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Automated, scalable plasma proteomics with P2 enrichment and iST technology on the Opentrons platform

Measho Abreha[1], Alessandro Brambilla[2], Katharina Limm[2], Boren Lin[3], Fran Slater[3]

[1]PreOmics Inc., Billerica, MA, USA | [2]PreOmics GmbH, Martinsried, Germany |[3]Opentrons Labworks Inc., Long Island City, NY, USA

INTRODUCTION

Blood plasma is a highly informative biofluid for biomarker discovery, reflecting systemic physiology and disease processes. However, plasma proteomics remains challenging due to its extreme protein dynamic range, biological variability between donors, and the need for highly reproducible sample preparation at cohort scale.

The P2 nanoparticle-based enrichment improves access to low-abundance proteins, but large-scale application requires robust automation to ensure consistency and scalability. Standardized and automated solutions are therefore essential for reliable, high-throughput plasma proteomics in biomarker research.

METHODS

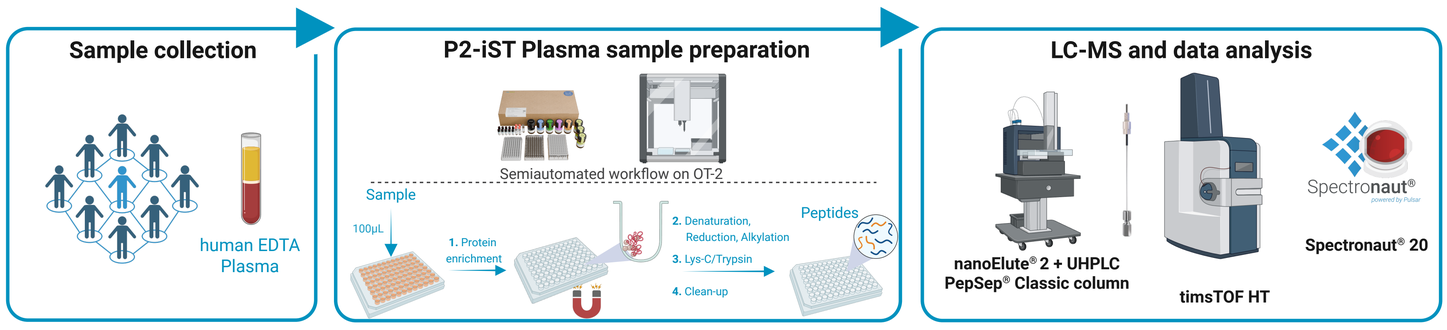
Input: Different commercial plasma samples
Human EDTA blood plasma, collected and pooled from 2 healthy donors (DS30) by DiaServe Laboratories GmbH, (centrifugation at 2000 × g), or pool from 4 healthy donors (InVent). 17 healthy single donors were purchased from BioIVT.

Sample preparation:
Multiple individual plasma samples, pooled plasma samples (performance testing), and sample blanks (cross-contamination testing) replicates of 100 µL plasma were processed using the P2–iST Plasma Kit (Biognosys Group) according procedure. All steps from plasma handling up to the addition of STOP were performed on the OT-2 platform (Opentrons Inc.).

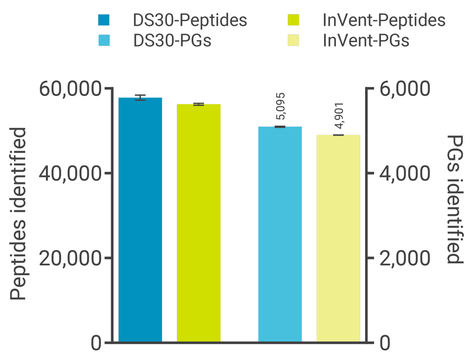
LC-MS analysis:
600 ng peptides loaded on a nanoElute® 2 (Bruker) equipped with a 15 cm PepSep® Classic column (Bruker) connected to the CaptiveSpray 2 emitter (20 µm). Data acquisition was performed on a timsTOF HT (Bruker) in DIA-PASEF® mode.

