

## Proof of performance

Orbitrap Exploris 240 mass spectrometer



## Plasma profiling—reliable large cohort analysis with a fully automated workflow

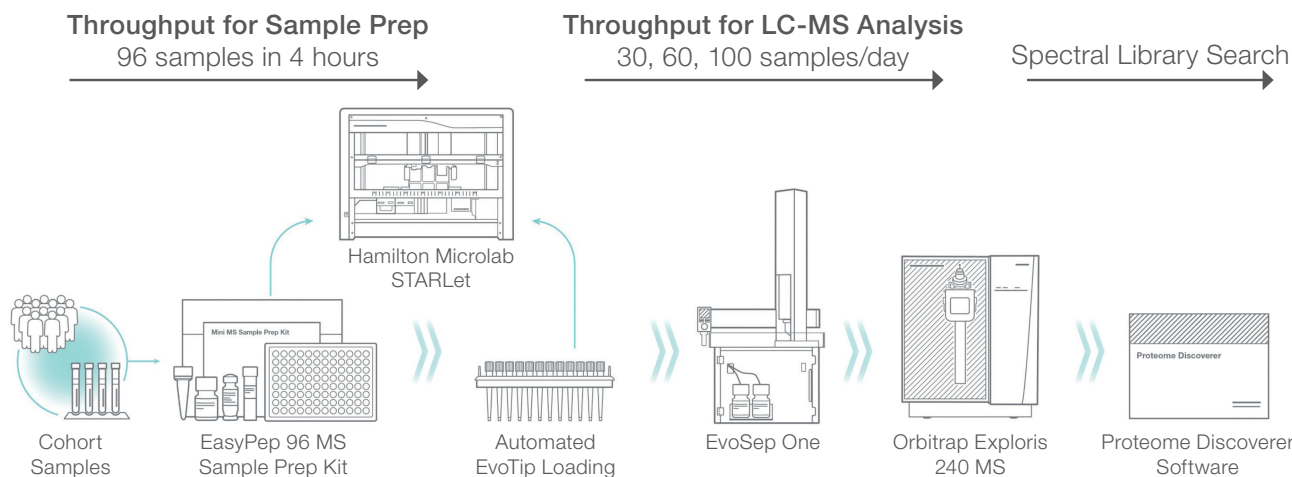
### Summary

Here we present an Ultra-High Throughput Plasma Protein Profiling (uHTPPP) workflow (Figure 1), from automated sample preparation to high-resolution, accurate-mass (HRAM) MS analysis. This workflow incorporates built-in QA/QC procedures within the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer. Up to 96 samples can be processed in 4 hours using the Thermo Scientific™ EasyPep™ 96 MS Sample Prep Kit combined with automation, while capillary flow liquid chromatography (LC) conditions on an EvoSep One LC system enables, 30, 60, or 90 samples to be analyzed per day.

We demonstrate:

1. Mass accuracy stability over 5 days of continuous analysis
2. Retention time stability
3. Reproducibility across the entire workflow with plate-to-plate peptide recovery, in terms of identification and dynamic range

**Best-in-class protein identification:** When throughput and standardization for translational proteomics is required, the Orbitrap Exploris 240 MS delivers an automated solution for plasma profiling. The workflow includes sample preparation and built-in QA/QC procedures that are reproducible and scalable, delivering rigorous and relevant results from large sample cohorts.



**Figure 1. Ultra-High Throughput Plasma Protein Profiling (uHTPPP) workflow for large-scale studies.** Thermo Scientific™ High Select™ Top14 Abundant Protein Depletion Resin was used to deplete pooled human serum and was processed using the EasyPep 96 MS Sample Prep Kit on a Hamilton Microlab® StarLet™ liquid handling system. Following loading onto disposable EvoTip™ trap columns, peptides were eluted by an EvoSep One LC by the pre-formed gradient and further separated at high pressure. High-resolution mass analysis was performed in data-dependent mode, with post-acquisition data analysis by Thermo Scientific™ Proteome Discoverer™ software, version 2.4.

