

# Targeted protein quantification

with high-resolution, accurate-mass MS

Highly selective • Very sensitive • Complex samples

**Thermo**  
SCIENTIFIC

# HR/AM

## A more complete quantitative proteomics picture

Targeted protein quantification is frequently applied to large sample sets to verify large numbers of targets such as putative biomarkers identified from earlier discovery experiments. It is also used to analyze protein–protein interaction networks in biological systems as part of signaling pathway studies. Selected-reaction monitoring (SRM) on triple quadrupole mass spectrometers has traditionally been considered the “gold standard” for targeted protein quantification. It can, however, present challenges when the goal is to quantify large numbers of targeted proteins in complex biological matrices.

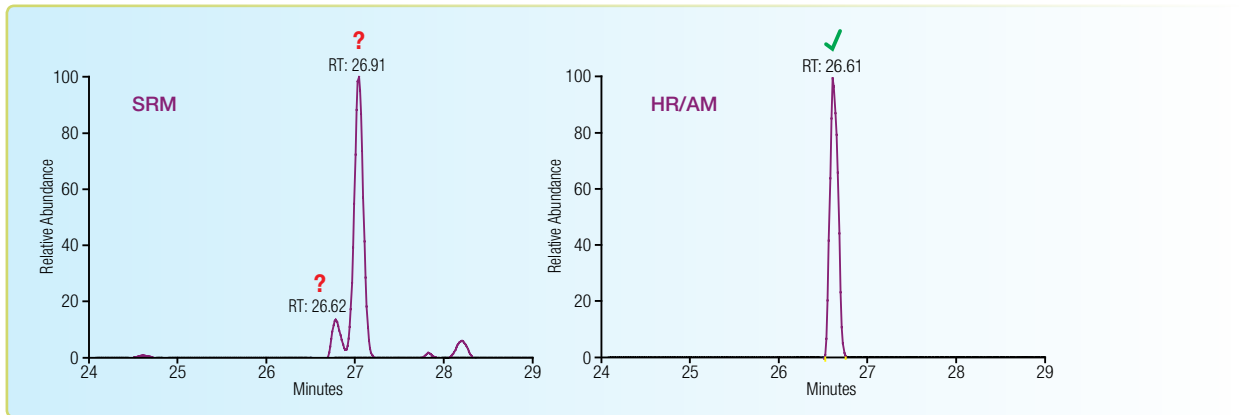
First, the lower mass resolution of quadrupoles limits the ability to distinguish targets from near-isobaric background ions. This can decrease the detection limit. Second, it is

slow and costly to develop an SRM assay, which may require hundreds to thousands of transitions for quantification of large numbers of peptides. Finally, some peptides such as glycopeptides, peptides with higher charge states, or peptides with higher mass-to-charge ratios, are difficult to analyze with SRM. Their poor fragmentation efficiency results in relatively few transitions that can be used for quantification.

Many of the limitations associated with traditional SRM assays for the verification stage of the proteomics workflow can be addressed using a high-resolution, accurate-mass (HR/AM) mass spectrometer. Thermo Scientific™ Orbitrap™-based mass spectrometers provide high resolving power and mass accuracy in both MS and MS<sup>2</sup> to speed research.

### Benefits of an HR/AM approach:

- High selectivity – resolves peptides differing in mass by as little as tens of parts per million (ppm)
- High sensitivity – detects low-abundance targets in complex samples
- High accuracy – quantifies targets accurately over a broad dynamic range
- High confidence – confirms sequences with HR/AM MS and MS/MS spectra
- High productivity – eliminates time-consuming selection of transitions and optimization of parameters
- High flexibility – targets any detectable peptide of interest



Quantification of the heavy peptide GISNEGQNASIK spiked in an *E. coli* digest mixture at a 10 amol concentration level.

The high resolving power of the Orbitrap mass analyzer enables selective and sensitive HR/AM quantitative assays for precise targeted protein quantification in complex matrices.



