

Proof of performance

Orbitrap Exploris 240 mass spectrometer



Data quality—confident results with highest data quality synonymous with Orbitrap MS technology

Summary

This document highlights why the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer is the best-in-class, high-resolution, accurate-mass (HRAM) Thermo Scientific™ Orbitrap™ instrument delivering high-quality data for confident results. With technology innovations and optimized methods, the Orbitrap Exploris 240 MS delivers complete quantitative workflows for the identification and quantitation of proteins without compromising accuracy, coverage or depth, while increasing sample throughput.

Here, we demonstrate that the Orbitrap Exploris 240 MS can deliver high-confidence proteomics solutions for interactomics via immunoprecipitation-mass spectrometry (IP-MS). These results are made possible by utilizing the fast

scan rate for data acquisition, exceptional mass accuracy for high-confidence identification, and the Thermo Scientific™ FAIMS Pro™ interface for increased precursor ion selectivity. Importantly, we assessed the reproducibility of spectra quality across multiple instruments, demonstrating both the spectra reproducibility, as well as method transferability across instruments. Additionally, we demonstrate the fragment ion peak area reproducibility which facilitates accurate quantitation across multiple platforms.

Best-in-class data quality: When high-quality data is key to obtaining high-confidence insights that are publishable and lead to actionable outcomes, the Orbitrap Exploris 240 MS with the FAIMS Pro interface deliver proven results synonymous with Orbitrap technology.

Experiment

Immunoprecipitation identification

Sample

- Multiplex pull-down of 12 proteins from the Thermo Scientific™ SureQuant™ AKT Pathway IP and MS Sample Preparation Module (A40081)
- 100 µg control lysate from the Thermo Scientific™ A549 (+hIGF-1) Control Lysate (Cat # A37960)

LC method

- Thermo Scientific™ EASY-Spray™ HPLC Column 150 mm × 75 µm, 3 µm particle (ES800)
- 15 min and 30 min gradient
- Flow rate 300 nL/min
- Mobile phase A: Water/0.1% formic acid (FA), Mobile phase B: 80% acetonitrile (ACN) in 0.1% FA

Time (min)	B%
0	4
1	4
16	34
17	98
22	98

LC Instrumentation

- Thermo Scientific™ EASY-nLC™ 1200 system (Cat # LC140)
- Thermo Scientific™ EASY-Spray™ ion source (Cat # ES081)
- FAIMS Pro interface (Cat # FMS02) (single compensation voltage: -60 V; two compensation voltages: -50 V/-70 V)

MS detection

- High-resolution, accurate-mass Orbitrap Exploris 240 mass spectrometer

Software

- Thermo Scientific™ Proteome Discoverer™ software, version 2.4

Parallel reaction monitoring experiment

Sample

- SureQuant AKT Pathway IP and MS Sample Preparation Module Kit (Cat # A40081)

LC method

- EASY-Spray HPLC Column 150 mm × 75 µm, 3 µm particle (ES800)
- 40 min gradient
- Flow rate 300 nL/min
- Mobile phase A: 0.1% formic acid (FA), Mobile phase B: 80% acetonitrile (ACN) in 0.1% FA

LC Instrumentation

- EASY-nLC 1200 system (Cat # LC140)
- EASY-Spray source (Cat # ES081)

