Proof of performance

Orbitrap Exploris 240 mass spectrometer

Quantitation–widest range of quantitative strategies that deliver biological insights

Summary

This document presents the versatility of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer to deliver comprehensive quantitative workflows. From label-free quantitation (LFQ) and quantitative multiplexing of proteomes for discovery proteomics studies, up to targeted quantitative proteomics, the Orbitrap Exploris 240 MS enables the deciphering of the true abundance of proteins in each sample – all with a new standard of sensitivity, accuracy and precision that allow the investigation of global protein kinetics.

Proteins are dynamic and interactive, and therefore quantitative proteomics is more complex than simply identifying proteins in a sample. The Orbitrap Exploris 240 MS utilizes high-resolution, accurate-mass (HRAM) Thermo Scientific™ Orbitrap™ technology to deliver high-quality, reproducible quantitation with the fast time to results. With technology and data acquisition innovations, this system delivers complete workflows that enable protein quantitation without compromising quantitative accuracy or precision.

Best-in-class quantitative workflows: When the highest quantitative accuracy and precision are key to your research goals, the Orbitrap Exploris 240 MS with the Thermo Scientific™ FAIMS Pro™ interface delivers high-quality, reproducible results. The Orbitrap Exploris 240 MS comes with the widest range of quantitative workflows and optimized methods that deliver the results you need with no compromise in sensitivity, depth or coverage.

Label-free quantitation

The Orbitrap Exploris 240 mass spectrometer utilizes HRAM to deliver high-quality data for LFQ. To demonstrate accuracy across a wide dynamic range of quantitation, we analyzed a two-proteome mixture containing yeast protein extract spiked in a ratio of 1:2, 1:5, or 1:10 into a constant amount of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (Figure 1a). Four technical replicates were measured for each sample, yielding both reproducible quantitation and a linear response across the amount of yeast protein spiked into the sample (Figure 1b).

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Precursor-based quantitation accurately quantified the expected ratios (Figure 1c), demonstrating the ability of the Orbitrap Exploris 240 MS to deliver accurate quantitation across a wide dynamic range.

Experimental conditions
Sample
- Pierce HeLa Protein Digest Standard (Cat # 88329)
- Promega™ MS Compatible Yeast Protein Extract, Digest (Cat # V7461)

LC method
- 25 cm IonOpticks™ Aurora™ series UHPLC emitter column (250 mm × 75 µm, 1.6 µm particle-integrated emitter)
- Flow rate 300 nL/min
- 120 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
73	19
101	29
121	41
124	95
131	95

Instrumentation
- Thermo Scientific™ EASY-nLC™ 1200 system (Cat # LC140)
- Thermo Scientific™ Nanospray Flex™ ion source (Cat # ES071) for IonOpticks Column
- Sonation Column Oven (PRSO-V2) operating at 40 °C
- FAIMS Pro interface (Cat # FMS02) for LFQ and TMT11-plex experiment

MS detection
- High-resolution, accurate-mass Orbitrap Exploris 240 MS
- Data-dependent acquisition (DDA)

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

Data

(a) HeLa/Yeast Spike

(b)