# Q Exactive Mass Spectrometer

The ideal choice for HR/AM protein quantification

While all Orbitrap-based instruments are capable of excellent targeted protein quantification, the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer (MS) is the most suitable Orbitrap platform for this application<sup>1,2</sup>. With its unique spectrum, multiplexing capabilities and faster scan speed, the Q Exactive MS provides enough scan points for precise quantification

even for narrow ultra-high-performance liquid chromatography (UHPLC) peaks. With resolving power up to 140,000, the Q Exactive MS consistently provides confident targeted peptide sequence confirmation with HR/AM MS and MS/MS spectra. The Q Exactive MS also provides SRM-comparable sensitivity for quantifying low-abundance proteins in complex biological matrices.





The Q Exactive MS enables a wide range of targeted quantification approaches with its unique design and features.

## Key Features:

- 140k max resolution
- <1 ppm mass accuracy
- 12 Hz HCD at 17.5k resolution
- Parallel filling and detection
- Spectrum multiplexing up to 10 events

Three targeted quantification methodologies using HR/AM

Parallel-reaction monitoring (PRM)

Selected-ion monitoring (SIM)

Data-independent acquisition (DIA)

# PRM

## for high selectivity

### How it works

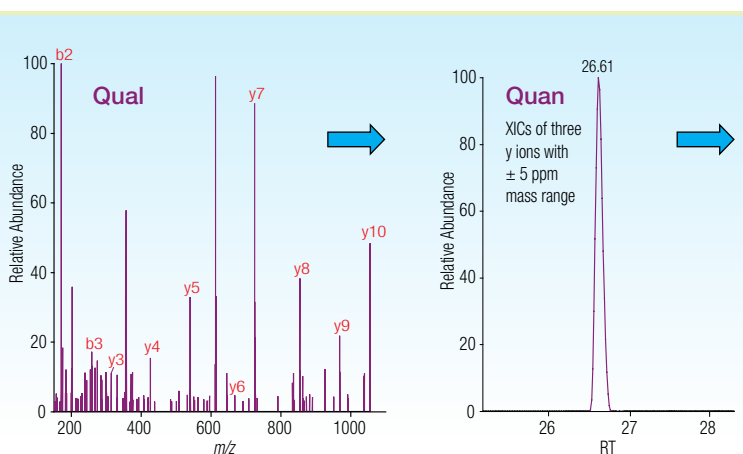
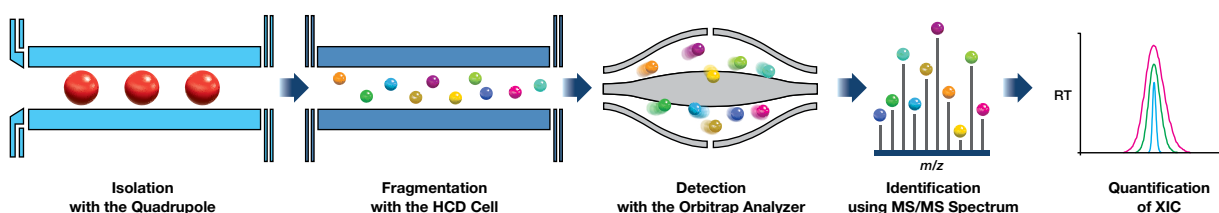
Parallel-reaction monitoring (PRM) provides high selectivity, high sensitivity, and high-throughput quantification with confident targeted peptide confirmation. It is most suitable for quantifying tens to hundreds of targeted proteins in complex matrices.

PRM methodology uses the quadrupole of the Q Exactive MS to isolate a target precursor ion, fragments the targeted precursor ion in the collision cell, and then detects the resulting product ions in the Orbitrap mass analyzer. Quantification is carried out after data acquisition by extracting one or more fragment ions with 5–10 ppm mass windows. PRM offers several advantages compared to traditional SRM quantification.

