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WORKS FOR ME 1

Nucleoside analysis with high performance liquid chromatography (HPLC)

COMMENTS 0

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.5jyl8jn39g2w/v1

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ABSTRACT

This protocol details the detection of modified nucleosides using HPLC.

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PROTOCOL CITATION

Atanas Radkov 2022. Nucleoside analysis with high performance liquid chromatography (HPLC) . **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8jn39g2w/v1

COLLECTIONS

Protocol collection: Phage DNA isolation and chemical analysis

CREATED

Sep 19, 2022

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OWNERSHIP HISTORY

Sep 19, 2022	 Megan Hochstrasser	Arcadia Science
Sep 20, 2022	Arcadia Science	
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PROTOCOL INTEGER ID

70252

PARENT PROTOCOLS

Part of collection

[Protocol collection: Phage DNA isolation and chemical analysis]

Prepare samples and solutions

1 Prepare the following **standards** at 100 µM by dissolving each in a small volume of HPLC-grade methanol and bringing up the volume as necessary with MQ water. Filter each solution through a 0.22 µm syringe filter (we used SLGSV255F - Millipore-Sigma), and keep solutions at 4 °C prior to HPLC analysis.

Note

2 Prior to HPLC analysis, adjust the total **experimental nucleoside sample** volume to 100 µL by diluting with MQ water and then centrifuge each sample at 21000 × g for 15 min to remove any debris.

Note

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Step 2 includes a Step case.
Long binary
Short isocratic

step case

Long binary

Using a 30 minute-long binary gradient to resolve nucleoside peaks.

3 Prepare solutions A and B. Filter solution A through a 0.22 µm filter (we used 10040-440 VWR) and then adjust pH with 1 M HCl.

Note

Run samples

4 Equilibrate the column with solution A. Once fully equilibrated, inject 10 µL of one of the **standards**.

Note

5 Begin the gradient at 100% solution A, then ramp up to 25% solution B over 16 min, then ramp down to 0% solution B in 1 min, and finally stay at 0% solution B for 13 min (30 min total method time). Flow rate for the entire run should be 0.5 mL per min. Detect elution of compounds at 260 nm.

6 Run in triplicate to obtain an average retention time and a standard deviation. Repeat for each **standard**.

7 Make sure the HPLC column is re-equilibrated in solution A, then inject 10 µL of your **experimental sample**. Run the same program used for the standards. Perform in triplicate to obtain an average retention time and a standard deviation.

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Analysis

8 Extract peak elution times from the HPLC software report. Common calculations can include calculating average elution time and standard deviation, if you have ran replicates. We also often plot the A_{260} signal to look at the shape of individual peaks.

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