High-Throughput Plasma Proteomics: Automated ENRICH-iST Workflow on the Biomek i7 Hybrid Workstation

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Keywords

Proteomics, biomarker discovery, high-throughput sample preparation, automated scalable workflows, plasma analysis, enrichment, dynamic range, protein abundance, iST technology, LC-MS, timsTOF, large sample cohorts.

Key takeaways

- **Automated LC-MS proteomics sample preparation** with reduced hands-on time and errors.
- **Fast, easy-to-use, and standardized workflow** for efficient plasma proteome processing.
- **High-throughput capability** enables processing of up to 96 samples per day, ideal for large-scale studies.
- **Optimized sample preparation** to reduce the high dynamic range in plasma, providing greater proteomic depth.
- **Enhanced protein coverage and identification** compared to manual methods, ensuring more comprehensive results.

Introduction

Liquid chromatography-mass spectrometry (LC-MS)-based proteomics has become a powerful tool for studying complex biological systems, enabling in-depth protein identification and quantification.[1] Despite its potential, key challenges remain, including the vast dynamic range of proteins in biological samples and the need for high-throughput processing to accommodate large-scale studies. Traditional sample preparation workflows are often labor-intensive, time-consuming, and susceptible to variability, limiting reproducibility and scalability.[2]

To overcome these limitations, we present the ENRICH-iST workflow automated on the Biomek i7 liquid handling platform **(Figure 1)**. This streamlined solution integrates magnetic bead-based enrichment with on-deck positive pressure technology to enhance throughput, consistency, and data quality. The automated workflow enables the processing of 96 plasma samples within 6 hours, significantly reducing hands-on time while increasing protein identifications. By addressing critical bottlenecks in LC-MS-based proteomics, this high-throughput, reproducible approach accelerates biomarker discovery and facilitates large-cohort proteomic studies.

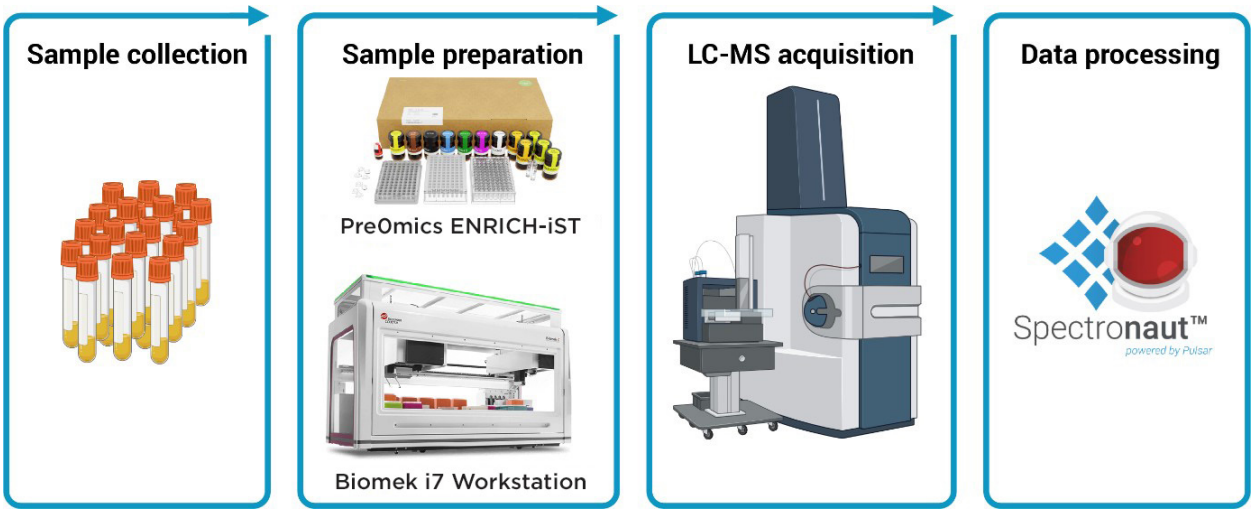

Figure 1. Automated ENRICH-iST workflow overview. Collected plasma samples were processed with the automated ENRICH-iST workflow on a Biomek i7 Workstation. Peptides were analyzed on the timsTOF HT system using the dia-PASEF® method. Data was processed by Spectronaut™ using directDIA+™.

