Fast, reproducible plasma proteomics with ENRICH-iST on Opentrons OT-2 platform

Authors: Zehan Hu[1], Katharina Limm[1], Alessandro Brambilla[1], Katrin Hartinger[1], Nils Kulak[1]

[1]PreOmics GmbH, Martinsried, Germany

Mass spectrometry (MS)-based plasma proteomics has become a powerful technology for biomarker discovery and translational research, yet the broad dynamic range of plasma protein concentrations and sensitivity to pre-analytical variability continue to challenge the reproducible detection of low-abundance proteins. Magnetic bead–based enrichment strategies, such as the ENRICH-iST, substantially improve proteome depth and biological interpretability. Manual workflows, however, remain labor-intensive, operator-dependent, and difficult to scale, limiting their routine adoption in high-throughput and multi-site studies.[1]

This technical note describes the automation-supported implementation of the ENRICH-iST workflow on the Opentrons OT-2 platform to address these challenges. The semi-automated protocol standardizes all magnetic bead handling, washing, and elution steps through precise, programmable liquid handling and timing control. It enables scalable processing of 8 to 96 samples (in increments of eight) and includes an integrated buffer volume calculator to support flexible experimental design.

Automation reduces hands-on time from approximately three hours to ten minutes while ensuring consistent processing across the entire plate. By integrating the ENRICH-iST plasma sample preparation workflow with accessible, modular automation, laboratories can enhance reproducibility, minimize operator-driven variability, and accelerate plasma proteomics from exploratory studies to large clinical cohorts.

Keywords

Plasma proteomics, automation, ENRICH-iST, Opentrons OT-2, magnetic-bead enrichment, reproducibility, data-independent acquisition (DIA), high-throughput sample preparation

Key takeaways

Reduced hands-on time through seamless automation of the ENRICH-iST workflow on the Opentrons OT-2

Equivalent proteome depth compared to manual preparation

No detectable cross-contamination between wells, ensuring clean and reliable high-throughput processing

Improved reproducibility, with lower peptide and protein CVs compared to manual preparation

Technical details

Product Specifications	Description / Values
System	
Opentrons OT-2	Benchtop liquid handler automation system.
Kit	
ENRICH-iST 96x HT	1–96 samples
Input	
Plasma, serum, CSF, or similar samples	For plasma and serum samples, 20 µL of sample is required. For other sample types, the input volume can be adjusted accordingly.

Materials and Methods

Sample preparation

Commercial human EDTA plasma (DiaServe #302590, Germany) and sample blanks were processed in parallel using the ENRICH-iST 96x HT kit (PreOmics GmbH) following either the manual or automated workflow. Each sample (20 µL input) was subject to magnetic bead–based plasma protein enrichment, lysis, digestion, and peptide cleanup according to the manufacturer's protocol.

Automation setup

Automation was implemented on the Opentrons® OT-2 equipped with a P300 8-Channel GEN2 pipette, Heater-Shaker GEN1 (module position 1), and Magnetic Module GEN2 (position 9). The PreOmics® ENRICH-iST_96.json and script controlled the entire magnetic-bead workflow, including enrichment, lysis, and digestion. In addition, a Post-digestion_96.json was provided as an optional script for automated peptide and liquid transfer after digestion. Peptide cleanup was performed on the Tecan A200 positive-pressure system. For the Quick Start Guide and additional information, please contact: info@preomics.com

Manual control samples were prepared using the standard ENRICH-iST protocol under identical reagent and incubation conditions.

LC–MS/MS and data analysis

Peptides were resuspended in LC-LOAD and separated on an Easy-nLCTM 1200 system (Thermo Fisher Scientific) equipped with a homemade UHPLC column (25×75 C18) coupled to a timsTOF Pro mass spectrometer (Bruker Daltonics) via a 30 min linear gradient. Data was acquired in data-independent acquisition (DIA) mode using dia-PASEF® settings. Raw data were analyzed using Spectronaut® 19 with default directDIA+ search parameters against the UniProt human proteome (SwissProt, 2024-02, reviewed entries). Protein-level quantification was performed using factory settings. The performance of the automated OT-2 and manual workflows was evaluated based on the total number of quantified proteins and coefficients of variation (CVs) across replicates.

Results and Discussion

Automated ENRICH-iST on OT-2 maintains identification depth and prevents cross-contamination

To evaluate the analytical performance, plasma and sample blanks were processed using either the manual or semi-automated ENRICH-iST workflows and analyzed by 30-minute DIA runs on the timsTOF Pro. Both workflows yielded highly comparable proteome coverage, identifying approximately 10,000 peptides and 1,300 proteins per plasma sample (Fig. 1).

The absence of significant identifications in both manual and OT-2 blank runs confirmed the lack of detectable carryover or cross-contamination between wells. These results demonstrate that automation of key workflow steps on the OT-2 platform preserves the sensitivity and selectivity of the ENRICH-iST workflow while supporting high-throughput sample processing.

Automation improves reproducibility of plasma proteome quantification

Reproducibility was assessed based on coefficients of variation (%CV) at peptide and protein levels across replicate plasma preparations (Fig. 2). The OT-2 automation-supported workflow achieved slightly lower median CVs compared to manual preparation, with 22.7% vs 24.1% at the peptide level and 13.4% vs 16.0% at the protein level. The narrower distribution of CVs indicates improved precision and reduced operator variability.

