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HPLC Analysis of Nucleotides

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Protocol status: Working

We use this protocol and it's working

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Abstract

Ion-pair reversed phase HPLC analysis of the nucleotide bound to PI3KC3-C1.

Attachments



[852-2201.pdf](#)

53KB

Materials

Materials

- PI3KC3-C1 sample
- Heat block
- Microcentrifuge
- Tubes
- 10 μ M ATP, ADP, GTP, GDP standards
- HPLC system with UV detector
- C18 reverse-phase HPLC column
- Mobile phase Buffer A: 100mM KH_2PO_4 , 5mM tetrabutylammonium bromide (TBA-B), pH 6.0, 1% acetonitrile (ACN)
- Mobile phase Buffer B: 100mM KH_2PO_4 , 5mM TBA-B, pH 6.0, 30% ACN
- Gradient elution program (Chromeleon)
- Wavelength set to 254 nm
- Pipettes and tips



Denaturation of PI3KC3-C1

10m

- 1 Heat the PI3KC3-C1 sample in a heat block at 90°C for 00:10:00 to denature the protein.

10m



Centrifugation

15m

- 2 After denaturation, centrifuge the sample at 21000 rpm, 00:15:00 to pellet any precipitated PI3KC3-C1.

15m



Supernatant Transfer

- 3 Carefully transfer the supernatant containing the released nucleotide to a clean tube, leaving behind the pellet.

Preparation of Nucleotide Standards

- 4 Prepare 10 micromolar (μM) stock solutions of ATP, ADP, GTP, and GDP standards in 25 millimolar (mM) HEPES pH 7.5, 150 millimolar (mM) NaCl, 2 millimolar (mM) MgCl_2 , and 2 millimolar (mM) TCEP.

HPLC Column Equilibration

- 5 Equilibrate the C18 reverse-phase HPLC column with the mobile phase Buffer A (and Buffer B according to the manufacturer's instructions.

HPLC Gradient Program

30m

- 6 Set up the HPLC system with the following parameters:
 - **Mobile phase:** gradient of Buffer A to Buffer B.
 - **Gradient duration:** 00:30:00 per run.
 - **Wavelength for detection:** 254 nm.

30m

Sample Injection



- 7 Inject  60 μL each of the nucleotide standards and the Protein nucleotide into the HPLC system.

Data Collection

- 8 Allow the HPLC system to run the samples through the column.
- 9 Record the retention times for each eluted nucleotide as they appear in the chromatogram.

Analysis

- 10 Compare the retention times of the eluted nucleotides in the sample to those of the known standards (ATP, ADP, GTP, GDP).
- 11 Identify the bound nucleotide(s) based on their retention times in the chromatogram.

