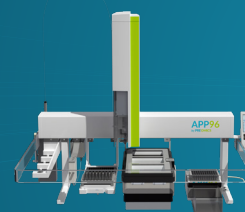


APP96™ iST automation platform: A Game-Changer in Proteomic Sample Preparation



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Introduction

Liquid chromatography-mass spectrometry (LC-MS)-based proteomics is crucial for deciphering complex biological processes, discovering novel biomarkers, and driving drug development.^{1,2} The introduction of the Automated ProteinPrep 96 (APP96) platform marks a significant leap forward in addressing key challenges in high-throughput applications, particularly those associated with traditional labor-intensive and time-consuming sample preparation methods prone to human error.

The APP96 platform, a compact liquid handling system, integrates the advanced in-StageTip (iST) technology to effectively overcome these difficulties while reducing contamination risks and sample loss, which are especially problematic in large-scale studies. By automating the preparation of up

to 96 samples within a single working day, APP96 significantly enhances scalability, efficiency, and reproducibility, ideal for high-throughput proteomics. Its robust and fully automated design ensures consistent results across various sample types, including cells and biological fluids. Additionally, its innovative design further reduces waste by eliminating disposable tips and streamlines the workflow with unique resin-bed tips (POPtips™), enabling sample cleanup without the need for large centrifuges, positive pressure, or vacuum devices.

In this application note, we benchmark the APP96 against traditional manual methods, demonstrating its superior performance and versatility, making it a valuable tool in diverse proteomic applications.

Keywords

APP96™ system, iST technology, high-throughput sample preparation, LC-MS proteomics, workflow automation, scalable proteomics, simplified proteomics, enhanced protein IDs, high reproducibility and standardization, biomarker discovery, drug development

Key takeaways

Fully automated iST sample processing for LC-MS-based proteomic analysis, reducing hands-on time and potential human error.

High throughput capability for processing up to 96 samples daily, ideal for large-scale studies.

Compatible with various sample types, including yeast, mammalian and insect cells, and biological fluids, ensuring broad applicability.

Improved protein coverage and identifications compared to manual methods, especially at low protein inputs.

Superior repeatability with lower intra-day and inter-day variability than manual workflows.

Materials and Methods

Hardware configuration

The APP96 hardware configuration (Figure 1) is equipped with a non-disposable, single-channel liquid-handling Robotic Arm connected to a Dilutor for liquid transfer and constant liquid flow. The system includes both a room temperature (RT) Tray Holder for the respective non-cooled buffers and the PreOmics Purification Tips (POPtips), as well as a refrigerated Tray Cooler for storing cooled buffers and eluted samples. Additionally, a Thermal Mixer is used for key processes such as lysing, reducing, alkylating, and digesting samples. Park Station and Waste Container hold tools and waste material, respectively. The system is operated via the handheld Terminal unit.

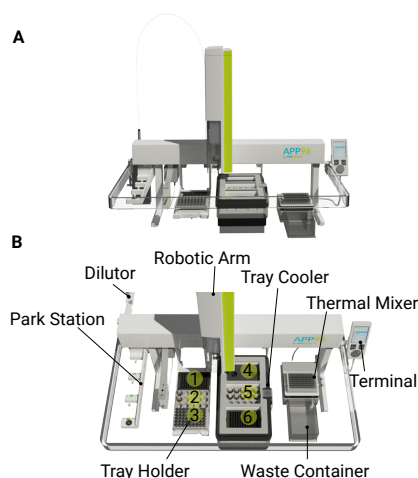


Figure 1 | Configuration of the APP96 platform. (A) Compact deck layout and (B) top view of APP96 with all elements and 6 tray positions indicated.

Samples tested

In this study, different types of biological samples were evaluated (Table 1): yeast cells (*Saccharomyces cerevisiae*), mammalian cells (HeLa and HEK293), insect cells (H5), and human blood plasma (EDTA; DiaServe).

Sample Preparation

The APP96 automated workflow integrates the iST technology for highly efficient bottom-up proteomics sample preparation. It comprises all sample preparation steps, including lysis, reduction, alkylation, digestion, and peptide cleanup. Cell samples were processed using the iST APP96 kit 96x, and human plasma samples were processed with the iST-BCT APP96 kit 96x (Table 1). In parallel, blank samples

were used to evaluate system carryover. As a reference workflow, the same samples were prepared manually using the respective iST or iST-BCT kits accordingly (Figure 2). After peptide elution, all samples were dried and resuspended in LC-LOAD buffer for LC-MS analysis.

