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

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Highlights

- Efficient, sustainable and cost-efficient strategy leveraged by the Evotip Pure.
- Robust and easy-to-use AssayMAP Bravo digestion workflow for processing 1 to 96 samples at the time
- Identification of more than 8,000 proteins with the Whisper Zoom 40 SPD method, demonstrates an automated highly sensitive workflow.

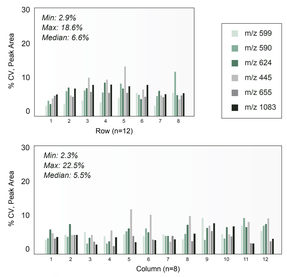
The need for streamlined solutions

Automated Evotip loading is the key enabler

The growing landscape of high-throughput proteomics requires standardization and simplified automated workflows to remove human errors and decrease data variation.

The Evotip can be loaded using an innovative and fully automated strategy with the Agilent AssayMAP Bravo. The liquid handler is equipped with 96 probe syringes, which enables very fast sample loading on Evotips, as a full box of Evotips are loaded in approximately four minutes with optimal performance.

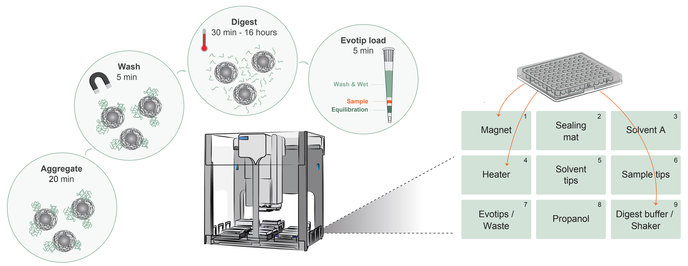
By precisely layering liquids within the Evotip and utilizing positive air pressure, this automated approach is highly effective. Moreover, the automated loading strategy minimizes manual intervention and reduces the potential for errors.



Reproducibility of peak areas across rows and columns with the automated AssayMAP Bravo loading protocol.

Complete AssayMAP solutions

The workflow strategy is minimized to reduce the sample starting material down to 1 ug protein lysate to save cost. The complete and automated sample preparation utilizes protein aggregation capture (PAC) on magnetic hydroxyl microparticles (Resyn Biosciences), using a single wash step with Isopropanol. Digestion was carried out with a protease mix of of 1:100 Lys-C to protein and 1:25 trypsin to protein ratio for 30 min to 16 hours, shaking at 37°C. A lid was placed on top of the sample plate during all incubation steps to reduce evaporation. Resulting peptides are loaded directly on Evotip Pure using the AssayMAP Bravo (VWorks 13), enabling nearly 100% sample utilization while reducing enzyme and sample consumption.

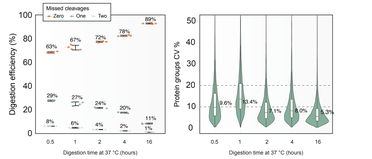


Schematic representation and deck layout of the fully automated digestion workflow for 1 to 96 samples

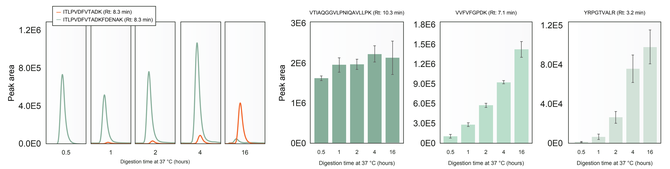
Simplified sample preparation allows for scalability

User-friendly flexibility

The standardized digestion workflow, integrated with the AssayMAP Bravo software (VWorks, v13), features a user-friendly interface where users can pre-select parameters, allowing for customization of throughput and proteome depth. These are number of samples (1-96) to process; the selected digestion time (0.5 – 16 hours); and the percentage of digest to load on the Evotip (20-100%).
Increased digestion time improves the digestion efficiency, which was tested based on digesting 1 ug of protein lysate at 37˚C from 30 minutes to overnight digestion. 40% of the resulting digest was loaded on two separate Evotips, where one was analyzed using dia-PASEF on a timsTOF Pro 2 (Bruker), while the other was analyzed using a targeted method on a QQQ 6495C (Agilent). 67% digestion efficiency was achieved after 30 minutes, which was increased to 89% for overnight digestion. The workflow demonstrated high reproducibility, with protein coefficient of variation (CV) below 15% at all time points. A targeted analysis revealed peptide specific digestion patterns as some peptides were fully digested after just 30 minutes, and other peptides required overnight digestion. This suggests the need to include target dependent optimization of digestion time to obtain reproducibility in the complete workflow.



