

Automated in-StageTip (iST)-based sample preparation for scaling up large cohort proteomics studies

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INTRODUCTION

Automation is a game-changer in proteomics. It speeds up the ability to unravel complex biological processes, discover new biomarkers, and assess the effectiveness of new drugs. To gain comprehensive insights, efficient preparation and analysis of multiple samples in parallel is crucial.

However, a key challenge is the need for a robust and rapid sample preparation workflow. To address this, we developed an innovative proteomics workflow that integrates the iST technology into a fully automated, compact liquid handling platform, the Automated ProteinPrep 96 (APP96™). The new system can process 1–96 samples daily with the innovative PreOmics Purification Tips POPtips™ and thereby eliminates the need for centrifuges, vacuum devices, or liquid handling disposable tips.

MATERIALS AND METHODS

Protein extraction

HeLa HEK H5 Yeast + LYSE
Plasma + LYSE-BCT

Sample preparation

Automated or Manual

LC-MS data acquisition

Data processing

MQ Spectronaut[®] DIA™

Sample type	Input amount	Kit	LC	Gradient [min]	MS	Acquisition	Data processing
Yeast	50 µg protein	iST	EASY-nLC™ 1200	30	timsTOF Pro/HT	dda-PASEF [®]	MaxQuant (v2.0.1.0)
HeLa	50 µg protein	iST	nanoElute [®] 2	30		dia-PASEF [®]	Spectronaut 17
Human EDTA plasma	2 µL liquid	iST-BCT		6			Spectronaut 18
HEK293	50 µg protein	iST	Evosep One	30			direct DIA™
High five	50 µg protein	iST		30			

