Nov 24, 2025

Metabolomics and Lipidomics Sample Preparation

DOI
dx.doi.org/10.17504/protocols.io.bf4tjqwn

Kevin Contrepois[1]

[1]Stanford University

Kevin Contrepois

DOI: https://dx.doi.org/10.17504/protocols.io.bf4tjqwn

Protocol Citation: Kevin Contrepois 2025. Metabolomics and Lipidomics Sample Preparation. **protocols.io https://dx.doi.org/10.17504/protocols.io.bf4tjqwn**

Protocol status: Working

Created: May 06, 2020

Last Modified: May 13, 2020

Protocol Integer ID: 36723

Keywords: HuBMAP, Metabolomics, Lipidomics, Stanford University, analyzed usin untargeted metabolomic, usin untargeted metabolomic, lipidomics sample preparation scope, targeted lipidomics approach, lipidomics approach, metabolite, biphasic separation with cold methyl, complex lipid, methanol, biphasic separation, cold methyl,

Abstract

Scope:
To describe the procedure to extract metabolites and complex lipids using a biphasic separation with cold methyl tert-butyl ether (MTBE), methanol and water.

Expected outcome/data:
Metabolites and complex lipids are prepared to be analyzed usin untargeted metabolomics and targeted lipidomics approaches, respectively.

Troubleshooting

1 Roughly 30 mg of frozen tissues were homogenized in 500 µl ice-cold methanol by bead beating (MP bioscience cat# 6913-100, Solon, OH) at 4°C (2 × 45 s).

2 1 ml of MTBE was added to 300 µl of the homogenate spiked-in with 40 µL deuterated lipid internal standards (Sciex, cat#: 5040156, lot#: LPISTDKIT-101).

3 The samples were then sonicated (3 × 30 s) and agitated at 4°C for 30 min.

4 After addition of 250 µl of ice-cold water, the samples were vortexed for 1 min and centrifuged at 14,000 g for 5 min at 20°C.

5 The upper organic phase contains the lipids, the lower aqueous phase contains the metabolites and the proteins are precipitated at the bottom of the tube. For quality controls, 3 reference plasma samples (40 µL plasma) and 1 preparation blank were prepared in parallel.

Metabolites

6 Proteins were further precipitated by adding 700 µl of 33/33/33 acetone/acetonitrile/methanol spiked-in with 15 labeled metabolite internal standards to 300 µl of the aqueous phase and 200 µl of the lipid phase and incubating the samples overnight at -20°C

7 After centrifugation at 17,000 g for 10 min at 4°C, the metabolic extracts were dried down to completion under a stream of nitrogen and resuspended in 100 µl 50/50 methanol/water.

Complex lipids

8 700 µl of the organic upper phase was dried down and resolubilized in 200 µl of methanol for storage at -20°C until analysis.

9 The day of the analysis, samples were dried down, resuspended in 300 µl of 10 mM ammonium acetate in 90/10 methanol/toluene and centrifuged at 16,000 g for 5 min at 24°C.