Application Note

EVOSEP

Evotip Pure brings innovative sample storage and introduction to high-sensitivity and scalable LC-MS workflows

Highlights

- Efficient sample capture and recovery streamlines high-sensitivity workflows
- Evotip Pure is the key to accelerate scalable single-cell proteomics

1. Introduction

The Evotip Pure technology has emerged as a key component of proteomics workflows enabling seamless integration of upstream sample preparation with LC-MS analysis by reducing sample handling steps and thereby efficiently minimizing sample losses to plasticware, which significantly improves recovery and reproducibility.
These properties are even more critical in single-cell proteomics (SCP), a rapidly advancing field, where recent innovations have greatly enhanced workflow robustness and sensitivity. Modern, highly sensitive mass spectrometers and cell sorters have paved the way for high-sensitivity proteomics workflows, where single cells routinely are lysed and digested in minimal volumes before undergoing LC-MS analysis.
Here, we assess the efficacy of capturing and recovering low-input samples using Evotip Pure and demonstrate its advantages for scalable high-sensitivity analyses.
Key features are highlighted, such as its ability to concentrate low-input samples while reducing sample handling steps, carryover, and storage complexities. Collectively, these capabilities enable the Evotip to support reproducible scaling in proteomic analysis across single-cell populations.

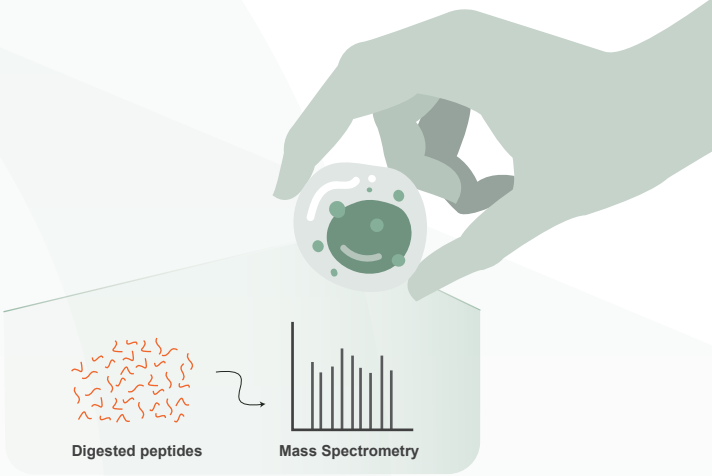

Figure 1: The Evotip solves analytical challenges in high sensitivity workflows.

2. Method details

HeLa and plasma digests were generated using protein aggregation capture-assisted digestion[1]. Peptides were desalted on sep-paks and the concentration was estimated using Nanodrop. Commercially available HeLa digest (Pierce) was used for all experiments, except loading volume.

For the loading volume experiment, Evotip Pure was loaded with 250 pg HeLa in differing volumes of solvent A.

For robustness experiments, one Evotip box was loaded with Pierce HeLa and plasma digests as visualized in Figure 3 and analyzed sequentially. Furthermore, 340 samples were analyzed for stability.

To assess the effect of n-dodecyl-β-D-maltoside (DDM), 250 pg HeLa digest was loaded on Evotips in quadruplicates with and without the presence of 0.015% DDM. Evotips were submerged in solvent A, stored at ambient temperature, and analyzed over three consecutive days. All samples were analyzed utilizing the Whisper™ Zoom 80 SPD or Whisper Zoom 120 SPD method on a timsTOF pro 2 mass spectrometer (Bruker) in dia-PASEF mode. Separation of samples was achieved with the Aurora Rapid 5x75 column (IonOpticks, operated at 50 °C). DIA spectra were collected over a mass range of 400-1000 Da and an ion mobility range of 0.64 to 1.37 1/K0, using 8x25 Da windows and 3 TIMS ramps per cycle, with ramp and accumulation times of 100 ms and a cycle time of 0.95 s. Data from each condition were processed independently using DIA-NN (version 1.8.1) in library-free mode against the reviewed human proteome (UniProt, Oct 2020, 20,600 entries excluding isoforms), with MBR (match-between-runs) enabled under default settings. Protein and precursor identifications were derived from unique entries in the DIA-NN matrix outputs.

3. Concentration of dilute samples

In contrast to most autosamplers which rely on a sample loop for injecting small sample volumes, the Evotip Pure accommodates larger loading volumes and facilitates the concentration of diluted samples. This capability not only allows for sample preparation in sizable volumes but also ensures consistent handling and transfer of minimal sample quantities to the Evotip and subsequent LC-MS analysis without sacrificing material or throughput during the transfer process. To illustrate this, we loaded 250 pg HeLa digest onto Evotip Pure in varying volumes (1-160 µl). For samples with less than 20 µl volume, an additional volume was deposited into the Evotip before loading for a total loading volume of 20 µl. The consistent precursor intensity readout from the analysis indicates no loss in signal intensities, despite the differing loading volumes demonstrating efficient concentration of very dilute samples on the Evotip. This allows precise and complete sample transfers in larger volumes without compromising throughput. Collectively, this reflects actual experimental conditions with a finite amount of sample, which is in contrast to repeat injections from the same vial, traditionally used to benchmark performance.

