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BAF_Protocol_009 Metabolomics: Lipid Extraction

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

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Abstract

This protocol uses a similar method to Protocol_008 but retains the chloroform phase containing lipids and other very hydrophobic molecules. Extractions are done in a way to make sure the final sample is mostly lipids. This type of sample will need to be run on a different column using different buffers from those used for the aqueous/methanol metabolites.

Guidelines

The dry down of this phase is best done using a stream of inert nitrogen to minimize oxidation of lipids and directly before analysis.

Materials

Microtubes 1.5 mL - SEAL-RITE® 1.5 ML MICROCENTRIFUGE TUBES color: natural, USA Scientific
Reinforced tubes 2 mL- with screw caps and O-rings, Fisherbrand™, White/Opaque, part number 5-340-162.
Stainless steel balls - OMNI International 2.4mm Metal Bead Media 500g SKU 19-640
Pipette tips - Fisherbrand™, yellow, part number: 02-681-151.
Water - Fisher chemical, W64, Optima LC/MS
FA - Fisher chemical, A117-50, Formic Acid, Optima LC/MS
Methanol - Fisher chemical, A456-212, Methanol, Optima LC/MS
Chloroform - Millipore sigma, CX1050P-1, Chloroform, HPLC grade
2 to 20 µL Micropipette - Gilson™ F144056MT
20 to 200 µL Micropipette - Gilson™ F144058MT
100 to 1000 µL Micropipette - Gilson™ F144059MT
VWR Analog Vortex mixer - CAT No: 58816-121
Thermo Scientific™ integrated Speedvac Concentrator CAT No: SPD1030-115
Eppendorf 5415D Digital Centrifuge
Thermo Scientific™ Thermal Mixer with blocks, Block, 24 x 2.0mL microtubes, CAT No: 13687713
Fisherbrand™ bead Mill 24 homogenizer CAT No: 15-340-163
Thermo Scientific™ Amber glass vials, 2mL, CERT5000-74W
SPLASH LipidoMIX™ Internal Standard - Part number 330707, Avanti Ploar Lipids, Inc.
WHEATON® MicroLiter® insert, 300uL, conical with spring Part number: 11-0000-100

Liquid Samples: Urine, Plasma, Culture Media

4h

1 To each sample containing 100 uL add 750 µL of -20°C cold Chloroform:methanol (2:1) mixture and vortex — 1m

2 Shake tubes vigorously for 30 min at 4°C in temperature temperature-controlled thermal shaker — 30m

3 Add 400 µL of water, shake vigorously, and centrifuge for 10 min at 10000 rpm for phase separation — 10m

4 Discard the top aq. methanolic phase (if interested in soluble metabolite, recover this phase. See BAF_Protocol_008) — 2m

5 Recover the Lower phase to new clean Eppendorf — 1m

6 To each tube with 500 µL of chloroform phase, add 500 µL of cold Chloroform:methanol (2:1) mixture — 1m

7 Vortex and shaken vigorously for 30 min at 4°C in temperature controlled thermal shaker — 30m

8 Add 200 µL of water, shake vigorously, centrifuge for 10 min at 10000 rpm and recover the lower organic phase as a lipid mixture in glass tubes and store at -80°C — 10m

9 Before running, add 10 µL of Avanti Splash Lipidomix to each sample as internal standard and dry samples under gentle stream of N2 using a Recti-VapTM Evaporator (Thermo Fisher Scientific) at 40°C — 2h

10 Reconstitue in 110 µL of methanol:isoproponal (1:1) — 1m

11 Transfer 100 uL to borosilicate glass inserts kept inside a screw-capped glass autosampler vials (Agilent) — 1m

Solid samples: Cell Media, Stool, Tissue

45m

12 Place the sample in reinforced tubes: Frozen tissue slice or lyophilized stools. For cell pellets – mix well with 50-100 uL of water transfer solution and suspended cells to reinforced tubes — 2m

13 To each sample add 5 stainless steel balls, 750 µL of -20°C cold Chloroform:methanol (2:1) mixture.
Disrupt cells/tissues with Fisher Bead Mill 24 (speed: 5m/s, time: 20 sec, number of cycle: 3, dwell/pause between runs: 10 sec). 1m

14 Shake tubes vigorously for 30 min at 4°C in temperature temperature-controlled thermal shaker 30m

15 Add 400 µL of water, shake vigorously, and centrifuge for 10 min at 10000 rpm for phase separation. Discard the top aq. methanolic phase (if interested in soluble metabolite, recover this phase. See BAF_Protocol_08) 10m

16 Recover the Lower phase to new clean Eppendorf - extract lipids. Perform steps 06-11 from the above protocol 2m