LC-MS and data analysis

200 ng of peptides from each sample were separated by various LC systems using different columns and gradients, as indicated in Table 1. For yeast samples, data acquisition was done in DDA-PASEF® mode on a timsTOF Pro, while all other samples were analyzed in dia-PASEF® mode on a timsTOF-Pro or -HT. DDA searches were performed using MaxQuant, and DIA searches were carried out by Spectronaut (Biognosys) using the library-free directDIA mode (Table 1).

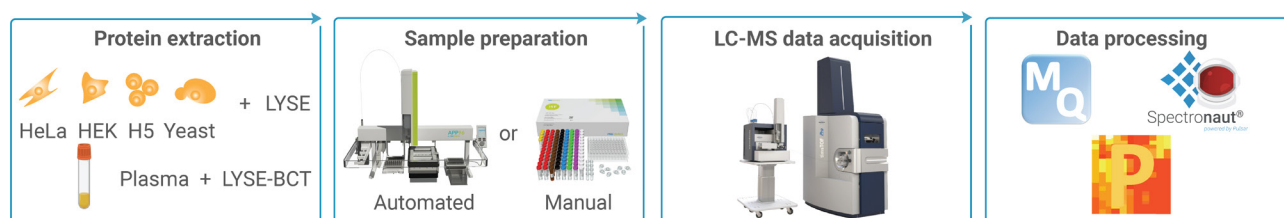


Figure 2 | Overview of the workflow comparing the APP96 and manual protocol.

Human EDTA plasma, yeast cells, mammalian cells (HeLa and HEK293), and insect cells (H5) were processed either by APP96 or manually using the respective iST or iST-BCT kits. Experimental conditions are described in Table 1.

Table 1 | Detailed information on sample types, input amounts, kits, LC setups, MS methods, and data processing software

Sample type	Input amount	Kit	LC (System, Gradient, and column)	MS (System, methods)	Data Processing
Yeast	1–50 µg protein	iST APP96 kit 96x	- EASY-nLC 1200 - 30 min - Self-packed column (25 cm, 75 µm, 1.9 µm C18)	TimsTOF Pro dda-PASEF	MaxQuant (v 2.0.1.0)
HeLa	0.4–100 µg protein	iST APP96 kit 96x	- nanoElute2 - 30 min - Ion Optics Aurora (25 cmx75µm, 1.6 µm C18)	TimsTOF HT dia-PASEF	Spectronaut 17
Plasma	2 µL liquid	iST-BCT APP96 kit 96x	- nanoElute2 - 6 min - Ion Optics Aurora (25 cmx75µm, 1.6 µm C18)	TimsTOF HT dia-PASEF	
HEK293	50 µg protein	iST APP96 kit 96x	- Evosep - 30 min - EVOSEP ENDURANCE EV1106 (15 cm, 150 µm, 1.9 µm C18)	TimsTOF HT dia-PASEF	Spectronaut 18
H5	50 µg protein	iST APP96 kit 96x			

Results and Discussion

High versatility in sample types

The APP96 versatility was demonstrated by processing five distinct sample types on the automation platform. Evaluation of protein group and peptide identifications revealed an excellent performance of the APP96 for preparing different mammalian cell types (HeLa and HEK293), insect cells (H5), yeast cells (*S. cerevisiae*), and human blood plasma (Figure 3), showcasing its compatibility with a wide range of input material.

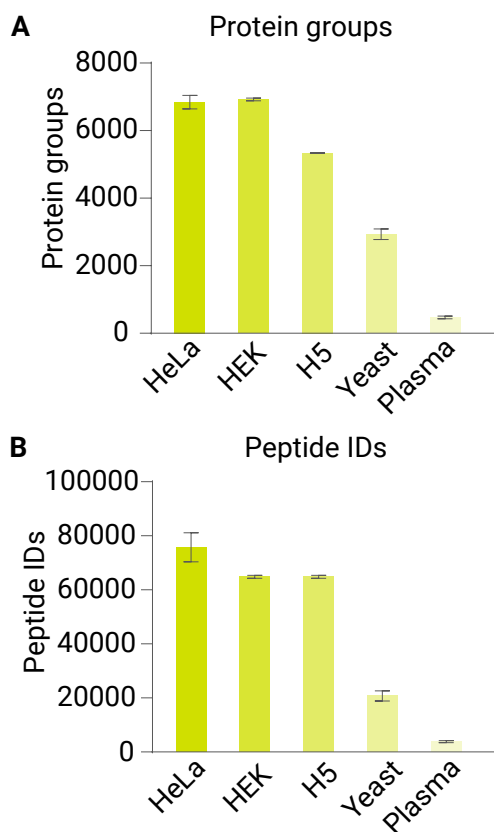


Figure 3 | APP96 system performance in processing various sample types. (A) Identified protein group IDs and (B) peptide IDs across sample types prepared using APP96. Samples analyzed: mammalian cells (HeLa and HEK), insect cells (H5), yeast, and human blood plasma (N=3, dia-PASEF for all samples except for yeast with dda-PASEF, input: 50 µg protein for cells and 2 µL for plasma).