Materials and Methods

Plasma samples were prepared using the ENRICH-iST sample preparation kit (PreOmics, Martinsried, Germany) automated on the Biomek i7 system (Beckman Coulter Life Sciences, Indianapolis, USA). The processed samples were then analyzed via liquid chromatography-mass spectrometry (LC-MS) using the nanoElute timsTOF HT system (Bruker, Billerica, MA, USA) for comprehensive proteomic profiling. Protein identification and quantification were performed using Spectronaut software (Biognosys, Newton, MA, USA).

Plasma preparation

Whole blood samples were drawn using EDTA as an anticoagulant. After collection, the samples were centrifuged at 2,000 x g, and the plasma fractions were transferred to fresh tubes for storage.

Study design

For automation validation, an intra-plate experiment was performed **(Figure 2A)**. Plasma samples and blanks were distributed in a checkerboard pattern on a deep-well plate (n = 48 for plasma samples and blanks). In comparison, quadruplicates of plasma samples and blanks were prepared manually **(Figure 2B)**. Eight plasma samples from the edge and center of the plate, along with four blanks from the center, were selected for LC-MS measurement. All manually prepared samples were measured by LC-MS.

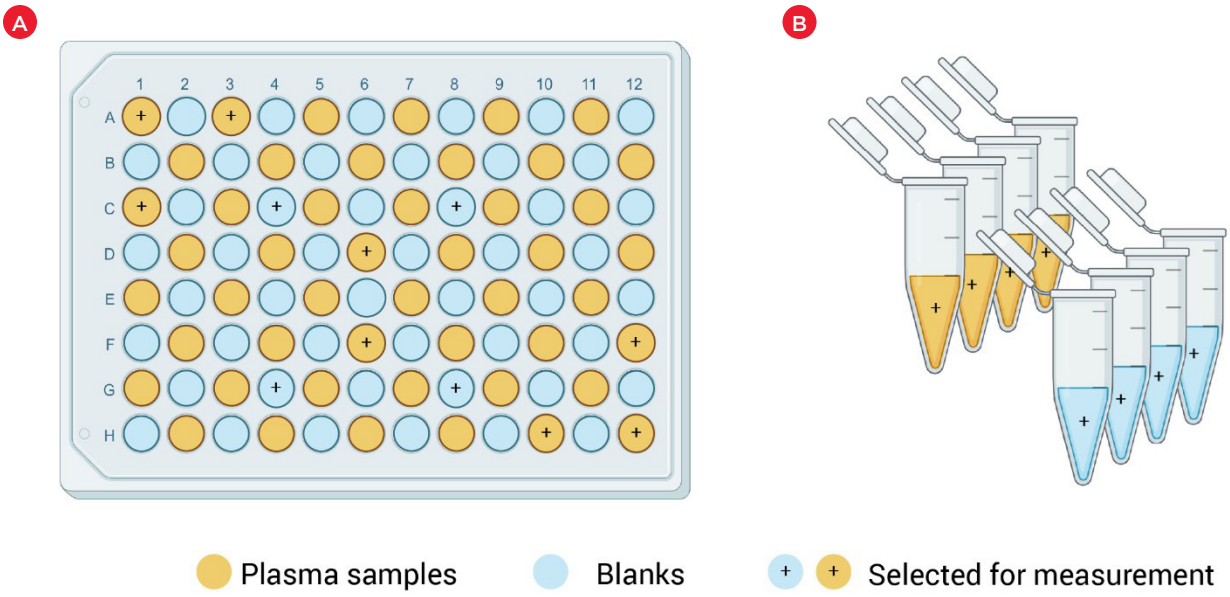

Figure 2. Overview of the study design. (A) Plate design for on-deck automated sample preparation. **(B)** Manually prepared samples.