These findings indicate that ENRICH-iST processing on the OT-2 platform reduces hands-on time while maintaining high reproducibility, supporting scalable plasma proteomics applications.

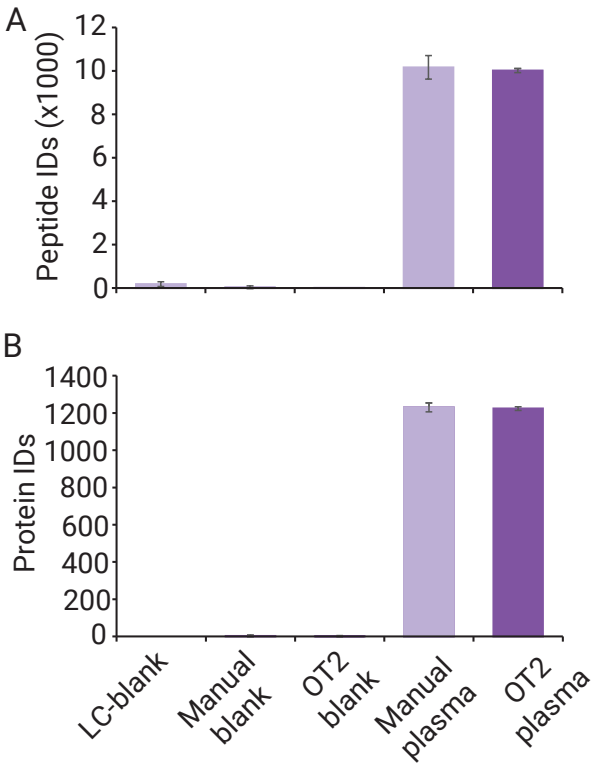

Figure 1 | Proteome depth and cross-contamination assessment of ENRICH-iST on the OT-2 platform. Number of identified (A) peptides and (B) proteins across LC blank, manual blank, OT-2 blank, manual plasma, and OT-2 plasma samples.

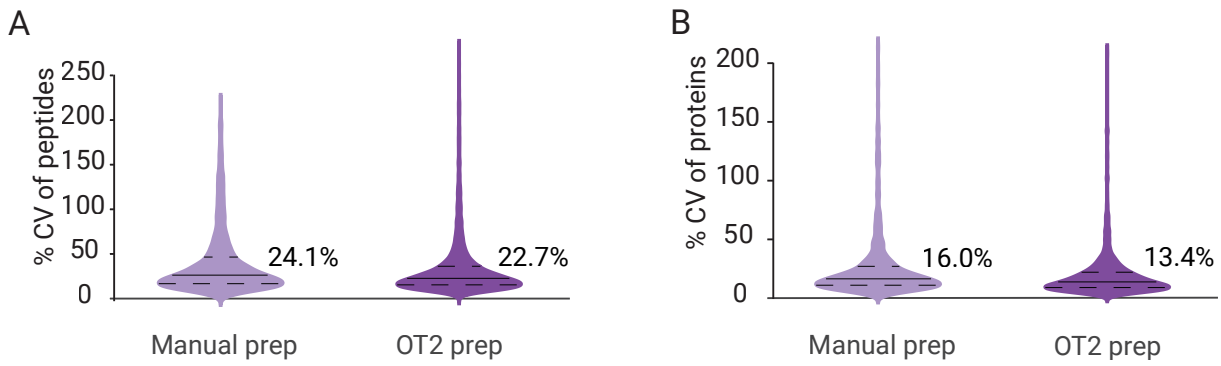

Figure 2 | Reproducibility of manual versus OT-2–based ENRICH-iST processing. Distribution of (A) peptide-level and (B) protein-level coefficients of variation (%CV) across replicate plasma samples processed manually or on the OT-2.

Conclusion

Implementation of the ENRICH-iST workflow on the Opentrons OT-2 platform enables standardized and scalable plasma proteomics with minimal user intervention. By automating the magnetic bead enrichment and liquid-handling steps, hands-on pipetting time is reduced from approximately three hours to about ten minutes while maintaining comparable protein identification depth and without evidence of detectable carryover or cross-contamination. Enhanced quantitative reproducibility further underscores the consistency of the workflow when implemented on the OT-2 platform.

The integration of ENRICH-iST plasma sample preparation with accessible benchtop liquid handling provides an efficient solution for laboratories seeking to scale from exploratory studies to large plasma cohorts. Straightforward implementation, flexible sample capacity (8–96 samples), and compatibility with standard LC–MS/MS setups make this approach particularly attractive for biomarker discovery, cohort studies, and clinical translational research.

Together, these results demonstrate that ENRICH-iST on the OT-2 platform maintains analytical performance while delivering substantial gains in reproducibility, throughput, and operational efficiency.

Products

Product	Manufacturer	Product Code
ENRICH-iST 96x HT	PreOmics GmbH	P.O.00165

Ordering information:

http://www.preomics.com/quote

order@preomics.com

References

1. King, C.D. et al. Mol Omics, 18(9):828-839 (2022).