May 01, 2020   Version 3

🌐 LC-MS/MS Label-Free Proteomic Data Acquisition V.3

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VU Biomolecular Multim...	Human BioMolecular Atl...

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DOI: dx.doi.org/10.17504/protocols.io.bft3jnqn

Protocol status: Working
We use this protocol and it's working

Created: April 30, 2020

Last Modified: October 18, 2023

Protocol Integer ID: 36443

Abstract

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

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 at a flow rate of 400nL/min over a 127 minute two-step gradient with initial conditions set to 98% 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.