Time (min)	B%
0	4
2.5	4
32.5	34
33.5	98
40	98

MS detection

High-resolution, accurate-mass Orbitrap Exploris 240 mass spectrometer

Software

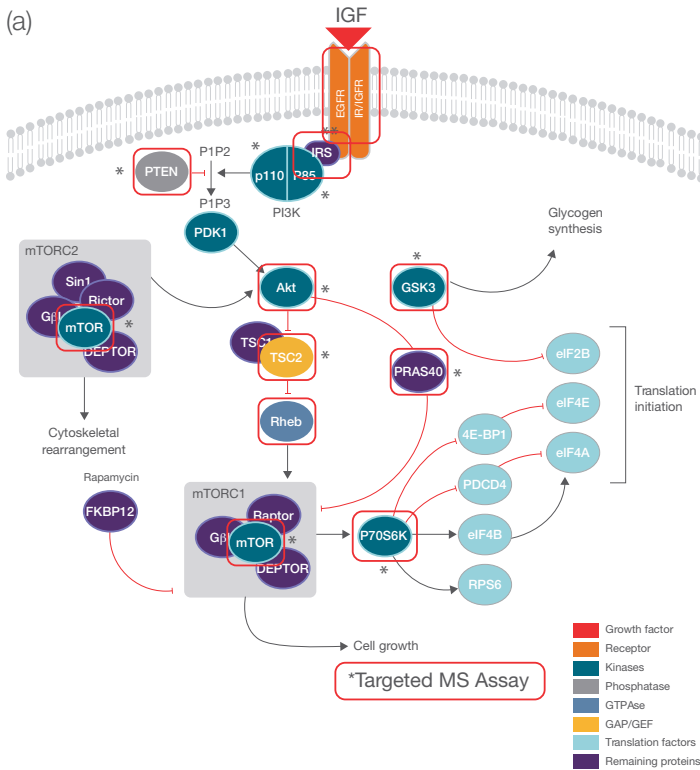
Proteome Discoverer software, version 2.4 with 1% PSM FDR

Data

Quantitative measurement of alterations in the expression of pathway proteins is necessary for classifying disease states, monitoring cancer progression and determining treatment response.

Results

- Identification of all 12 target proteins from a multi-protein pull-down assay (IP-MS) resulted in sequence coverage up to 50% for the short 15 min acquisition time (Figure 1a & b).



- High spectral quality and peak area reproducibility across Thermo Scientific™ Orbitrap Exploris™ instruments deliver portability and consistency in identification across platforms (Figure 2a & b).

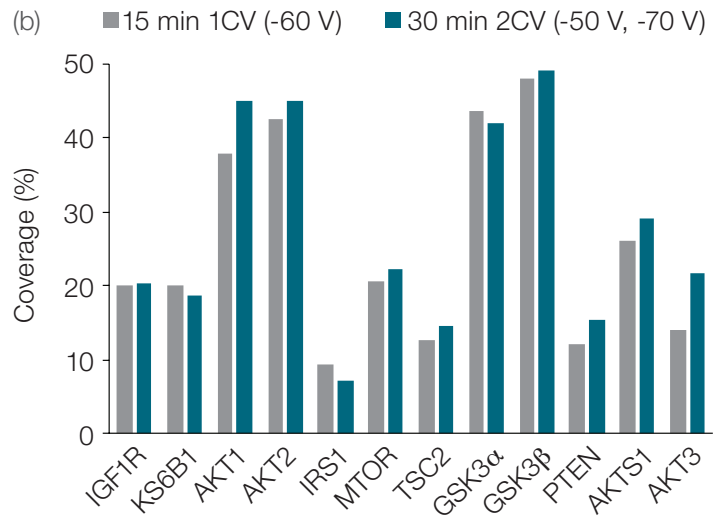


Figure 1. (a) We performed a multi-protein pull-down of the AKT/mTOR pathway which is crucial to many aspects of cell growth and survival. **(b)** In either 15 min or 30 min gradient time we identified all 12 target proteins in the pull-down assay.