Biomek i7 workstation configuration

For automation, a Biomek i7 Hybrid Liquid Handling Workstation (Beckman Coulter Life Sciences, Indianapolis, USA) was used. Integrated into the workstation were a microplate shaker (Shaking Peltier ALP, Inheco, Martinsried, DE), an incubator (Inheco, Martinsried, DE), and a positive pressure unit (Amplius, Rostock, DE), which together automate the purification process using an ENRICH-iST sample preparation kit.

For bead processing, a magnetic plate (Magnum FLX, Alpaqua, Beverly, USA) was employed. The plasma samples (Sigma Aldrich, St. Louis, USA) were provided in 96-well protein LoBind PCR plates on the workstation deck. Custom racks were developed by the Center for Life Science Automation (celisca; Rostock, DE) for the preparation of the bead vials, digest vials, and buffer vials from the ENRICH-iST kit. Extraction from the special vials was performed using the Span-8 Pod. The multichannel pod was used in the method sequence to accelerate the liquid transfer processes.

Figure 3 shows the integrated special devices and deck layout. Figure 4 shows the developed custom racks.

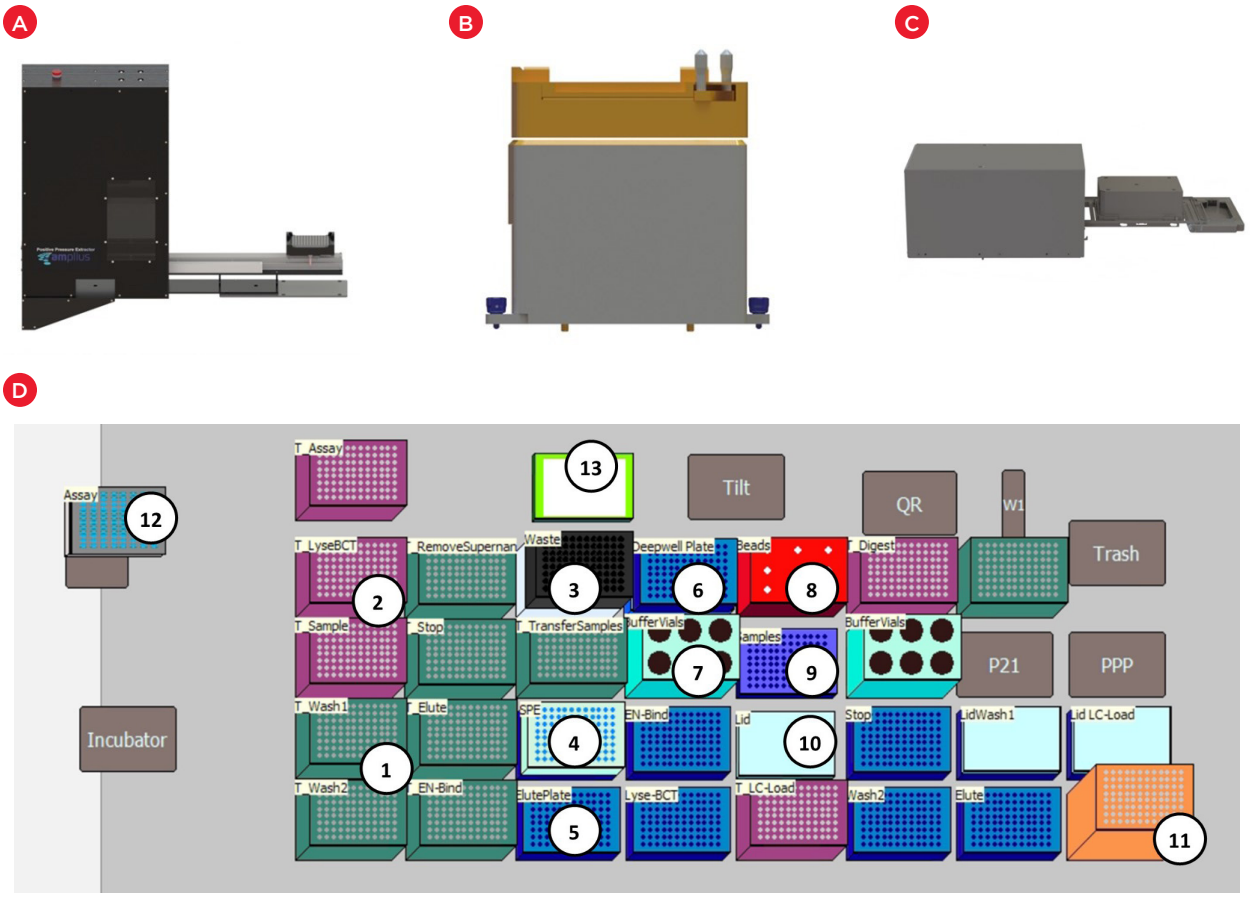

Figure 3. Deck configuration: (A) Positive Pressure Unit, **(B)** Shaking Peltier ALP, **(C)** Incubator, **(D)** Deck layout for PreOmics ENRICH-iST Kit: (1) tip boxes 230 µL, (2) tip boxes 90 µL, (3) reservoir, (4) 96-well sample purification (SPE) plate, (5) 96-well deep-well plates, (6) stack of the magnetic plate and deep-well plates, (7) rack with buffer vials, (8) rack with beads and digest vials, (9) sample plate, (10) lid, (11) tip box 1070 µL, (12) flat bottom 96-well microplate with lid, (13) tissue holder for blotting.

