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

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Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS)

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ABSTRACT

Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS)

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1 The data acquisition by LC-MS/MS was adapted from a published procedure

CITATION

Seyfried NT, Dammer EB, Swarup V, Nandakumar D, Duong DM, Yin L, Deng Q, Nguyen T, Hales CM, Wingo T, Glass J, Gearing M, Thambisetty M, Troncoso JC, Geschwind DH, Lah JJ, Levey AI (2017). A Multi-network Approach Identifies Protein-Specific Co-expression in Asymptomatic and Symptomatic Alzheimer's Disease..

https://doi.org/10.1016/j.cels.2016.11.006

- Derived peptides were resuspended in a loading buffer (0.1% trifluoroacetic acid, TFA) and separated on a Water's Charged Surface Hybrid (CSH) column (150 μm internal diameter x 15 cm; particle size: 1.7 μm).
- The samples were run on an EVOSEP liquid chromatography system using the 15 samples per day preset gradient (88 min) and were monitored on a Q-Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific).
- The mass spectrometer cycle was programmed to collect one full MS scan followed by 20 data-dependent MS/MS scans. The MS scans (400-1600 m/z range, 3 x 10⁶ AGC target, 100 ms maximum ion time) were collected at a resolution of 70,000 at m/z 200 in profile mode.
- The HCD MS/MS spectra (1.6 m/z isolation width, 28% collision energy, 1 x 10⁵ AGC target, 100 ms maximum ion time) were acquired at a resolution of 17,500 at m/z 200. Dynamic exclusion was set to exclude previously sequenced precursor ions for 30 seconds. Precursor ions with

+1, and +7, +8, or higher charge states were excluded from sequencing.