(c)
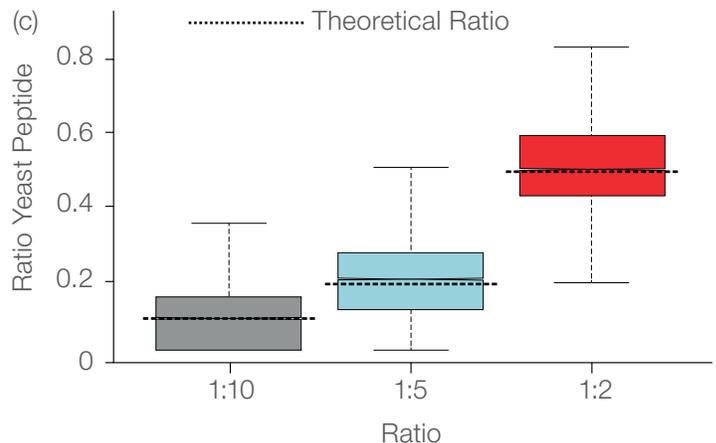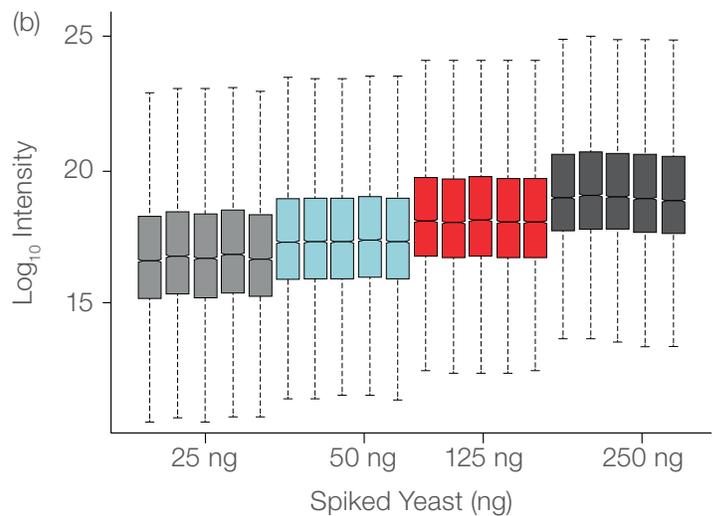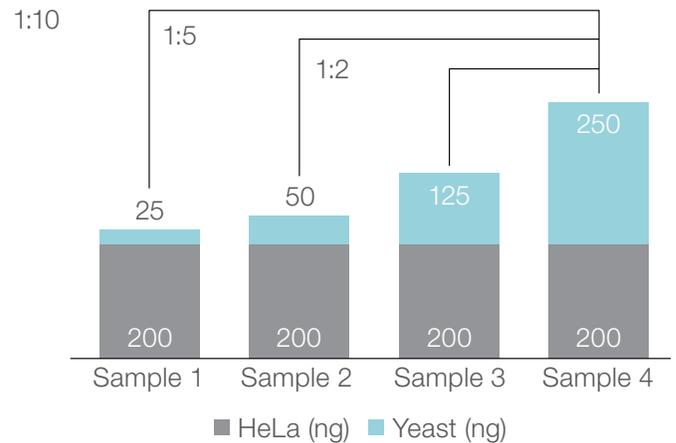
| Theo. Ratio	1/10	1/5	1/2
Exp. Ratio	0.10	0.21	0.51

Figure 1. Label-free quantitation: (a) Experimental setup for a mixed proteome analysis to evaluate quantitative precision and accuracy. (b) Box plots of the peptide intensities for 5 technical replicates for each of the samples. Intensities were $\log_{10}$ transformed. (c) Box plots of the peptide ratios for yeast derived proteins demonstrating excellent precision and accuracy.

TMT multiplexing

The FAIMS Pro interface with the Orbitrap Exploris 240 MS increases quantitative performance and sampling efficiency with the use of Tandam Mass Tags™ (TMT™), with the Thermo Scientific™ TMT 11plex Label Reagent Set or the Thermo Scientific™ TMTpro™ 16plex Label Reagent Set.

Multiplexed quantitation strategies using TMT deliver precise and accurate measurements of peptides or protein abundances from multiple samples in a single LC-MS run, improving the analysis of large sample sets for applications such as thermal shift assays to single cells. Isobaric tagging strategies using TMT or TMTpro allows up to 11 or 16 samples, respectively, to be multiplexed in a single high-resolution LC-MS experiment. However, co-isolation of ion interference can suppress ratio quantification and thereby mask true differences in protein abundance across samples.

The Orbitrap Exploris 240 MS delivers best-in-class TMT MS^2-level proteome quantitation and is designed to address the conventional challenges associated with TMT quantitation, including co-isolated interferences. Incorporating the FAIMS Pro interface into the workflow increases precursor selectivity and reduces interference using gas-phase fractionation, resulting in greater accuracy for TMT-based quantitation. Importantly, the Orbitrap Exploris 240 MS has the resolving power and speed capabilities to perform TMT 11plex and TMTpro 16plex experiments with unrivaled confidence and no compromise in coverage or depth.

