LC-MS/MS Label-Free Proteomic Data Acquisition V.4

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1 Works for me
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ABSTRACT

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

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1 Label-free proteomic samples were analyzed on a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer in line with an EvoSep One HPLC system.

2 Samples,1 µL at a 1 µg/µL, were loaded onto EvoTips and diluted with 19 µL of mobile phase A (100% water, 0.1% formic acid). Samples were processed on tip according to the manufacturer's recommended protocol. EvoTips were placed onto the EvoSep One, and samples were eluted from the EvoTips onto a Dr. Maisch C18 AQ, 100 micron inner diameter, 15 cm long column with 3 micron particles (EV1074) and analyzed using the 30 sample per day method settings.

3 Peptides were separated at a flow rate of 500nL/min over a 44 minute gradient according to the 30 samples/day settings. Mobile phase A was 100% water, 0.1% formic acid, and mobile phase B was 100% acetonitrile, 0.1% formic acid.

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 12 data-dependent acquisition mode.

6 Fourier transform mass spectra (FTMS) were collected using 60,000 resolving power, an automated gain control (AGC) target of $1e^6$, and a maximum injection time of 50 ms over the mass range of 375-1500 *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 1.2 *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 60 ms, and 120 *m/z* was set for the first scan mass to enable detection of the lysine residue fragmented ion.

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