

Dec 16, 2024

Lipidomic Analysis of Neuronal Lipid Droplets

 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn99kpl5d/v1>

Mukesh Kumar¹, Justin Knapp², Kallol Gupta^{3,2,4}, Timothy A. Ryan^{1,3}

¹Department of Biochemistry, Weill Cornell Medicine, New York, NY 10065;

²Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06520, USA;

³Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland 20815, USA;

⁴Nanobiology Institute, Yale University, West Haven, CT 06516, USA



Mukesh Kumar

Weill Cornell Medical College

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.14egn99kpl5d/v1>

Protocol Citation: Mukesh Kumar, Justin Knapp, Kallol Gupta, Timothy A. Ryan 2024. Lipidomic Analysis of Neuronal Lipid Droplets. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.14egn99kpl5d/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: November 27, 2024

Last Modified: December 16, 2024

Protocol Integer ID: 115703

Keywords: ASAPCRN, lipidomic analysis of neuronal lipid droplet, lipidomic analysis, neuronal lipid droplet, purified lipid droplet, quantification of lipid, detailed analysis of constituent lipid, lipid droplet, constituent lipid, lipid, details of extraction

Funders Acknowledgements:

NIH

Grant ID: NS036942

NIH

Grant ID: NS11739

ASAP

Grant ID: ASAP-024404

Abstract

This protocol provides details of extraction, identification, and quantification of lipids from purified lipid droplets using advanced LC-MS techniques, enabling detailed analysis of constituent lipids.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

- LD samples: Collected as per LD purification protocol
- MTBE (Methyl tert-butyl ether)
- Avanti Splashmix (Internal standards)
- 1-Butanol
- Mobile Phase A: 60% Acetonitrile, 40% H₂O, 7.5 mM Ammonium Acetate
- Mobile Phase B: 90% Isopropanol (IPA), 10% Acetonitrile, 7.5 mM Ammonium Acetate
- MS-DIAL software (version 4.7)

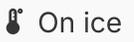
Equipments

- Centrifuge capable of processing organic phase extractions
- LC-MS system with gradient capability
- Agilent Poroshell 120 EC-C18 column (2.7 μm, 1000 bar, 2.1 × 100 mm)
- Agilent 1290 Infinity II LC system
- Agilent Quadrupole Time-Of-Flight 6546 mass spectrometer

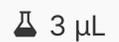
Troubleshooting



Lipid Extraction

- 1 Prepare the LD samples for extraction by ensuring they are thawed  and homogenized. 
- 2 Add MTBE to the LD samples along with spiked Avanti Splashmix (internal standards) to normalize lipid quantification.
- 3 Vortex the mixture thoroughly to ensure complete mixing. 
- 4 Centrifuge the mixture to separate the organic phase. 
- 5 Collect the organic phase carefully to avoid contamination from the aqueous phase.
- 6 Dry the collected organic phase under a gentle stream of nitrogen or in a vacuum concentrator.

Lipid Resuspension

- 7 Resuspend the dried lipid extract in 1-Butanol.
- 8 Vortex the sample to ensure uniform suspension. 
- 9 Load  of the lipid mixture onto the LC-MS system. 

LC-MS Analysis

17m 30s

- 10 Set up the Agilent Poroshell 120 EC-C18 column (2.7 μm , 2.1 \times 100 mm) on the Agilent 1290 Infinity II LC system.
- 11 Prepare the following mobile phases:

Mobile Phase A (60% Acetonitrile, 40% H₂O, 7.5 mM Ammonium Acetate),
Mobile Phase B (90% IPA, 10% Acetonitrile, 7.5 mM Ammonium Acetate).

12 Use the following gradient for lipid separation:

12m 30s

1. Start at 85% A (15% B) and decrease to 70% A over  00:02:00 .
2. Further decrease to 52% A over  00:00:30 .
3. Gradually reduce to 18% A over  00:05:00 .
4. Reduce to 1% A within  00:01:00 and hold for  00:04:00 .

13 Restore gradient to 85% A and wash the column for  00:05:00 .

5m



14 Perform the analysis on the Agilent Quadrupole Time-Of-Flight 6546 mass spectrometer for high-resolution detection of lipid species.

Data Analysis

15 Process the LC-MS data using MS-DIAL software (version 4.7; PMID: 25938372).

16 Identify lipid species based on the mass spectra and retention times.

17 Quantify each lipid species using the moles of corresponding class-specific lipid standards present in the Splashmix.

Note

- Ensure the organic phase is free from aqueous contamination to avoid interference in LC-MS.
- Handle MTBE and other organic solvents with care in a fume hood to prevent exposure.
- The gradient setup must be precise to ensure reproducible lipid separation and identification.