Here, we analyzed the Thermo Scientific™ Pierce™ TMT11plex Yeast Digest Standard, a TMT quality control standard used to assess:

1. Co-isolation interference and quantitation accuracy

2. Reproducibility in identification across quantified, unique peptide groups and quantified protein groups while maintaining a 1% FDR

3. Accuracy of TMT quantitation using the FAIMS Pro interface according to the interference free index (IFI)

Thus, the combination of the FAIMS Pro interface and the Orbitrap Exploris 240 MS provides exceptional sample throughput for TMT 11plex or TMTpro 16plex reagents with high quantitation accuracy.

Experimental conditions

Sample
• Pierce TMT11plex Yeast Digest Standard

LC method
• Thermo Scientific™ EASY-Spray™ HPLC Column ES803 (500 mm × 75 µm 3 µm particle)

• 120 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	5
110	25
120	40
130	95
140	95

Instrumentation
• EASY-nLC 1200 system (Cat # LC140)

• Thermo Scientific™ EASY-Spray™ Source (Cat # ES081)

• FAIMS Pro interface (Cat # FMS02)

MS detection
• High-resolution, accurate-mass (HRAM) Orbitrap Exploris 240 mass spectrometer

• Data-dependent acquisition (DDA)

Software
Proteome Discoverer software, version 2.4 with 1% PSM FDR

Data

The Pierce TMT11plex Yeast Digest Standard was analyzed to assess co-isolation interference and quantitation accuracy (Figure 2a). Three replicates were analyzed to demonstrate reproducible identification and quantitation of unique peptide groups (Figure 2b) and quantified protein groups (Figure 2c) while maintaining a 1% FDR, yielding extremely similar numbers of quantified identifications between replicates. The accuracy of TMT quantitation was assessed using the IFI, where any signal detected in the three genetic knockout proteins, respectively, Met6, His4, Ura2 is due to co-isolation interference. An IFI of 1.00 represents the best quantitation accuracy. Using the FAIMS Pro interface on the Orbitrap Exploris 240 MS quantitation was superb for MS^2 method with an average IFI of 0.89 (Figure 2d).
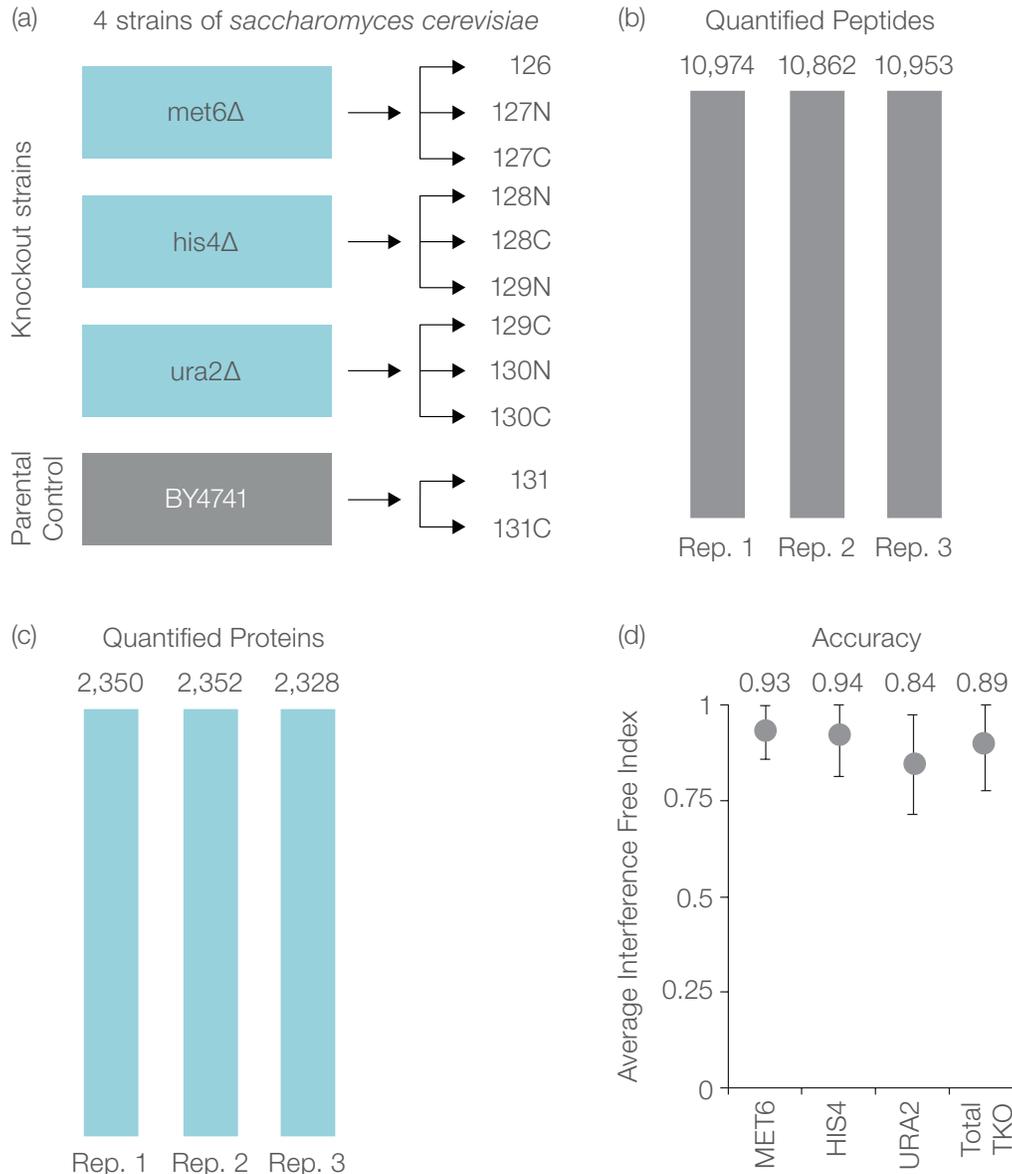