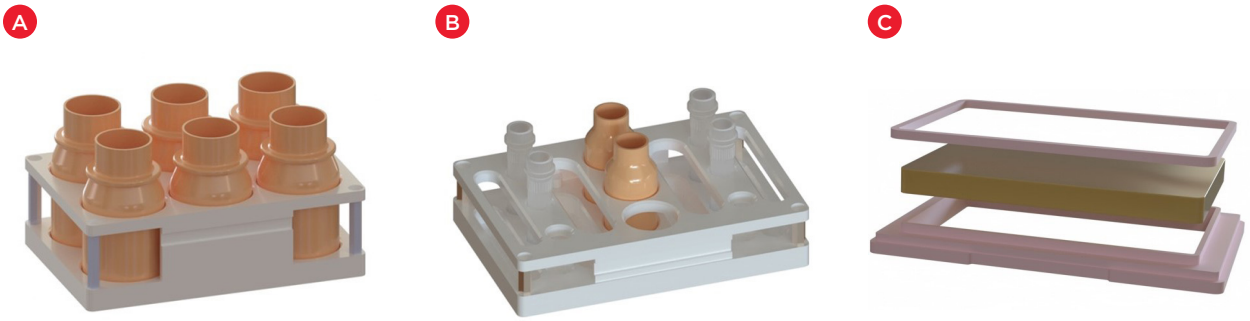

Figure 4. Customized labware: (A) rack with buffer vials, **(B)** rack with beads and digest vials, and **(C)** tissue holder with tissue for blotting.

Automated ENRICH-iST workflow

Step 1: Beads Preparation

In the first step, the beads were resuspended by shaking them on the shaker for 1 minute, followed by thorough homogenization using in-pipette mixing **(Figure 5A)**. Next, 25 µL of beads were transferred to the wells of the 96-deep-well plate (Eppendorf, Hamburg, DE). The beads were then washed three times with 200 µL of EN-Wash buffer per wash. After adding the buffer, the deep-well plate was covered with a lid and shaken for 1 minute at 1,200 rpm. The deep-well plate was then placed on the magnetic plate and left for 1 minute to allow the beads to form a pellet. The supernatant was removed and discarded into a full reservoir (Thermo Fisher Scientific, Waltham, USA). After removing the supernatant, the process was repeated twice more.

Step 2: Enrichment

For the enrichment step, 80 µL of EN-Bind buffer was added to the beads, followed by the addition of 20 µL of plasma for sample loading. The plate was then covered with a lid (Sigma Aldrich, St. Louis, USA) and incubated for 30 minutes at 1,200 rpm and 30°C in the incubator. After incubation, the plate was placed on the magnetic rack for 1 min. The supernatant was removed, and 100 µL EN-Bind buffer was added. The plate was shaken for 1 minute at 1,200 rpm and placed on the magnetic rack again for 1 minute. This process of adding the EN-Bind buffer and removing the supernatant was repeated two more times.

