Cost efficient, fully automated high throughput sample preparation with Evokits and Evosep Lupo for large scale discovery proteomics

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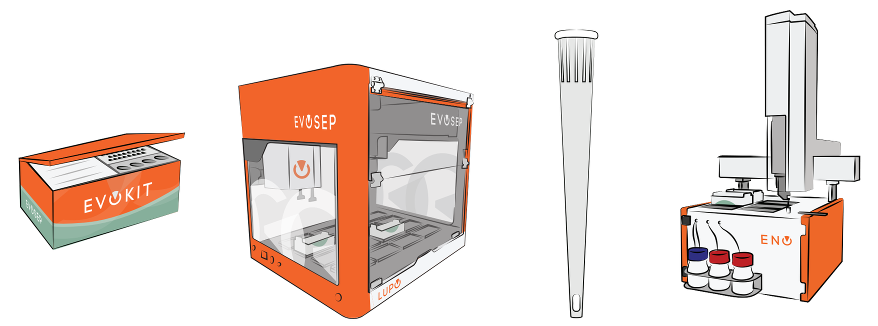
Highlights

- Evosep Proteomics enables a high-performance Sample to Separation solution for standardized and scalable mass spectrometry.
- Evokits with built-in quality control samples, executed through Evosep Lupo with automated verification of setup and consumables for seamless sample preparation.
- Robust protein quantification within the ecosystem with <13% protein CVs across ten commonly used sample matrices using the Evosep Eno 500 SPD method .

Robust sample preparation is the key to scalable proteomics

An ecosystem from sample to separation

Evosep Proteomics extends standardization upstream by integrating sample preparation into the same controlled ecosystem as LC separation. The combination of Evokits, Evosep Lupo, and the Evosep Eno creates a unified workflow with Evotip Pure as the crucial link between sample preparation and LC-MS analysis, ensuring each stage deliver consistent and optimized outputs. Together, these components form an integrated ecosystem where processes are controlled as a single system rather than independent steps.



Evokit – *Standardized kits with defined protocols*

Evosep Lupo – *Dedicated platform to execute Evokits*

Evotip Pure – *Sample desalting and storage*

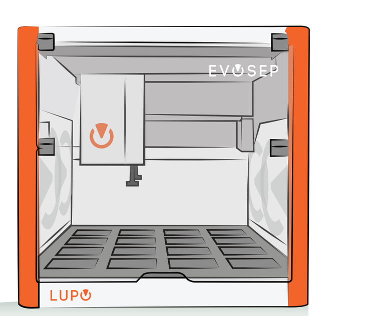
Evosep Eno – *Robust, high-throughput LC-MS separation*

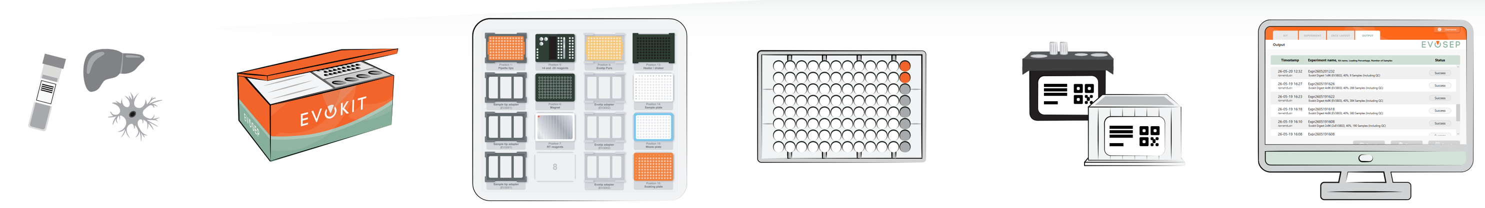
Reproducible results with built-in controls and walk-away automation

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.

Each Evokit incorporates built-in quality controls, including:
- Process control samples that monitor the full sample preparation workflow
- System suitability controls that verify LC–MS performance over time

Automation through the Evosep Lupo platform further reduces 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.*

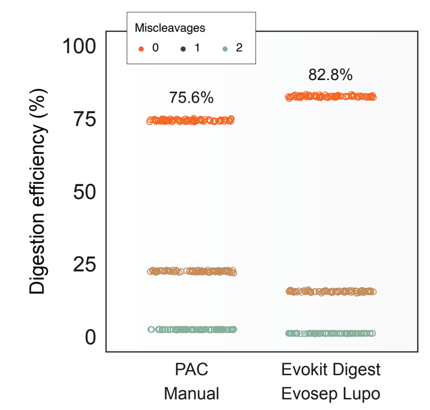
Full traceability – *Batch tracking. Easy access to Certificate of Analysis.*

