

DDA High-throughput proteomics for complex samples using the Aurora Rapid column

FEATURED PRODUCTS:



5cm Aurora Series packed emitter column with CaptiveSpray Insert
(5cm x 150µm ID, 1.6µm C18)
Part No. AUR2-50150C18A-CSI



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INTRODUCTION

Ultra-sensitive, high-throughput proteomics

IonOpticks' 5cm Aurora series packed emitter columns featuring our revolutionary nanoZero® fitting, allow for simplified plug-and-play high-throughput proteomics. These columns are capable of analysing 180 samples per day with a 5 min gradient. Tryptic digested HeLa cell lysate was injected and separated using our 180 samples per day method and data dependent acquisition using PASEF resulting in 3,600 protein identifications (16,000 unique peptide identifications) [Fig1] with highly reproducible peptide retention times between runs [Fig 2].

Fig 1 Number of unique peptide and protein IDs

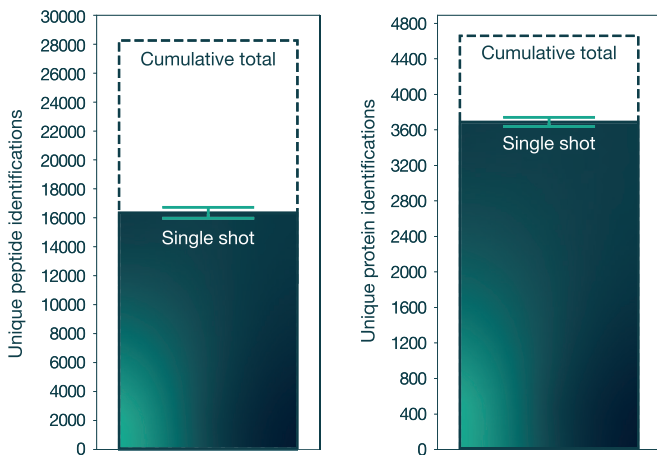


Fig 2 Retention time comparison for 9 different peptides across 180 samples

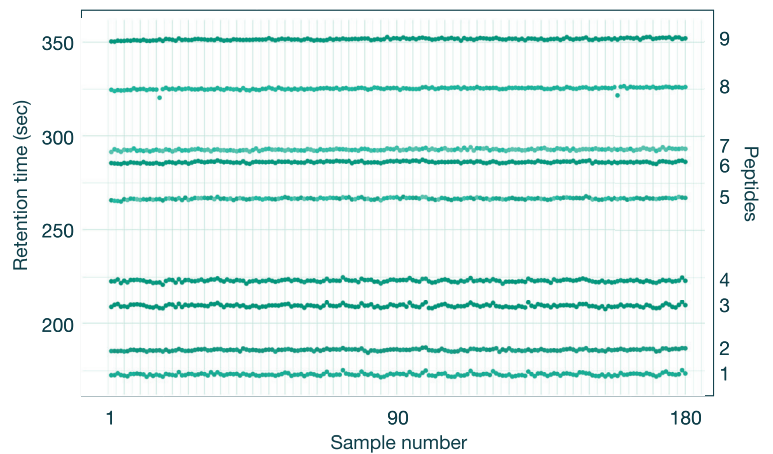


Fig 3 Pearson correlation plot of peptide intensities

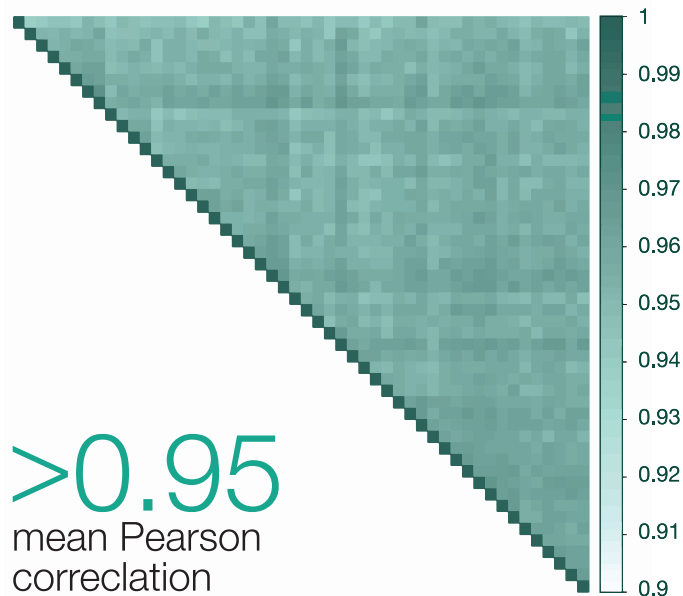
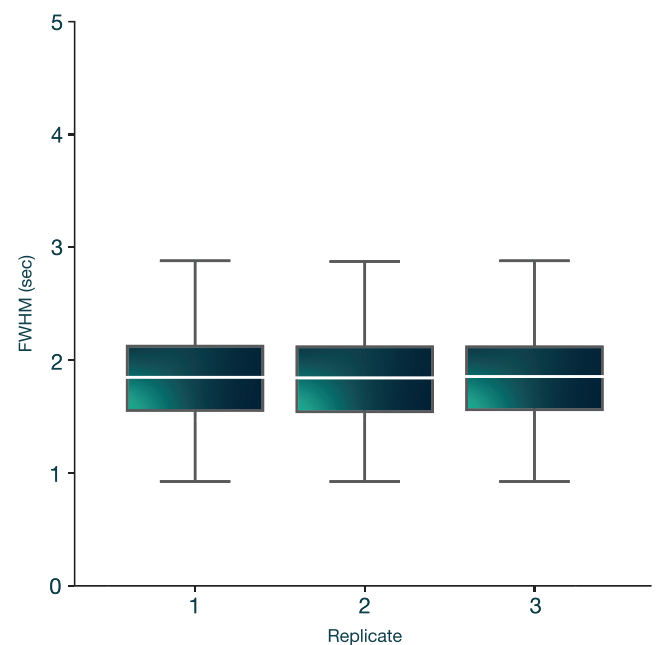



