Feb 03, 2025

Targeted LC–MS metabolomics analyses

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.261ger4zdl47/v1

Maria Jose Perez J.[1]

[1]Institut Imagine

Team Deleidi

Federico Bertoli
ASAP

DOI: https://dx.doi.org/10.17504/protocols.io.261ger4zdl47/v1

Protocol Citation: Maria Jose Perez J. 2025. Targeted LC–MS metabolomics analyses. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ger4zdl47/v1

Protocol status: Working
We use this protocol and it's working

Created: November 26, 2024

Last Modified: February 03, 2025

Protocol Integer ID: 112830

Keywords: m, lc

Funders Acknowledgements:
ASAP

Abstract

Targeted LC–MS metabolomics analyses

1 Detach 5 × 10^5 iPSC-derived microglia using Accutase

2 Centrifuge the cells and immediately flash-freeze them in collection tubes on dry ice

3 Store samples at -80°C until metabolomics analysis

4 Prepare the extraction solution comprising 50% methanol, 30% acetonitrile (ACN), and 20% water. Use 1 mL of extraction solution per 1 × 10^7 cells

5 Add the extraction solution to the samples, vortex for 5 minutes at 4°C, and centrifuge at 16,000 × g for 15 minutes at 4°C

6 Collect the supernatants and store them at -80°C until further analysis.

7 Collect the supernatants and store them at -80°C until further analysis.

8 Calibrate the mass spectrometer externally using a standard calibration mixture every seven days as per the manufacturer's instructions.

9 Inject 5 µL of the sample onto a ZIC-pHILIC column (150 mm × 2.1 mm; i.d. 5 µm) with a guard column (20 mm × 2.1 mm; i.d. 5 µm) (Millipore) for LC separation.

10 Use the following chromatographic gradient at a flow rate of 0.200 µL/minute:
- 0–20 minutes: linear gradient from 80% to 20% of buffer B.
- 20–20.5 minutes: linear gradient from 20% to 80% of buffer B.
- 20.5–28 minutes: maintain 80% buffer B.

11
- Prepare buffers:

- Buffer A: 20 mM ammonium carbonate and 0.1% ammonium hydroxide (pH 9.2).
- Buffer B: ACN.

12 Operate the mass spectrometer in full scan, polarity-switching mode with these parameters:
- Spray voltage: 2.5 kV.
- Heated capillary temperature: 320°C.

- Sheath gas flow: 20 units.
- Auxiliary gas flow: 5 units.
- Sweep gas flow: 0 units.
- Mass range: 75–1,000 m/z at 35,000 resolution (at 200 m/z).
- Automatic gain control (AGC) target: 10^6.
- Maximum injection time: 250 ms.

13 Use lock mass technique to ensure mass accuracy below 5 ppm.

14 Acquire data with Xcalibur software (Thermo Fisher Scientific).

15 Determine metabolite peak areas with TraceFinder software (Thermo Fisher Scientific) using exact mass and retention times.

16 Perform data analysis with MetaboAnalyst (version 6.0, **https://www.metaboanalyst.ca/**, RRID: SCR_016723):

- Statistical analysis module: conduct t-tests (unpaired, equal variance, FDR < 0.05), PCA, and heatmap generation.
- Enrichment analysis module: perform MSEA using the Small Molecule Pathway Database (SMPDB; RRID: SCR_004844) with metabolite sets containing at least 2 entries.
- Joint pathway analysis module: integrate metabolomics and bulk RNA-seq data using:
- Hypergeometric Test for enrichment analysis
- Degree Centrality for topology measure
- Combine p-values (unweighted) for the integration method.