Figure 2. Tandem Mass Tags multiplex protein quantitation: (a) Experimental setup for a mixed proteome analysis to evaluate quantitative precision and accuracy using three strains with specific protein knock outs and a parental control. Bar plots highlighting the number of peptides (b) and proteins (c) quantified per run across the different samples. (d) Plot highlighting the average IFI for each of the knock-out proteins.

Parallel reaction monitoring

Targeted protein quantitation experiments focus on the quantification of analytes with the highest level of performance in terms of accuracy, precision, dynamic range and reproducibility. For targeted experiments, parallel reaction monitoring (PRM) is an ideal reference method which can be optimized to maintain high data quality and scale, depending on how much of the instrument acquisition time is dedicated to the measurement of each individual analyte.

Typical PRM experiments involve retention time scheduling to maximize data acquisition and quantitation accuracy.

In order to demonstrate the high quantitative precision as well as the quantitative dynamic range, 30 peptides from the Thermo Scientific™ SureQuant™ AKT Pathway IP and MS Sample Preparation Module were analyzed at different concentrations. Results demonstrate that the Orbitrap Exploris 240 MS achieves high-confidence quantitation for targeted proteomics.

Experimental conditions

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

LC method
- Thermo Scientific™ EASY-Spray™ HPLC Column ES800 (150 mm × 75 µm 3 µm particle)

- Flow rate 300 nL/min

- 40 min gradient

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

Instrumentation
- EASY-nLC 1200 system (Cat # LC140)

- EASY-Spray Source (Cat # ES081)

MS detection
- High-resolution, accurate-mass Orbitrap Exploris 240 MS

- PRM

Software
Skyline software (skyline.org)