RESULTS

Fully automated iST sample preparation

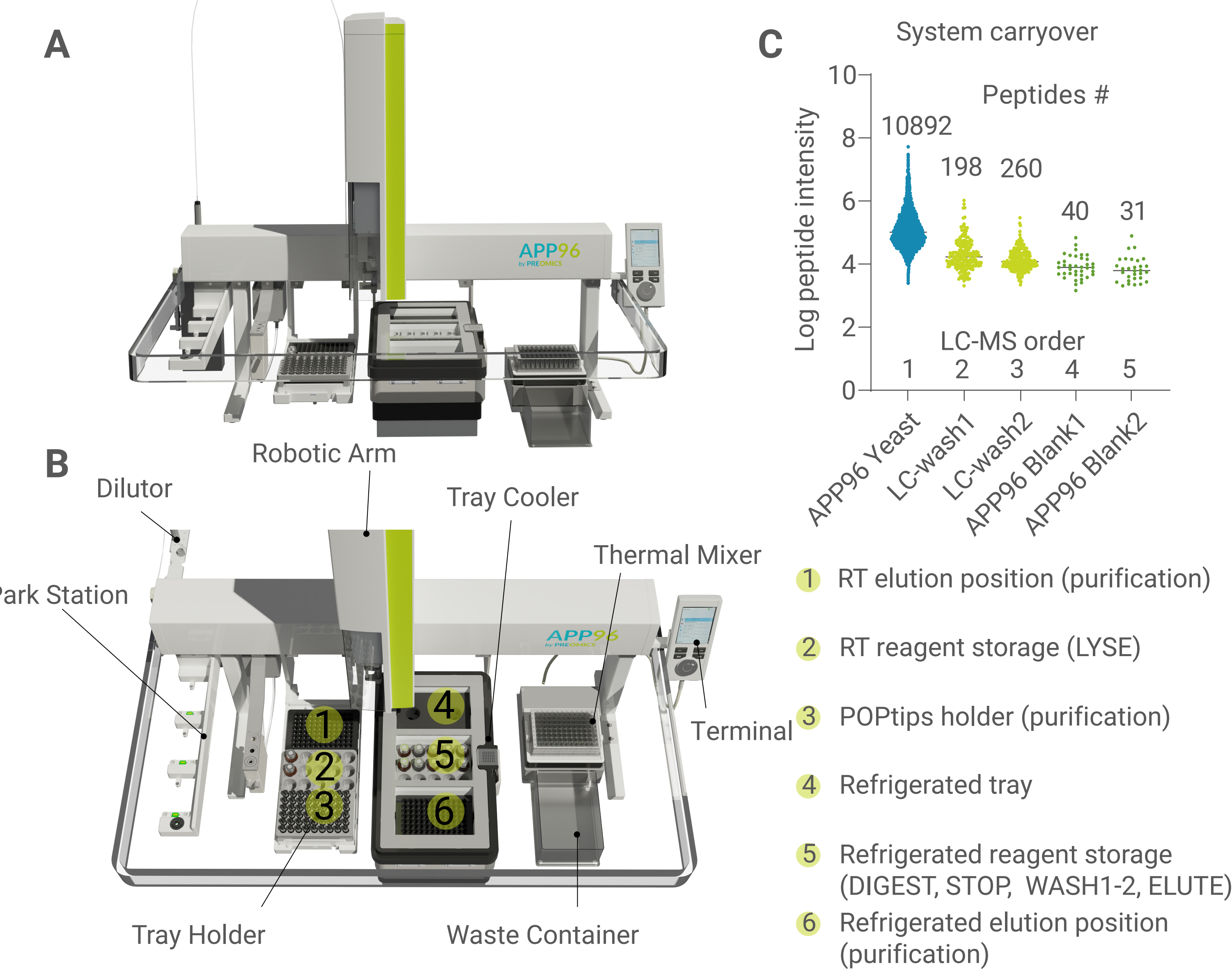


Figure 1. Robust configuration of the APP96 platform. The deck layout (A) and top view (B) showcase the compact, all-in-one APP96 system, which offers a straightforward, standardized, and flexible automation solution for processing 1 to 96 LC-MS samples using the iST technology, including lysis, reduction, alkylation, digestion, and purification. System carryover assessment (C) shows no evidence of cross-contamination between samples.

Innovative POPtips for peptides purification

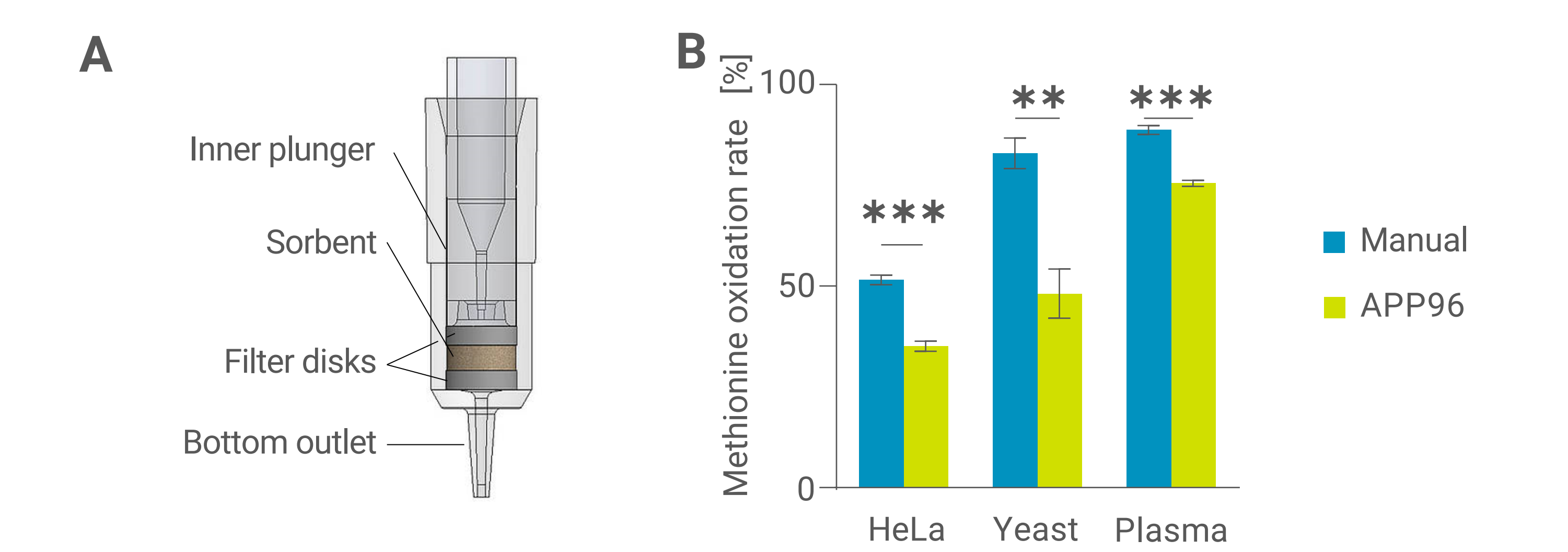


Figure 2. Reduced methionine oxidation achieved by POPtips. The innovative design of the seamless POPtips setup (A) minimizes air passing through the sorbent, leading to a notable reduction in methionine oxidation rates compared to the manual iST workflow (B, N=9).

