Standardized, fully automated neat plasma and Mag-Net enrichment workflows enabled by the Evotip Pure

Joel Vej-Nielsen[1], Magnus Huusfeldt[1], Ian D. Shoemaker[2], Stoyan Stoychev[1,3], Laurent Rieux[1], Dorte B. Bekker-Jensen[1], Nicolai Bache[1]

[1]Evosep Biosystems, Denmark, [2]Beckman Coulter, United States, [3]Resyn Biosciences, South Africa

Highlights

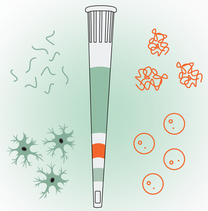
- Scalable high-throughput proteomics enabled by Evotip Pure integration on the Biomek i5 liquid handler
- Fully automated sample preparation of up to 576 samples in parallel.
- Showcasing four sample preparation workflows for robust analysis of large clinically-relevant sample cohorts.

The need for standardized solutions

A selection of scalable workflows directly integrated with Evotip Pure

As proteomics studies become larger and analyses become more routine, the demand for standardized and scalable solutions is increasing. To address this need, we introduce four fully automated workflows developed for the Biomek i5 Automated liquid handler, seamlessly integrated with Evosep technology to ensure efficient and reproducible end-to-end sample preparation.

Evotip Sample Loading
This workflow uses a specialized prototype sealing adapter enables automated desalting and loading on the Evotip for efficient sample storage prior to downstream LC-MS analysis.



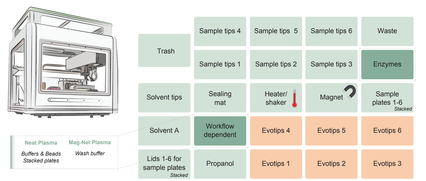
Neat Plasma workflow
Combining steps for reduction and alkylation, this workflow utilizes PAC digestion as a streamlined approach for processing large plasma sample cohorts.

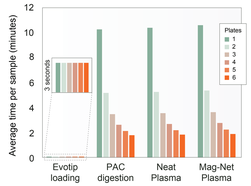
Digestion workflow
The PAC digestion workflow leverages protein aggregation on magnetic beads, followed by direct on-bead digestion of reduced and alkylated protein lysate.

Mag-Net Plasma workflow
For deep plasma proteomics, the Mag-Net workflow combines enrichment of membrane-bound vesicles with PAC digestion.

Designed for large-scale analysis

For efficient analysis of large sample cohorts, the workflows are designed to minimize both cost- and time per sample. Enzyme and sample consumption is minimized with online Evotip loading, as peptides are efficiently captured and stored on Evotip Pure allowing utilization of close to 100% of the sample. With efficient deck space usage, 576 samples can be processed simultaneously, resulting in less than two minutes of total processing time per sample for each workflow.





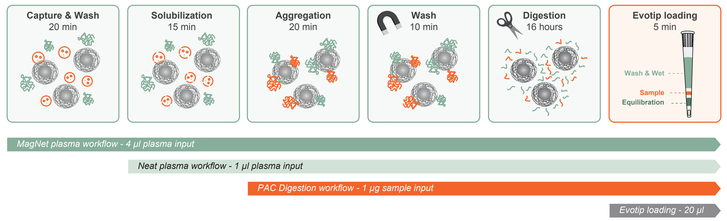
Schematic representation of deck layout on the Biomek i5 and an overview of how much time on is spent on average per sample when 1-6 plates are processed with each workflow.

Seamless integration with Evotip Pure technology

Versatility through modular sample preparation

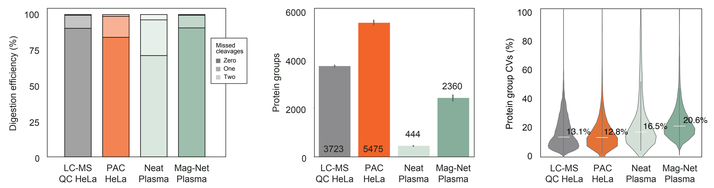
Automated sample loading on Evotips is the cornerstone of this modular sample preparation strategy. As the final step in each workflow, it facilitates sample clean-up and stable storage on the Evotip until LC-MS analysis. The entire process is fully automated and can process up to 576 samples at the time.

Each workflow includes an additional sample processing module for PAC digestion[1], neat plasma or Mag-Net[2] plasma respectively. These modules are interchangeable, allowing for easy adaptation to various proteomics applications.



Automation without compromise

The combination of Evosep technology and the Biomek i5 liquid handler seamlessly integrates scalabe sample preparation with high-throughput LC-MS analysis, ensuring high-quality and reproducible results. All four workflows exhibit excellent performance using the standard 200 SPD method on the timsTOF HT. Overnight digestion at 37˚C with trypsin and Lys-C ensures high digestion efficiency. This approach results in great proteome coverage with high precision for both HeLa and plasma samples.



