

# Proof of performance

Orbitrap Exploris 240 mass spectrometer



## Diverse applications for your core lab research and clients

### Summary

Core labs require systems that are robust, versatile and easy to switch from one application to another.

This document demonstrates how the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer delivers utmost flexibility from protein identification, shotgun proteomics to gel-band protein identification, and quantitative applications from label-free quantitation to multiplexed proteome quantitation (see XX-65866 proof of performance note).

Protein identification experiments can vary from several sample loads or gradient lengths depending on the goal. Here we demonstrate expected performance for analytical gradients of 30 min, 60 min, 90 min or 120 min. Thus, you can choose the appropriate gradient length and sample load to achieve the desired proteome coverage and throughput for your experimental design.

We demonstrate the performance of the Orbitrap Exploris 240 MS with the Thermo Scientific™ FAIMS Pro™ Interface for:

1. 200 ng of Thermo Scientific™ Pierce™ HeLa Protein Digest Standard on four different gradient lengths identifying >6,355 unique protein groups from 52,000 peptide groups while maintaining a 1% False Discovery Rate (FDR).
2. SDS-PAGE (Thermo Scientific™ Pierce™ 6 Protein Digest Standard)-based protein separation, followed by gel-band protein extraction and identification by mass spectrometry (MS) using either a 15 min or 30 min gradient. For both 15 min and 30 min gradient conditions, all 6 target proteins were identified with individual sequence coverage up to 75%. This workflow maximizes productivity enabling the analysis of up to 96 gel bands and simplified protein mixtures per day.

**Best-in-class protein identification:** When flexibility for proteomics is required, the Orbitrap Exploris 240 MS with the FAIMS Pro interface deliver leading performance and the ability to quickly switch between applications.

### Gradient curves for the protein digest studies

#### Sample

- Pierce HeLa Protein Digest Standard (Cat # 88329) 200 ng
- Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (Cat # 88321) 10 fmol

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

30 min gradient		60 min gradient	
Time (min)	B%	Time (min)	B%
0	3	0	3
1	3	1	3
19	19	37	19
26	29	51	29
31	41	61	41
34	95	64	95
41	95	71	95

90 min gradient		120 min gradient	
Time (min)	B%	Time (min)	B%
0	3	0	3
1	3	1	3
55	19	73	19
76	29	101	29
91	41	121	41
94	95	124	95
101	95	131	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)

#### Gel spots

#### Sample

- Pierce 6 Protein Digest Standard (Cat # 88342) 250 fmol

#### LC method

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

15 min gradient		30 min gradient	
Time (min)	B%	Time (min)	B%
0	4	0	4
1	4	2.5	4
16	34	32.5	34
17	98	33.5	98
22	98	40	98

#### Instrumentation

- EASY-nLC 1200 system (Cat # LC140)
- Thermo Scientific™ EASY-Spray™ Source (Cat # ES081)

#### MS detection

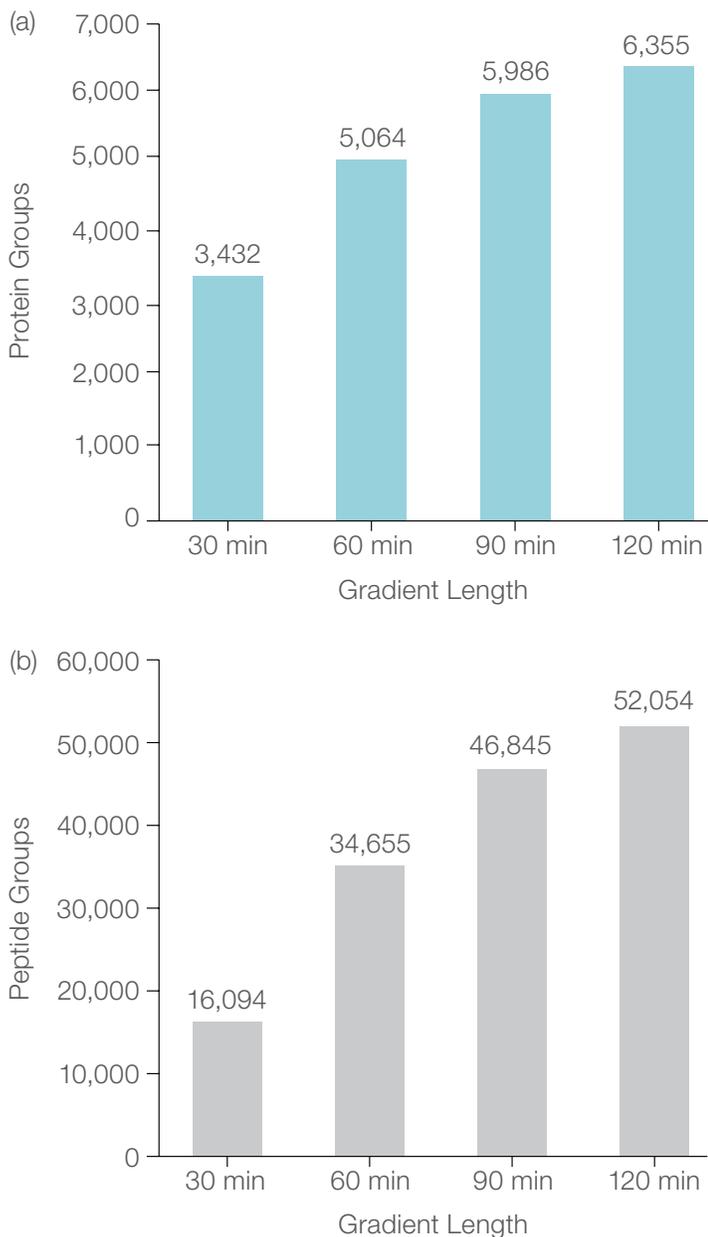
- High-resolution, accurate-mass (HRAM) Orbitrap Exploris 240 mass spectrometer
- Data-dependent acquisition (DDA)