## Experimental conditions

### Sample

- 200 ng pooled serum with 50 fmol Thermo Scientific™ Pierce™ Peptide Retention Time Calibration (PRTC) Mixture
- Thermo Scientific™ High Select Top14 Abundant Protein Depletion Mini Spin Columns

### LC method

- All samples resuspended in 50 µL by Hamilton StarLet Liquid Handler
- EvoTip Loading and 60 Samples Per Day LC method

- Thermo Scientific™ EASY-Spray™ HPLC column ES806 (150 mm × 150 µm 3 µm particle)

### Instrumentation

- EvoSep One, 21-minute method

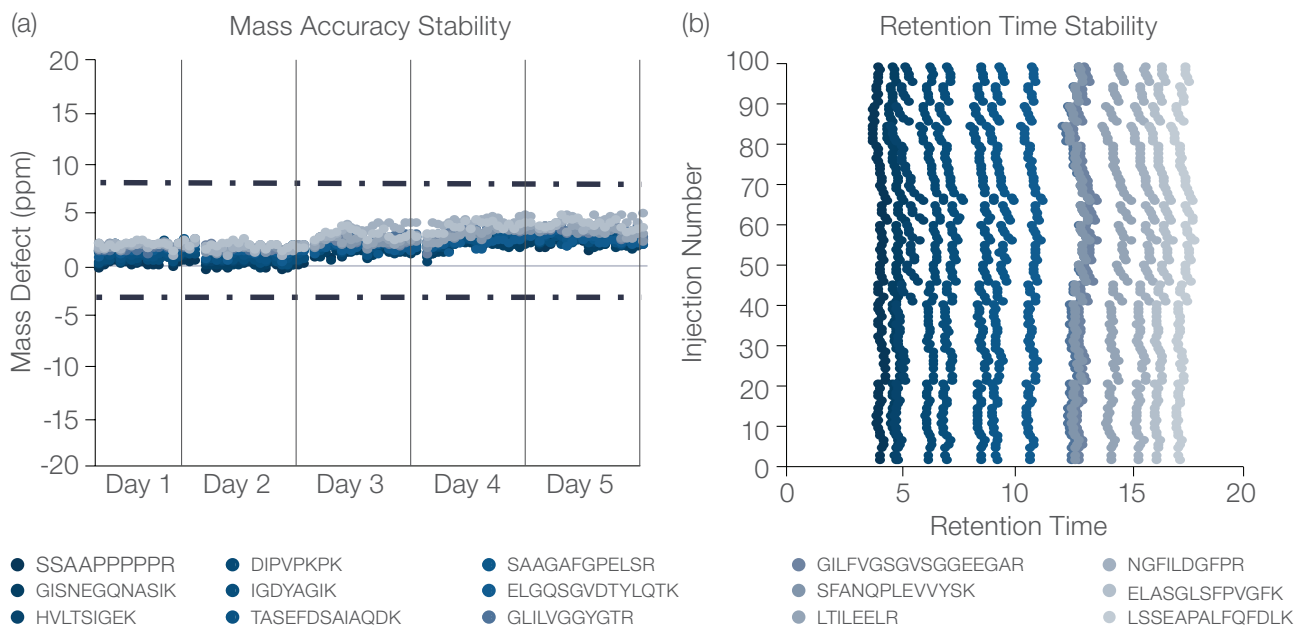
### MS detection

- High-resolution, accurate-mass (HRAM) Orbitrap Exploris 240 mass spectrometer
- Data-dependent acquisition (DDA)

