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UC Davis - Metabolomics: Sample preparation for Lipidomics

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Mouse Metabolic Phenotyping Centers

Metabolomics Protocols & Workflows

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Protocol status:

Working
We use this protocol and it's working

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21071

Keywords: Lipidomics

ABSTRACT

Summary:

This SOP describes sample extraction and sample preparation for lipid profiling by liquid chromatography / quadrupole time of flight mass spectrometry (LC-QTOF) or nanoelectrospray ion trap-FTICR MS.

MATERIALS

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☒	Centrifuge Eppendorf Centrifuge Catalog #5415 D
☒	Calibrated pipettes 1-200 ul and 100-1000ul Contributed by users Catalog #1-200ul and 100-1000ul
☒	Eppendorf tubes 1.5 mL uncolored Eppendorf Centrifuge Catalog #022363204
☒	ThermoElectron Neslab RTE 740 cooling bath at – 20°C Contributed by users Catalog #RTE 740 cooling bath
☒	MiniVortexer VWR Scientific Catalog #58816-121
☒	Orbital Mixing Chilling/Heating Plate Torrey Pines Scientific Instruments
☒	Speed vacuum concentration system Labconco Centrivap cold trap
☒	Eppendorf tips for organic solvents such as acetonitrile methanol and MTBE Contributed by users
☒	Glass Amber Vials National Scientific Catalog #C4000-2W
☒	Glass Inserts Supelco Catalog #27400-U
☒	Blue Tops for Vials Agilent Technologies Catalog #5182-0717
☒	Crushed ice Contributed by users
☒	Nitrogen line with pipette tip Contributed by users
☒	Pure water Contributed by users
☒	MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL) Sigma Aldrich Catalog #34875-100ML
☒	Methanol: J.T. Baker LC/MS Grade (9830-03) JT Baker Catalog #9830-03
☒	CUDA (12-[[[(cyclohexylamino)carbonyl]amino]- dodecanoic acid) Cayman Chemical Company Catalog #10007923

Note:

Sigma-Aldrich [RRID:SCR_008988](#)

Cayman Chemical [RRID:SCR_008945](#)

- 1 **Starting material:**
Plasma/serum: 30 µl sample volume or aliquot

- 2 **Sample Preparation:**
Switch on bath to pre-cool at –20°C (±2°C validity temperature range)

Extraction solvents

- ◆ Purge both MeOH and MTBE for 5 min with nitrogen.
- ◆ Store solvents in the –20°C freezer to pre-chill

Homogenization and extraction

- ◆ Thaw plasma on ice, and gently rotate or invert the blood samples for about 10s to obtain a homogenized sample.
- ◆ Take out 60 μ L and add 220 μ L cold MeOH. Add 5 μ L of QC mix as internal standard (see SOP “QC mix for LC-MS lipid analysis”).
- ◆ Vortex each sample for 10s, keeping the rest on ice
- ◆ Add 750 μ L MTBE
- ◆ Vortex for 10s
- ◆ Shake for 6min at 4°C
- ◆ Add 187.5 μ L distilled water
- ◆ Vortex for 20s
- ◆ Centrifuge for 2 min @ 14000 rcf
- ◆ Remove supernatant, splitting into two aliquots of 300 μ L, keeping one at –20°C for backup
- ◆ Dry samples to complete dryness in the speed vacuum concentration system

Preparation for analysis

- ◆ Re-suspend dry samples in 70 μ L MeOH containing CUDA (10 μ M), degassed using the above method.
- ◆ Transfer 30 μ L to two separate amber glass vial with micro-insert. Cap vials with Agilent blue top.
- ◆ Use independent vials for positive and negative mode acquisitions.

3 Quality assurance

- ◆ For each sequence of sample extractions, perform one blank negative control extraction by applying the total procedure (i.e. all materials and plastic ware) without biological sample.
- ◆ Use one commercial plasma/serum pool sample per 10 authentic subject samples as control. If no suitable commercial blood sample is available, prepare a large pool sample during the thawing/mixing step by aliquoting 100 μ L per 1 ml plasma sample, and aliquot such pool sample for 1 pool extract per 10 authentic subject samples.
- ◆ Prepare at least six NIST plasma extracts in the same manner as positive controls

Final Protocol

IMPORTANT: To prevent contamination disposable material is used. To prevent inhalation of toxic ether vapor, use fume hood during lipid extraction.

DISPOSAL OF WASTE: Collect all chemicals in appropriate bottles and follow the disposal rules. Collect residual plasma / serum samples in specifically designed red 'biohazard' waste bags.