Comparison of the workflows carried out using 50 ng QC HeLa digest (Automated loading, n=96), 1 µg HeLa lysate (PAC, n=40), 1 µl plasma (Neat, n=40), and 4 µl plasma (Mag-Net, n=40), respectively. All digestions were carried out at 37˚C overnight (16 hours). The figures showcase, digestion efficiency, mean identified proteins, and protein CVs, respectively on the timsTOF HT.

References

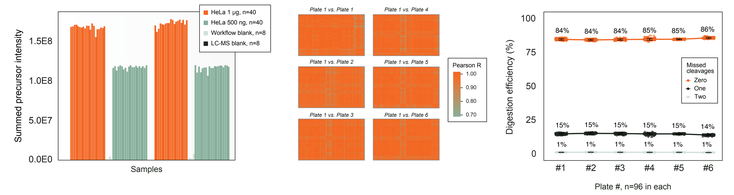
1. Batth TS., Tollenaere M., Rüther P., Gonzalez-Franquesa A., Prabhakar BS., Bekker-Jensen S., Deshmukh AS., Olsen JV (2019) Protein Aggregation Capture on Microparticles Enables Multipurpose Proteomics Sample Preparation. Mol Cell Proteomics., mcp.TIR118.001270

2. Wu CC., Tsantilas KA., Park J., Plubell D., Naicker P., Govender I., Buthelezi S., Stoychev S., Jordaan J., Merrihew G., Huang E., Parker ED., Riffle M., Hoofnagle AN., MacCoss MJ (2023) Mag-Net: Rapid enrichment of membrane-bound particles enables high coverage quantitative analysis of the plasma proteome. BioRxiv., https://www.biorxiv.org/content/10.1101/2023.06.10.544439v1.full.pdf

Outstanding plasma proteomics

Preservation of sample and cohort integrity

In large-scale applications, maintaining sample integrity is crucial. To examine this, we processed a single plate with varying HeLa input amounts and two types of blanks with the PAC workflow. The consistent intensity within conditions and negligible intensity in the blanks confirm the absence of cross-contamination, positional bias, and significant carry-over during analysis. Furthermore, 6 plates with 576 1 µg HeLa samples were processed with the PAC workflow and the 500 SPD method. The entire processing from lysate to data took 47 hours and resulted in an average of 3,500 protein identifications. The consistent digestion efficiency and high Pearson correlation across plates demonstrate minimal inter-plate variation.

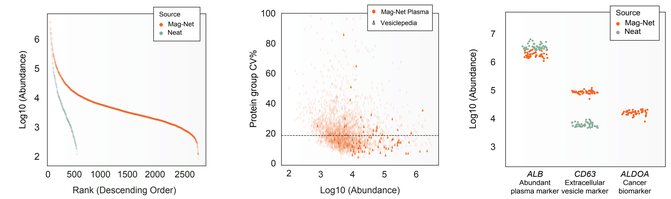


Summed intensities for one plate with 1 µg HeLa, 500 ng HeLa, workflow blanks, and LC-MS blanks, digested overnight at 37˚C. Samples are shown in their run order during acquisition. 6 plates with 1 µg HeLa lysate in each well were prepared simultaneously with overnight digestion at ambient temperature. The heatmaps show protein-level Pearson correlation and the jitter plot shows digestion efficiency across the six plates.

Robust quantification

The Evosep is an ideal platform for handling plasma and other challenging sample types. More than 500 unique proteins were identified from just 1 µl of neat plasma using the 200 SPD method. With the Mag-Net workflow, the dynamic range was successfully compressed yielding 2,700 unique protein identifications with the same 200 SPD method. Additionally, 40 proteins listed in Vesiclepedia were identified in the Mag-Net enriched plasma, with most showing CVs below 20%, validating the method.

Plasma is a rich source of potential biomarkers for minimally invasive monitoring, making plasma proteomics crucial in clinical research. We examined three proteins across 40 replicates: The abundant plasma protein (ALB), the EV marker (CD63), and a known cancer biomarker (ALDOA). Each protein represents a different abundance level in plasma. Both workflows result in good quantification with little variation in abundance within each condition. The identification of 40 proteins listed in Vesiclepedia, most with CVs below 20%, with the Mag-Net workflow validates the approach.



Dynamic range plot for Neat plasma (n=40) and Mag-Net enriched plasma (n=40). A scatter plot showing all identified proteins across all 40 Mag-Net samples. The dashed line marks 20% CV and proteins listed in Vesiclepedia are highlighted with triangles. A jitter plot showcasing the abundance of ALB, CD63, and ALDOA across 40 replicates of Neat and Mag-Net enriched plasma.