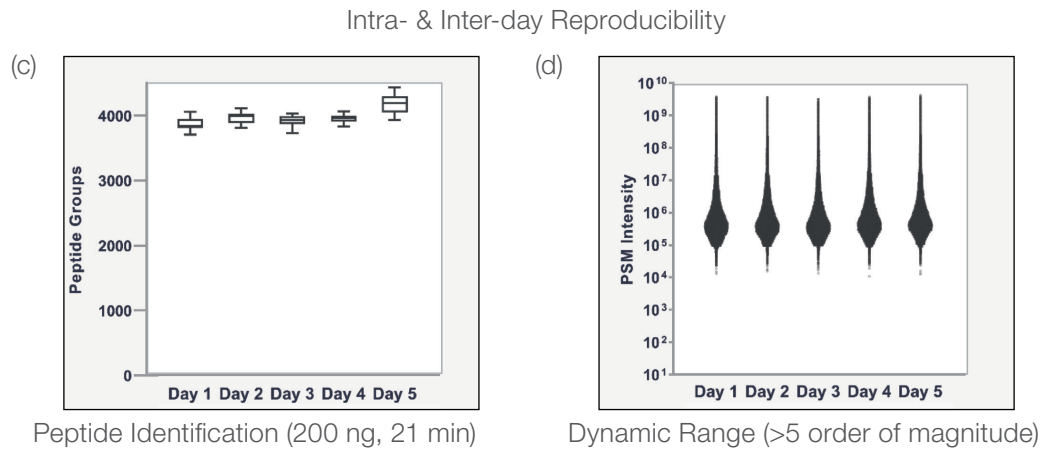
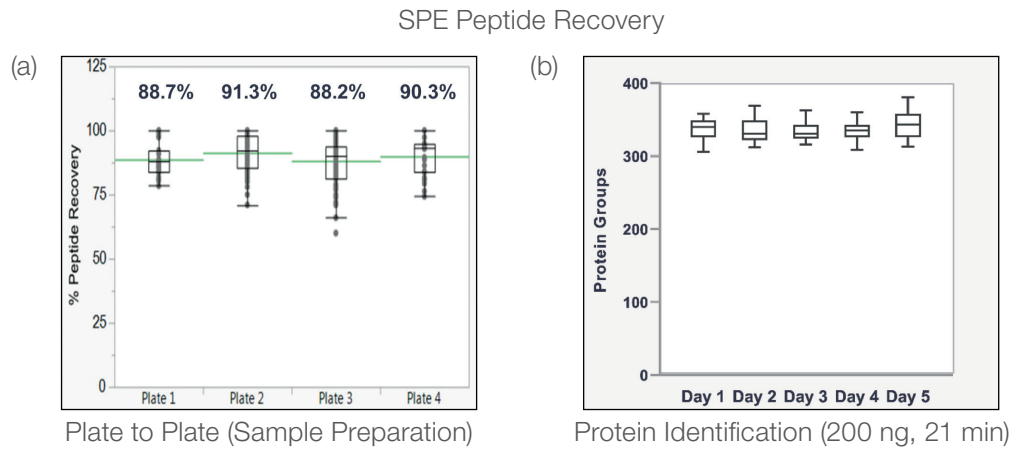
### Software