#### Software

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

## Data

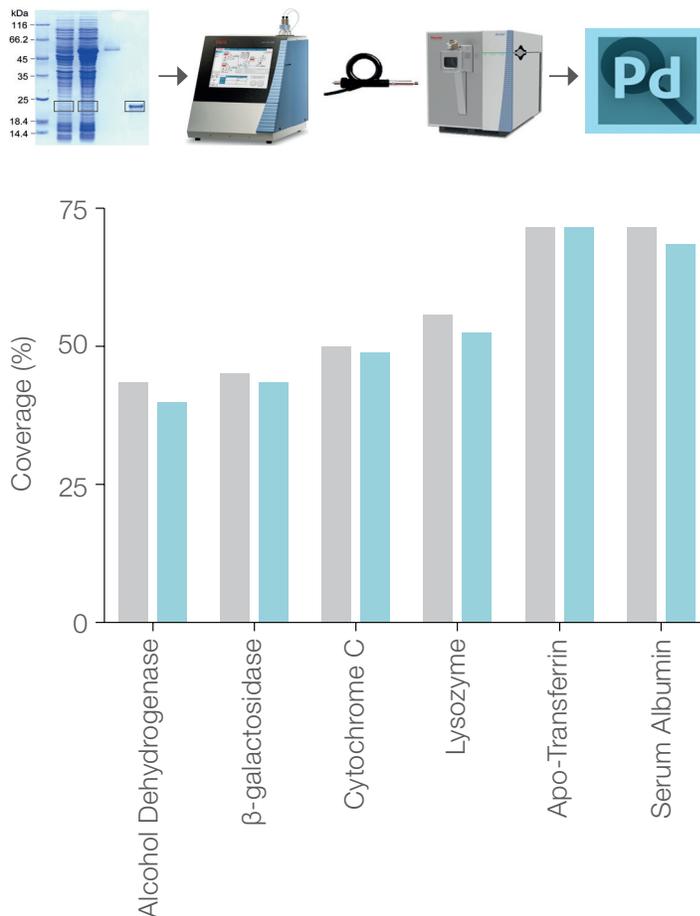
Over 6,355 unique Protein groups (Figure 1a) from 52,000 Peptide groups (Figure 1b) were identified while maintaining a 1% FDR.



**Figure 1. Performance benchmark for complex proteome analysis using different gradient lengths:** Histograms of the number of (a) Protein groups and (b) Peptide groups identified per run as a function of the gradient length from a HeLa digest sample. All the identifications are derived from a high-confidence peptide spectrum match.

SDS-PAGE-based protein separation followed by gel-band protein extraction and identification by MS continues to be a powerful tool for identifying specific proteins of interest (Figure 2). Here, we analyzed gel-bands using either a 15 min or 30 min gradient. We identified all 6 target

proteins with individual sequence coverage up to 75% (Figure 2 & Table 1). This workflow maximizes productivity enabling the analysis of up to 96 gel bands and simplified protein mixtures per day.



**Figure 2. Performance benchmark for LC-MS analysis of simple protein mixtures, such as gel bands.** A mixture of Pierce 6 Protein Digest standard was analyzed using different gradient lengths with no difference in the results. A 15-minute gradient is sufficient to identify all proteins in the sample with excellent protein coverage, enabling the analysis of up to 96 samples per day.

**Table 1. All proteins were identified using either a 15 min or 30 min gradient with greater than 75% sequence coverage.**

6 protein mix	15 min	30 min
Serum Albumin	✓	✓
Cytochrome C	✓	✓
Lysozyme	✓	✓
$\beta$ -galactosidase	✓	✓
Apo-Transferrin	✓	✓
Alcohol Dehydrogenase	✓	✓

## Results

- High protein and peptide identifications across gradient conditions from 30 min up to 120 min, allowing flexibility to balance deep proteome coverage with higher sample throughput (Figure 1).
- Identification of all 6 target proteins from a gel-band protein extraction resulting in sequence coverage up to 75% in only a 15 min acquisition (Figure 2 and Table 1).

## Outlook

The Orbitrap Exploris 240 MS delivers on workhorse reliability and flexibility for proteomics that enables you to switch quickly between applications designed to fit the diverse needs of a core lab while delivering results for your research and clients.

## Conclusion

The Orbitrap Exploris 240 MS delivers utmost flexibility from protein identification, shotgun proteomics to gel-band protein identification, and quantitative applications from label-free quantitation to multiplexed proteome quantitation.

Find out more at

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

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