Increased protein identifications

The APP96 efficiency was compared to manual sample processing in identifying protein groups across a dilution series of input protein amounts, ranging from 1–100 µg for yeast and 0.4–50 µg for HeLa. In both yeast and HeLa samples, APP96 consistently identified more protein groups than the manual workflow at every level of protein input. The logarithmic trend lines indicate that APP96 exhibits a steeper increase at lower protein inputs, highlighting its higher sensitivity in this range (Figure 4).

For instance, at a yeast input of 10 µg, approximately 2,200 protein groups were identified in the APP96 samples compared to 1,900 for the manually processed samples.

Similarly, at a HeLa input of 2 µg, around 6,200 protein groups were identified in the APP96 samples, whereas about 5,900 were identified in the manually processed samples. The maximum number of protein groups identified by APP96 in yeast and HeLa reached approximately 2,900 and 6,800, respectively, compared to 2,500 and 6,600 identified by the manual method. These results demonstrate the superior performance of APP96, especially at low-input conditions. The data from the dilution series underscore that APP96 is more efficient, sensitive at low protein inputs, and delivers enhanced protein identification, making it a more advantageous method for comprehensive protein analysis.

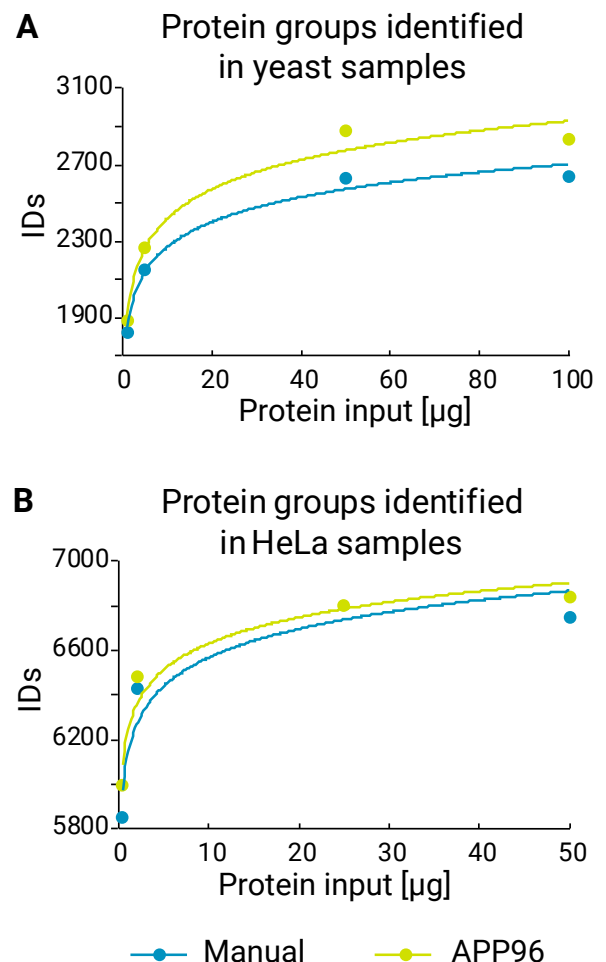


Figure 4 | Comparison of protein groups identified with APP96 and manual sample preparation. (A) For yeast, 1–100 µg protein input and (B) for HeLa, 0.4–50 µg protein input was processed and analyzed (N=3). Data acquisition was done in dda-PASEF for yeast and dia-PASEF mode for HeLa. Scatter plots: average IDs of triplicates. Trendline: logarithmic.

Enhanced repeatability

To evaluate the overall performance of iST automated sample preparation on the APP96 system, it was compared to the manual iST workflow using a diverse set of sample types, including HeLa and yeast cells (each with 50 µg protein input) and human plasma samples (with 2 µL input). The results indicated an equal or slightly higher number of protein group identifications for all APP96 samples compared to the manual samples, with a notable overlap between the two workflows (Figure 5A, C). Specifically, more than 98% of the 6,800 protein groups identified in HeLa cells were common to both APP96 and manual sample

preparation. Additionally, all three sample types prepared by APP96 demonstrated improved digestion efficiency and repeatability compared to the manual workflow (Figure 5B). The intra-day coefficient of variation (CV) improved from 8–10% with manual preparation to 5–8% with the automated method, while the inter-day CV was reduced from 23% with manual preparation to 17% with the automated samples (Figure 5D).

