Application Note

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Evotip Pure for end-to-end workflows:
A cost-efficient, sustainable and scalable proteomics solution

Highlights

- Complete end-to-end automation, from protein samples directly to LC-MS analysis

- Sustainable and cost-efficient scalability

1. End-to-end scalability

As proteomics expands into clinical and pharmaceutical applications, the need for robust and scalable solutions is driving the development of automated sample preparation workflows. While automation enhances throughput and reproducibility of digestion workflows, conventional liquid handling platforms still face challenges in automating post-digestion processing.

Evotip Pure, a unique sample introduction device for LC-MS, efficiently addresses these challenges by eliminating the need for traditional in-solution peptide handling. Our automated Evotip-loading solutions integrate peptide desalting into automated workflows, enabling immediate clean-up and efficient storage of ready-to-analyze samples. Designed for seamless integration into protein digestion workflows, the automated Evotip loading

process facilitates single-platform, hands-free, and end-to-end sample preparation. Integrating Evotip loading directly into the digestion workflows improves scalability, especially for the analysis of large sample cohorts. When processing thousands of samples, the consumption of pipette tips, digestion enzyme, and manual sample transfer steps significantly impact sustainability and cost-efficiency. This application note highlights the key benefits of integrating Evotip Pure into liquid handler-based automated workflows for more efficient and sustainable sample preparation.
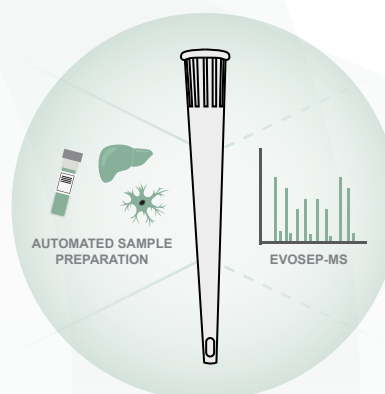

AUTOMATED SAMPLE PREPARATION   EVOSEP-MS

Figure 1: The Evotip is the central part of a complete integrated workflow.

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2. Method details

For information about how to set up end-to-end protocols on various liquid handlers, please visit www.evosep.com/support/automation. The experiments in this application note were carried out using the AssayMAP Bravo liquid handler as previously described[1].

In brief, HeLa cells were harvested in boiling lysis buffer (5% sodium dodecyl sulfate (SDS), 5 mM tris(2-carboxyethyl)phosphine (TCEP), 10 mM chloroacetamide (CAA), 100 mM Tris, pH 8.5). The lysate was subsequently boiled for 10 min, sonicated, and the concentration was estimated by BCA.

Protein aggregation capture (PAC)-assisted digestion was performed on the AssayMAP Bravo liquid handler. Protein aggregation was initiated with the addition of 5 µL MagReSyn hydroxyl beads (Resyn Biosciences) and isopropanol to a final concentration of 80%, followed by mixing. After aggregation, samples were washed once in isopropanol. Digestion was performed overnight at ambient temperature using a 1:25 trypsin:protein ratio (T6567, Sigma Aldrich) and a 1:100 Lys-C:protein ratio (129-02541, Wako Fujifilm). Peptides were loaded directly on Evotips using the AssayMAP, and stored in 0.1% formic acid at 4°C, unless stated otherwise. Samples were analyzed on a timsTOF HT mass spectrometer (Bruker Daltonics) using the 100 SPD method with an EV1109 Performance column (Evosep) operated at 40°C using the default "short gradient dia-PASEF MS method". Data was analyzed with DIA-NN (version 1.9.1) in library-free mode against the human proteome database (Uniprot, Oct 2020, 20,600 entries without isoforms) with trypsin/P as protease allowing 2 missed cleavages. All conditions were searched separately with match-between-runs enabled across replicates within the same condition.

