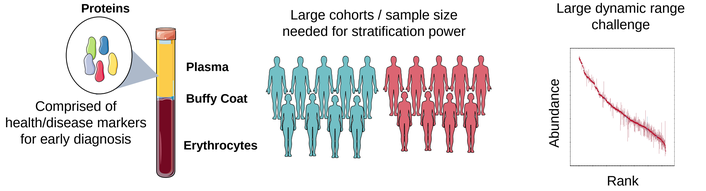
Standardized, high-throughput platform for automated, rapid, and extensive plasma proteome characterization

Claudia Martelli[1*]; Fabian Wendt[2*+]; Andreas Schmidt[3]; Katrin Hartinger[4]; Gary Kruppa[5]; Nils A. Kulak[4]; Manuel Bauer[2]

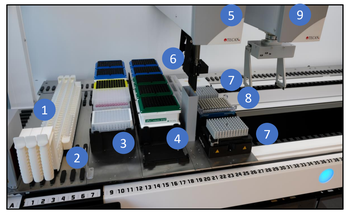
[1]Bruker Switzerland AG, Fällanden, Switzerland; [2]Tecan, Männedorf, Switzerland; [3]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [4]PreOmics GmbH, Martinsried, Germany; [5]Bruker S.R.O., Brno, Czech Republic

*These authors contributed equally to the work, [+] Presenting author

Promises & Challenges of Plasma Proteomics

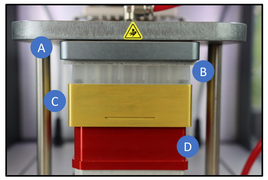


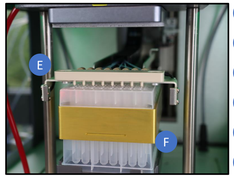
Simplified and scalable plasma proteomics including an in-depth analysis is required to meet challenges of pre-clinical research.



1. Fluent ID™ with 1.5 ml Ependorf tube runners
2. BEADS & DIGEST storage in 2 ml Sarstedt tubes
3. Assay Plate & 96 well SPE plate
4. 1000 µl, 200 µl, and 50 µl Disposable Tips
5. Flexible Channel Arm ™ (AirFCA)
6. 25 ml & 100 ml troughs for WASTE & Buffer storage
7. Bioshake, 37 °C, 95 °C (1200 rpm, 3 mm orbital)
8. Ring-Magnet (Alpaqua FLX)
9. Robotic Gripper Arm (RGA)

Fluent® workdeck layout. 8-channel pipetting (AirFCA) and automated plate transfer (RGA). Liquid level detection facilitates process supervision. On-deck heating/shaking for lysis & tryptic digest. Full reagent & buffer on-deck storage. Starting from Eppendorf tube stored samples, 2 - 96 samples can be prepared fully automated including magnetic bead capture, wash and elution without user intervention.





A. Positive Pressure Module
B. PreOmics SPE PLATE
C. Adapter for 96-well plate
D. Waste guide
E. Dispenser head
F. Collection plate

Automated peptide-clean up on the Resolvex® A200. Zoom onto workdeck, depicting positive pressure-based liquid displacement (left) and 8-channel guided dispensing of up to 11 buffers (right). Integrated version of A200 enables full end-to-end automation.

Interest? Questions? Please contact us!

Sample preparation: Katrin.Hartinger@preomics.com

Mass spectrometry: Claudia.Martelli@bruker.com

Automation / Liquid Handling: Fabian.Wendt@tecan.com

Plasma samples

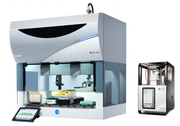
Plasma Biobank

Plasma 1 – Single donor (EDTA)
Plasma 2 – Single donor (EDTA)
Plasma 3 – Single donor (EDTA)

Sample preparation*

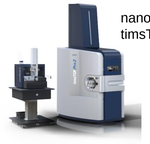
PreOmics ENRICH-iST kit

96 samples ~5 hours



Tecan Fluent® & Resolvex® A200

Data acquisition



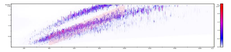
nanoElute 2
timsTOF HT

96 samples ~2.5 days

20 min gradient

dia-PASEF

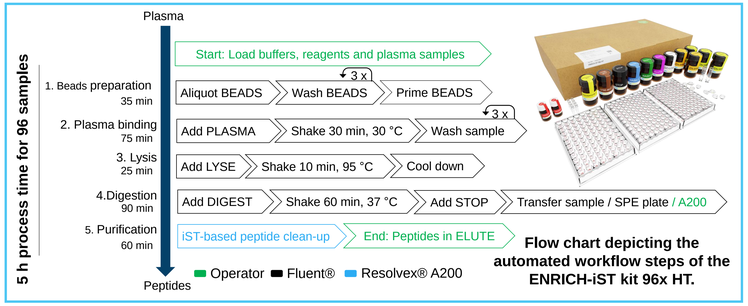
3x8x25Da window scheme



Data processing

directDIA+™ analysis, Spectronaut 17

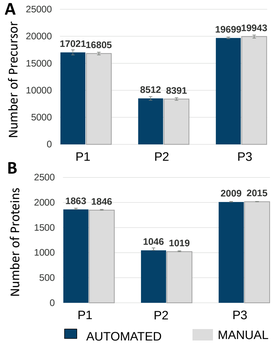
*For research use only. Not for use in diagnostic procedures.