Fig 4 Median and standard deviation of FWHM for peptides



More than
3600
proteins per run
DDA-PASEF


180
times per day

Integrated
nanoZero
high-pressure
fitting 

1.9 secs
Full-width
half-maximum

METHODS

HeLa Protein tryptic digest standard (Pierce, Thermo Fisher) was reconstituted in 2% acetonitrile/1% formic acid in MilliQ water to a concentration of 80 ng/μL. Samples were analysed on a M-class (Waters, USA) coupled to a timsTOF Pro (Bruker) equipped with a CaptiveSpray source. 80ng (1μl) of peptides were separated on a 5cm X 150μm Aurora column, using a constant flow rate of 2μl/min. The column was maintained at room temperature. Sample was injected into a sample loop which takes approximately 0.5min. Mobile phase at 100% buffer A continues to flow over the analytical column during this period facilitating column equilibration. The sample loop was switched on-line for 1min at 100% buffer A. A recommended sample gradient is outlined in Figure 6.

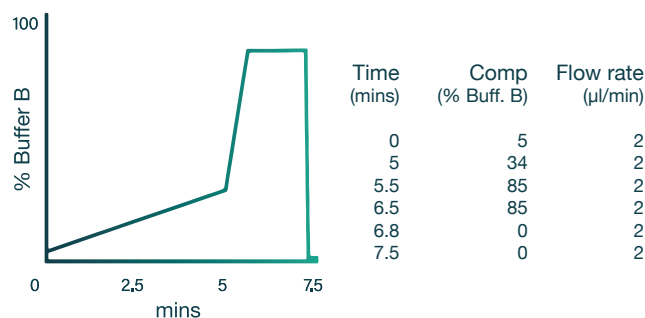
Separately, 20μg of a HeLa tryptic digest (Pierce, Thermo Fisher) was resuspended in 10mM Ammonium Formate pH 10. Peptides were separated into 12 fractions using a stage-tip containing 4 X C18 plugs. Fractions were lyophilised to dryness using a CentriVap (Labconco) before reconstitution in 2% ACN, 1% FA prior to analysis. Fractions were analysed using the same LC-MS method as the single shot samples.

Raw data processing and analysis. Data was analyzed by MaxQuant software using the integrated Andromeda search engine and searched against the human Uniprot Reference Proteome and matched to a high pH fractionation library using the match-between-runs feature.

Aurora Rapid 5x150, 5 minute gradient

(5cm x 150μm ID, 1.6μm C18) Part No. AUR2-50150C18A

Example gradient



Robust reproducibility

- High-throughput analysis
- Maximum protein IDs
- Minimum peak widths
- Consistence performance over large sample cohorts

FURTHER READING

For further resources and technical support, visit our Help Centre at helpcentre.ionopticks.com.

To view other application notes, read the latest publications featuring Aurora Series columns, or view the full range of IonOpticks products, visit our website at www.ionopticks.com

Find these additional documents in our help centre:



Aurora Series UHPLC packed emitter column user guide

ABOUT IONOPTICKS

IonOpticks produces high-performance chromatography solutions for the global research community. We specialise in the development and manufacture of columns for analytical applications in liquid chromatography with mass spectrometry (LC-MS) and high-end proteomics. Our highly reproducible methods provide a unique ability to enhance the sensitivity of mass spectrometry sample analysis, enabling scientists and clinicians to discover more from their samples. Our team are experts in a broad array of LC-MS platform technologies and are driven by the need to improve chromatographic performance in order to achieve data quality and deep proteome coverage on a whole new scale.