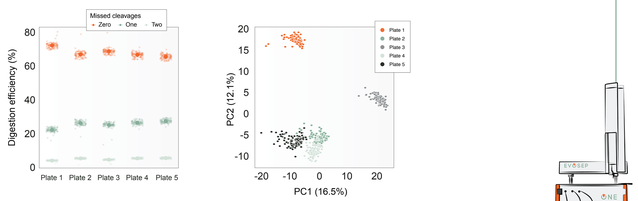
Digestion efficiency for 30 minutes to 16 hours digestion and corresponding protein group CVs per condition. ~40% of each peptide digest was loaded on Evotips and analyzed with 100 SPD.



Peak area of targets at each digestion time. 1 ug protein lysate was digested and ~40% of the peptide digest was loaded on Evotips and analyzed with 100 SPD using dia-PASEF on a timsTOF Pro 2.

Maximiized utilization by digesting ~500 samples in a workday

The efficient processing of nearly 500 samples in a single workday is achieved using multi-purposing deck positions, including heater and shaker modules, ensuring consistent digestion at 37˚C in just 30 min. With the fast 500 SPD method, 480 samples were processed from protein lysate to Evotips ready-to-inject in approximately 24 hours. Globally, the combination of short digestion time at 37˚C with fast acquisition (500SPD) resulted in less than 7% CV digestion efficiency. Low intra-plate variation validated the robustness of the workflow.

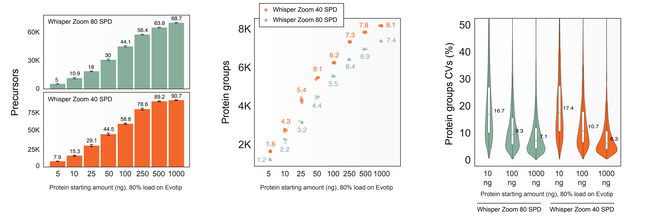


Digestion efficiency (30 min, 37˚C) across five plates and a PCA analysis to measure variation across the plates. Samples were analyzed with the 500 SPD method.

Complete automation with high sensitivity

Comprehensive proteome coverage with the Whisper Zoom 40 SPD method

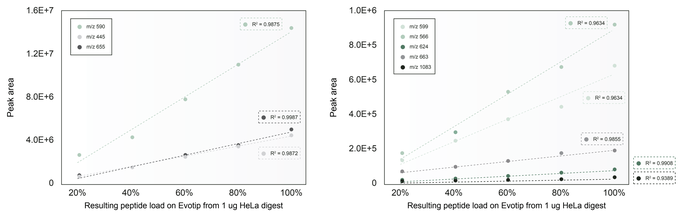
The workflow is designed for scalability, while considering the protein starting amount and cost-per-sample for the entire workflow. High sensitivity is ensured throughout the workflow, particularly due to the direct sample loading on Evotip post digestion and the seamless integration of the Evotip with the subsequent LC-MS analysis. To showcase this, a serial dilution of HeLa lysate from 1000 ng to 5 ng starting material was used to perform eight replicate digestions overnight at 37°C. 80% of the resulting peptides were loaded on Evotips and analyzed using the Whisper Zoom (40 & 80 SPD) methods coupled with the timsTOF HT. We identified more than 90,000 precursors and 8,000 proteins from 1000 ng input material with the Whisper Zoom 40 SPD method. By increasing the throughput to 80 SPD, nearly 70,000 precursors and more than 7,200 proteins from 1000 ng input material. Importantly, the protein CVs were outstanding with less than 20% for 10 ng input and ~6-7% for 1000 ng input for both Whisper Zoom methods.



Identification of precursors, proteins, and CVs of a protein lysate dilution curve, n=4. ~80% of each peptide digest was loaded on Evotips and analyzed with Whisper Zoom 40 & 80 SPD.

Sensitivity of the digestion workflow

The sensitivity was also assesed through a targeted experiment, where 1 ug of HeLa lysate was digested overnight at 37˚C. Various amounts of the resulting peptide digests were loaded on the Evotip with 20%, 40%, 60, 80% and 100% respectively. By monitoring a set of peptides using a targeted method on the 6495C with the 100 SPD method, calibration curves were calculated from the peak area across eight workflow replicates. Excellent linearity was obtained for the targets spanning low and high abundancies across the gradient. This is enabled by high sensitivity in all parts of the workflow and especially the direct loading on the Evotip post digestion, ensures seamless integration and optimal storage conditions in preparation for LC-MS analysis.



Peak area of targets from digestion of 1 ug HeLa lysate and loading 20%, 40%, 60%, 80, 100% peptide digest on the Evotip, respectively. Samples were analyzed with the 100 SPD method.