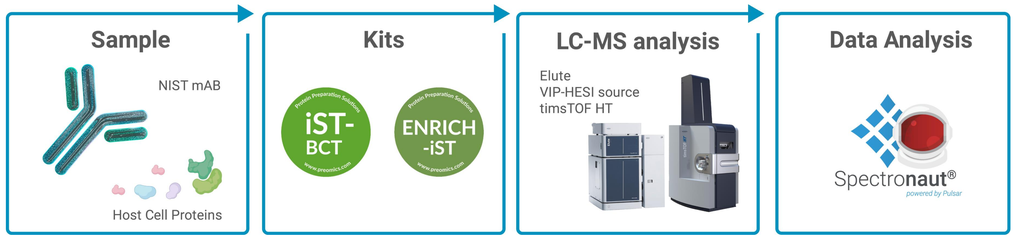
Enhanced and Reproducible Detection and Quantification of Host Cell Proteins using Protein Enrichment and Data-Independent Acquisition LC-MS

Konrad Winkels[1], Oskar Knittelfelder[1], Karin Yeoh[2], Juliane Weißer[1], Zuzana Demianova[2], Guillaume Tremintin[3] and Christian Albers[1]

[1]Bruker Daltonics GmbH, Bremen, Germany,
[2]PreOmics GmbH, Martinsried, Germany,
[3]Bruker Scientific LLC, San Jose, CA, USA



Introduction

Host Cell Proteins (HCPs) are residual proteins from host cells present in biopharmaceutical drug preparations that require monitoring to ensure product efficacy and safety. LC-MS has emerged as a flexible alternative to traditional ELISA methods, offering the ability to analyze samples from different cleanup stages, cell lines, and drug products with the same experimental setup. Additionally, LC-MS measurements can deliver individual HCP abundances, enabling more accurate risk assessments. However, the large dynamic range between drug and low-abundant HCPs poses analytical challenges. Strategies for sample preparation that enrich low-abundant HCPs can help overcome these challenges. In this study, we evaluate the ENRICH-iST and iST-BCT kits (PreOmics) for their effectiveness in identifying and quantifying HCPs using LC-MS.

Methods

NISTmAb (200 µg for iST-BCT, 300 µg for ENRICH-iST) was processed in four process replicates per kit. Samples were analyzed in triplicate (25% per injection) on a Bruker timsTOF HT with VIP-HESI, coupled to an Elute UHPLC with an Acquity UPLC CSH™ C18 column (2.1 × 100 mm, 1.7 µm; Waters). An active gradient of 15 min (2–35% B) was used at a flow rate of 200 µL/min. Acquisition was performed in dia-PASEF mode (see Table 1). Data were processed in Spectronaut 19 (directDIA+). For detailed LC-MS settings: Appnote LCMS #235, bruker.com.

dia-PASEF Settings	
Mass Width	50 Da
Cycle Time Estimate	0.85 s
Number of MS1 Ramps	1
Number of MS/MS Ramps	7
Number of MS/MS Windows	14
Mass Range	338.8 to 1038.8 Da
Mobility Range	0.63 to 1.26 1/K_0

Table 1: dia-PASEF Method Settings.

Results

Benchmarking Process Replicates iST-BCT vs. ENRICH-iST

Process replicates were analyzed in Spectronaut, with "Method Evaluation" enabled. In iST-BCT samples 19 HCPs were identified with at least two peptides across all replicates. An additional nine HCPs were identified in at least two out of four process replicates. The ENRICH-iST kit depletes highly abundant proteins, thereby reducing the dynamic range within the sample. In this case, 60 HCPs were identified in all replicates, with 22 additional HCPs identified in at least three out of four process replicates. The consistency in protein identification underscores the robustness and reproducibility of the workflow.

Figure 1: Protein Group identifications per run and HCP overlap across process replicates (Single Hit Proteins excluded).

