Application Note

EVOSEP

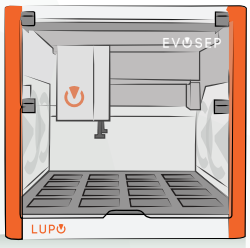
Standardized sample preparation at scale with Evokits and Evosep Lupo

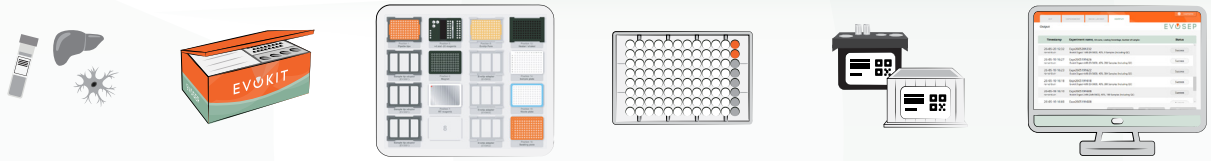
Highlights

- Evosep Proteomics enables high-performance Sample to Separation solutions for mass spectrometry.
- Evokits with built-in quality control samples, executed through Evosep Lupo.
- Automated verification of setup and consumables for seamless sample preparation.

1. Introduction

For proteomics to operate reliably in large-scale and regulated environments, reproducibility must be continuously verified – not assumed. This requires integrated quality control, full traceability, and automation that reduces risk at every step. Evosep Proteomics embeds these capabilities directly into the workflow using standardized Evokits with incorporated quality controls to monitor the full sample preparation workflow and verify LC-MS performance. Evokits are executed on Evosep Lupo further reducing variability and operational risk. The system incorporates intelligent checks for consumables, plate positioning, and workflow execution, minimizing human error. Combined with a simplified interface and predefined workflows, this allows true walk-away operation, enabling users to generate reproducible, high-quality data without specialized expertise.





Starting point
Protein lysate, reduced and alkylated
≤ 1 µg protein lysate
≤ 0.5% SDS final conc.
≤ 5 µl sample volume

Standardized kits
No preparation needed.
Consumables stored under required conditions.

Error-free setup
Built-in verification of correct position, orientation and expiry.

Built-in QC
Automated monitoring kit and LC-MS performance.
Supports additional study QCs.

Full traceability
Batch tracking.
Easy access to Certificate of Analysis.

MS ready export
Tailored .csv file for your setup.
Controlled run order via Evotip positions.

Figure 1: Overview of how Evokits are executed through Evosep Lupo.

2. Method details

K562 cell lysates prepared using 1% SDS. For one experiment, the samples were spiked with common lysis-buffer contaminants. Samples were processed using Evokit Digest, 1x96 (EV3802) together with built-in control samples on a Evosep Lupo instrument. 1 µg sample input was aliquoted into an Evokit sample plate and inserted into the Evosep Lupo, where the protocol associated to the Evokit was selected with a choice of loading 30% of the peptide digest on the Evotip.

Evotips were processed with the 500 SPD and 200 SPD methods on an Evosep Eno. An Evosep Performance column (EV1182) was used at 40 °C with Evosep Pods (EV1187 for Thermo EasySpray, EV1189 for Waters NanoLockSpray). Discovery analysis was performed on an Orbitrap Astral MS (Thermo Fisher Scientific) with EasySpray (1.9kV spray voltage, funnel RF level 40, 275 °C). DIA full MS scans were acquired at 240,000 resolution over 380-980 m/z and with an injection time (IT) of 3 ms. Isolation windows were set to 4 m/z, and HCD fragmentation was performed at 25% NCE with 3ms IT and 0.6 s cycle time. Raw files were analyzed with DIA-NN (v1.9.2) in library-free mode against the reviewed human proteome (UniProt, Oct 2020, 20,600 entries) using trypsin/P with up to two missed cleavages, and default settings. Conditions were searched separately, with match between runs enabled within replicates.

Targeted analysis was performed on a Waters Xevo TQ-Absolute XR with a NanoFlow ESI source (150 °C; 3 kV capillary, 33 V cone; gas 100 L/h for purge and 50 L/h for cone). Analyzer settings were: LM Res1 2.63, HM Res1 15.01, LM Res2 2.65, and HM Res2 14.98. Data were acquired in MassLynx using an MRM assay for 17 K562 proteotypic peptides, with automatic dwell time optimization. Skyline was used to optimize collision energies and cone voltages for MRM method development and transition filtering, refining the method to three transitions per peptide for specificity.