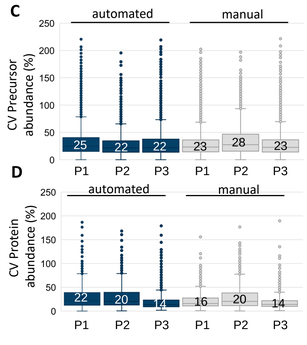


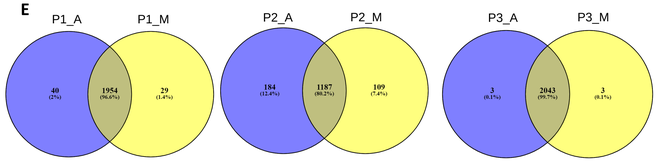
Flow chart depicting the automated workflow steps of the ENRICH-iST kit 96x HT.

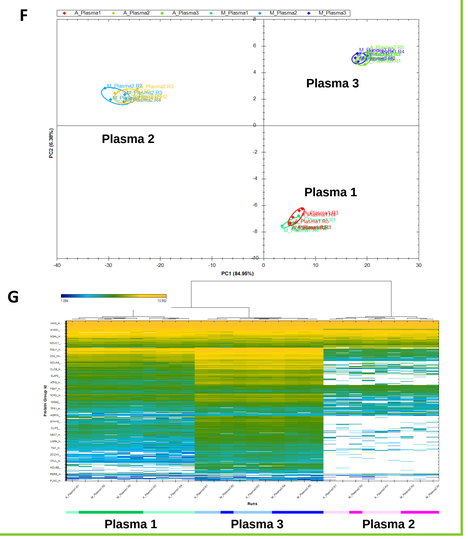
Automated ENRICH-iST workflow demonstrates excellent quantitative plasma protein characterization with on par performance to its manual counterpart.

A: Precursor identifications. > 19000 precursors have been observed for plasma samples **B: Protein identifications. >** 2000 protein groups were observed. **C**: **Coefficient of variation** (CV, %) of the precursor abundance. **D: CV** (%) of the protein abundance. **E: Overlap** of identified protein groups between (i) Plasma 1 automated vs Plasma 1 manual is > 96 % (ii) Plasma 2 automated vs Plasma 2 manual is > 80 % (iii) and Plasma 3 automated vs Plasma 3 manual is > 99 %. **F: Principal component analysis** reduces sample space to three clusters, driven by the plasma-origin. Moreover, automated and manual samples cluster per donor together. Thus, the manual and automated workflow preserve the same biological variability in terms of protein abundances. **G: Hierarchical clustering** depicted in heatmap shows high consistency of identifications within the same plasma sample.









Materials & Methods

Sample collection: Plasma samples were collected using EDTA from 3 healthy donors. All samples were provided in 60 µl as quintuplicates in order to evaluate the automation method reproducibility.

Sample preparation: Per replicate, 20 µl plasma was processed both manually and with the automation platform following the ENRICH-iST kit 96x HT (PreOmics) instructions. The automated was developed using the Tecan Fluent liquid handling platform in combination with Tecan`s positive pressure module Resolvex® A200. The automated protocol covers the protein enrichment, the iST-based protein denaturation and digestion, together with peptide clean-up for a total of 96 samples/run within 5 hours.

LC-MS/MS analysis: Peptide samples were separated on a 10 cm column (10 cm x 75 µm, 1.5 µm, PepSep) on the nanoElute 2 nanoflow liquid chromatography system (Bruker) coupled to the Bruker's timsTOF HT via a CaptiveSpray ionization source. The sample amount injected was 400 ng in 1 µl of LC-LOAD and the separation was performed using a 20-min ACN gradient. For the dia-PASEF acquisitions, a window placement scheme consisting of 8 TIMS ramps with 3 mass ranges per ramp spanning from 400 – 1000 m/z and from a mobility range of 0.64 – 1.37 1/K0 with a cycle time of 0.5 seconds, including one MS1 frame was applied.

Data analysis: All dia-PASEF data were elaborated using Spectronaut® (v17, Biognosys®) selecting the library-free directDIA+ workflow. Factory's default settings were applied the spectra were searched against Uniprot human reviewed protein sequence database (April. 2022).

The automated platform brings simplicity and profound insights to the challenging analysis of plasma empowering to find the underlying biology.

- ENRICH technology for efficient dynamic range compression coupled to robust iST-BCT sample preparation provides improved protein identification and quantification with excellent technical variability.
- High-throughput sample preparation (5 h for 96 samples) with short-gradient MS-based analysis (~ 45 samples / day) allows to scale for large cohorts.
- Manual and automation workflow show equivalent performance in terms of protein recovery, precursors/proteins ids, quantification reproducibility.

Future work:

- Automation of peptide concentration measurements & their normalization reduces sample loading time.
- Full package automating PreOmics` application portfolio (iST-BCT, iST-PSI, SP3-iST and ENRICH-iST) will be available soon.