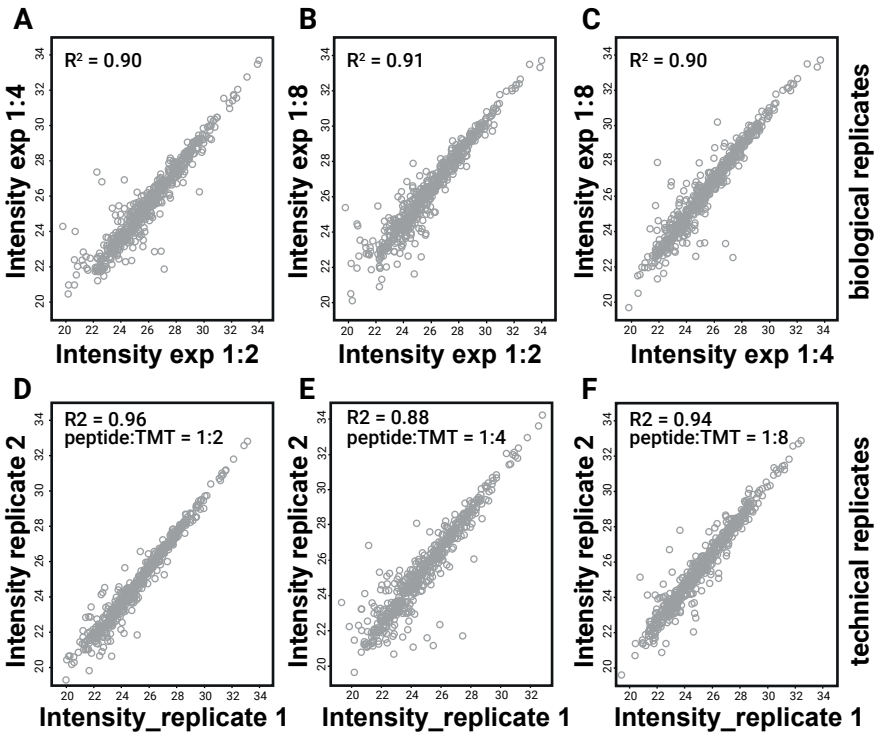

Figure 3 | Intensity correlations across biological (A-C) and technical (D-F) replicates of the distinct TMT:peptide ratio experiments. Reproducibility is displayed as R2.

Conclusion

Here, we show that the iST-NHS kit provides a streamlined solution for robust and reproducible sample preparation compatible with chemical labeling such as iTRAQ or TMT, achieving excellent labeling efficiency and reproducibility required for robust quantification. The chemical labeling is directly integrated within the intuitive four-step protocol of the iST-NHS kit. In addition, the iST-NHS method is fully compatible with 96well plate formats, as well as automated liquid handling platforms, enabling high-throughput chemical labeling experiments.

References

(1) Rauniyar N, Yates JR 3rd. Isobaric labeling-based relative quantification in shotgun proteomics. J Proteome Res (2014) 13(12):5293-309. doi: 10.1021/pr500880b.
(2) S Tyanova, T Temu and J Cox. The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. Nat Protocols (2016) 11(12):2301-19. doi: 10.1038/nprot.2016.136

PREOMICS

Products

Product	Quantity	Manufacturer	Product Code
iST-NHS kit 12x	12 reactions	PreOmics GmbH	P.O.00026
iST-NHS kit 96x	96 reactions	PreOmics GmbH	P.O.00030

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