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Electrospray ionization analysis of eluted nucleotides

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ABSTRACT

This protocol describes the denaturation of PI3KC3-C1, and subsequent analysis of eluted nucleotides by electrospray ionization mass spec.

ATTACHMENTS

852-2200.pdf

MATERIALS

Materials

- PI3KC3-C1(VPS15-TSF) and PI3KC3-C1(mCherry-ATG14|VPS15-TSF) protein samples
- 5 mL PD-10 column (Cytiva)
- 0.5 M $NH_4CH_3CO_2$ buffer
- Nanodrop spectrophotometer
- Liquid chromatography (LC) system (Agilent 1200 series)
- LTQ-Orbitrap-XL mass spectrometer with ESI source (Thermo Fisher Scientific)
- Ammonium acetate (≥98% purity)
- Methanol (Optima LC-MS grade, ≥99.9% purity)
- Purified water (resistivity of 18.2 MΩ·cm)
- Ultra C18 column (length: 150 mm, inner diameter: 2.1 mm, particle size: 3 µm)
- Pierce LTQ ESI positive ion calibration solution (Thermo Fisher Scientific)
- Xcalibur software (version 2.0.7, Thermo Fisher Scientific)

Buffer Exchange

1 Wash a 5 mL desalting (PD-10) column with [M] 0.5 Molarity (M) $NH_4CH_3CO_2$.

2 Load PI3KC3-C1(VPS15-TSF) and PI3KC3-C1(mCherry-ATG14|VPS15-TSF) protein samples onto a 5-mL PD-10 column.

3 Exchange the protein sample buffer by passing the protein through the column.

Protein Denaturation

10m

4 Heat the buffer-exchanged protein samples to 90 °C for 00:10:00 to denature the proteins. 10m

Centrifugation

15m

5 After denaturation, centrifuge the samples at 21000 x g, 00:15:00 to separate out any precipitated protein. 15m

Nucleotide Concentration Assessment

6 Measure the A260 absorbance of the denatured samples using a Nanodrop spectrophotometer.

Setup of LC-MS System

7 Connect the LC system (Agilent 1200 series) to the LTQ-Orbitrap-XL mass spectrometer equipped with an ESI source (Thermo Fisher Scientific).

LC Column Equilibration

8 Equilibrate the Ultra C18 column with the LC mobile phase solvents according to the manufacturer's instructions.

Mobile Phase Preparation

9 Prepare mobile phase solvents A and B:

- **Solvent A**: Water with [M] 10 millimolar (mM) ammonium acetate.
- **Solvent B**: Methanol with [M] 10 millimolar (mM) ammonium acetate.

LC Elution Program

25m

10 Set up the LC system with the following gradient program:

10.1 Isocratic flow at 0.5% (volume/volume) B for 00:02:00. 2m

10.2 Linear gradient to 99.5% B over 00:01:00. 1m

10.3 Isocratic flow at 99.5% B for 00:04:00. 4m

10.4 Linear gradient to 0.5% B over 00:00:30. 30s

10.5 Isocratic flow at 0.5% B for 00:17:30 . 17m 30s

11 Maintain a flow rate of 150 µL /min and a column temperature of 40 °C .

12 Inject 20 µL of the sample into the LC system.

Mass Spectrometer Calibration

13 Perform external mass calibration in the positive ion mode using the Pierce LTQ ESI positive ion calibration solution.

Data Acquisition

14 Acquire full-scan, high-resolution mass spectra in the positive ion mode over the range of mass-to-charge ratio (m/z) = 300 to 1000 using the Orbitrap mass analyzer.

15 Set the mass resolution to 60,000 (at m/z = 400, FWHM).

Data Analysis

16 Analyze the acquired data using Xcalibur software (version 2.0.7, Thermo Fisher Scientific) for peak identification and interpretation.