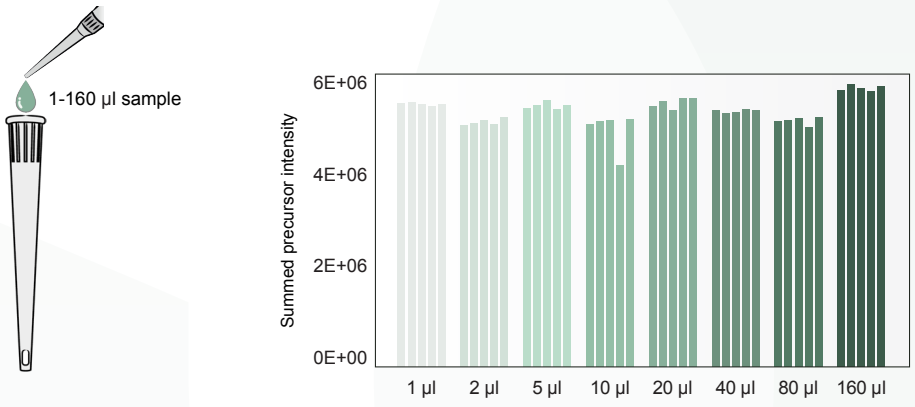

Figure 2: Summed precursor intensities of 250 pg HeLa loaded on Evotips in 1-160 µl solvent A.

4. Versatility with precision

The high-sensitivity Evotip Pure workflow was evaluated for reproducibility and quantitative precision by sequentially injecting single-cell equivalents of HeLa (250 pg) along with high-load plasma (50 ng) and HeLa digest (50 ng) mixed with blank Evotips to monitor carryover, as illustrated below, using the Whisper Zoom 80 SPD method. High reproducibility was observed across all samples with good quantitative precision with median CVs of less than 7% for both 50 ng plasma and 50 ng HeLa. Blank samples were analyzed following the different sample types to probe carryover, which was low independent of the sample type. Specifically, the carryover in single-cell equivalent (250 pg HeLa) samples was 0.6% following 50 ng plasma and 0.02% following 50 ng HeLa. Carryover in the blank injections was 0.2% following both 50 ng plasma and HeLa samples. The low carryover is achieved by the Evotip because of its single-use and disposable trap column functionality.

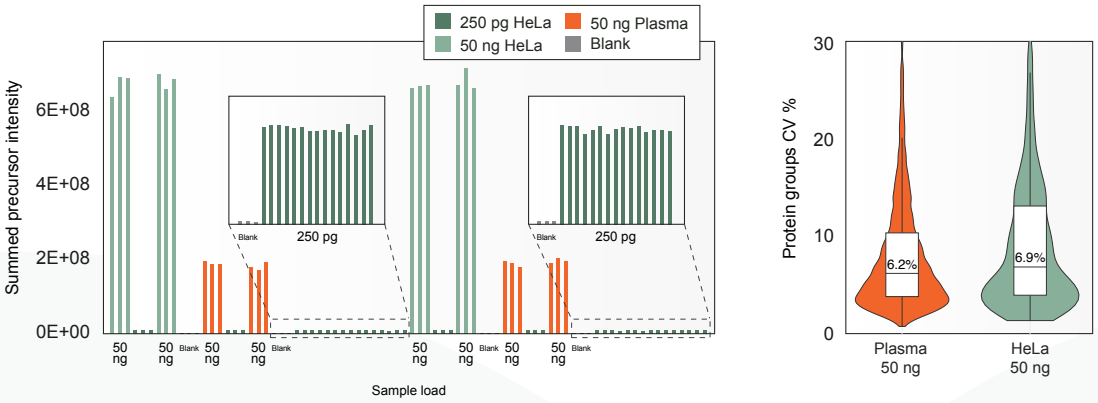

Figure 3: Summed precursor intensity of 50 ng HeLa, 50 ng plasma, 250 pg HeLa and blanks and CVs quantified for 50 ng HeLa and 50 ng plasma. Samples were analyzed with Whisper Zoom 80 SPD.

5. Robustness yields reproducibility

The Evosep One is robust by design, leveraging Evotip technology to enhance column longevity through partial elution, single-use trap columns, and low carryover. To demonstrate this, we monitored the stability across 340 samples with the Whisper Zoom 120 SPD method. The HP pump pressure remained constant throughout the analysis, showing that the analytical column is well-protected by the partial elution and the low carryover enabled by the Evotip Pure. Monitoring five HeLa peptides across the single-cell equivalent samples (250 pg HeLa), demonstrated excellent run-to-run reproducibility for the Whisper Zoom 120 SPD method with an average standard deviation of 2 seconds.