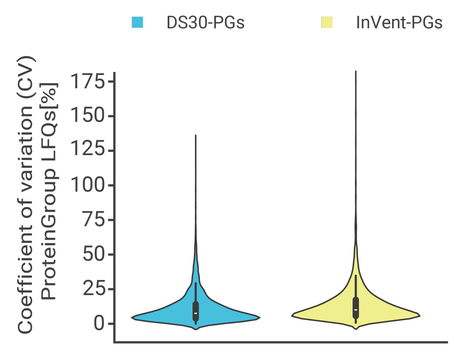
Data processing:
Data was processed using Spectronaut® 20 software in directDIA+™ mode (Biognosys). For visualization, the search-engine-agnostic visualization tool ProteoVision™ and GraphPad Prism were used.

RESULTS

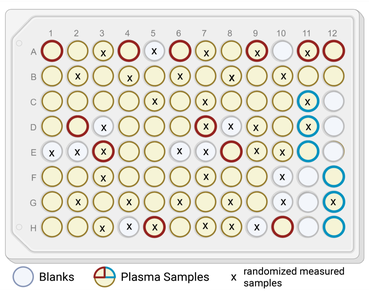


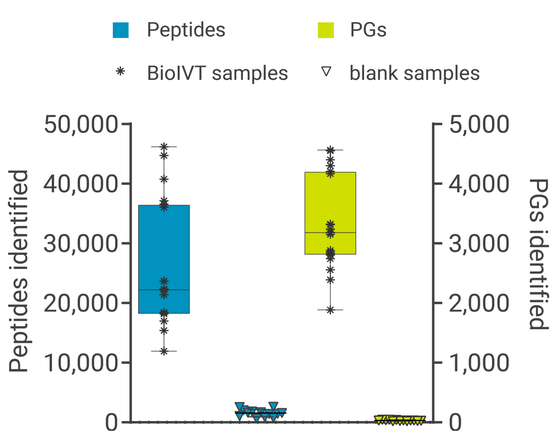
Semi-automated P2-iST Plasma workflow on Opentrons OT-2 enabling reproducible large-cohort sample preparation for LC–MS-based plasma proteomic analysis.