Step 3: Lysis

In this step, 50 µL of LYSE BCT was added to the bead pellets, and the samples bound to the beads were incubated for 10 minutes at 1,200 rpm and 80°C in the preheated incubator. Any volume lost through evaporation during this process was replaced with 20 µL of water (Sigma Aldrich, St. Louis, USA). The samples were then cooled to room temperature for 10 minutes.

Step 4: Digestion

To resuspend the lyophilized digest enzymes, 5 mL of Resuspend-BCT was pipetted into the digest vial. The vial was then shaken on the shaker for 10 minutes and further homogenized using in-pipette mixing **(see Figure 5B)** to ensure complete dissolution of the powder. Next, 50 µL of the digest solution was added to the samples. The deep-well plate was incubated for 1 hour at 1,200 rpm at 37°C. The reaction was stopped by adding 100 µL of stop solution, and the plate was shaken for 1 min at 1,200 rpm.

Step 5: Purification

After loading the samples, the solution containing the beads was completely removed from the deep-well plate and transferred to the SPE cartridges. The SPE plate was then placed on the positive pressure unit for loading, washing, and eluting **(Figure 5C)**. Detailed pressure profiles are described in Table 5. To prevent cross-contamination, the bottom of the SPE plate was blotted on the tissue holder beforehand **(Figure 3C)**.

