A complete and automated end-to-end sample preparation strategy for high-throughput and standardized proteomics with high sensitivity

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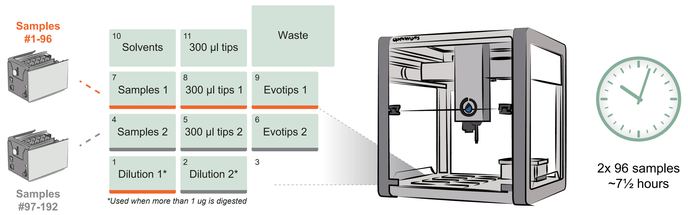
Highlights

- Towards standardized workflows with integration of peptide loading on Evotips with a fully automated end-to-end protocol on the Opentrons platform.
- Optimized for low sample input to ensure maximum utilization of digested material and cost efficient analysis of large sample cohorts.
- Robust and high sensitivity workflow working efficiently down to 1 ng protein starting amount.

A fast and efficient, fully automated end-to-end workflow

Protein aggregation capture protocol on the Opentrons (OT-2)

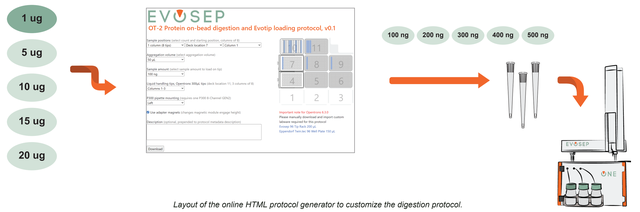
Here, we introduce a fast and efficient, fully automated sample handling protocol on the OT-2 utilizing protein aggregation capture (PAC) on magnetic microparticles in an end-to-end standardized hands-off workflow, starting from protein lysate to peptide loaded on Evotips. The presented protocol can digest and load up to 192 samples on Evotips in less than eight hours, enabling a throughput of up to 384 samples prepared in a work day.



Layout of the Opentrons deck for automation of protein aggregation capture.

Easy-to-use HTML form to customize the protocol

The protocol is designed to digest an unusually small amount of protein starting material, i.e. from 1 to 20 µg and therefore, it exemplifies a very cost-efficient sample preparation strategy for deep proteome profiling. It is available in an easy-to-use HTML form that generates complete scripts that can be directly imported into the Opentrons app.

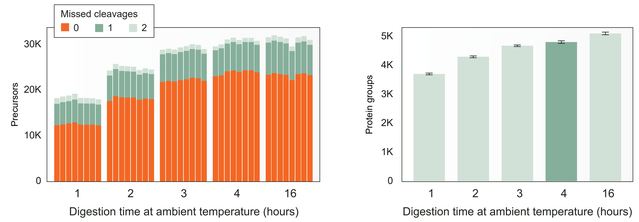


Layout of the online HTML protocol generator to customize the digestion protocol.

Short digestion time yields highly reproducible data

Digestion time

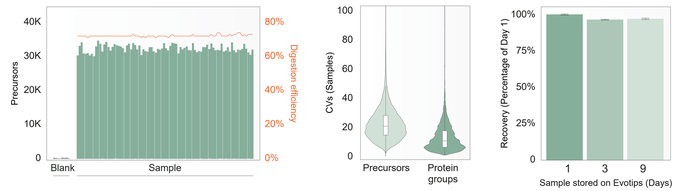
The digestion efficiency and proteome coverage of this automated sample preparation strategy was assessed with 1, 2, 3, and 4 hours of digestion time against a standard overnight digestion at ambient temperature. The different digestion times were evaluated based on the number of identified precursors, protein groups and the digestion efficiency, as estimated by the number of missed cleavages at precursor level. It was determined that a digestion time of 4 hours yielded the optimal ratio of time to digestion efficiency and recovery.



Identifications and digestion efficiency, measured at different time points based on 1 µg lysate starting amount. ~20% of resulting peptides loaded on Evotips and analyzed with 100 SPD.

Reproducibility

The reproducibility and sample-to-sample carry-over of the protocol was evaluated by digesting a full plate of samples with eight randomized blanks. The analysis reproducibly identified over 30,000 precursors across all samples and a digestion efficiency of 73% fully cleaved peptides. The precision in the sample data was great with median CVs of 22% and 13% at the precursor and protein group levels, respectively. Finally, samples from the same digestion were stored for ten days at 4 °C post Evotip loading and analyzed to demonstrate the stability of peptides once loaded on the Evotip Pure.

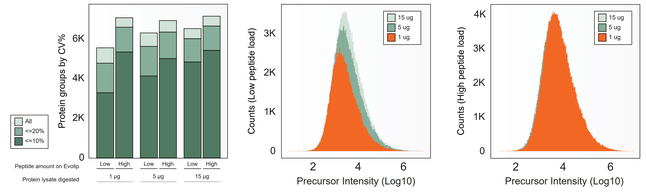


Identifications, CVs from replicate digestions of 1 µg HeLa and effect of storage of digested samples on the Evotip. ~20% of each peptide digest was loaded on Evotips and analyzed with 100 SPD. Recovery was calculated based on total number of precursors in relation to Day 1.

More than 7,000 proteins identified from as little as 125 ng

Maximum Depth

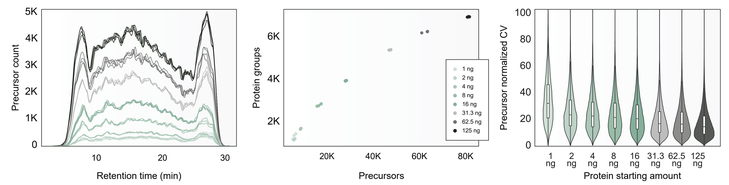
The protocol is optimized for excellent proteome depth, while keeping the sample input low in the range of 1 µg - 20 µg protein. To assess the achievable proteome depth at low sample input material, 1 µg, 5 µg, and 15 µg of HeLa lysate were digested in triplicates. From 1 µg of starting material, ~20% (low load) and ~50% (high load) of the resulting peptides were loaded onto Evotips. The same relative amounts based on volume, were loaded from the 5 and 15 µg digestions. All samples were analyzed with the 30 SPD method. When low peptide amount is loaded, it is attractive to digest more material, whereas this effect is balanced once a higher peptide amount is analyzed.



Proteome coverage and precursor abundance distribution from 1, 5 and 15 µg protein starting amount with 30 SPD.

Sensitivity

Through the digestion of 1 ng to 125 ng of HeLa lysate and subsequent analysis of 70% of the resulting peptides with the Whisper 40 SPD method, the performance of the automated workflow with very low sample amounts is showcased. With more than 1,000 and 7,000 proteins identified at 1 ng and 125 ng input material, respectively, the protocol shows outstanding performance for sensitive applications. The reduction in the number of identified precursors in diluted samples occurred evenly across the gradient indicating that the performance is not biased towards peptides with specific chemical properties at reduced loads. These results showcase the potential of the protocol for high sensitivity applications with robust quantification of precursors as evidenced by median CVs below 25% for all samples where more than 1 ng was digested.



Precursor identifications across the gradient, total number of identified precursor and protein groups and precursor CVs. ~70% of the resulting peptide is analyzed with Whisper 40 SPD.