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Responsible:	
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Extraction of Cells

1. References:

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

2.Starting material:

- Blood plasma/serum: 5-30ul sample volume (or aliquot) (validated for plasma)
- Other body liquids: 5-30ul sample volume (or aliquot) such as liquor, urine (not validated)

3. Equipment:

- Centrifuge (Eppendorf 5415 D)
- Calibrated pipettes 1-200µl and 100-1000µl
- Eppendorf tubes 2ml, uncoloured (Cat.No. 022363204)
- ThermoElectron Neslab RTE 740 cooling bath at –20°C
- MiniVortexer (VWR)
- Orbital Mixing Chilling/Heating Plate (Torrey Pines Scientific Instruments)
- Speed vacuum concentration system (Labconco Centrivap cold trap)

4. Chemicals

- Acetonitrile, LCMS grade (JT Baker; Cat. No.9829-02)
- Isopropanol, HPLC (JT Baker; Cat. No. 9095-02)
- Crushed ice
- pH paper 5-10 (EMD Chem. Inc.)
- Nitrogen line with pipette tip
- 18 MΩ pure water (Millipore)

5. Preparation of extraction mix and material before experiment:

1. Switch on bath to pre-cool at –20°C (±2°C validity temperature range)
2. Check pH of acetonitrile and isopropanol (pH7) using wetted pH paper
3. Make the extraction solution by mixing acetonitrile, isopropanol and water in proportions 3 : 3 : 2
4. Rinse the extraction solution mix for 5 min with nitrogen. Make sure that the nitrogen line was flushed out of air before using it for degassing the extraction solvent solution

6. Sample preparation:

1. After quenching the cells add 1 X 10^6 dried cells to 1.5 ml eppendorff tube.
2. Place the eppendorf tube with cells on dry ice for 20 minutes or til the cells are completely frozen and ice for 20 minutes, till they are completely thawed. Repeat this step twice.
3. Add 1ml of extraction solvent which has been pre-chilled using the ThermoElectron Neslab RTE 740 cooling bath set to -20°C. to the ependorff tube with cells.
4. Repeat step **2** twice with the extraction solvent.
5. Vortex the sample for 10s and shake for 5min at 4°C using the Orbital Mixing Chilling/Heating plate. Centrifuge samples for 2min at 14000 rcf using the centrifuge Eppendorf 5415 D.
6. Aliquot two 500µL portions of the supernatant. One for analysis and one for backup. Store one aliquot in the -20°C freezer as a backup.
7. Evaporate one 500µL aliquot of the sample in the Labconco Centrivap cold trap concentrator to complete dryness.

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8. Submit to derivatization.

The residue should contain membrane lipids because these are supposedly not soluble enough to be found in the 50% acetonitrile solution. Therefore, this 'membrane residue' is now taken for membrane lipidomic fingerprinting using the nanomate LTQ ion trap mass spectrometer. Likely, a good solvent to redissolve the membrane lipids is e.g. 75% isopropanol (degassed as given above). If the 'analysis' aliquot is to be used for semi lipophilic compounds such as tyrosine pathway intermediates (incl. dopamine, serotonine etc, i.e. polar aromatic compounds), then these are supposedly to be found together with the 'GCTOF' aliquot. We can assume that this mixture is still too complex for Agilent chipLCMS. Therefore, in order to develop and validate target analysis for such aromatic compounds, we should use some sort of Solid Phase purification. We re-suspend the dried 'GCTOF' aliquot in 300 □l water (degassed as before) to take out sugars, aliphatic amino acids, hydroxyl acids and similar logP compounds. The residue should contain our target aromatics .We could also try to adjust the pH by using low concentration acetate or phosphate buffer. The residue could then be taken up in 50% acetonitrile and used for GCTOF and Agilent chipMS experiments. The other aliquot should be checked how much of our target compounds would actually be found in the 'sugar' fraction.

7. 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.

8. Disposal of waste

Collect all chemicals in appropriate bottles and follow the disposal rules.