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

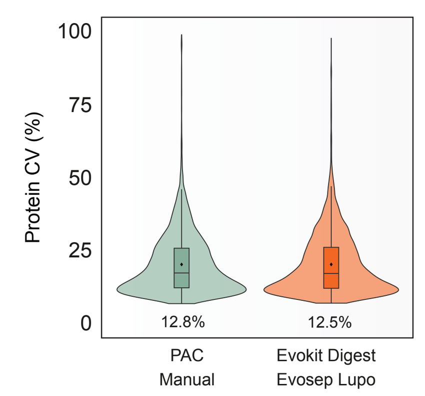
Sample preparation at scale with Evokits and Evosep Lupo

Consistent performance with standardized Evokit Digest

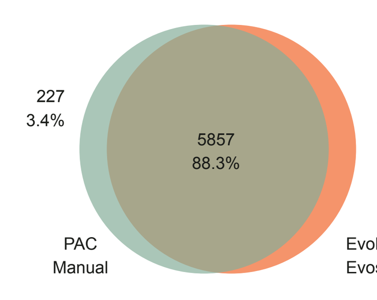
Evokit Digest performance with Evosep Lupo was assessed across 88 digestions of K562 samples (1 µg input, 30% loaded on Evotips), relative to manual PAC digestion. Reassuringly, comparable performance was observed based on coverage with slightly improved digestion efficiency for the Evokit, resulting in enhanced data completeness across samples.



Digestion efficiency (4h at ambient temp.) 500 SPD, Evosep Eno. Thermo Orbitrap Astral (n=88).



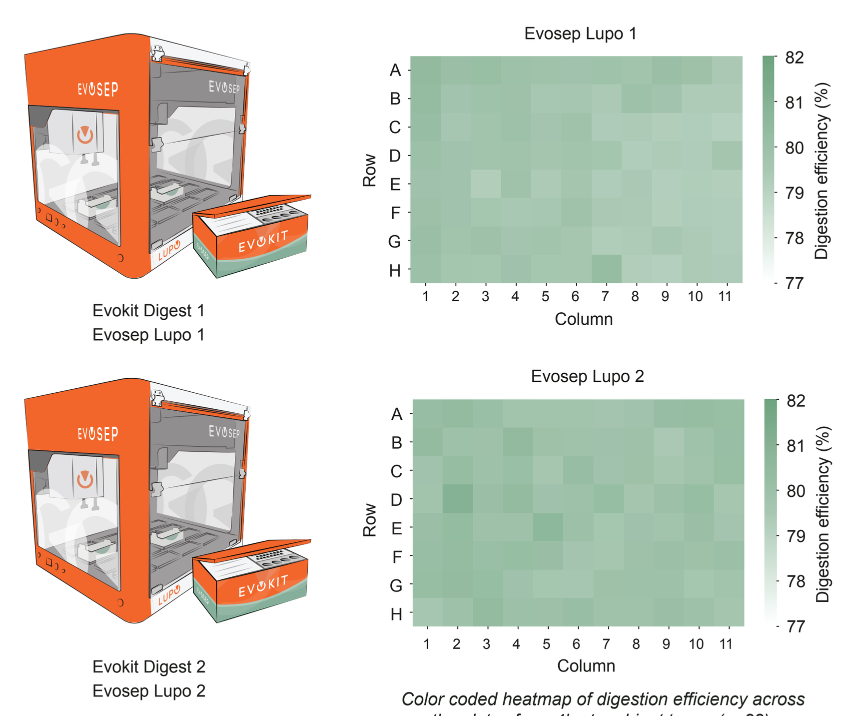
Protein CV%. 500 SPD, Evosep Eno. Thermo Orbitrap Astral (n=88).



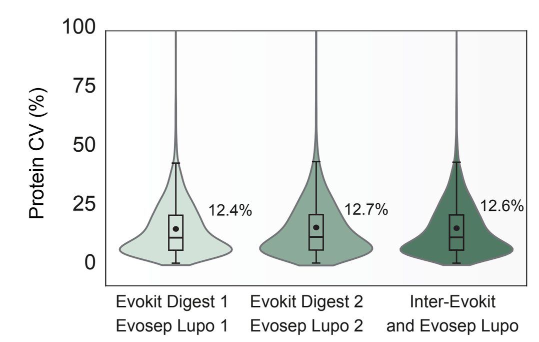
Overlap of protein groups. (n=88).

Reproducibility across plates and instruments

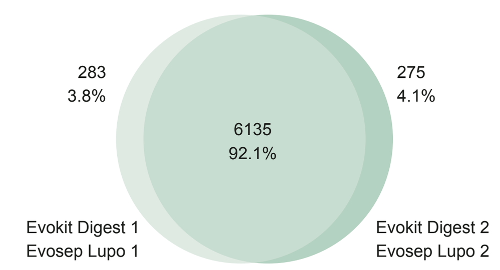
Reproducibility was further assessed across two different plates processed on two different Evosep Lupo systems to evaluate transferability and robustness at scale. The comparison showed a high degree of overlap in identified proteins between plates and platforms, as visualized by the Venn diagram, while closely aligned CV distributions confirmed consistent quantitative performance across runs.