3. Next generation sample storage

The scalability of any liquid handler-based sample preparation can be significantly enhanced by the integration of automated Evotip loading. It provides a complete solution for peptide processing that enables miniaturization of the workflow and true end-to-end automation on the liquid handler. The automated Evotip loading relies on surface tension to form distinct liquid layers within the Evotip, which are then loaded using positive air pressure. This can be applied to any upstream sample preparation worfklow, which can be automated. In this experiment, 1 µg HeLa protein lysate was digested and 40% of the resulting peptide digest was loaded on Evotips. These were submerged in 0.1% formic acid, and showed no significant sample loss for up to nine days. This flexibility enables users to process large sample batches over multiple days without the need for additional storage processes. Ultimately this minimizes sample loss, the requirement for extensive storage space, and plastic consumption. By integrating Evotip Pure into workflows, users can achieve greater scalability while maximizing the high-throughput capabilities of the Evosep One. This results in more efficient LC-MS analysis with minimal hands-on time, ensuring seamless and reliable sample processing.
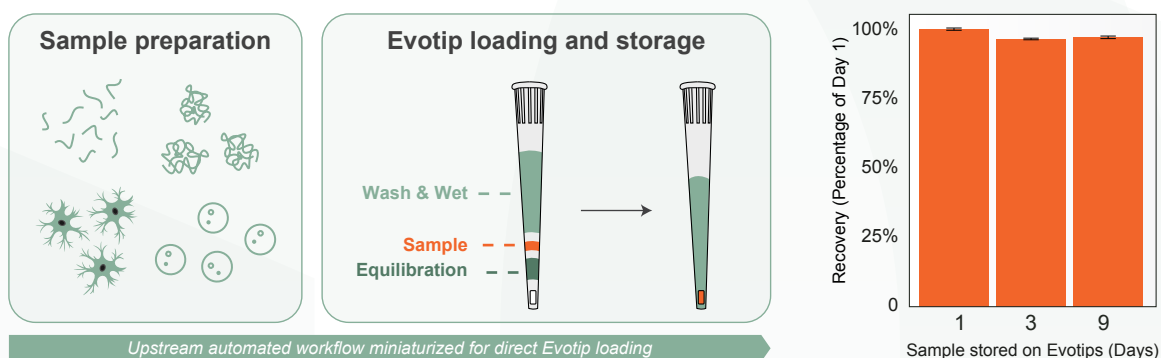

Figure 2: Automated and direct Evotip based sample storage preparation workflow. High sample recovery was observed after nine days of storage on the Evotip.

4. Direct loading enhances recovery

Automated Evotip loading streamlines the sample preparation workflow, saving both time and consumables while significantly reducing the number of sample handling steps. As hydrophobic peptides tend to adhere to plastic surfaces, minimizing sample transfer directly improves peptide recovery.

To assess the effect of peptide adsorption, the PAC digestion workflow was applied to 2 µg of HeLa lysate using the AssayMAP Bravo liquid handler. After digestion, half of the samples were loaded directly onto Evotips from the sample plate, mimicking the fully automated, end-to-end workflow. The remaining samples were dried in a vacuum centrifuge and resuspended in 0.1% formic acid before Evotip loading, simulating traditional post-digestion peptide handling without Evotips. The results demonstrate that samples directly loaded onto Evotips have increased summed precursor intensities compared to reconstituted samples. Notably, when comparing direct loading (10%) to reconstituted samples (20%), direct loading preserves more of the hydrophobic peptides that elute late in the gradient. The increased intensity and number of identifications with direct Evotip loading is a direct effect of the reduced sample handling achieved with Evotip-based sample preparation. By integrating Evotip loading directly in an automated workflow, users can enhance peptide recovery, particularly for hydrophobic peptides, ensuring more accurate and comprehensive proteomics analysis. Moreover, as desalting is integrated with peptide elution, less protein starting material is required as compared to a traditional workflow, where offline desalting and elution is needed, ultimately driving a more cost-efficient workflow.
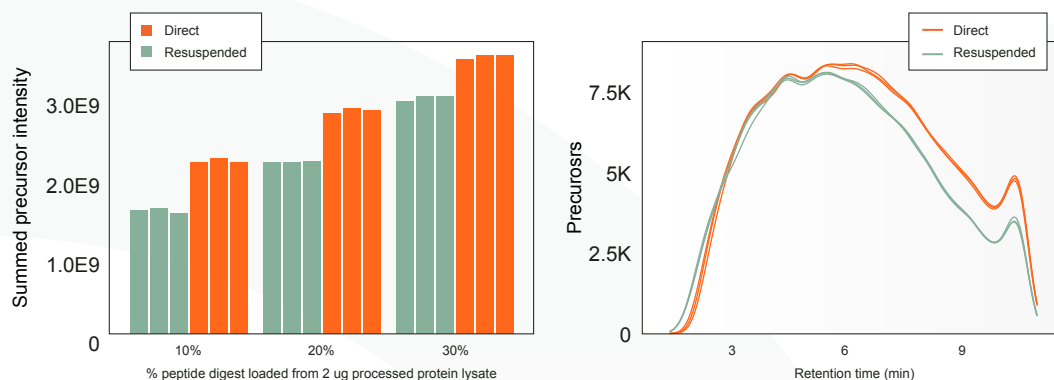