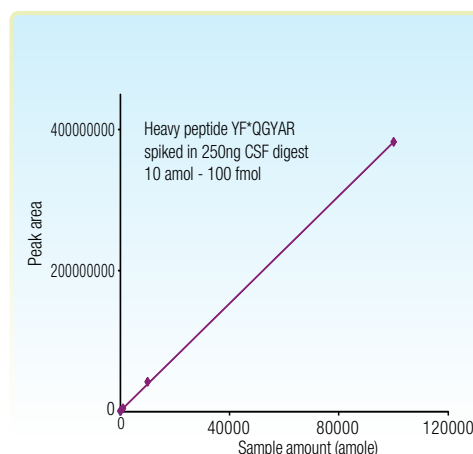
### Benefits of PRM

- Eliminates most interferences, providing more accuracy and attomole-level limits of detection and quantification
- Enables the confident confirmation of the peptide identity with spectral library matching
- Reduces assay development time since no target transitions need to be preselected
- Ensures UHPLC-compatible data acquisition speeds with spectrum multiplexing and advanced signal processing

#### PRM with the Q Exactive mass spectrometer



Increased sensitivity of HR/AM PRM eliminates interferences from background.



PRM provides precise quantification over a wide linear dynamic range.

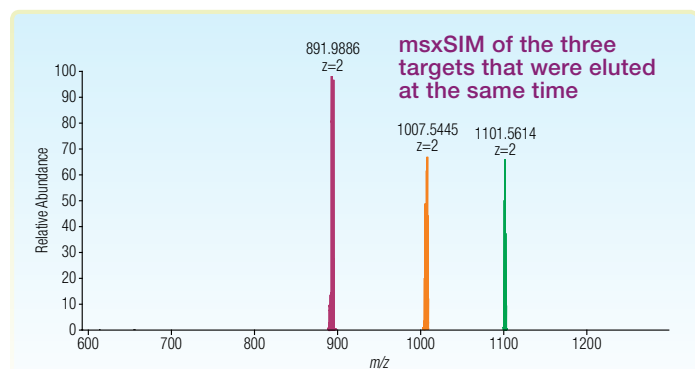
# SIM

## for flexibility

### How it works

Selected-ion monitoring (SIM) provides the simplest method set up and the most selective and sensitive quantification. It is most suitable for quantifying tens of proteins in samples of medium complexity. SIM also provides higher sensitivity for quantification of labile peptides which do not fragment efficiently.

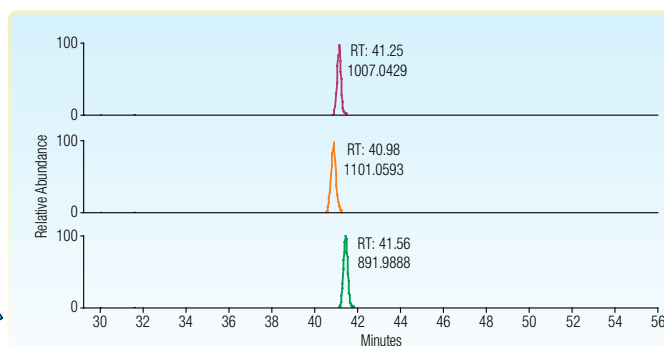
The SIM methodology uses the quadrupole of the Q Exactive MS to isolate a target ion. Only the selected target ion is transferred to the Orbitrap mass analyzer for detection. There is no fragmentation. Confirmation of the targeted peptide is accomplished using accurate-mass measurements in combination with elution-time information.