Color coded heatmap of digestion efficiency across the plates from 4h at ambient temp. (n=88).



Protein CV% from two preparations with Evokit Digest. 500 SPD, Evosep Eno. Thermo Orbitrap Astral (n=88).



Overlap of protein groups from two preparations with Evokit Digest. (n=88).

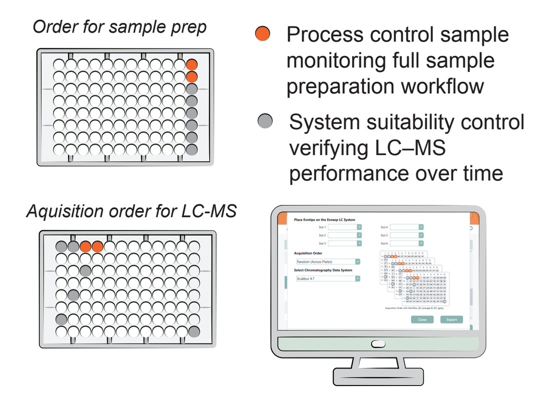
Technical details

All samples analyzed with 60 and 100 SPD methods were analyzed using an EV1109 column, while the 200, 300 and 500 SPD methods were analyzed using an EV1182 column. All columns were kept at a stable temperature of 40°C using the Evosep Pod for the EasySpray source with the Orbitrap Astral MS. MS full scan were acquired at a resolution of 240K with a precursor scan range of 380-980 m/z. MS 2 scans were acquired with isolation windows of 4 m/z for the three fast methods, whereas 2 m/z was used for the 60 and 100 SPD methods. Both methods used 3 ms injection time. Data was searched using DIA-NN 1.9.2.

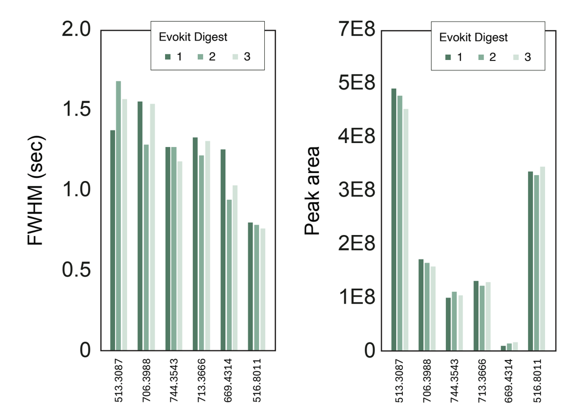
Controlled, consistent results across every workflow step

Built-in quality controls incorporated with each Evokit

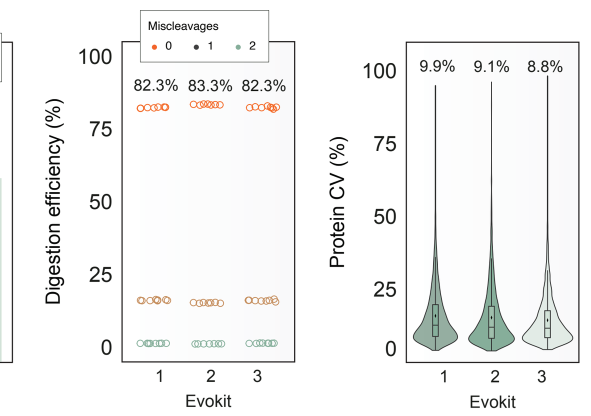
For proteomics to operate reliably in large-scale and regulated environments, reproducibility must be continuously verified. This requires integrated quality control, full traceability, and automation that reduces risk at every step. While, the QC samples are processed in the last column of each plate, they are analyzed across all samples during LC-MS. Predefined run order setups are easily integrated through an easy-to-use software interface.



Experimental setup for Evokit Digest.



