Proof of performance

Orbitrap Exploris 240 mass spectrometer

Sensitivity–leading level sensitivity for the analysis of limited and precious samples

Summary

In proteomics, sensitivity is essential for studying samples that either have very limited total protein amount, or when biology is driven by proteins that are expressed in low abundance. High sensitivity is needed not only for the detection and identification of proteins, but also for quantitative studies that reveal their biological significance.

This document presents results that demonstrate the high sensitivity and selectivity of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer equipped with the Thermo Scientific™ FAIMS Pro™ interface to analyze low sample amounts. Thermo Scientific™ Orbitrap™ technology has proven in peer reviewed publications to demonstrate the sensitivity to analyze true single cells[1]. This new level of sensitivity is essential for limited samples and for single cells or rare cells, such as circulating tumor cells.

Here, we demonstrate that the Orbitrap Exploris 240 MS can deliver high performance for shotgun proteomics experiments from low sample amounts of 10 ng, to higher loads of 200 ng, for both peptide and protein identification.

Additionally, we present data on five replicate injections of 10 ng of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard, spiked with the Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (PRTC). In such experiments, standard peptides spiked in at 10 fmol dominate the chromatogram, the combination of the Orbitrap Exploris 240 MS and FAIMS Pro interface enables reproducible identification of a low abundant peptide precursor.

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Best-in-class sensitivity: When the highest sensitivity data is key to obtain insights that are actionable, the Orbitrap Exploris 240 MS with the FAIMS Pro interface delivers increased sensitivity and selectivity that enable the reproducible identification of low abundant proteins or low level samples.

Experimental conditions
Sample
- Pierce HeLa Protein Digest Standard (Cat # 88329) 10 ng

- 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 Column Oven (PRSO-V2) 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
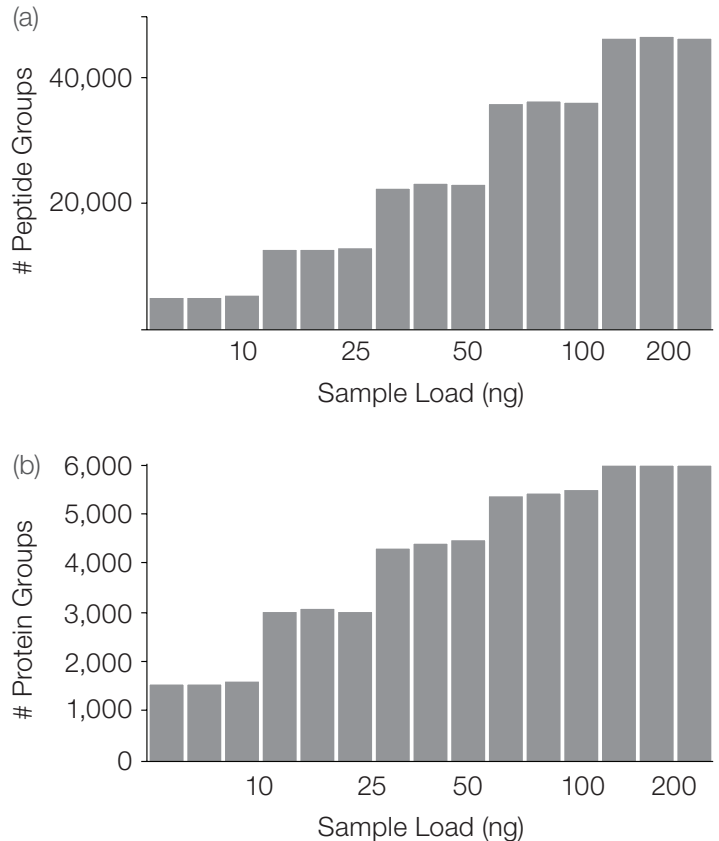
Data

