Proof of performance

Orbitrap Exploris 240 mass spectrometer

Industry-leading performance for protein identification depth and breadth of coverage

Summary

This document presents results that demonstrate the deep protein coverage achievable on the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer equipped with the Thermo Scientific™ FAIMS Pro™ interface. Proteome coverage is essential for studying biological systems, not only for the identification of the expressed proteins at a given time, but also for quantitative studies that monitor proteome expression across time and/or space that reveal more detailed insights about the biological system under study. It is important to consider both the proteome depth across a wide range of sample amounts and the corresponding analysis time to achieve those results.

Here, we demonstrate that the Orbitrap Exploris 240 MS delivers high performance for shotgun proteomics experiments from low sample amounts of 10 ng to higher loads of 1,000 ng. Protein identification spans five orders of dynamic range. The Orbitrap Exploris 240 MS also enables high-throughput experimental or large cohort studies while maintaining proteome depth. High quality data is maintained throughout, featuring high resolution of precursors for accurate label-free quantitation (LFQ), exceptional mass accuracy for high-confidence identification, and strict control of False Discovery Rate (FDR) of 1% for both peptide groups and protein group.

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Best-in-class protein identification: When high performance for proteomics is required, the Orbitrap Exploris 240 MS with the FAIMS Pro interface delivers higher protein identifications for shotgun proteomics.

Experimental conditions
Sample
- Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (Cat # 88329) 10 ng to 1000 ng

- Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (Cat # 88321) 10 fmol

LC method
- IonOpticks™ Aurora™ series UHPLC emitter column (250 mm × 75 μm, 1.6 μm particle-integrated emitter)

- Flow rate 300 nL/min

- 90 min gradient

- Mobile phase A: Water/0.1% formic acid (FA), Mobile phase B: 80% acetonitrile (ACN) in 0.1% FA

Time (min)	B%
0	3
1	3
55	19
76	29
91	41
94	95
101	95

Instrumentation
- Thermo Scientific™ EASY-nLC™ 1200 system (Cat # LC140)

- Thermo Scientific™ Nanospray Flex™ ion source (Cat #ES071)

- Sonation™ PRSO-V2 column oven operating at 40 ˚C

- FAIMS Pro interface (Cat # FMS02) (compensation voltage: -50 V/-70 V)

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
High performance of the Orbitrap Exploris 240 mass spectrometer for shotgun proteomics is demonstrated below, from low sample amounts of 10 ng to higher loads of 1,000 ng for both peptide (Figure 1a) and protein identifications (Figure 1b).
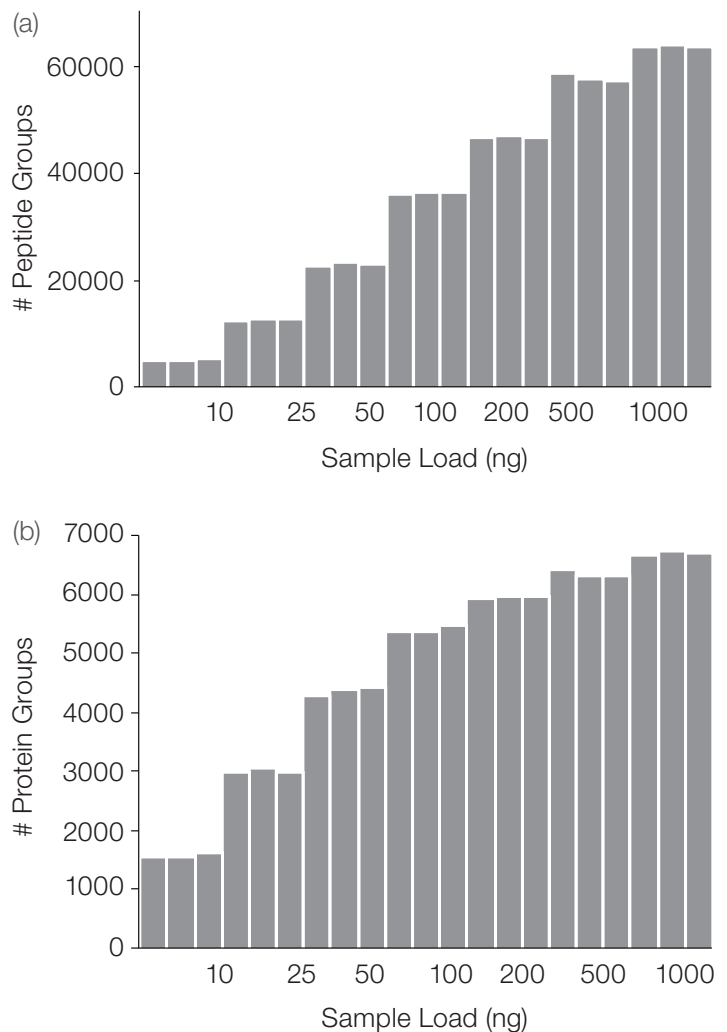

