ApplicationNote iST-BCT Kit
Biological fluids & therapeutic proteins

PREOMICS

INTRODUCTION

Sample preparation is an often overlooked yet very important component of the overall mass spectrometry-based protein characterization and remains to be a limiting factor for streamlined analyses.
Based on our effective iST technology, we have further optimized our workflow specifically for the demands of reproducible sample preparation of therapeutic proteins (e.g. antibodies, drug substances) and biological fluids that require enhanced denaturing conditions for efficient sample preparation.
The iST-BCT (inStageTip-Biopharmaceutical) kit was developed to enable such robust processing of biological fluids, HCPs and therapeutic proteins, and has been optimized to minimize artificial modifications (e.g. de-amidation, oxidation) and increase the alkylation rate. The kit provides an all-in-one solution for processing samples in less than two hours and with less than 30 minutes hands-on-time. In the regular iST-BCT workflow, samples are denatured, reduced and alkylated within 10 minutes at 95°C, followed by a one hour enzymatic digestion using LysC and trypsin, and a dual peptide cleanup step for one hour at room temperature.

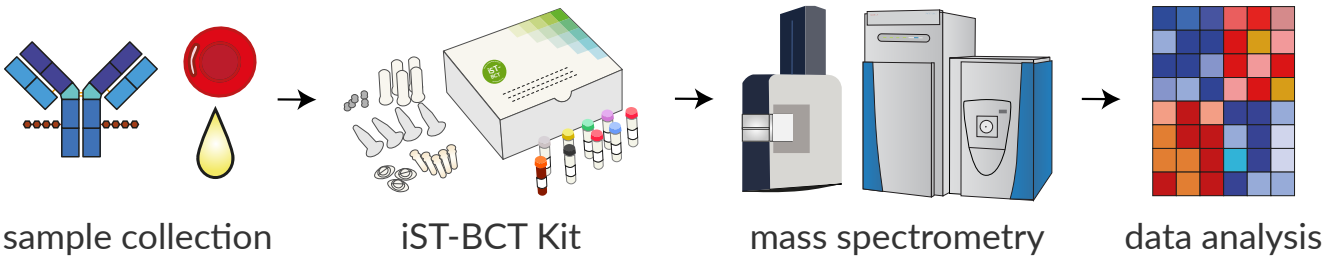

Fig. 1 | iST-BCT workflow

In this Application Note, we compare the performance of the new iST-BCT kit against other sample preparation workflows including our regular iST kit that has been optimized for processing intact cells. To this end, we use different sample types includig undepleted human plasma, the NIST mAB reference standard and selected single proteins. Furthermore, we assess identification of host cell proteins (HCPs), process-related impurities encountered in biotherapeutic drug development. We demonstrate superior performance and reduced artifical modifications for the iST-BCT kit when using biological fluids, therapeutic proteins and for HCP analyses.

AUTHORS

[1]Katrin Hartinger, [2]Stuart Pengelley, [1]Fabian Hosp and [1]Nils Kulak

[1]PreOmics GmbH, 82152 Martinsried, Germany | [2]Bruker Daltonik GmbH, Bremen, Germany