Enhanced performance of iST sample preparation

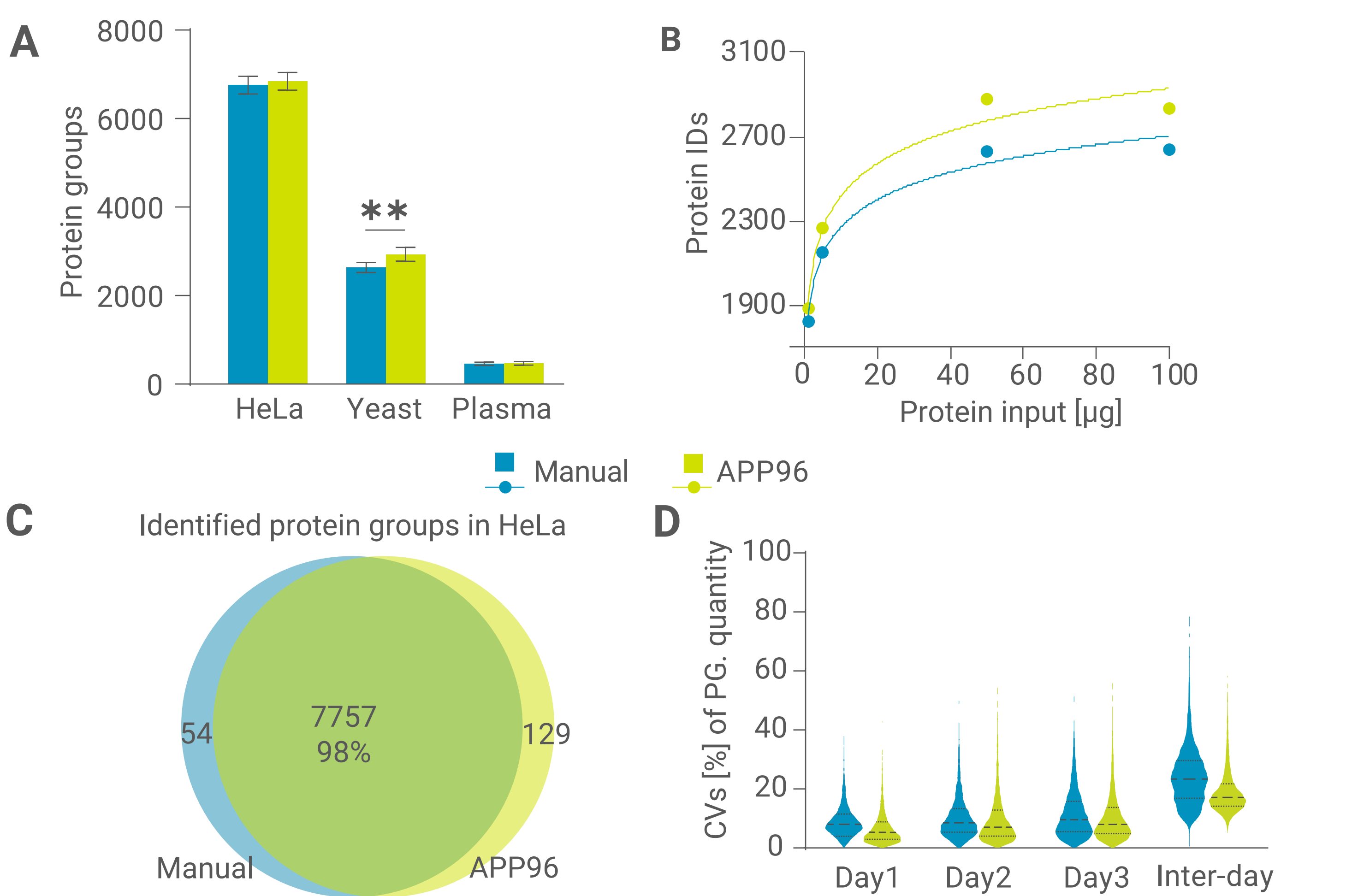


Figure 3. Improved protein identification and quantification. Compared to the manual workflow, different samples types processed on the APP96 demonstrate a slight increase in protein identifications (A, N=9). Additionally, 1–100 µg yeast protein samples were processed and analyzed. APP96 exhibits a steeper increase at lower protein inputs, highlighting its higher sensitivity in this range (B, N=3, scatter plots: average IDs of triplicates; trendline: logarithmic). An extraordinary overlap (>98%) is observed between the automated and manual approaches (C, HeLa, N=3). Improved intra-day ($CV_M=8-10\%$ vs. $CV_A=5-8\%$) and inter-day ($CV_M=23\%$ vs. $CV_A=17\%$) repeatability in protein quantification (D) are also observed. (C and D were generated using HeLa cells; N=3 for intra-day and N=9 for inter-day)