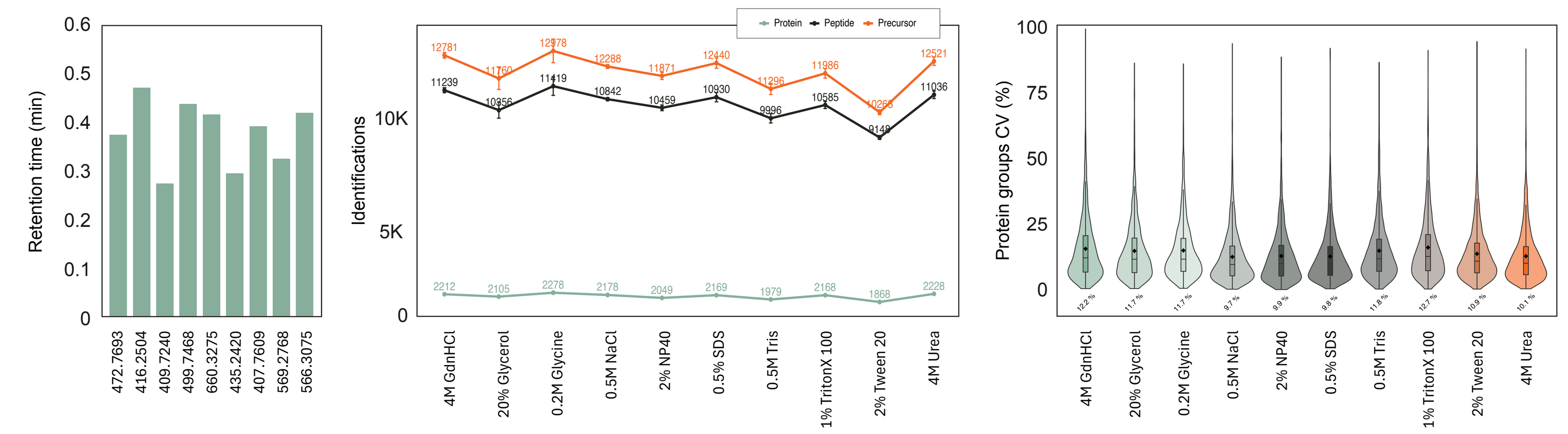
System Suitability Control samples across 3 Evokit Digest. FWHM and peak area. 500 SPD, Evosep Eno. Thermo Orbitrap Astral (n=6).



Process Control samples across three Evokit Digest. Digestion efficiency and protein CV. 500 SPD, Evosep Eno. Thermo Orbitrap Astral (n=6).

Consistent performance with Evokit Digest across diverse sample matrices

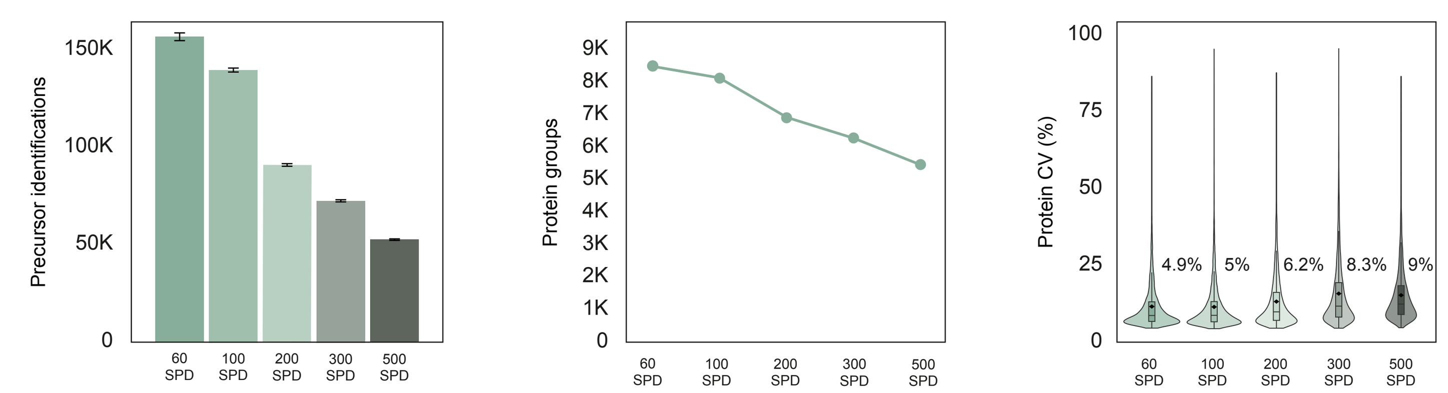
The workflow was tested against a number of commonly used sample matrices for preparing samples for proteomics analyses. The performance maintains robustness as shown by retention time stability, precursor identifications and quantitative precision using 1 µg bone tissue lysate as starting amount. 30% of the digest was loaded on Evotips.



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). Orbitrap Astral 500 SPD.

Evokit Digest performance with Evosep Eno standard methods

The Evokit Digest performance was assesed using the Evosep Eno standard methods on the Orbitrap Astral MS, providing good proteome coverage and protein CVs. 1 µg K562 lysate was processed, and 30% of the resulting digest was loaded on Evotips.



Precursor identifications, number of protein groups, and protein group CVs from 1 µg lysate samples, 30% digest loaded on Evotip. Evosep Eno 500 SPD, Orbitrap Astral (n=6).