Figure 1. Performance benchmark for protein identification: Histograms of the number of (a) peptide groups and (b) protein groups identified per run as a function of the peptide amount loaded on column. All the identifications are derived from at least one high-confidence peptide spectrum match.
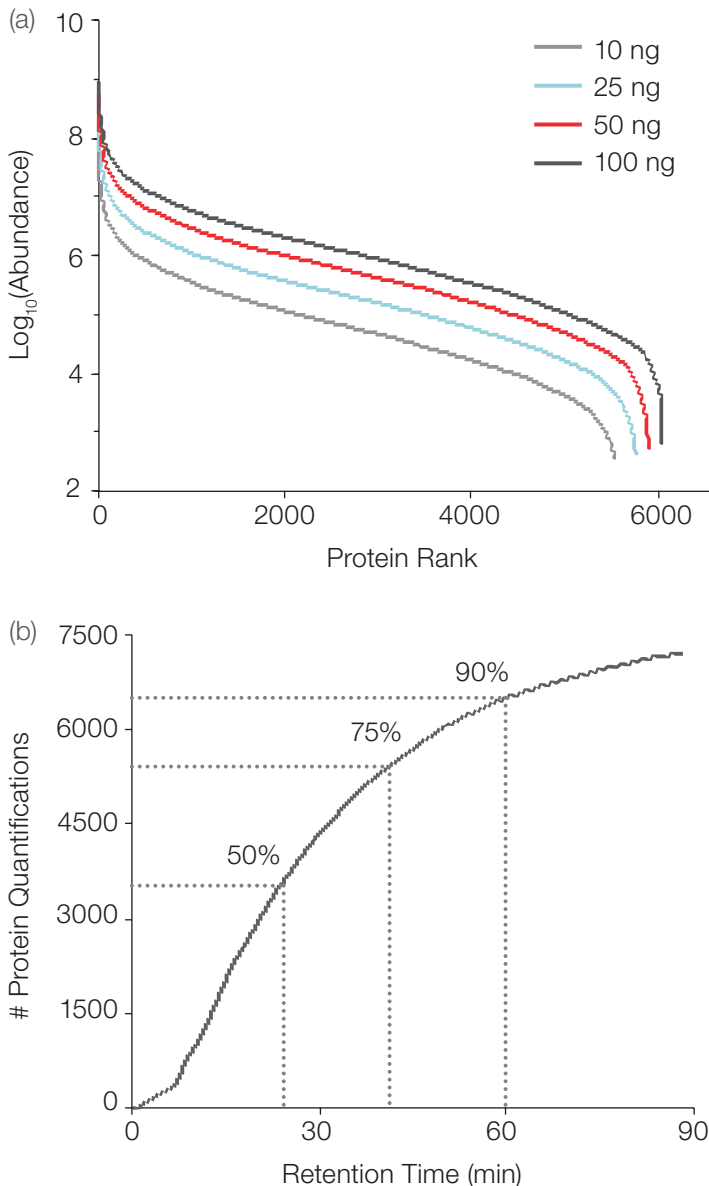
(a)

(b)

Figure 2. Performance benchmark for protein quantitation: (a) Protein rank as a function of protein abundance. Protein abundance is calculated as the median of the intensity for all the peptide intensities from a given protein. Trends were plotted for 10 ng up to 100 ng peptide loads highlighting over 6 orders of quantitative dynamic range while maintaining high sensitivity. (b) Number of proteins quantified per minute across the LC-MS gradient for the whole experiment.

Results

- Deep proteome coverage on wide range of sample amounts:

  – Very sensitive measurements at only 10 ng sample amount resulting in high-confidence identifications, averaging 1,600 protein groups and 5,300 peptide groups per dataset identified by at least one peptide spectrum match.

  – Extensive proteome coverage at 1,000 ng with an average of 6,700 protein groups and 63,000 peptide groups, the Orbitrap Exploris 240 MS delivers excellent depth for proteome coverage (Figure 1a & b).

- Measure with confidence across a wide dynamic range of protein expression, spanning five orders of magnitude, enabling the identification of low abundance peptides (Figure 2a).

- High-throughput DDA measurements with high proteome depth from 30 to 90 min analytical gradient length resulting in identifications of 3,500 to 6,700 protein groups, with extremely fast acquisition speed. The Feature Mapper node within Proteome Discoverer software links identified peptides to their corresponding precursor feature in the MS^1 scan and maps them across all datasets, improving the overall number of peptides quantified and reduces missing values. Applied to our experiment, it allows for high quantitation rates of 6,500 protein groups/hour and a total of almost 7,500 proteins quantified in the experiment.

Outlook
The Orbitrap Exploris 240 mass spectrometer combined with the FAIMS Pro interface delivers best-in-class balance of throughput and sensitivity for proteome coverage, allowing the study of limited sample amounts to an unprecedented depth in less time.

Conclusion
High performance proteomics: Together with FAIMS technology, the Orbitrap Exploris 240 MS delivers deep single shot proteome coverage with high-confidence identifications from 10–1,000 ng amounts of sample.

Find out more at
thermofisher.com/OrbitrapExploris240Proof

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