3. Built-in quality controls

For proteomics to operate reliably in large-scale and regulated environments, reproducibility must be continuously verified—not assumed. This requires integrated quality control, full traceability, and automation that reduces risk at every step. Evosep Proteomics addresses this by embedding two complementary quality controls: a process control sample that monitors the entire sample preparation workflow, and a system suitability control that verifies LC–MS performance over time. Executed as part of the routine workflow, these controls enable continuous quality monitoring and support early detection of deviations without increasing operational complexity.

QC samples are positioned in the last column of each 96-well plate, while data acquisition is designed to analyze them alongside all study samples during LC–MS analysis. This is enabled by predefined run-order setups, which can be easily accessed through an intuitive software

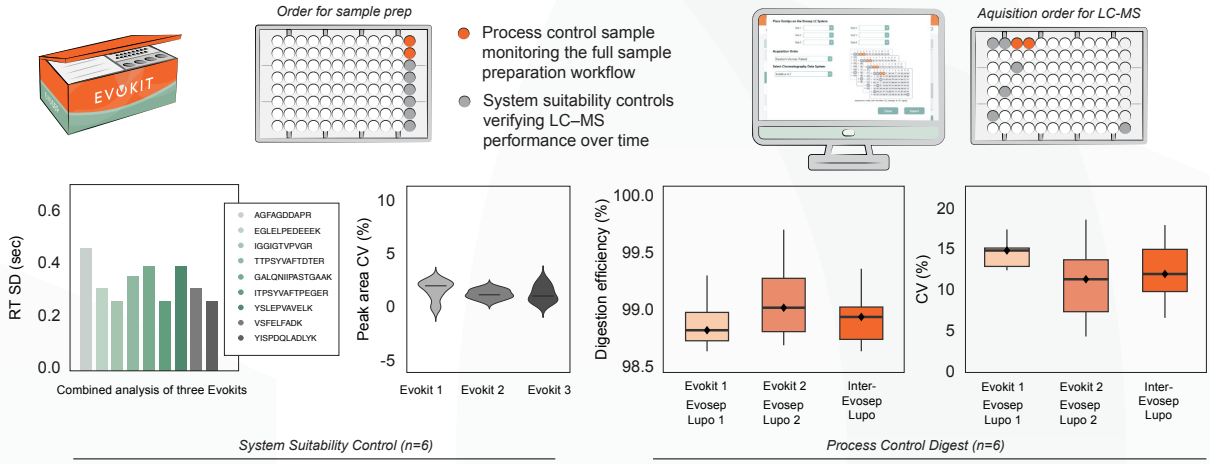


Figure 2: Performance of Process Control Digest and System Suitability Control samples across Evokits. The Evosep Eno 200 SPD method was used with a Waters Xevo TQ-Absoute XR in MRM mode.

interface, ensuring seamless integration of quality assessment into routine operation.

Reporter peptides from the system suitability sample are used to assess LC-MS performance on the Evosep Lupo instrument by monitoring chromatographic and signal stability across Evokits. Retention time, peak shape, peak width, and signal intensity remains consistent across Evokits, injections, and days, with low RT variability (<1 s). The Evokit workflow on Evosep Lupo demonstrates high technical reproducibility, with strong agreement across systems over the full dynamic range. While LC-MS variability remains below 5% (SSC), overall workflow variability (~10–15% CV) highlights excellent performance across the complete sample preparation and analysis pipeline. Digestion efficiency, calculated from the ratio of fully cleaved to missed cleavage peptides, remains consistently high (~99%), confirming robust and reliable front-end processing.

4. Robustness between runs

Workflow transferability and inter-platform consistency were assessed by evaluating reproducibility across two independent plates processed on separate Evosep Lupo systems. The analysis showed a 92% overlap in identified proteins between plates and systems, calculated across all identified proteins without applying missing-value filtering, as visualized by the Venn diagram, while closely aligned CV distributions confirmed consistent quantitative performance across runs. Together, these data demonstrate that the workflow delivers reproducible and transferable performance across independent plates and automated platforms.

