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Mass-Spectrometry analysis of ATP13A2 samples

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ABSTRACT

Preparing mass-spectrometry samples to analyze polyamine content in purified ATP13A2 samples

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MATERIALS TEXT

MS Buffer
25 mM Tris pH 7.5
100 mM NaCl
1 mM EDTA
1 mM DTT
0.03% DDM/ 0.006% CHS

1 Pool purified ATP13A2 sample after SEC (sample should be in MS buffer after SEC)

2 Concentrate the protein to ~1.2 mg/mL using an Amicon Ultrafilter (cut-off 100kDa) at **4 °C**

3 Prepare standards of spermidine (Sigma-Aldrich) and spermine (Sigma-Aldrich) at a concentration of 10 µM in MS buffer

4 Flash-freeze samples in liquid nitrogen and store at −80 °C until use

5 Dilute each sample 1:1 into acetonitrile with 1% formic acid (volume/volume)

6 Analyze by nanoelectrospray ionization (nanoESI) high-resolution mass spectrometry, using an LTQ-Orbitrap-XL mass spectrometer (Thermo Fisher Scientific)

6.1 Performed by QB3/Chemistry Mass Spectrometry Facility (University of California, Berkeley)

6.2 Mass spectrometer was equipped with a nanoESI source and operated in the positive ion mode

6.3 Mass spectra were acquired at a mass resolution setting of 100,000, as measured at mass-to-charge ratio (m/z) = 400, full width at half-maximum

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peak height

6.4 Mass spectrometry data acquisition and processing were performed using Xcalibur software (version 2.0.7, Thermo)

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