MATERIAL AND METHODS

100 µg undepleted plasma (*H.sapiens*, ethical consent obtained); 50 µg NIST antibody standard (NIST Monoclonal Antibody Reference Material 8671); 50 µg carbonic anhydrase (Sigma-Aldrich, #C3666); 50 µg glycogen phosphorylase (Sigma-Aldrich, # P4649); 50 µg apomyoglobin (Sigma-Aldrich, #A8673). Samples were processed with either iST or iST-BCT kits according to manufacturer`s instructions. Samples processed with the AccuMAP Low pH Protein Digestion Kit (Promega) or the EasyPep Mini MS Sample Prep kit (ThermoFisherScientific) were prepared according to manufacturer`s instructions. Samples processed with the urea workflow were mixed with urea denaturation buffer (6 M urea, 2 M thiourea, 10 mM Hepes) and alkylated by consecutive incubation with dithiothreitol and iodoacetamide. Pre-digestion of samples with LysC was performed at room temperature for 3 hours. Next, urea concentration was diluted to less than 2 M urea with 50 mM ammonium hydrogen carbonate and overnight digestion with trypsin was performed at room temperature. Digestion was stopped by acidifying samples with trifluoroacetic acid. Samples processed with the AccuMAP kit and urea-based protocol were desalted using the Pierce™ peptide desalting columns (ThermoFisherScientific) according to manufacturer`s instructions. NIST antibody data was acquired on the timsTOF Pro using the PASEF MS/MS scan mode, coupled to the nanoElute LC system via the Captive-Spray ion source (all Bruker Daltonics). 200 ng sample was separated using an Aurora 25 cm x 75 µm ID, 1.6 µm C18 column (ionopticks), 2-37% acetonitrile gradient in 100 mins with a 400 nL/min flow rate. PASEF scans were searched against the mouse SwissProt database at 1% FDR using Mascot in BioPharma Compass software (Bruker Daltonics). Plasma and single protien data was acquired on the LTQ-Orbitrap XL coupled to an EASY-nLC 1200 (all ThermoFisherScientific). Peptides were eluted with a linear 45 min gradient using a selfmade 20 cm x 75 µm ID, 1.9 µm C18 column. Raw files were analyzed using MaxQuant software v.1.6.0.16, the false discovery rate was set to 0.01 for both proteins and peptides with a minimum length of seven amino acids, and was determined by searching a reverse database. Enzyme specificity was set as C-terminal to arginine and lysine, using trypsin as the protease, and a maximum of two missed cleavages were allowed in the database search. Statistical analysis was performed using Perseus software.

SUMMARY

We show that the new iST-BCT kit has enhanced performance over our regular iST kit and other sample preparation workflows when processing bodyfluids such as plasma, or therapeutic proteins such as the NIST mAB reference and for HCP analysis. At the same time, the iST-BCT kit reduces undesired artifical modifications such as methionine oxidation and demidation.
The iST-BCT kit will be integrated into the menu of kits to run on the PreON automation platform soon.

PRODUCTS

Product	Quantity	Manufacturer	Product Code
iST-BCT kit 8x	8 reactions	PreOmics GmbH	P.O.00084
iST-BCT kit 96x	96 reactions	PreOmics GmbH	P.O.00099