Data

Peptides were analyzed from the SureQuant AKT Pathway IP and MS Sample Preparation Module Pierce Kit to demonstrate the high precision obtainable across all target peptides (Figure 3a). Importantly, 83% of peptides at the lower limit of quantitation (LLOQ) were measured with a coefficient of variation (CV) of less than 10%. The average CV across all peptides quantified delivered exceptional precision with an average CV of 1.36% (Figure 3b). Furthermore, accurate quantitation was achieved across five orders of dynamic range (Figure 3c).
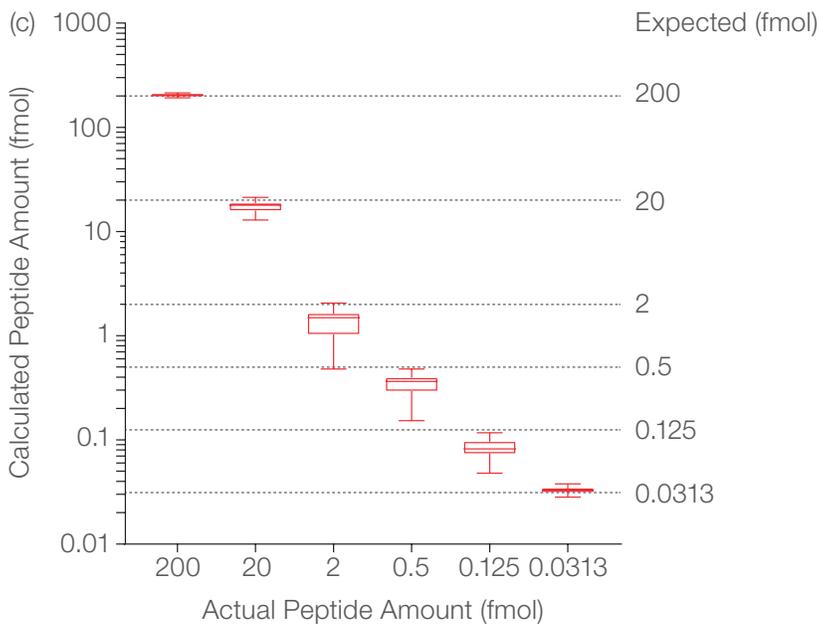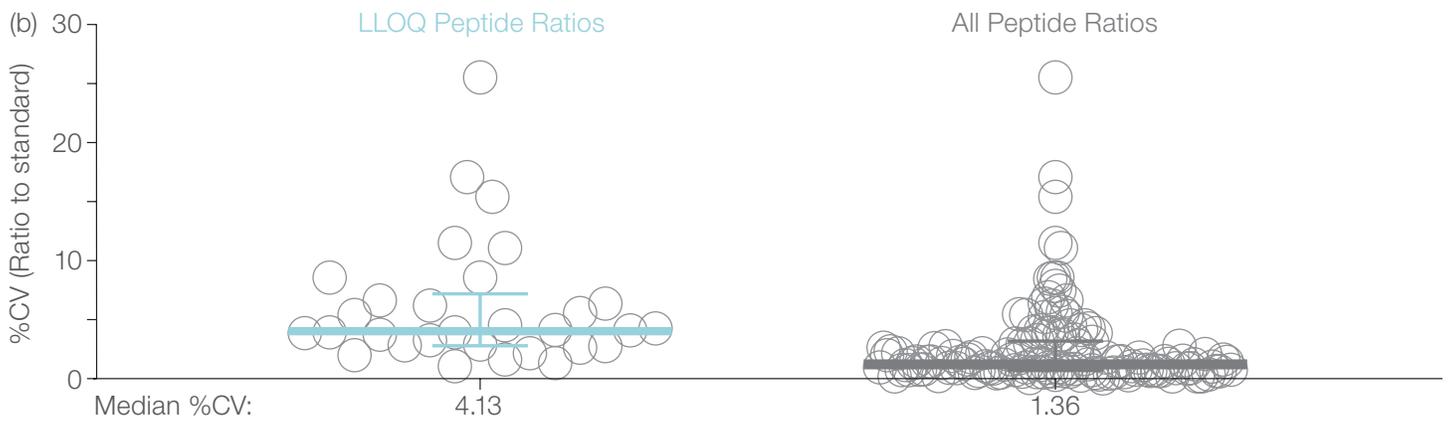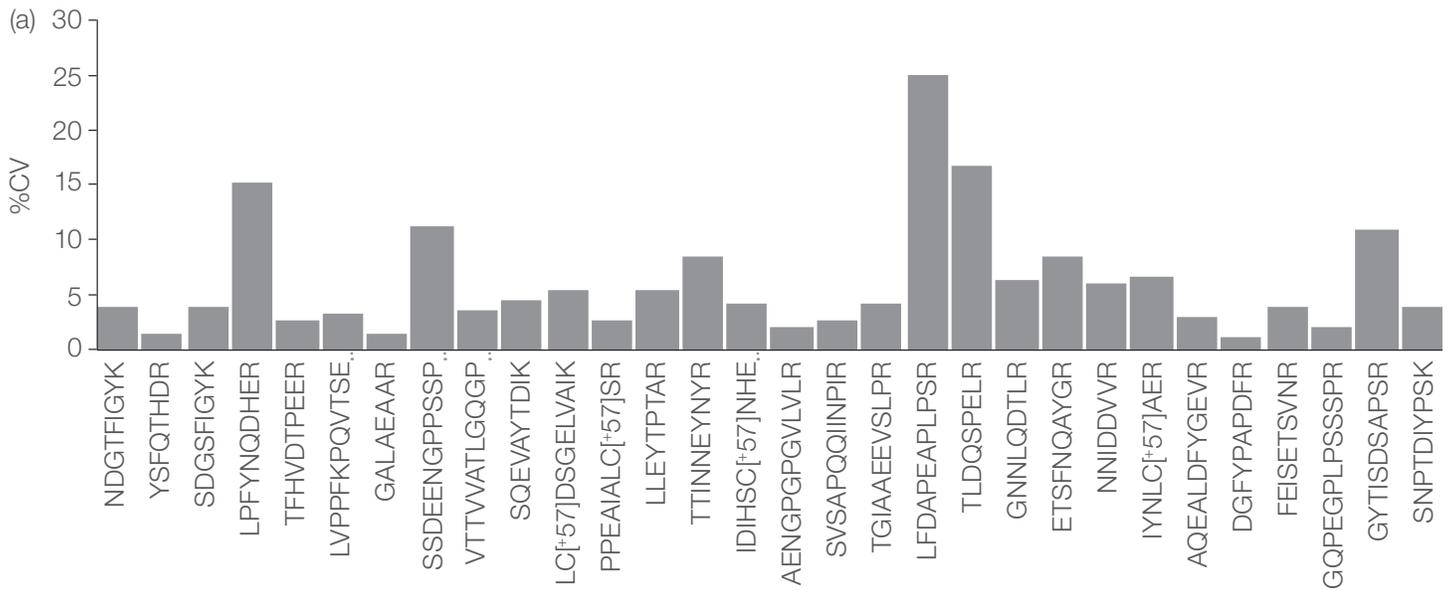
Figure 3. Target protein quantitation: PRM assay monitoring 30 peptides from 12 proteins from the AKT/mTOR pathway. (a) Bar diagram from the coefficients of variation at the most challenging measurement, the LLOQ of each peptide in the dilution curve. b) Box plots from each peptide's CV at LLOQ and for all measurements in the dilution curve. (c) Box plots of accuracy of the dilution curve measurements from 200 fmol down to low attomole level.

