Issued: 02-25-2019 Valid from: 02-27-2019	Validity area: UC Davis Genome Center, Metabolomics Core and Research Laboratories
Responsible: Oliver Fiehn	Secondary: Luis Valdiviez
This SOP supersedes: extraction for GCTOF	Approved: Oliver Fiehn

Sample preparation of blood plasma or serum samples for GCTOF analysis

1. Purpose:

This SOP describes sample extraction and sample preparation for primary metabolism profiling by gas chromatography/time-of-flight mass spectrometry (GCTOF).

2. References:

Fiehn O, Kind T (2006) Metabolite profiling in blood plasma. In: Metabolomics: Methods and Protocols. Weckwerth W (ed.), Humana Press, Totowa NJ.

Fiehn, O. Metabolomics by gas chromatography - mass spectrometry: combined targeted and untargeted profiling. 2016. *Curr. Protoc. Mol. Biol.* 114:30.4.1-30.4.32. doi: 10.1002/0471142727.mb3004s114.

3. Starting material:

Plasma/serum: 30 µL sample volume or aliquot

4. Equipment:

- Centrifuge Eppendorf 5415 D
- Calibrated pipettes 1-200µL and 100-1000µL
- Multi-Tube Vortexer (VWR VX-2500)
- Orbital Mixing Chilling/Heating Plate (Torrey Pines Scientific Instruments)
- Speed vacuum concentration system (Labconco Centrivap cold trap)
- Nitrogen line with Pasteur pipette

5. Chemicals and consumables:

Product	Manufacturer & Part Number
Eppendorf tubes 1.5 mL, uncolored	Eppendorf 022363204
Crushed ice	UC Davis
Water, LC/MS Grade	Fisher Optima W6-4
Acetonitrile, LC/MS Grade	Fisher Optima A955-4
Isopropanol, LC/MS Grade	Fisher A461-4
pH paper 5-10	Millipore Sigma 1095330001
Bioreclamation human plasma (disodium EDTA)	Bioreclamation HMPLEDTA

6. Sample Preparation:

Preparation of extraction solvent

1. For 1 L of extraction solvent, combine 375 mL of acetonitrile, 375 mL of isopropanol, and 250 mL water in a 1 L bottle conditioned with the aforementioned chemicals. If a different total volume of extraction solvent is needed, simply mix acetonitrile, isopropanol, and water in volumes in proportion 3:3:2.
2. Purge the extraction solution mix for 5 min with nitrogen with small bubbles. Make sure that the nitrogen line is flushed out of air before using it for degassing the extraction solvent solution.
3. Store at -20°C until use. Note: if solvent freezes, sonicate until thawed and mix before use.

Extraction

1. Thaw raw samples at room temperature (or in the refrigerator at 4˚C) and vortex 10 sec at low speed to homogenize.
2. Aliquot 30 µL of plasma sample into a 1.5 mL Eppendorf tube. Keep all samples on ice.
3. Add 1 mL 3:3:2 (v/v/v) ACN:IPA:H2O extraction solvent (prechilled in a -20°C freezer).
4. Vortex the sample for 10 sec.
5. Shake for 5 min at 4°C using the Orbital Mixing Chilling/Heating Plate. Continue to keep all extracted samples on ice.
6. Centrifuge samples for 2 min at 14000 rcf.
7. Aliquot two 450 µL portions of the supernatant into 1.5 mL Eppendorf tubes (one for analysis and one as a backup sample).
8. Transfer 100 µL of the remaining supernatant from each sample to a 2, 15, or 50 mL tube for pools, depending on number of samples in the study.
9. Evaporate one 450 µL aliquot of the sample in the Labconco Centrivap cold trap concentrator to complete dryness. Proceed with cleanup or store tubes at -20°C until cleanup.

Pooling

10. Transfer multiple 475 µL aliquots of pooled samples to 1.5 mL Eppendorf tubes, one aliquot for every 10 samples in the study. If there is still pool remaining, prepare additional aliquots for backup.
11. Centrifuge pool samples for 2 min at 14000 rcf.
12. Remove 450 µL supernatant to new 1.5 mL Eppendorf tube.
13. Evaporate to complete dryness in the Labconco Centrivap cold trap concentrator. Proceed with cleanup or store tubes at -20°C until cleanup.

Cleanup

14. Resuspend the dried aliquot with 500 µL 50:50 (v/v) ACN:H2O (degassed as given above) and vortex for about 10 sec.
15. Centrifuge for 2 min at 14000 rcf.
16. Remove 475 µL supernatant to a new 1.5 mL Eppendorf tube.
17. Evaporate the transferred supernatant to complete dryness in the Labconco Centrivap cold trap concentrator.
18. Submit to derivatization (see SOP “Derivatization of GC Samples & Standards”) or store at -20°C until ready for analysis.

7. Quality assurance

- For every 50 samples, perform one method blank negative control extraction by applying the total procedure (i.e. all materials and plastic ware) without biological sample.
- If no combined pool was made from the extracted samples, use one commercial plasma/serum pool sample per 10 authentic subject samples as control instead.

8. 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.