http://www.preomics.com/products

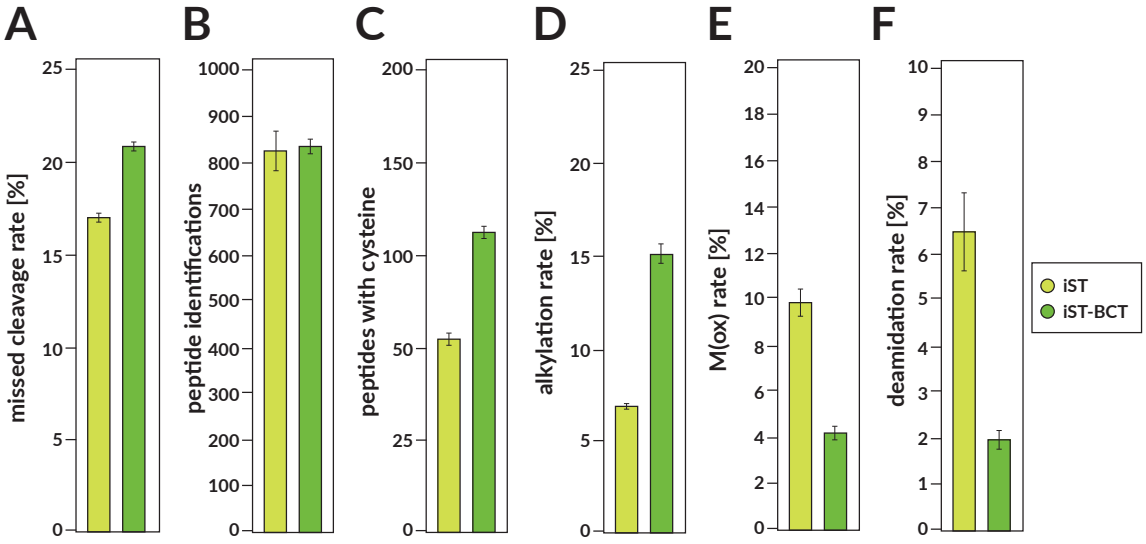

Figure 2 | Performance of iST and iST-BCT kits for processing undepleted human plasma. (A) Missed cleavages. (B) Peptide identifications. (C) Peptides with cysteine identifications. (D) Alkylation rate. (E) Methionine oxidation rate. (F) Deamidation rate.

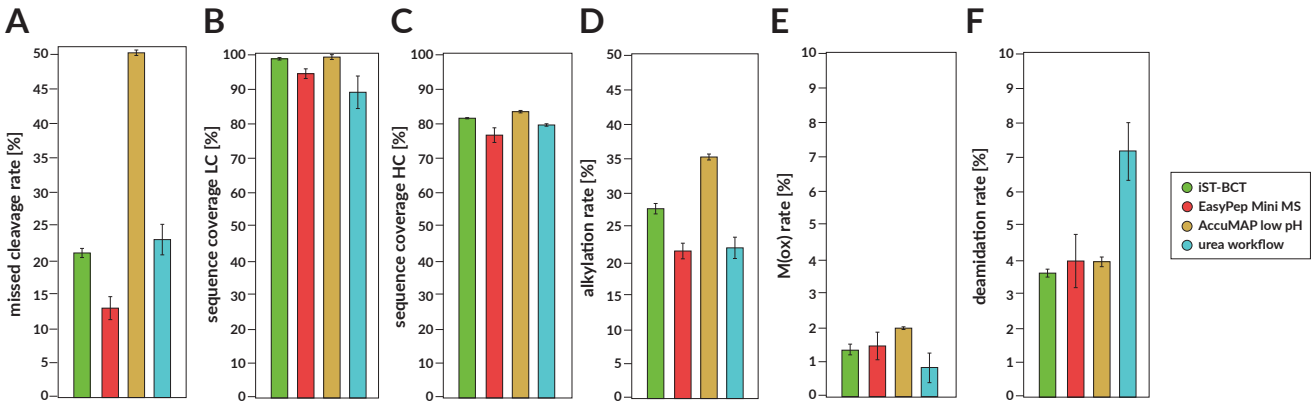

Figure 3 | Performance of iST-BCT and other sample preparation methods for processing NIST mAB reference standard. (A) Missed cleavages. (B) Sequence coverage light chain. (C) Sequence coverage heavy chain. (D) Alkylation rate. (E) Methionine oxidation rate. (F) Deamidation rate.

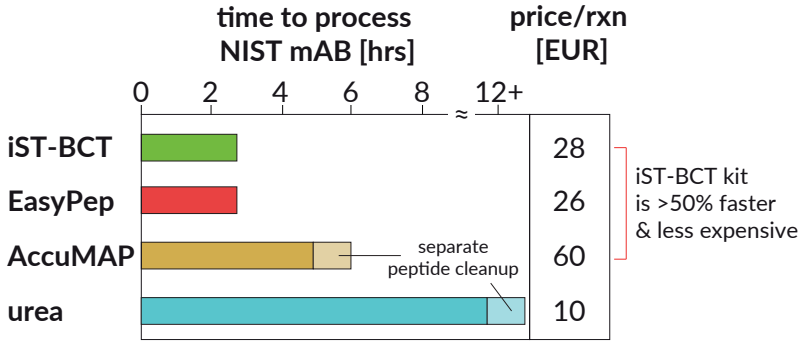

Figure 4 | Processing time & cost comparison of iST-BCT and other sample preparation methods for preparing the NIST mAB reference standard.

