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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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Protocol Integer ID: 98244

Keywords: baf-protocol-009 metabolomic, lipid extraction, similar method to protocol-008, containing lipid, protocol-008, methanol metabolite, lipid, extraction, methanol

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 × 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 Polar 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 μ L 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 μ L 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 μ L of chloroform phase, add 500 μ L 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 μ L 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 μ L 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 μ L 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 2m



reinforced tubes

- 13** To each sample add 5 stainless steel balls, 750 μ L 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 μ L 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