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

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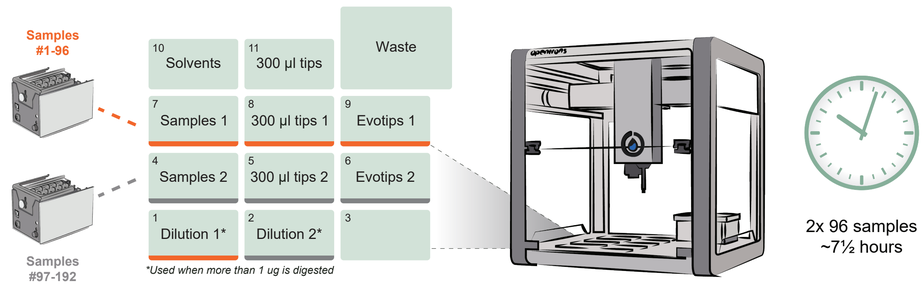
Highlights

- Towards standardized workflows with integration of peptide loading on Evotips with a fully automated end-to-end protocol on the Opentrons OT-2.
- 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 for efficient analysis down to 1 ng protein starting amount.

An efficient, fully automated end-to-end workflow

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

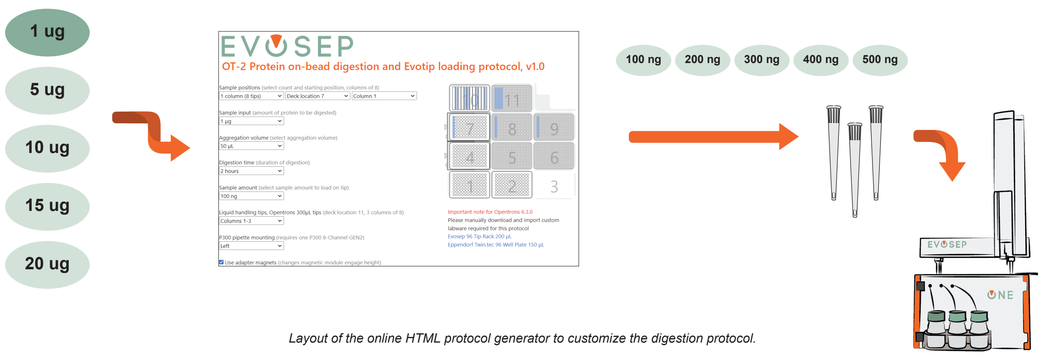
Here, we introduce a fast and efficient, fully automated sample handling protocol on the OT-2 utilizing protein aggregation capture (PAC) on magnetic microparticles in an end-to-end standardized hands-off workflow. The presented protocol carries out digestion of up to 192 samples of protein lysate and loading of the resulting peptides 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 utilizes the efficient storage capabilities of the Evotip Pure for a low waste digestion strategy. With sample inputs in the range of 1 to 20 µg over half of the resulting peptides can be utilized for cost-efficient deep proteome profiling. The protocol is available in an easy-to-use HTML form that generates complete scripts that can be directly imported into the Opentrons app.

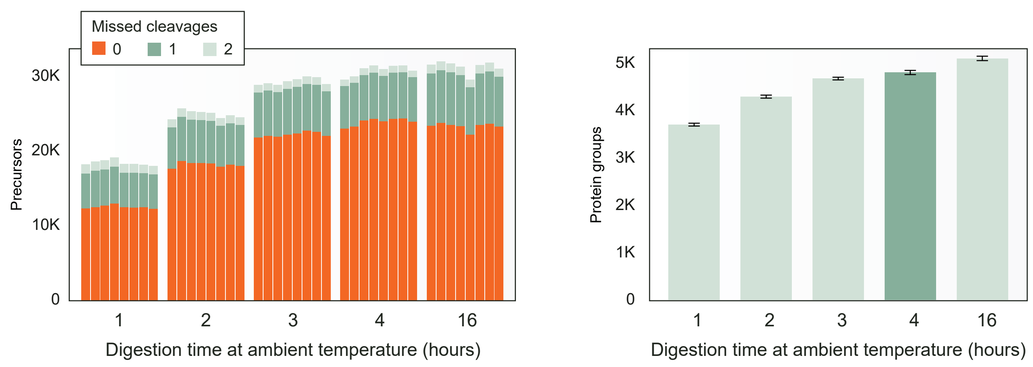


Layout of the online HTML protocol generator to customize the digestion protocol.