Quantitative Evaluation - Process Replicate 3

Employing the ENRICH-iST kit not only increases the total number of protein identifications but also substantially improves quantification depth, raising the number of quantifiable HCPs from 21 to 73 (CV < 20%, Top2-3 Quantification).

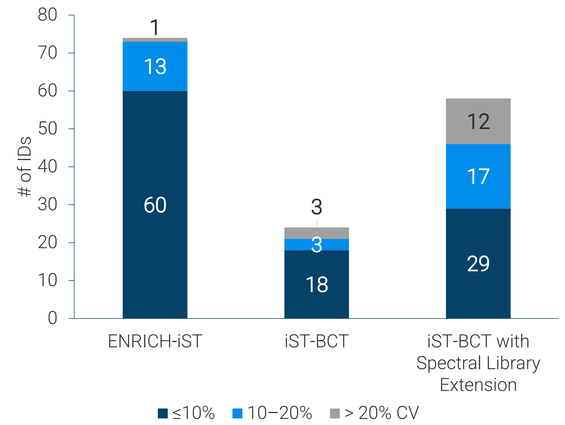


Figure 2: Number of quantifiable HCPs in ENRICH-iST and iST-BCT measurements in single process replicate.

Revealing Synergies from Both Kits - Process Replicate 3

Many users aim to report HCP abundances relative to the drug substance (e.g., in ppm or ng/mg). Therefore, enrichment or depletion strategies that alter protein abundance profiles may not always be ideal for accurate quantification.

A promising solution to maintain quantification accuracy while enhancing HCP coverage is the use of spectral libraries. To achieve this, the three ENRICH-iST measurements were added as Library Extension Runs to the iST-BCT processing pipeline in Spectronaut.

As shown in Figure 2, this strategy enabled the accurate quantification of 46 HCPs (CV < 20%), effectively increasing coverage while preserving the original dynamic range of the sample.

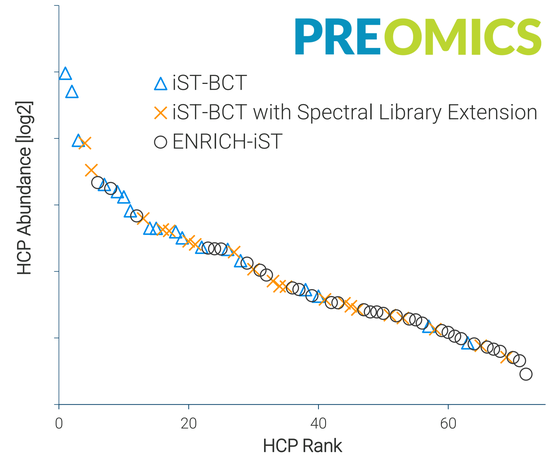


Figure 3: Abundance ranking of HCPs quantified across different analytical workflows. Ranking is based on Protein Group Quantity reported in the ENRICH-iST results. Only HCPs with a CV below 20% are included.

Figure 3 illustrates the capability of the three approaches to comprehensively profile the HCP landscape of NISTmAb. While the iST-BCT workflow alone primarily quantifies relatively abundant HCPs, the addition of spectral libraries significantly enhances sampling depth—particularly improving detection in the mid-abundance range.

Although library extension runs can increase HCP coverage in non-depleted samples, only proper enrichment strategies, such as ENRICH-iST, provide the ultimate sensitivity required to detect low-abundance HCPs.

Conclusion

- High reproducibility across process replicates
- ENRICH-iST significantly enhances HCP detection
- ENRICH-iST as a fast-track spectral library source
  - ➢ Increase HCP coverage while relying on unbiased quantification

Robust Sample Preparation Strategies for Enhanced HCP Profiling

COI Disclosure: KW, JW, OK, CA, and GT are employees of Bruker, which manufactures the HPLC system and mass spectrometer used in this work. ZD and KY are employees of PreOmics, which produces kits used in this work.