Figure 2: Summed precursor intensities and precursor identifications across the gradient for directly loaded (10% load) and reconstituted (20% load) PAC digested HeLa samples

5. Sustainable sample preparation

As proteomics scales to handle larger sample cohorts, it is essential to reevaluate traditional sample preparation methods. Evotip Pure-based workflows are designed with sustainability in mind, reducing the use of both reagents and consumables at every stage. By integrating direct Evotip loading with automated PAC digestion as example, the complete workflow can be processed by using as few as 1.25 pipette tips per sample, significantly reducing plastic consumption. The reduction in material waste, combined with enhanced sample recovery due to decreased peptide adsorption on plastic surfaces allows for more sophisticated sample processing. Laboratories can now digest only the required amount of sample, minimizing waste. With automated Evotip loading, sample preparation requires as little as 1 µg of protein input for complete processing, a significant reduction compared to the commonly used input of 100-200 µg. This not only lowers resource usage and operational costs but also promotes eco-friendly practices in high-throughput sample preparation.

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By integrating Evotip Pure directly in a complete, end-to-end workflow, you achieve:

Sustainability by using just ~1.25 tips per sample

20% increased recovery through fewer transfer steps

Up to 80% utilization of the processed sample

Cost-efficiency by minimizing use of reagents ~100x

6. Conclusion

Automated Evotip loading is the cornerstone of an easy-to-implement solution for automated end-to-end proteomics workflows, combining scalability, cost-efficiency, and sustainability in a single integrated platform. Evotip Pure facilitates seamless peptide desalting and storage, preserving sample integrity while ensuring flexible scheduling for workflow execution. By streamlining sample preparation, minimizing peptide loss, and improving sample recovery, automated Evotip loading allows efficient processing of large sample cohorts with minimal manual intervention. This innovative proteomics approach not only increases throughput and reproducibility but also reduces resource consumption, including reduction of plastic waste and reagent use. The ability to store samples on Evotips for extended periods without compromising analytical quality enhances operational efficiency and the cost-efficiency of the workflow. Evotip Pure-based workflows offer a practical, sustainable solution for laboratories aiming to boost efficiency, improve performance and minimize environmental impact. By incorporating automated Evotip loading directly with automated workflows, labs can expand their proteomics capabilities and deliver more reliable results while adopting sustainable practices.

Evosep One is for General Laboratory Use.

Evosep assumes no responsibility and shall have no liability for any damage or loss of samples, material and hardware that may arise from use of or in connection with Evosep recommended protocols and SOP's.

References

1. Application note AN-033, Evosep
   Efficient proteomics with an automated sample preparation strategy leveraged by
   Evotip Pure and the AssayMAP Bravo

2. Application note AN-022, Evosep
   Evotip Pure simplifies workflows with excellent reproducibility storage and recovery

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