Short digestion time yields highly reproducible data

Digestion time

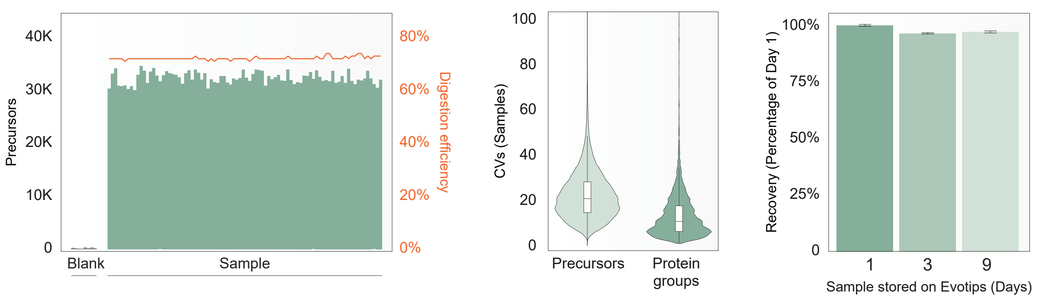
The digestion efficiency and proteome coverage of this automated sample preparation strategy was assessed with 1, 2, 3, and 4 hours of digestion time against a standard overnight digestion at ambient temperature. The different digestion times were evaluated based on the number of identified precursors, 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

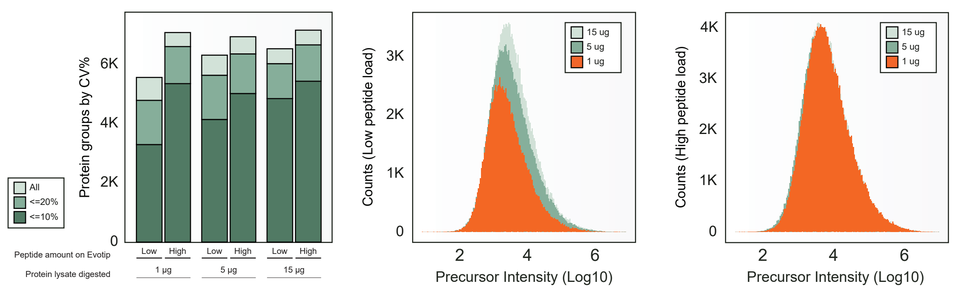
The reproducibility and sample-to-sample carry-over of the protocol was evaluated by digesting a full plate of samples with eight randomized blanks. The analysis reproducibly identified over 30,000 precursors across all samples and a digestion efficiency of 73% fully cleaved peptides. The precision in 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.

Maximum depth achieved at just 1 ug starting amount

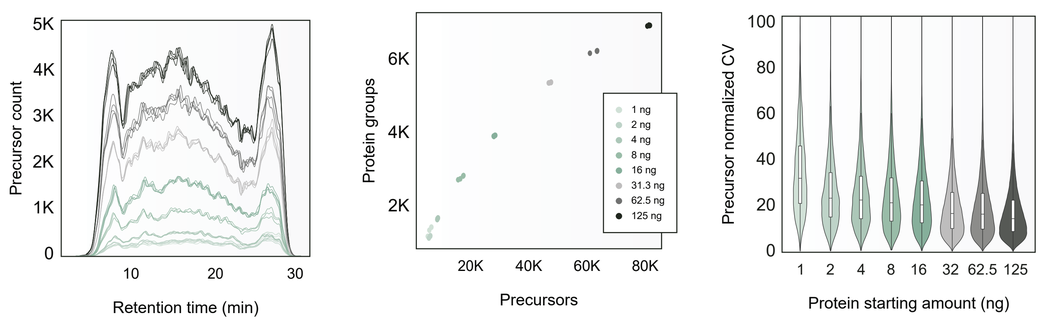
The protocol is optimized for excellent performance with low sample inputs to reduce enzyme and sample consumption. The digestion of 1 µg, 5 µg, and 15 µg of HeLa lysates showed that the same proteome depth is achievable with low sample input when a higher amount of the resulting peptides was loaded on Evotips. High and low loads correspond to 20% and 50%, respectively, of the resulting peptides from 1 µg and the same relative amounts were loaded from 5 µg and 15 µg based on volume.



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

More than 7,000 proteins identified from just 125 ng

Digestion of 1 ng to 125 ng HeLa lysate and analysis with the Whisper 40 SPD method, yielded outstanding proteome depth with more than 1,000 and 7,000 identified proteins, respectively. The even reduction in the number of identified precursors across the gradient showed that no bias was introduced with lower loads and low CVs showcased the robustness of quantification. 66% of the resulting peptides from each digestion were loaded for analysis.



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