- Thermo Scientific™ Proteome Discoverer™ software, version 2.4 with 1% PSM FDR

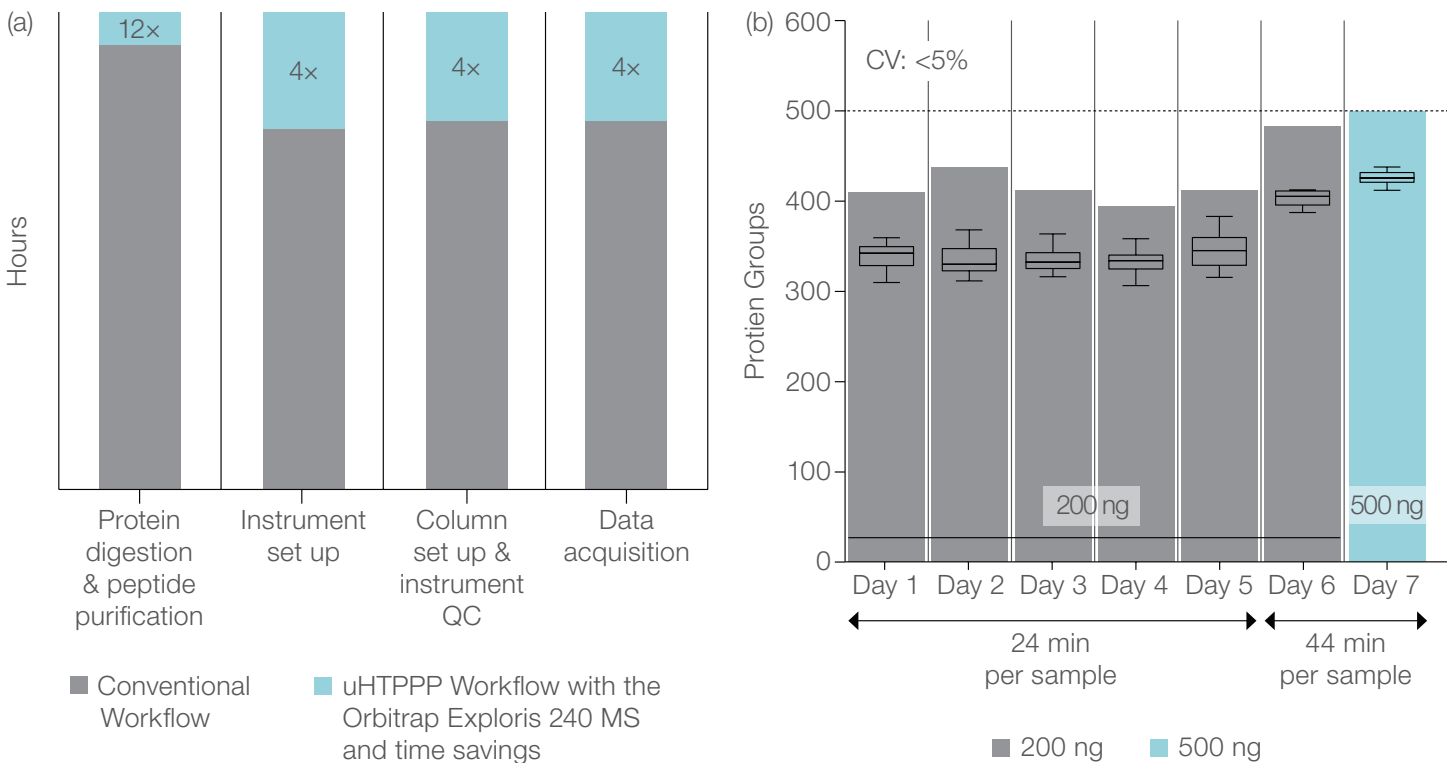
## Data



**Figure 2. Mass accuracy and retention time stability over 5 days:** 100 depleted serum samples were analyzed over 5 days of continuous operation. Mass accuracy and retention time stability of Peptide Retention Time Calibration (PRTC) peptides spiked into each sample were monitored.



**Figure 3. Reproducibility of the uHTPPP workflow:** To determine the reproducibility of the workflow for large-scale studies, peptide recovery efficiency from multiple plates was determined to assess the plate-to-plate consistency. Over 5 orders of magnitude of dynamic range were observed for label-free quantification of more than 100 injections of depleted pooled human serum samples.



**Figure 4. (a)** Automated sample preparation (uHTPPP) coupled with the Orbitrap Exploris 240 MS provides a high-throughput workflow delivering reproducibility and time savings. **(b)** The Orbitrap Exploris 240 MS showed consistent and precise plasma protein characterization over 150 injections (130 samples and 20 QCs). Box plot shows the averaged number of proteins identified. Total proteins identified and quantified are highlighted in bars.