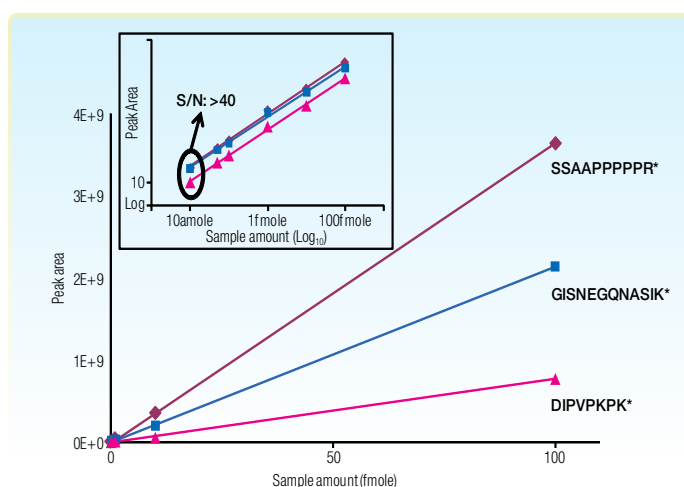
Sensitivity and linear dynamic range using msxSIM

### Benefits of SIM

- Increases sensitivity 5- to 50-fold compared to full-scan MS
- Enriches targeted precursor ions in the narrow mass range
- Provides higher selectivity and sensitivity with maximum resolving power of 140K
- Improves throughput using multiplexed SIM capability in combination with scheduled time windows



Multiplexed SIM (msxSIM) can significantly increase throughput



# DIA

## for high throughput

### How it works

Data-independent acquisition (DIA) provides a high-throughput method for comprehensive quantification with qualitative confirmation. It is most suitable for quantifying large numbers of mid- to high-abundance proteins in samples of simple to medium complexity. In DIA experiments, a precursor mass range is selected, usually one that covers the masses of most enzymatic peptides. That range is then divided

into a series of relatively wide isolation windows: for example, 25  $m/z$  each. MS/MS data is acquired from all detected precursor ions in the first isolation window. That is repeated for each consecutive, adjacent isolation window until the entire precursor mass range is covered.<sup>3</sup> MS/MS spectral libraries are used to identify peptides of interest from the acquired data.

### DIA

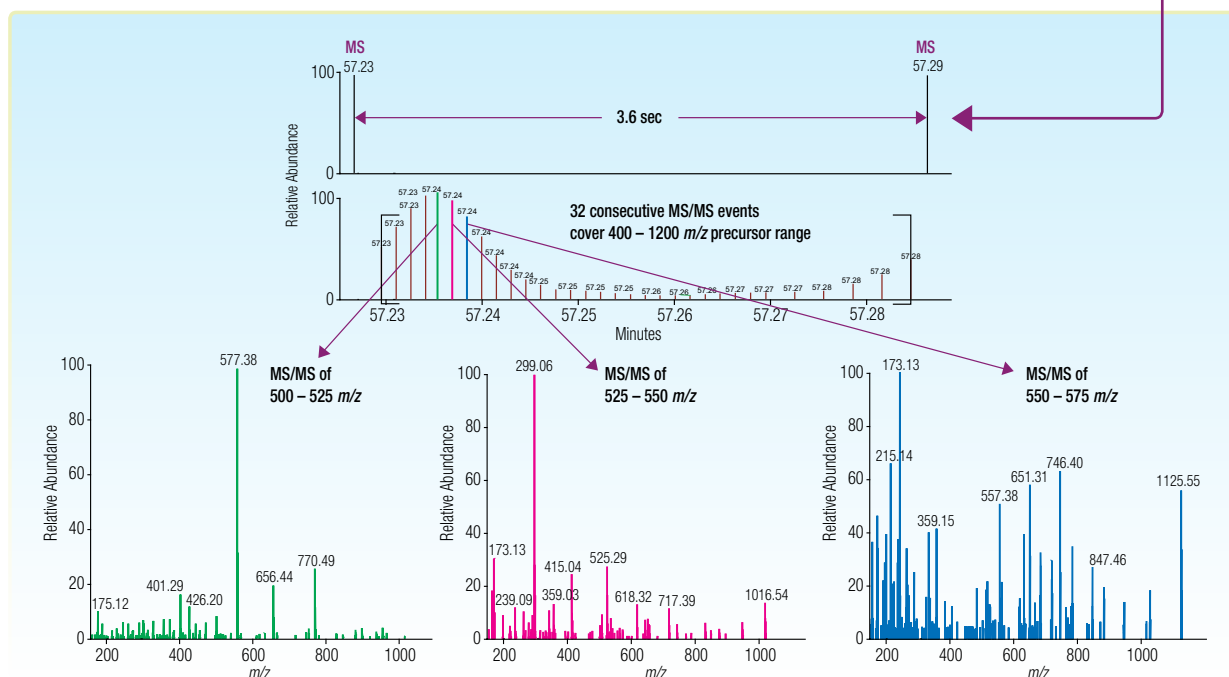
DIA significantly increases coverage and reproducibility by acquiring MS/MS data from all detected precursor ions. This also makes possible retrospective data analysis. Because no prior knowledge of expected precursors is required, DIA method development is very simple.

