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WORKS FOR ME 1

Nucleoside analysis with liquid chromatography–tandem mass spectrometry (LC–MS/MS)

COMMENTS 0

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.q26g7yrq1gwz/v1

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ABSTRACT

This protocol details the detection of modified nucleosides using LC–MS/MS

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PROTOCOL CITATION

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COLLECTIONS

Protocol collection: Phage DNA isolation and chemical analysis

KEYWORDS

LC–MS, LC-MS, LS-MS/MS, MS/MS, nucleosides, dna, chemical modification, modification, modified, phage, genome, phages, mass spec

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OWNERSHIP HISTORY

Sep 19, 2022	 Megan Hochstrasser	Arcadia Science
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70255

PARENT PROTOCOLS

Part of collection

[Protocol collection: Phage DNA isolation and chemical analysis]

Chromatography

1 Perform online separation of nucleoside mixtures using a liquid chromatography (LC) setup paired to a mass spectrometer.

Note

1.1 Prepare 20 µL of nucleoside digest containing approximately 1 µg of nucleoside mixture. Inject 10 µL of digest per run.

1.2 Separate nucleosides using a C18 column and binary solvent gradient of HPLC-grade water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B).

1.3 Set LC flow rate at 150 µL/min.

Note

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1.4 Create a chromatography method using the following 30 min gradient as a guide:

- 0–0.5% B over 2 min
- 0.5–30% B over 4 min
- 30–95% B over 0.5 min
- Hold at 95% B for 4 min
- 95–0.5% B over 0.5 min
- Hold at 0.5% B over 19 min

Note

1.5 Enable column heater at a constant 45 °C.

Mass spectrometry

2 Acquire data using a mass spectrometer capable of MS/MS experiments (we used a Thermo LTQ Orbitrap XL outfitted with an API source, below are recommended settings).

Note

2.1 Acquire data in positive mode with lock-mass enabled (targets: 391.284290, 413.266230).

2.2 Adjust MS1 scan settings to target ions in the 200–800 m/z range at 100,000 resolution selecting for charge states +1 and +2 for MS/MS.

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2.3 Follow each MS1 scan with 7 data-dependent MS2 scans collected also at 100,000 resolution. Set ion isolation window at 4 m/z.

2.4 Activate ions by CID at NCE of 35% with a minimum signal threshold of 3e4.

2.5 Enable dynamic exclusion with a maximum repeat count of three times within 30 s with an exclusion duration of 30 s.

Data processing

3 Convert Thermo .raw files obtained after tandem mass spectrometry analysis to .mgf format using MSConvert 3.0.22031 (a component of the Proteowizard open source mass spectrometry bioinformatics software package) under generic default presets for .mgf file extraction.

Analyte identification

4 Use the Jupyter notebook linked below to identify candidate nucleosides based on .mgf files extracted during the previous step.

Note

Our GitHub repository with "nucleoside finder" script is available here: https://github.com/Arcadia-Science/nucleoside-finder/tree/v1.0

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