Less is more: Avoiding artificial modifications in proteomic sample preparation for pharmaceutical and clinical applications

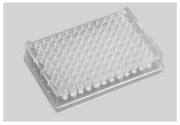
Katrin Hartinger, Sebastian Johansson, Katharina Scheck, Fabian Hosp, Nils A. Kulak | PreOmics GmbH, 82152 Martinsried, Germany

Katrin Hartinger

1. Features of the iST-BCT workflow

- iST-BCT workflow: Robust, high-performance iST-based workflow for pharmaceutical and clinical applications
- Features:
  - Minimizes artificial modifications (deamidation, oxidation)
  - Increases alkylation rate
  - Sample preparation in < 2 hours with < 30 minutes hands-on time
  - High reproducibility and excellent sample quality
- Sample types: biological fluids such as blood plasma, antibodies, single proteins

3. Applications & Conclusions



96 well format

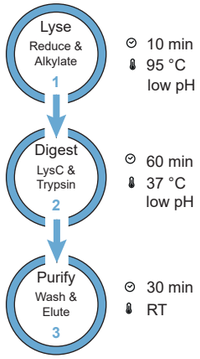
- Adaptable to 96 well format for high-throughput applications
- Combinable with iST-fractionation e.g. for HCP analysis (coming soon)
- Combinable with on-beads digestion for highly diluted samples
- Compatible with full automation platforms such as PreON

PREON by PREOMICS

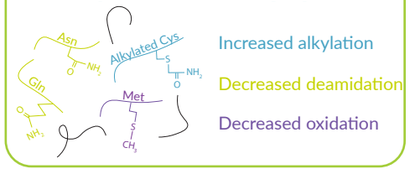
Sample types

Biological fluids | Antibodies | Proteins

iST-BCT workflow

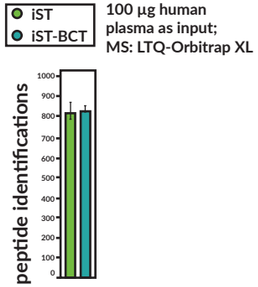


Ready-to-measure peptides

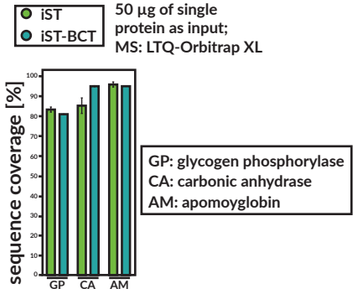


2. Performance of iST-BCT workflow

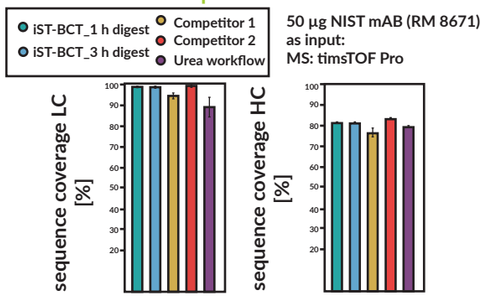
Plasma



Single proteins

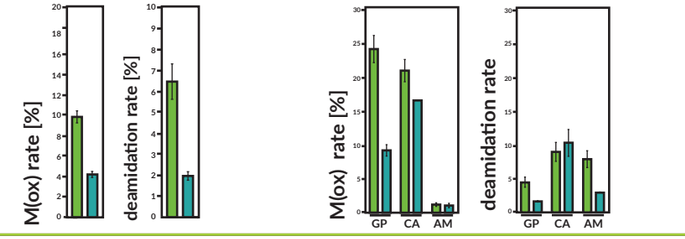


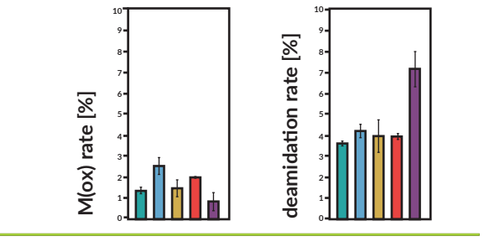
NIST mAB - compared to various workflows



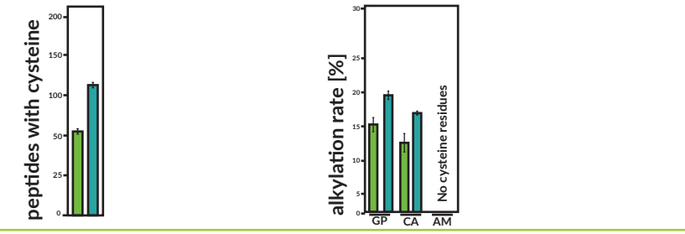
Overall performance

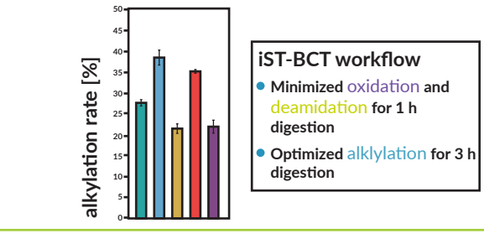
Decreased artificial modifications





Increased alkylation





iST-BCT workflow

- Minimized oxidation and deamidation for 1 h digestion
- Optimized alkylation for 3 h digestion

Materials & Methods

Sample preparation was performed with the following kits or workflows: iST kit from PreOmics; Competitor kit 1; Competitor kit 2; Home-brew urea sample preparation; Newly developed iST-BCT workflow. The iST-BCT workflow is based on the iST-technology and optimized for pharmaceutical and clinical applications. By employing a new detergent that is stable at alkaline pH, lysis and digestion is performed at low pH to suppress artificial modifications. Additionally, alkylation efficiency was improved by using a stronger reducing agent and optimizing the ratio of buffer components.
The following sample types were analyzed: Human plasma; NIST mAB (RM 8671); Carbonic anhydrase isozyme II from bovine erythrocytes; Glycogen phosphorylase from rabbit muscle; Apomyoglobin from equine skeletal muscle.
MS runs were performed in DDA mode using instruments as indicated in the figures, resulting MS/MS spectra were analzed using MaxQuant software.