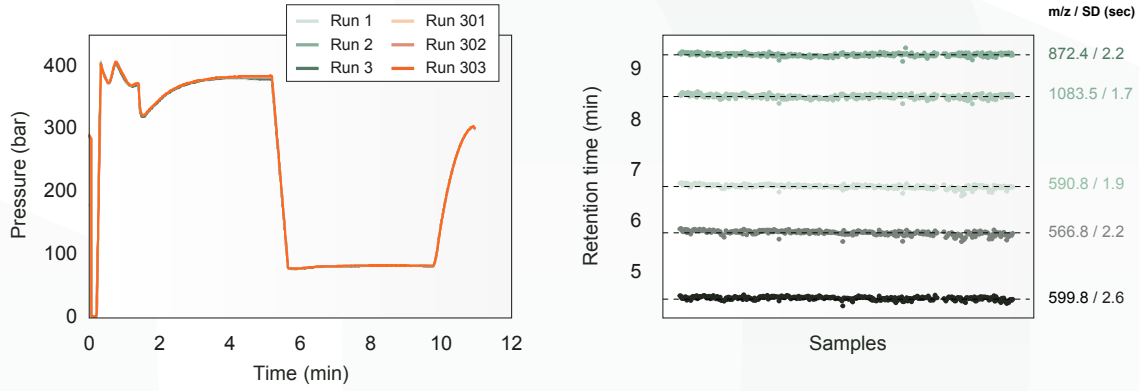

Figure 4: Pressure traces across 340 runs with the Whisper Zoom 120 SPD method and retention times of 5 monitored HeLa peptides.

6. Sensitive sample storage

Sample storage prior to LC-MS analysis is often required and typically done in tubes, plates, or HPLC vials. Storage can result in varying degrees of sample loss due to additional transfer steps and peptide adsorption to plastics and is especially problematic with low-input samples. Here, we evaluated the sample loading capabilities of DDM in single-cell levels of peptides (250 pg HeLa digest) and found that DDM increases overall precursor identifications. In both cases, more than 90% of precursor identifications are preserved after 48 hours of storage on the Evotip, whereas close to 75% is preserved following 72 hours of storage. This is essential for robust and scalable single-cell proteomics, as it enables the preparation and execution of experiments over extended periods. Furthermore, we evaluated the impact of DDM for sample loading through a dilution series of HeLa and found that DDM has a positive effect for low sample loads until saturation is observed at higher loads. Altogether the results demonstrate that DDM increases overall precursor identifications and quantitative precision for low loads without impacting storage capability. In conclusion, this shows that the beneficial effect from DDM impacts the sample preparation workflow upstream of the Evotip.

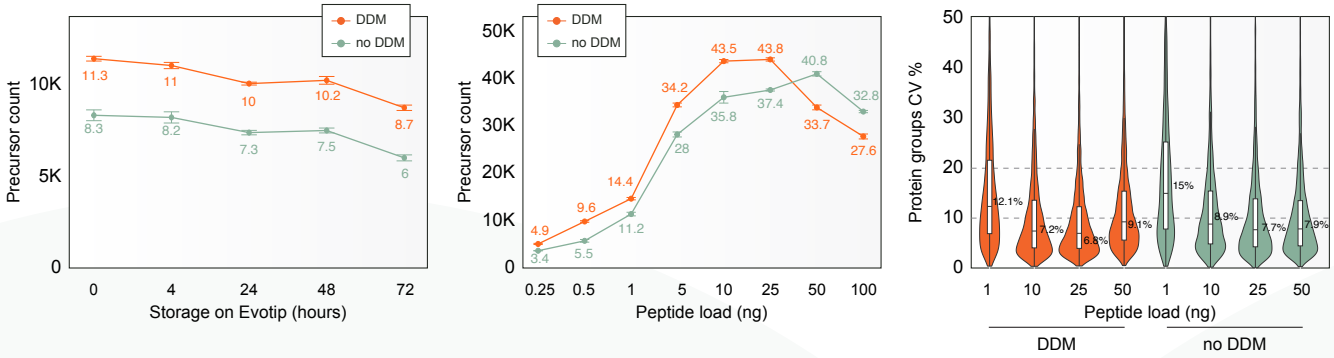

Figure 5: Storage of 250 pg HeLa loaded +/- DDM. Precursors and protein group CVs from HeLa dilution series loaded +/- DDM. Samples were analyzed with Whisper Zoom 120 SPD, n = 4.

7. Conclusion

The Evotip Pure is the core technology of Evosep One workflows, seamlessly coupling front-end sample preparation with liquid chromatography and mass spectrometry analysis. The Evotip Pure enables consistently low carry-over, reduces sample losses, and allows for long-term capture and storage of samples, while the partial elution from the Evotip Pure retains unwanted materials on the tip and protects the analytical system. These key features are relevant to a wide range of sample types but are particularly important concerning the handling of low-amount samples. Furthermore, the sensitivity of the Evotip workflow is increased by using DDM for loading low-amount samples. Finally, Evotip loading can be seamlessly integrated with automated workflows collectively paving the way for future advancements in scalable high-throughput single-cell proteomics.

Evosep One is for General Laboratory Use.

References

1. Batth TS., Tollenaere M., Rüther P., Gonzalez-Franquesa A., Prabhakar BS., Bekker-Jensen S., Deshmukh AS., Olsen JV (2019) Protein Aggregation Capture on Microparticles Enables Multipurpose Proteomics Sample Preparation. Mol Cell Proteomics., mcp.TIR118.001270