Figure 1. Performance benchmark for protein identification at low peptide levels: 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 from a HeLa cell digest sample. All the identifications are derived at least from a high-confidence peptide spectrum match. Base peak chromatograms for 5 different injection replicates of 10 ng injection, and extracted ion chromatogram for a low intensity peptide.
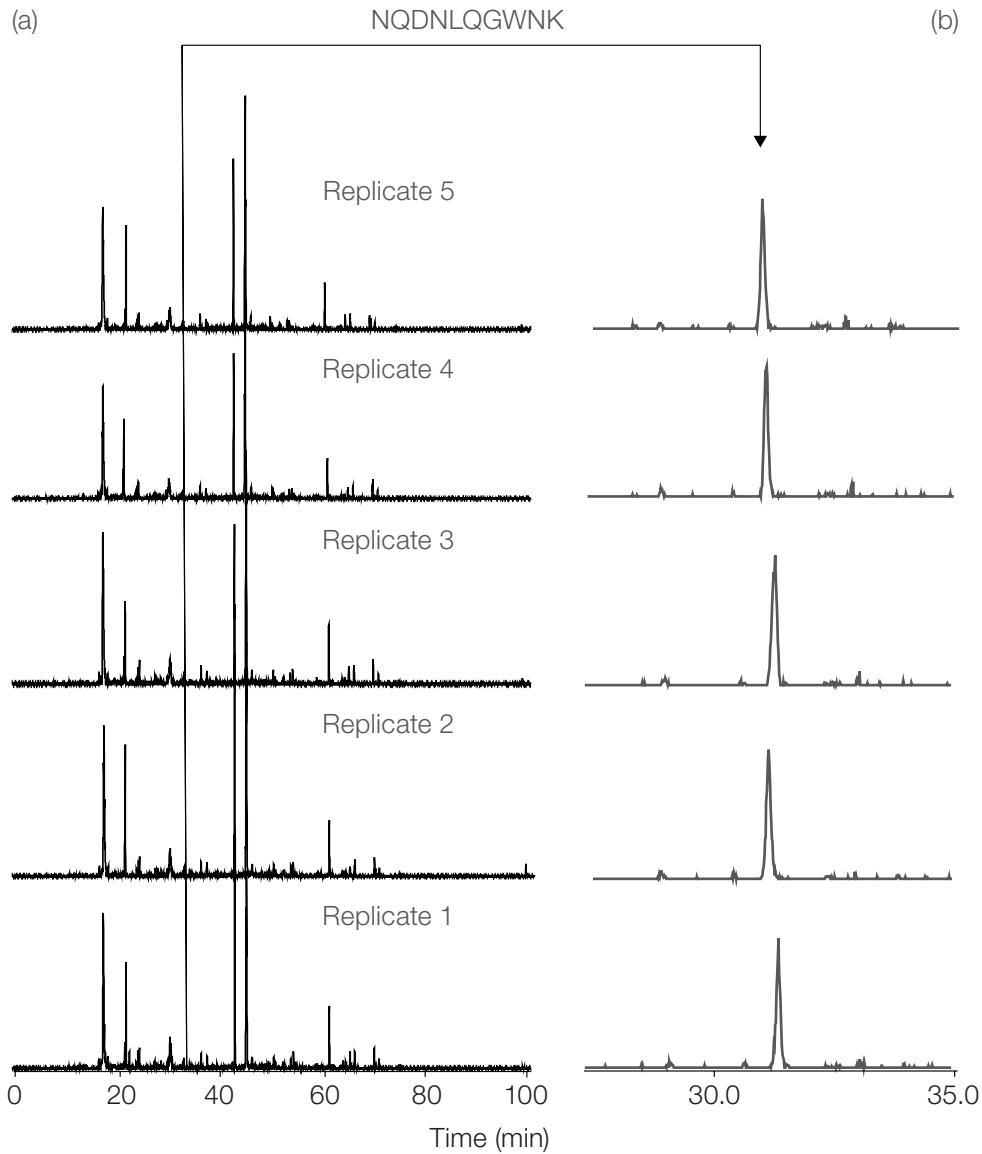
Figure 2. Five replicate injections of 10 ng Pierce HeLa Digest standard spiked with the Pierce Peptide Retention Time Calibration mixture. (a) While the standard peptides spiked in at 10 fmol dominate the chromatogram, (b) the combination of the Orbitrap Exploris 240 MS and FAIMS Pro interface enable reproducible identification of a low abundant peptide precursor shown in the insert.

Results

- Deep proteome coverage on low amounts of sample: From very sensitive measurements at only 10 ng sample amount resulted in high-confidence identification of 1,600 protein groups and 5,300 peptide groups on an Orbitrap Exploris 240 mass spectrometer (Figure 1a & b).

- Extremely reproducible identification of peptide precursors were obtained across five replicates of the 10 ng sample (Figure 2b).

Outlook

New levels of sensitivity are essential for the analysis of limited samples, and for single cells or rare cells such as circulating tumor cells. The Orbitrap Exploris 240 mass spectrometer delivers best-in-class sensitivity required to identify and quantify proteins that reveal biological insights leading to actionable outcomes.

Conclusion

With the FAIMS Pro interface, the Orbitrap Exploris 240 mass spectrometer delivers the high sensitivity needed to identify low abundant proteins from limited samples with reproducibility while maintaining proteome coverage and depth.

Reference

1. Yongzheng Cong, Yiran Liang, Khatereh Motamedchaboki, Romain Huguet, Thy Truong, Rui Zhao, Yufeng Shen, Daniel Lopez-Ferrer, Ying Zhu, Ryan T. Kelly. Improved Single-Cell Proteome Coverage Using Narrow-Bore Packed NanoLC Columns and Ultrasensitive Mass Spectrometry. Anal. Chem. 2020, 92, 3, 2665-2671

Find out more at
thermofisher.com/OrbitrapExploris240Proof

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