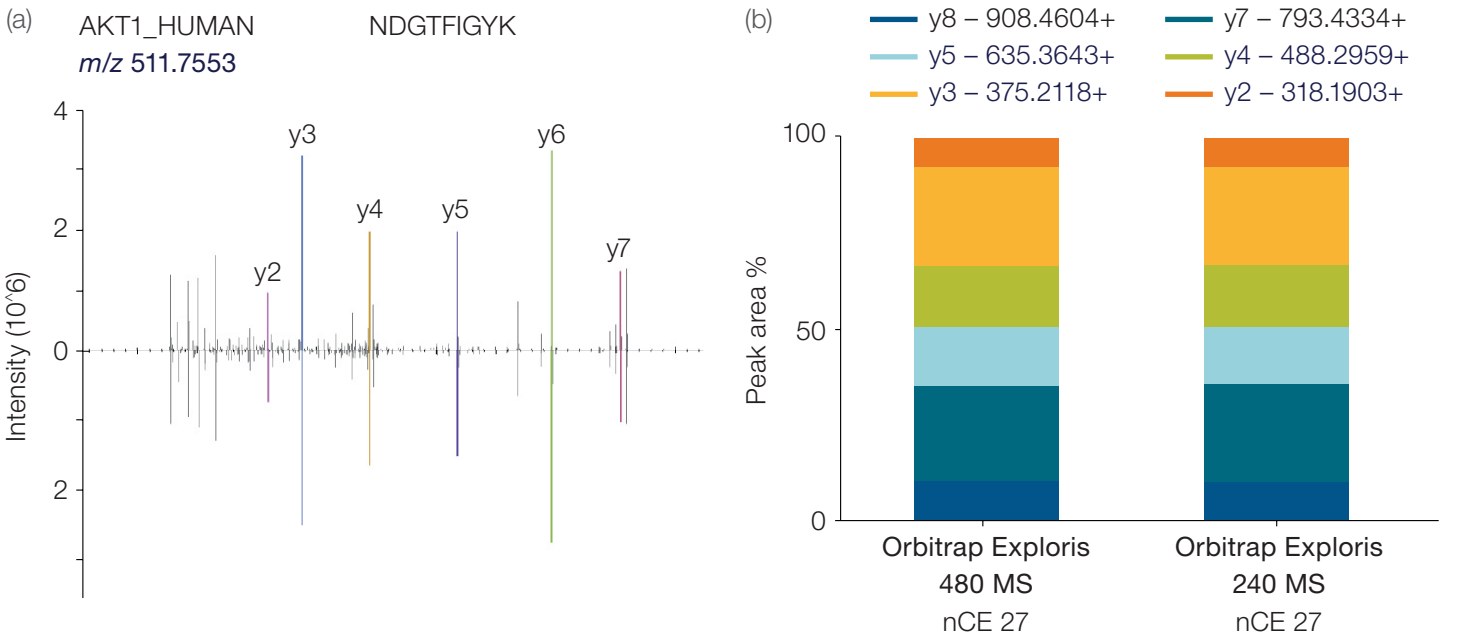


Figure 2. Spectral Quality: (a) Overlay of the MS² spectra for peptide NDGTFIGYK from AKT1 protein acquired with a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer and Orbitrap Exploris 240 mass spectrometer. (b) Bar graph showing the contribution to the peak area for each of the y-ions generated during fragmentation spectra for each of the instruments. Results are of equally high quality across both instruments.

Results

- Identification of all 12 target proteins from a multi-protein pull-down assay (IP-MS) resulted in sequence coverage up to 50% for the short 15 min acquisition time (Figure 1a & b).
- High spectrum and peak area reproducibility across Orbitrap Exploris instruments deliver portability and consistency in identification across platforms (Figure 2a & b).

Outlook

The Orbitrap Exploris 240 mass spectrometer provides best-in-class, high-quality data for high-confidence results that are publishable and lead to actionable outcomes.

Conclusion

Together with FAIMS Pro interface technology, the Orbitrap Exploris 240 MS delivers high spectral quality, high-resolution, and high mass accuracy that increases confidence in identification and quantification that is synonymous with Orbitrap mass spectrometer data, and sets this instrument apart from Quadrupole Time-of-Flight (Q-TOF) analyzers.

Find out more at

thermofisher.com/OrbitrapExploris240Proof

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