Altogether, the APP96 automated workflow exhibited superior performance in terms of protein identification, digestion efficiency, and repeatability compared to the manual iST workflow.

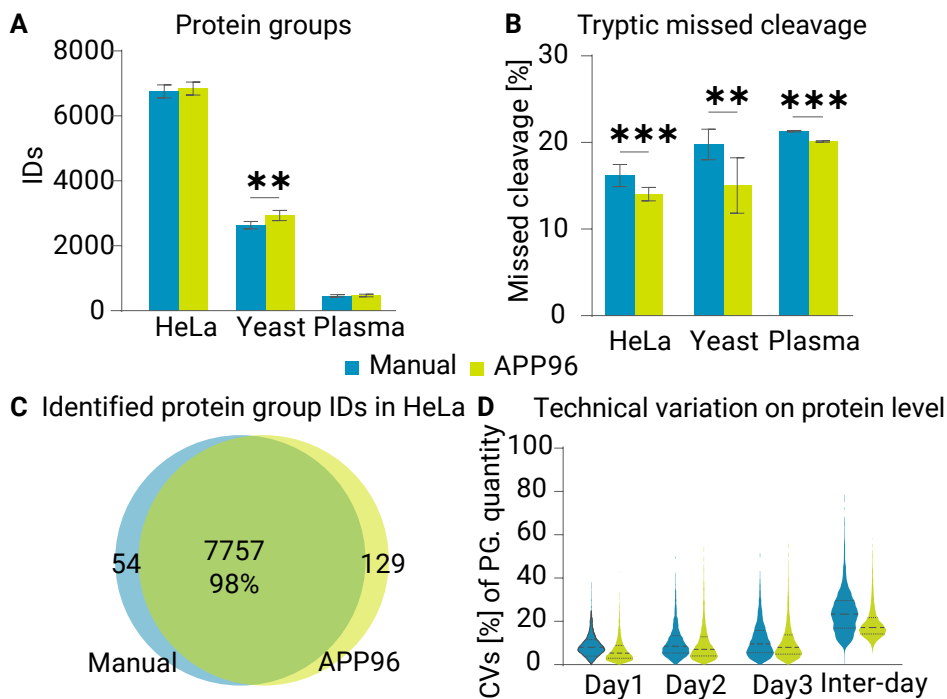


Figure 5 | Comparison of various cell types processed with APP96 and the manual workflow. HeLa and yeast cells (both 50 µg protein input) and human plasma samples (2 µL) were processed and evaluated regarding (A) protein group identifications (N=9, t-test, **p<0.01); (B) tryptic missed cleavage rates (N=9, t-test, **p<0.01, ***p<0.001); (C) protein groups overlap between manual and automated workflow for HeLa cells; and (D) intra-day and inter-day repeatability for HeLa cells (N=3 for intra-day and N=9 for inter-day).

Conclusion

The APP96 system represents a major advancement in proteomic sample preparation, addressing the limitations of traditional methods. By integrating iST technology into a compact, fully automated liquid handling platform, APP96 offers a scalable, efficient, and reliable solution for high-throughput proteomics, capable of processing up to 96 samples per day across various applications, from small to large-scale studies.

This streamlined platform eliminates the need for large centrifuges or complex vacuum systems and avoids waste from disposable tips. It is versatile and compatible with a wide range of sample types, such as mammalian, insect, and yeast cells, as well as human blood plasma.

Compared to manual methods, the APP96 enhances sensitivity and protein identification, even with low protein inputs, and ensures high repeatability with lower intra-day and inter-day variability.

Overall, the APP96 powered by the iST technology provides a robust, fully automated, and scalable solution that improves the efficiency, reliability, and reproducibility of proteomic studies, enabling more accurate and comprehensive protein analyses.

Products

Product	Workflow	Manufacturer	Product Code
APP96 instrument	Automation	PreOmics GmbH	P.O.00139
iST APP96 kit 96x	Automation	PreOmics GmbH	P.O.00141
iST-BCT APP96 kit 96x	Automation	PreOmics GmbH	P.O.00184
iST 8x	Manual	PreOmics GmbH	P.O.00001
iST-BCT 8x	Manual	PreOmics GmbH	P.O.00084

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Acknowledgments

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