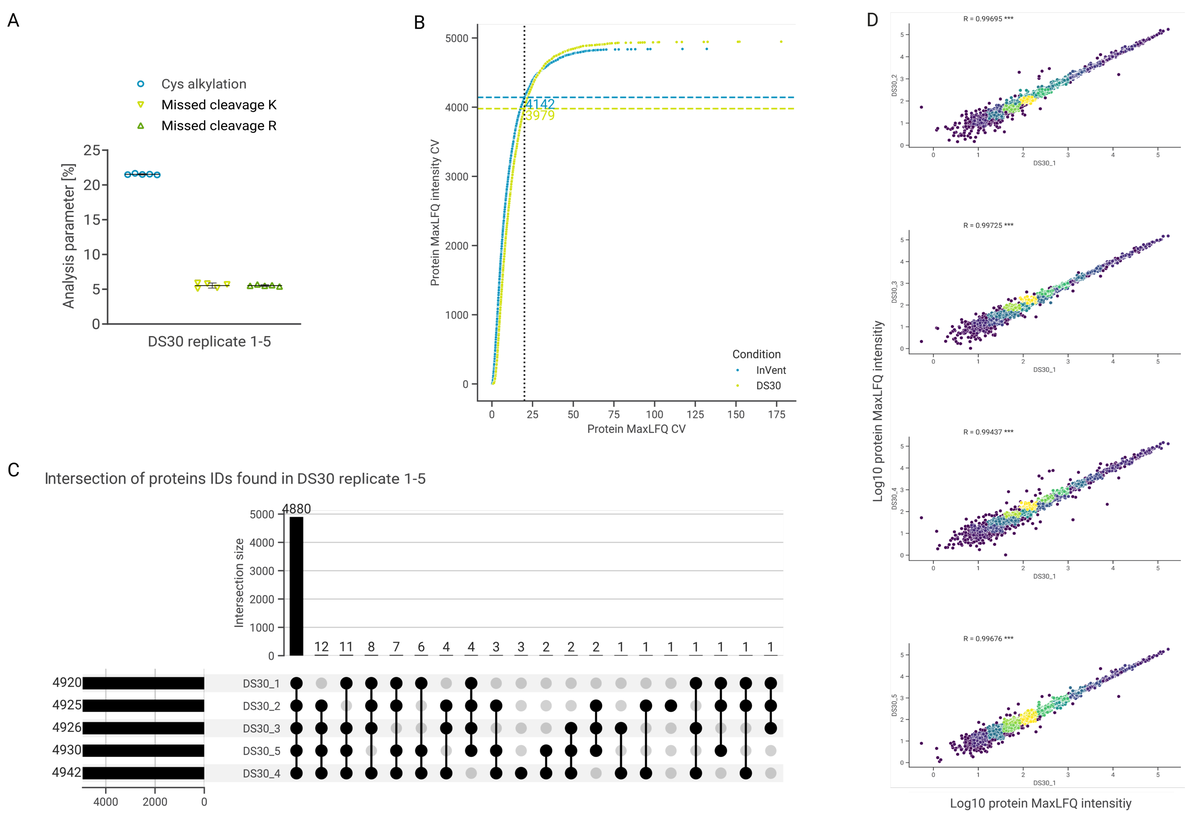


Intra-plate robustness assessment. Pooled plasma samples were distributed across a 96-well plate to evaluate positional effects on processing. Commercial plasma controls (DS30, red circles, n=5; InVent, blue circles, n=3) were used to monitor plate-level performance and reproducibility. Peptide and protein group identifications were highly consistent across the plate, with low coefficients of variation (CVs) of 10% for DS30 and 7.5% for InVent.





Evaluation of plate position effects and background signals. Alongside pooled plasma controls, 17 individual human EDTA plasma samples and sample blanks (no plasma matrix) were distributed across the plate to assess cross-contamination and variability under realistic conditions. Individual samples yielded up to 57,000 peptides and 1,800–4,700 protein groups. Blank samples showed minimal background, mainly from trypsin/LysC autodigestion and trace keratin peptides likely introduced prior to automated handling.



Robustness of automated P2-iST Plasma sample preparation. A detailed evaluation of sample preparation consistency was performed using DS30 control plasma. **(A)** High alkylation efficiency and very low missed cleavage rates at lysine and arginine residues indicated efficient denaturation, reduction, alkylation, and consistent digestion. **(B)** Coefficients of variation below 20% demonstrated excellent quantitative reproducibility and reliable plasma protein quantification. **(C)** Overlap analysis of five technical replicates showed high data completeness (>90%) with only a small proportion of single-hit proteins. **(D)** Comparison of LFQ intensities across DS30 replicates (Pearson correlation > 0.99) revealed highly consistent enrichment on P2 nanoparticles and reproducible peptide cleanup, underscoring the robustness of the overall workflow.

KEY TAKEAWAYS

P2-iST Plasma enables deep plasma proteome coverage
The P2-iST Plasma kit provides access to thousands of low-abundance plasma proteins from small sample inputs using a streamlined workflow that can be performed manually or automated within a single day.

PepSep® UHPLC columns deliver high-performance separations
The separate column and emitter configuration achieves increased protein and peptide identifications for P2-iST-prepared samples and improves peak shape, while maintaining strong quantitative performance. Flexible gradient compatibility supports both high-throughput and deep-profiling applications.

Together, these technologies enable an efficient end-to-end plasma proteomics workflow
Combined with advanced mass spectrometry and data analysis solutions, they provide a robust pipeline from sample preparation to biological insight.

CONTACT & MORE

PepSep®

P2-iST Plasma

Contact:
Measho.Abreha@preomics.com

Conflict of Interest Disclosure
Abreha, M. is employed by PreOmics Inc.
Brambilla, A.; Limm, K. are employed by PreOmics GmbH.
Lin, B.; Slater, F. are employed by Opentrons Labworks Inc.