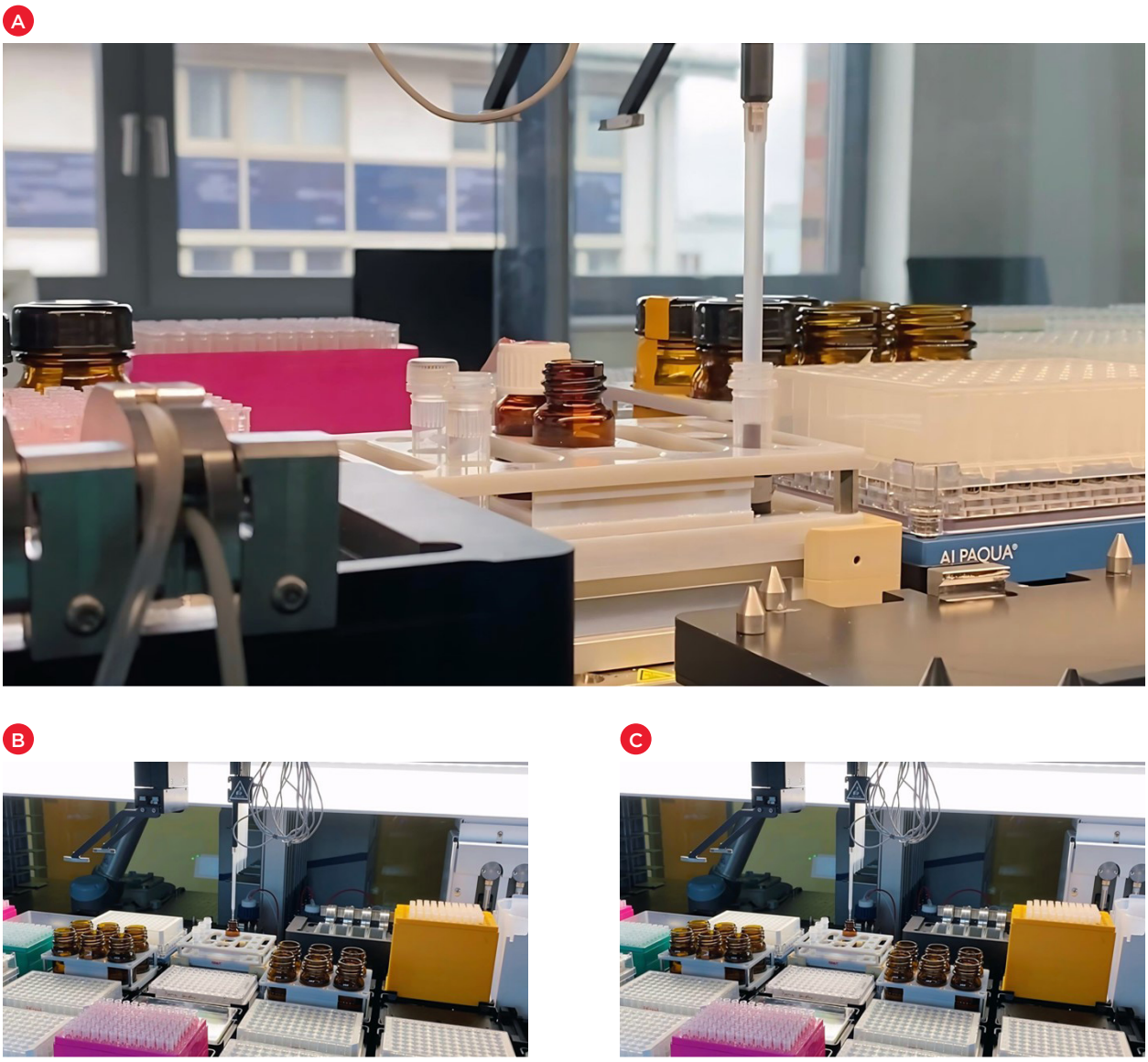

Figure 5. Photos of processes on deck. (A) Homogenization of beads, **(B)** Pipetting on the Digest Vial, and **(C)** SPE plate in the Positive Pressure Unit.

Step 6: Evaporation and reconstitution

After purification, the samples were evenly split into two plates and manually placed under an N2-evaporator (in-house system) for 1 hour until completely dry. For analysis by LC-MS, the samples were reconstituted with 15 µL LC-Load buffer from the ENRICH-iST kit. Alternatively, the samples in the other plate were reconstituted in 15 µL water, and the peptide content was quantified using a fluorometric assay.

Step 7: Fluorometric peptide assay

The fluorometric peptide assay can be performed automatically. For optical measurement, flat, 96-well microplates with black borders (Greiner Bio-One, Frickenhausen, DE) were used. A volume of 10 µL of the reconstituted sample was transferred into the wells. Next, 70 µL of assay buffer from the Pierce Quantitative Fluorometric Peptide Assay kit (Thermo Fisher Scientific, Waltham, USA) and 20 µL of assay reagent were added. After a 5-minute incubation, the measurement was performed in the PHERAstar microplate reader (BMG LABTECH, Ortenberg, Germany). The transfer from the Biomek i7 workstation to the PHERAstar can be carried out using a Universal Robot UR5.

Table 1 summarizes the entire process plan. Up to 96 samples can be prepared using the described method and deck setup shown in Figure 2. Tables 4 and 5 outline the instruments and labware used.

