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We use this protocol and it's working

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# HPLC Analysis of Nucleotides

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## ABSTRACT

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

## ATTACHMENTS

852-2201.pdf

## MATERIALS

### Materials

- PI3KC3-C1 sample
- Heat block
- Microcentrifuge
- Tubes
- 10 µM 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 [M] 10 micromolar (µM) stock solutions of ATP, ADP, GTP, and GDP standards in [M] 25 millimolar (mM) HEPES pH 7.5, [M] 150 millimolar (mM) NaCl, [M] 2 millimolar (mM) $MgCl_2$, and [M] 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: 30m

- **Mobile phase**: gradient of Buffer A to Buffer B.
- **Gradient duration**: 00:30:00 per run.
- **Wavelength for detection**: 254 nm.

## 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.