## Results

- The Orbitrap Exploris 240 MS enables a uHTPPP workflow for large-scale studies (Figure 1).
- The Orbitrap Exploris 240 MS delivers reliable and reproducible high-quality qualitative and quantitative data for plasma, with stable LC retention time and mass accuracy for up to 5 days of continuous operation (Figure 2 a and b).

- Over 100 depleted lung cancer serum samples were processed and analyzed with high peptide recovery and protein identification with CV of less than 5% while spanning a dynamic range of five orders of magnitude (Figure 3).
- uHTPPP workflow delivers time savings of 12× (sample preparation) to 4× (for data acquisition and processing), enabling faster results (Figure 4).

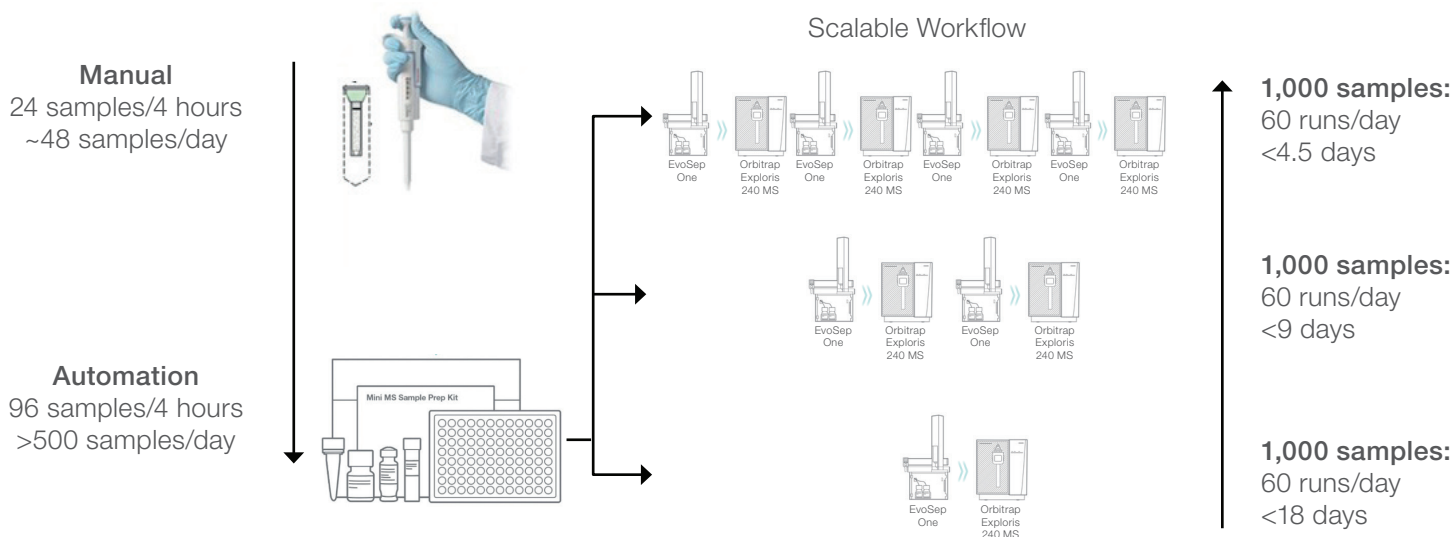


Figure 5. Versatility and scalability of the uHTPPP workflow.

## Outlook

The uHTPPP workflow can be scaled up to deliver remarkable time savings and rigorous, relevant results for plasma profiling. The Orbitrap Exploris 240 MS enables scaling up for large sample cohorts with automated sample preparation and a compact footprint that saves valuable bench space (Figure 5).

## Conclusion

Demonstrating that when you require higher-confidence results for translational and clinical proteomics research, Thermo Scientific™ Orbitrap™ technology smoothly plugs in with automated sample preparation workflows delivering a complete end to end solution.

Find out more at

[thermofisher.com/OrbitrapExploris240Proof](https://thermofisher.com/OrbitrapExploris240Proof)

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