Step	Description
1	Resuspension of beads using the Shaking Peltier ALP and In-Pipette Mixing
2	Transfer 25 µL of beads to the deep-well plate (DWP)
3	Transfer 200 µL EN-Wash buffer to DWP
4	Shake for 1 min at 1,200 rpm
5	Place DWP on the magnetic rack for 1 min to form a bead pellet
6	Remove the supernatant and discard it into the reservoir
7	Repeat steps 3-6 2x
8	Transfer 80 µL EN-Bind to DWP
9	Transfer 20 µL sample to DWP
10	Cover DWP with a lid
11	Incubate DWP for 30 min at 30°C and 1,200 rpm
12	Place DWP on the magnetic rack for 1 min to form a bead pellet
13	Remove the supernatant and discard it into the reservoir
14	Transfer 100 µL EN-Bind to DWP
15	Shake for 1 min at 1,200 rpm
16	Remove the supernatant and discard it into the reservoir
17	Repeat steps 14-16 2x
18	Transfer 50 µL Lyse-BCT to DWP
19	Incubate DWP for 10 min at 80°C and 1,200 rpm
20	Transfer 20 µL of water
21	Cool down to room temperature for 10 min
22	Resuspend Digest with 5 mL Resuspend-BCT
23	Shake Digest Vial for 10 min and homogenize with In-Pipette Mixing
24	Transfer 50 µL Digest to DWP
25	Incubate DWP for 1 h at 37°C and 1,200 rpm
26	Transfer 100 µL Stop-Solution to DWP
27	Shake DWP for 1 min at 1,200 rpm
28	Transfer sample solution from DWP to SPE plate
29	Filter SPE plate using a positive pressure ramp from pF/pC 300/2000 mbar for 10 sec to pF/pC 800/2400 mbar for 240 sec
30	Transfer 200 µL Wash 1 buffer to the SPE plate
31	Filter SPE plate using a positive pressure ramp from pF/pC 300/2000 mbar for 10 sec to pF/pC 800/2400 mbar for 240 sec
32	Transfer 200 µL Wash 2 buffer to the SPE plate
33	Filter SPE plate using a positive pressure ramp from pF/pC 300/2000 mbar for 10 sec to pF/pC 800/2400 mbar for 120 sec
34	Place the SPE plate on the tissue holder to remove any adhering droplets
35	Place the SPE plate over a new DWP to collect the eluate
36	Transfer 100 µL Elute buffer to the SPE plate
37	Filter SPE plate using a positive pressure ramp from pF/pC 300/2000 mbar for 10 sec to pF/pC 400/2000 mbar for 60 sec
38	Repeat steps 35-36 2x

Step	Description
39	Stop automated method
40	Place the elute plate in the evaporator manually for 1 h
41	Restart automated method
42	Reconstitute in 15 µL LC-Load buffer for LC-MS analysis or in 15 µL water for optic analysis (used in this application note)
43	Transfer 10 µL of reconstituted sample to flat-bottom microplate
44	Transfer 70 µL of assay buffer to the flat microplate
45	Transfer 20 µL of assay reagent to the flat microplate
46	Incubate flat microplate for 5 min
47	Transport flat microplate to microplate reader using the Universal Robot UR5
48	Measurement at Ex/Em 390 nm / 475 nm

Table 1. Summary of the automated processing workflow.

A detailed description of the method can also be found in the instructions supplied with the ENRICH-iST kit from PreOmics.

LC-MS and data analysis

Peptides were resuspended in LC-LOAD. For LC-MS analysis, 300 ng of peptides were analyzed using a nanoElute2 HPLC system (Bruker Daltonics, Billerica, MA, USA) equipped with an Aurora™ Ultimate CSI 25×75 C18 UHPLC column (IonOpticks) and coupled to a timsTOF HT mass spectrometer (Bruker Daltonics, Billerica, MA, USA) in dia-PASEF® mode with a 30-minute gradient. MS raw files were analyzed using Spectronaut® 19 (Biognosys, Newton, MA, USA) in directDIA+™ mode against the Swiss-Prot FASTA database of Homo sapiens (2024-02; without isoforms) using factory settings.

Results

Robust sample preparation for plasma proteomic analysis

The performance of the automated bottom-up plasma proteomic workflow was assessed by comparing the results of manual and automated preparation. Automation of the ENRICH-iST technology on the Biomek i7 workstation resulted in a higher number of protein identifications **(Figure 6A)**. The intra-plate variation was comparable to that of the manual workflow **(Figure 6B)**. Additionally, the total protein intensity in blanks prepared using the Biomek i7 workstation was negligible compared to plasma samples **(Figure 6C)**, indicating no cross-contamination during automation. Overall, these findings demonstrate the robustness of the workflow, underscoring its reliability for high-throughput plasma proteomics applications and ensuring reproducible sample processing and peptide quantification.