Results

- LFQ enables deep proteome coverage for a wide dynamic range of quantitation (Figure 1).

- The FAIMS Pro interface delivers high quantitation accuracy for TMT or TMTpro multiplexing reagents (Figure 2).

- PRM enables sensitive targeted protein quantitation down to the LLOD (Figure 3).

- High data quality (resolution, mass accuracy, spectral quality) improves quantitation and imparts confidence and certainty for further validation studies.

Outlook

The Orbitrap Exploris 240 MS system is designed for proteomics scientists in research and core laboratories looking to increase sample throughput with higher quantitation confidence and rigor across multiple methods that lead to impactful publications and results. As biology is quantitative, the confidence of your quantitative data, with a new standard of sensitivity, accuracy and precision, enables you to further understand global protein kinetics for biological understanding.

Conclusion

The Orbitrap Exploris 240 MS enables HRAM measurements of proteins and peptides with confidence, including for PRM targeted protein quantitation, with high precision, even at the lower limit of detection.

The increased sensitivity and resolution of the instrumentation when combined with FAIMS Pro interface further improves proteome depth of coverage for LFQ. This is especially important for TMT multiplexing strategies, where resolution is not high enough on Q-TOF analyzers to achieve higher multiplexing and accurate quantitation.

Find out more at
thermofisher.com/OrbitrapExploris240Proof

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