High versatility in sample types

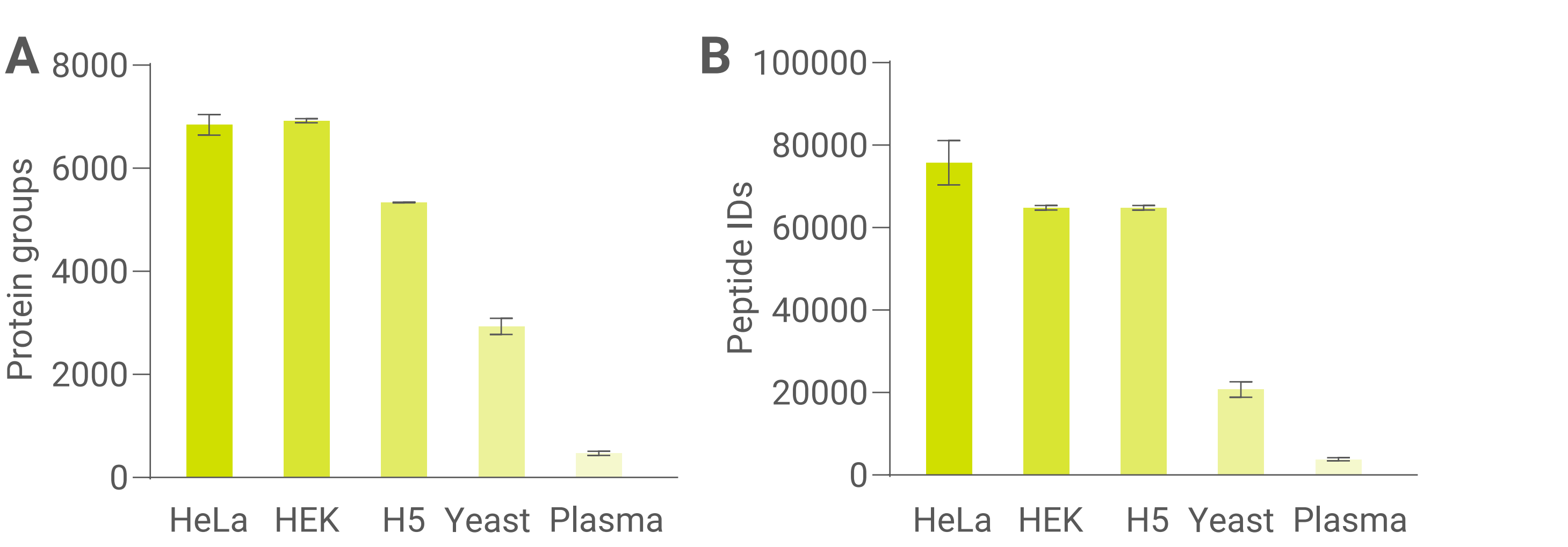


Figure 4. APP96 system performance in processing various sample types. Protein groups (A) and peptide IDs (B) were evaluated across various sample types prepared using APP96, including mammalian cells (HeLa and HEK), insect cells (H5), yeast, and human blood plasma. (N=3)

Interpretation: **: 0.001 < p-value < 0.01; ***: p-value < 0.001.

KEY TAKEAWAYS

Improved performance.

The APP96 workflow offers several advantages over the manual iST method, including reduced methionine oxidation, improved performance at lower input ranges, comparable identification, and enhanced repeatability.

Enhanced workflow.

The fully automated iST sample preparation processes 1–96 samples per day with the option for overnight operation. The introduction of POPtips eliminates the need for a centrifuge, positive pressure, or vacuum device, offering an eco-friendly solution that reduces plastic waste.

Versatile technology.

The APP96 workflow is highly compatible with a wide range of samples, including mammalian cells (HeLa and HEK), insect cells (H5), yeast, and human blood plasma.

