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LC-MS/MS Label-Free Proteomic Data Acquisition V.2

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We use this protocol and it's working

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Abstract

Description of settings used to acquire LC-MS/MS data from label-free proteomic samples.

Troubleshooting

1 Label-free proteomic samples were analyzed on a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer in line with a Thermo Scientific Easy-nLC 1000 UHPLC system.

2 Samples, 2 µL, were injected via the autosampler and loaded onto a fused silica pulled-tip C_{18} UHPLC column (100 µm i.d. x 350 mm length,) packed with Waters C18 BEH resin (1.7 µm particle size, 130 Å pore size), with 0.1% formic acid in water (mobile phase A).

3 Peptides were separated over a 127 minute two-step gradient with initial conditions set to 100% mobile phase A for 2 minutes before ramping to 20% mobile phase B, 0.1% formic acid in acetonitrile, over 100 minutes and then 32% mobile phase B over 20 minutes. The remainder of the gradient was spent washing at 95% mobile phase B and returning to initial conditions.

4 Eluted peptides were ionized via positive mode nanoelectrospray ionization (nESI) using a Nanospray Flex ion source (Thermo Fisher Scientific).

5 The mass spectrometer was operated using a top 17 data-dependent acquisition mode.

6 Fourier transform mass spectra (FTMS) were collected using 120,000 resolving power, an automated gain control (AGC) target of $1e^6$, and a maximum injection time of 100 ms over the mass range of 400-1600 *m/z*.

7 Precursor ions were filtered using monoisotopic precursor selection of peptide ions with charge states ranging from 2 to 6. Previously interrogated precursor ions were excluded using a 30 s dynamic window (± 10 ppm).

8 Precursor ions for tandem mass spectrometry (MS/MS) analysis were isolated using a 2 *m/z* quadrupole mass filter window and then fragmented in the ion-routing multipole via higher energy dissociation (HCD) using a normalized collision energy of 35%.

9 Ion trap fragmentation spectra were acquired using an AGC target of 10,000 and maximum injection time of 35 ms, and 120 *m/z* was set for the first scan mass to enable detection of the lysine residue fragmented ion.