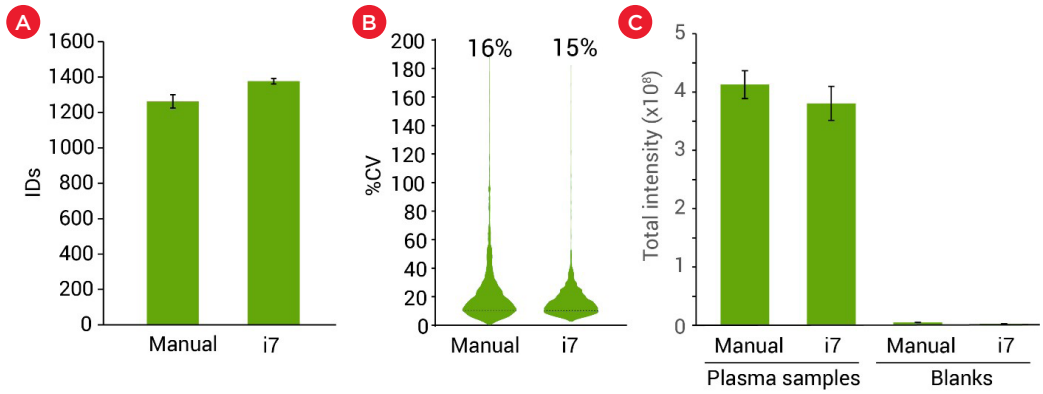

Figure 6. Performance of plasma proteomics sample preparation following manual and automated workflow. Comparison of (A) protein IDs, **(B)** coefficient of variation, and **(C)** cross-contamination between manual and Biomek i7 automated workflows.

Conclusion

This application note showcases the successful automation of the ENRICH-iST sample preparation technology on the Biomek i7 workstation. By integrating the magnet module and on-deck positive pressure module, the workflow could be fully automated until the clean peptide stage, accelerating plasma sample preparation while reducing manual intervention. This enables the processing of 96 samples within 6 hours, yielding higher protein identifications, comparable precision, and no cross-contamination, highlighting the robustness of the system. Overall, the automated ENRICH-iST workflow provides a high-throughput and streamlined solution for plasma proteome preparation, facilitating molecular insights and biomarker discovery in both small- and large-scale studies.

References

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Compound	Vendor	Order No.
ENRICH-iST Kit	PreOmics, Martinsried, DE	P.O.00165
Pierce Quantitative Fluorometric Peptide Assay	Thermo Fisher Scientific, Waltham, USA	23290
Plasma (lyophilized powder)	Sigma Aldrich, St. Louis, USA	P9523
Water	Sigma Aldrich, St. Louis, USA	8483339010

Table 2. Chemicals and reagents.

Labware	Vendor	Order No.
Nunc full reservoir	Thermo Fisher Scientific, Waltham, USA	1200-1300
96-deep-well plate, protein LoBind, PCR Clean, 500 µL	Eppendorf, Hamburg, DE	0030504100
96-well plate, twin.tec PCR plate, 150 µL	Eppendorf, Hamburg, DE	0030128770
Lids	Sigma Aldrich, St. Louis, USA	L4537
Flat bottom 96-well plate	Greiner Bio-One, Frickenhausen, DE	655096
90 µL tips	Beckman Coulter Life Sciences, Indianapolis, USA	B85884
230 µL tips	Beckman Coulter Life Sciences, Indianapolis, USA	B85903
1070 µL tips	Beckman Coulter Life Sciences, Indianapolis, USA	B85945
Magnetic Plate Magnum FLX	Alpaqua, Beverly, USA	A000400
Rack buffer	celisca, Rostock, DE	-
Rack beads & digest	celisca, Rostock, DE	-
Tissue holder	celisca, Rostock, DE	-

Table 3. List of labware.

Instruments	Vendor
Biomek i7 Hybrid Workstation	Beckman Coulter Life Sciences, Indianapolis, USA
Shaking Peltier ALP	Inheco, Martinsried, DE
Single Plate Incubator	Inheco, Martinsried, DE
Positive Pressure Unit	amplius GmbH, Rostock, DE
Evaporator	celisca, Rostock, DE
Universal Robot UR5	Universal Robots, Odense, DK
PHERAstar	BMG Labtech, Ortenberg, DE

Table 4. List of instruments.

	Time (s)	Pressure pF/pC (mbar)
Loading	10	300/2000
	240	800/3200
Wash 1	10	300/2000
	240	800/2400
Wash2	10	300/2000
	120	800/2400
Elution	10	300/2000
	60	400/2000

Table 5. Pressure profile for the purification step.

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