By increasing or decreasing the mass range, the user can balance mass range coverage with mass resolution. Due to the wide isolation windows used in DIA, MS/MS spectra of interfering background ions may be acquired along with the spectra from peptides of interest. Thus,

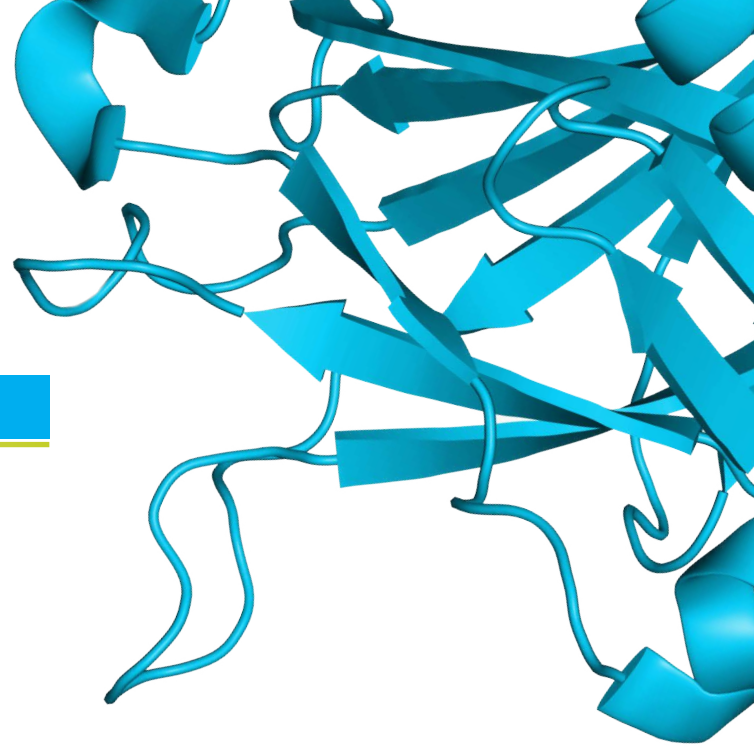
the dynamic range, accuracy, and precision of targeted quantification by DIA may be limited in complex samples. DIA experiments do not provide the same sensitivities and low quantitative limits as PRM and SIM.

The unique ability of the Q Exactive MS to reconstruct a precursor ion during DIA analysis is crucial.

### Principles of DIA







## Benefits of DIA

- Enables large-scale targeted proteomics studies with qualitative confirmation
- Allows simple and universal acquisition method development without detailed sample knowledge prior to data acquisition
- Increases coverage and reproducibility with a complete record of quantitative data
- Facilitates retrospective mining of additional analytes