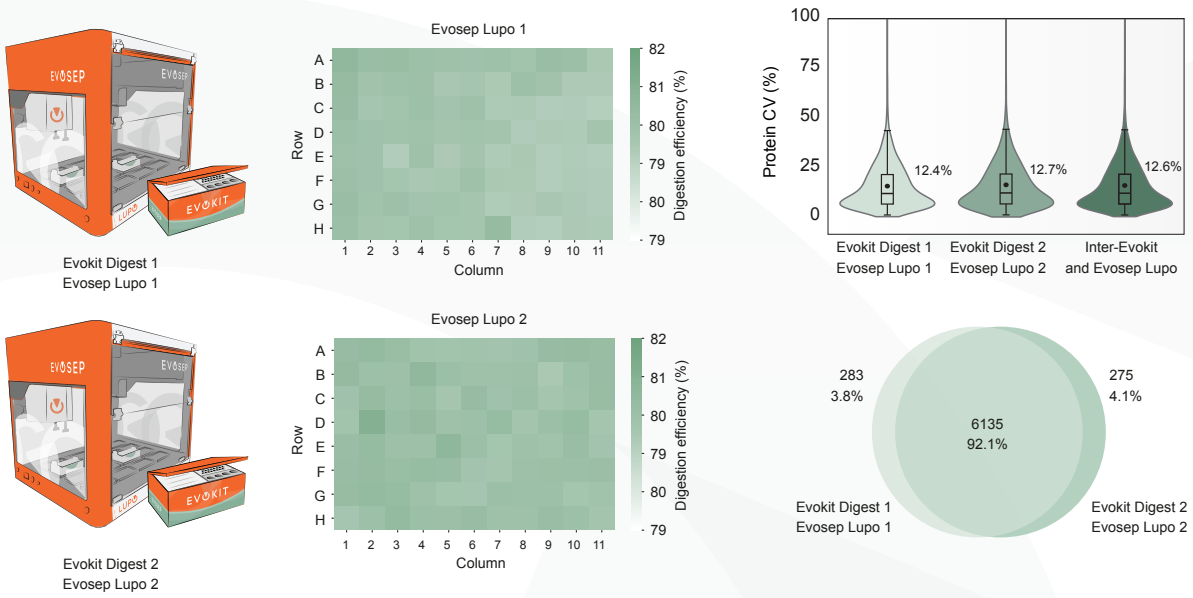


Figure 3: Inter-robot performance of two individual Evokits monitoring digestion reproducibility across the plates, quantitative precision using the 500 SPD Evosep Eno method on the Orbitrap Astral MS and overlap between identified proteins on the two platforms.

5. Diverse sample matrices

The workflow was challenged using a complex bone tissue matrix spiked with detergents, salts, and chaotropic reagents to reflect realistic sample conditions encountered in routine proteomics workflows. Even in the presence of contaminants that typically challenge LC–MS sample preparation and analysis, the workflow maintained stable retention times (SD <0.5 s for all measured peptides), consistent precursor identifications, and comparable CV distributions across conditions, demonstrating robust contaminant removal and reproducible performance across diverse sample inputs.

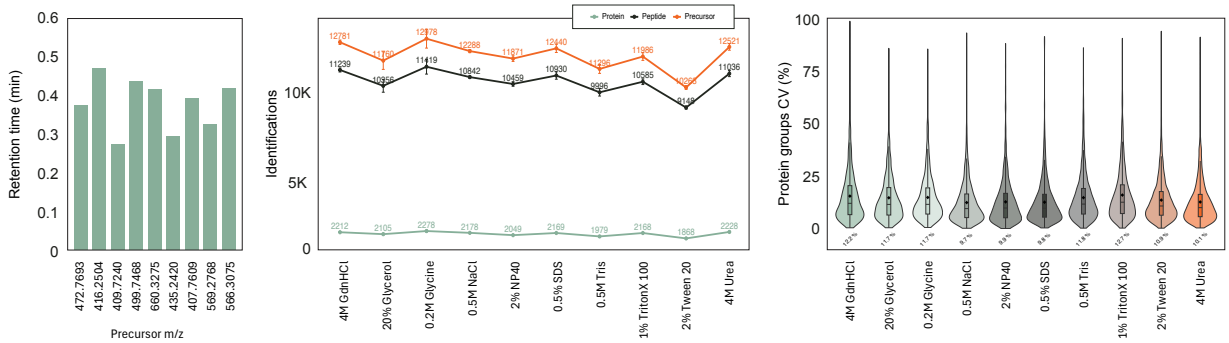


Figure 4: Retention time stability, identifications (protein, peptide, precursor), and protein group CV across sample matrices using 1 µg bone tissue as starting material, 30% digest loaded on Evotip (n=8 per sample matrix). Orbitrap Astral 500 SPD.

6. Conclusion

Evosep Lupo combined with Evokit Digest provides a standardized, scalable, and traceable approach to proteomics sample preparation designed for routine operation rather than expert-dependent execution. The workflow demonstrates robust quantitative precision and highly reproducible performance across plates, Evosep Lupo systems, and challenging sample matrices, highlighting strong transferability under conditions relevant to high-throughput proteomics.

Importantly, the integration of built-in quality controls extends this solution beyond automation alone by enabling continuous monitoring of both sample preparation and LC–MS performance. These complementary controls support early detection of deviations and continuous verification of reproducibility, strengthening analytical confidence in large-cohort and regulated environments.

Together with simplified setup, guided execution, and digital process traceability, these features enable reliable and scalable implementation of proteomics workflows with reduced operational complexity. Overall, this integrated sample-to-separation solution establishes a strong foundation for standardized, operational proteomics from research to applied settings.