

# HRMS1-DIA workflow for high-throughput, precise, and comprehensive large-scale quantitative proteomics analysis

## Benefits

- A novel high resolution MS1 based quantitation data-independent acquisition (HRMS1-DIA) workflow
- Improved system robustness through capillary LC
- High-throughput and precise quantitation

To successfully elevate discovery proteomics to translational research in the pipeline of precision medicine, large-cohort studies are essential in discovery and verification of protein biomarkers. Apart from sensitivity and specificity, to quantify large numbers of proteins in different laboratories reproducibly and reliably remain challenges. To address these challenges, a high-throughput and streamlined analytical workflow using high resolution MS1-based quantitative data-independent acquisition (HRMS1-DIA) mass spectrometry is developed. The system includes a capillary flow LC, coupled to a high-resolution accurate mass (HRAM) mass spectrometer (Q Exactive HF, Q Exactive HF-X, Orbitrap Fusion Lumos, Orbitrap Fusion).

## Keywords

HRMS1-DIA, large-cohort study, translational medicine, capillary LC, data-independent acquisition, Q Exactive HF, Q Exactive HF-X, Orbitrap Fusion Lumos, Orbitrap Fusion, Easyspray



**UltiMate 3000  
RSLC (CapLC)**



**Q Exactive HF**

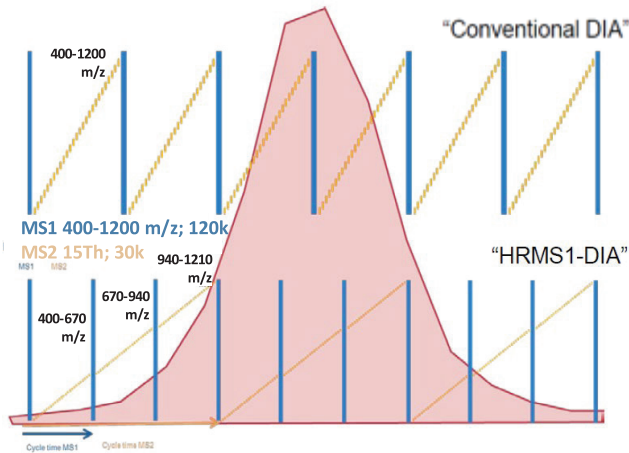
150µm \* 15cm EASY-Spray  
Column

- Sensitivity, robustness and throughput
- 1 hour of LC gradient
- 2µg loading

System set-up of high resolution MS1 based quantitation data-independent acquisition (HRMS1-DIA) workflow.

HRMS1-DIA acquisition cycle

Conventional DIA is comprised of one full MS and 20 fragment scans in a cycle. HRMS1-DIA Method contains three full scans and 54 fragment scans in one cycle.



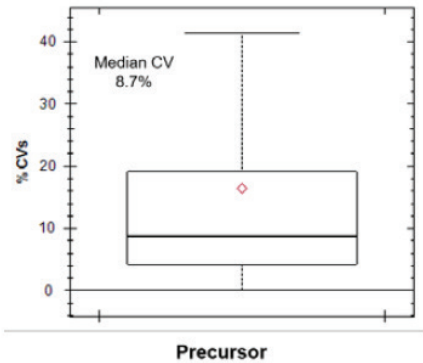
HRMS1-DIA outperforms conventional DIA >20% in terms of protein ID, as well as providing better quantitation precision.

DIA method	Peak point MS1	Peak point MS2	Peptides	Proteins	Median CV
Conventional DIA	6	6	36596	4523	9.6%
HRMS1-DIA	10	3	44084	5301	7.6%

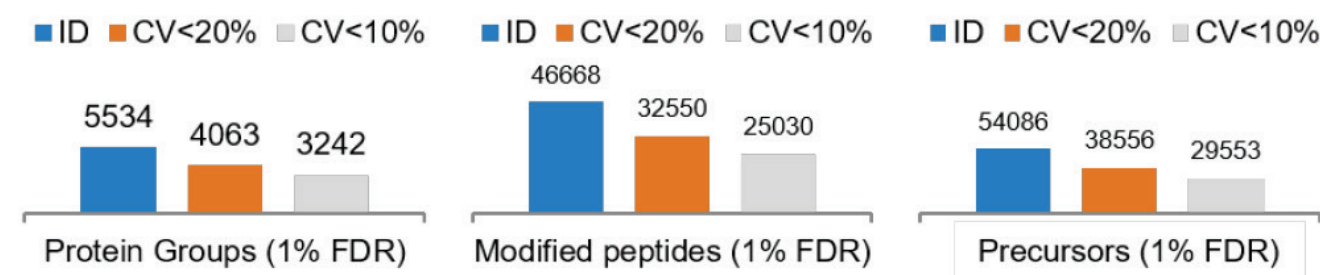
High-throughput and robust label-free quantitation by HRMS1-DIA

The HRMS1-DIA workflow is standardized with well-defined experimental steps and systematically applied to a set of standard test samples.

With one hour gradient, >5000 proteins is identified from 2µg HeLa digest. The median CV of precursors is 8.7%.



CV distribution of precursors.



Protein groups, modified peptides and precursors ID with 1% FDR analyzed with 1hr capLC-HRMS1-DIA from QC sample (2µg HeLa digest).



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