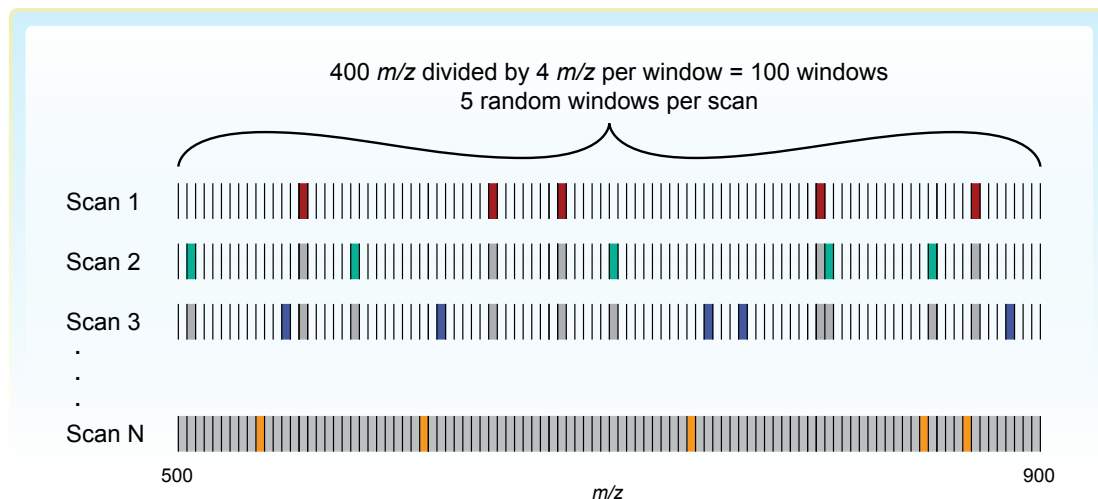
## msxDIA

Multiplexed DIA (msxDIA) is a variation of the DIA technique. It can reduce interference from background ions and improve selectivity while retaining the comprehensive coverage of DIA. In msxDIA, the mass range is divided into much narrower precursor isolation windows, each typically about 4  $m/z$

wide. In each scan cycle, five of these windows are chosen at random and MS/MS data is acquired from the precursor ions in those windows. This is repeated until the list of isolation windows is empty. The MS/MS spectra are assembled and demultiplexed using a non-negative least squares technique

that helps remove interferences from the spectrum and thus provides higher selectivity than standard DIA.<sup>4</sup> Skyline software<sup>5</sup> is required to manage the selection of isolation windows and process the data.

## Multiplexed DIA process



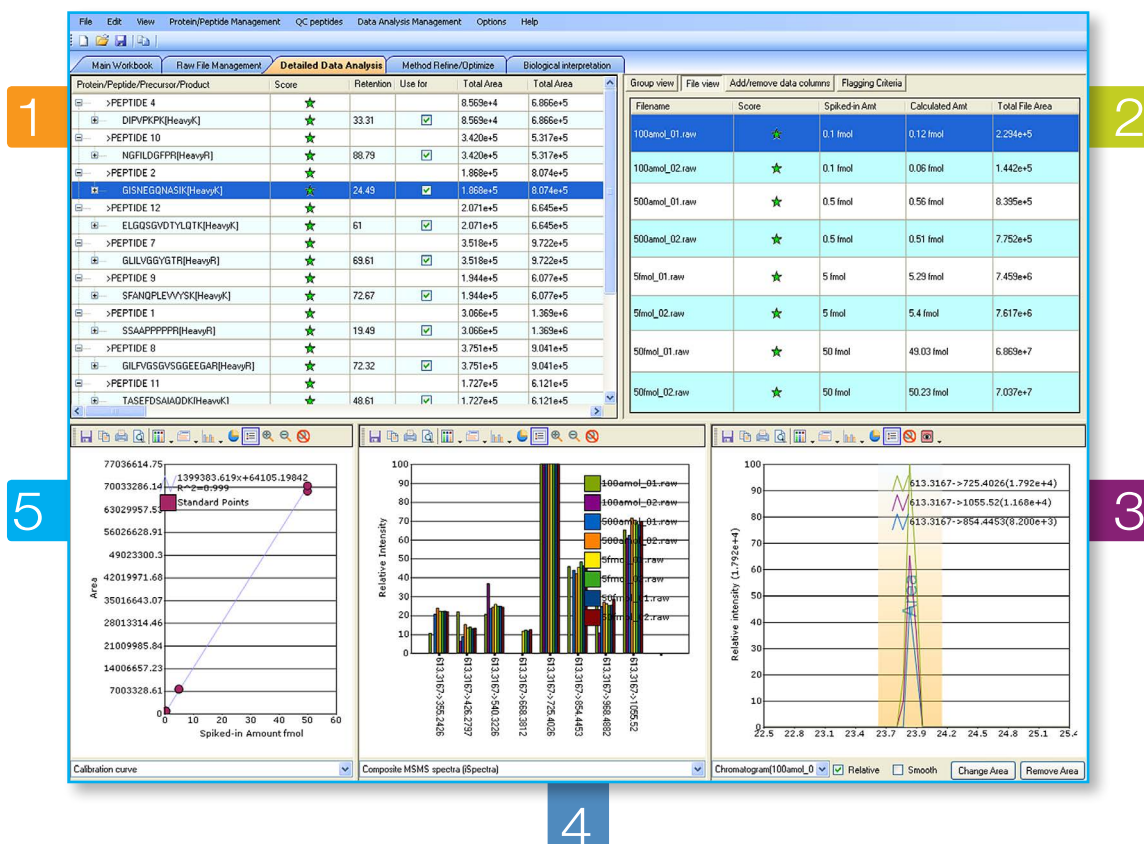
# Accelerating targeted quantification with software