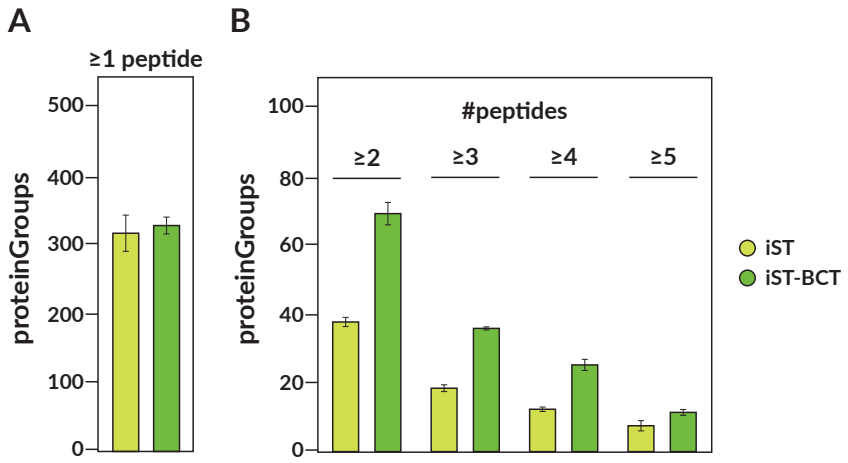

Figure 4 | Performance of iST and iST-BCT kits for host cell protein identification. (A) Average number of HCPs identified by ≥1 peptide. (B) Average number of HCPs identified by ≥2 to ≥5 peptides.

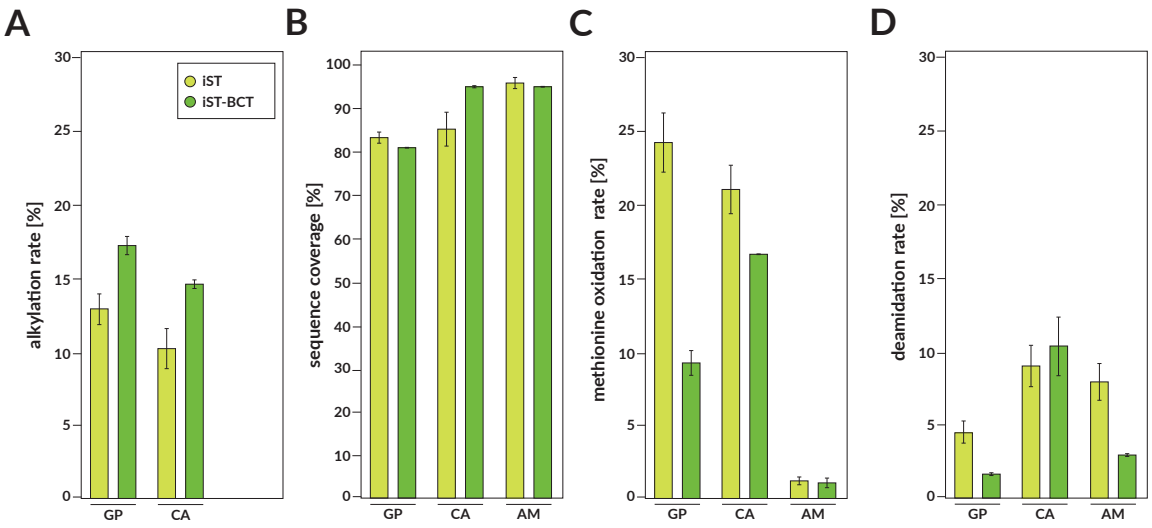

Figure 5 | Performance of iST and iST-BCT kits for single protein processing. GP: glycogen phosphorylase; CA: carbonic anhydrase; AM: apomyoglobin. (A) Alkylation rate. (B) Sequence coverage. (C) Methionine oxidation rate. (D) Deamidation rate.

For further information please contact us at:
PreOmics GmbH
Am Klopferspitz 19
D-82152 Martinsried
Germany
Tel: +49-89-2314-1630
info@preomics.com