Thermo Scientific™ Pinpoint™ software facilitates targeted protein quantification by providing rapid method development and automatic qualitative and quantitative data processing.

- Leverages discovery data from local and global repositories
- Builds MS/MS spectral libraries using previously acquired discovery data
- Develops a targeted list of proteins of interest
- Analyzes qualitative and quantitative results simultaneously with HR/AM PRM, SIM, or DIA data

Pinpoint software displays, in a single view, the results of a DIA experiment

- 1 Targeted list of proteins
- 2 Quantification results for highlighted peptide
- 3 Integrated peak area for highlighted peptide in each sample
- 4 Spectral library match results for highlighted peptide in each sample
- 5 Calibration curve for highlighted peptide



## Related products

As a leader in serving science, we offer a full range of Thermo Scientific products – from QC peptide mixtures and sample enrichment kits to UHPLC systems and nanoelectrospray sources – to support the entire targeted protein quantification mass spectrometry workflow.

### The Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano system

delivers continuous flow from 20 nL/min to 50  $\mu$ L/min at up to 800 bar. The system is designed with application flexibility in mind. The RSLCnano system can be operated at nano, capillary, and micro flow rates, configured for a large range of 1D and 2D workflows.



### The Thermo Scientific™ EASY-nLC 1000™ system

is designed for maximum ease-of-use and provides a fully integrated, split-free, nano-LC system working at pressures up to 1000 bar. The flow range is 50 nL/min to 1  $\mu$ L/min for peptide separation.



### The Thermo Scientific™ EASY-Spray™ source

provides an integrated, temperature-controlled, column-emitter design. It uses a single Thermo Scientific™ Dionex™ nanoViper™ connection between the LC and the EASY-spray source to reduce the opportunity for error. By offering plug-and-spray simplicity with state-of-the-art performance, the EASY-Spray source ensures excellent repeatability and reproducibility.



### The Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture

enables reversed-phase LC and LC-MS users to optimize and confirm correct operation of their equipment.



### The Thermo Scientific™ HeavyPeptide™ Custom Synthesis Standards

are custom-made, isotopically labeled, AQUA-grade peptides for the relative and absolute quantification of proteins of interest.



### The Thermo Scientific™ HeavyPeptide™ IGNIS Prime Quantitation Kit

enables absolute quantitation of target peptides in less time by using the properties of isotopologues to generate the calibration curve and perform quantification in a single LC-MS run.



# References

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