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Integrated Extraction for Cells

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This liquid-liquid extraction for cells enables the simultaneous generation of lipidomics and metabolomics samples for HILIC and C18 chromatography.

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This extraction should be performed in a fume hood.

Equipment

- Qiagen Bead Beater
- Centrifuge
- Mixmate Shaker
- Sonicator
- Centrifugal evaporator
- P200 Micropipette
- P1000 Micropipette
- P200 Micropipette tips
- P1000 Micropipette tips

Consumables

- Stainless steel balls 2.3 mm
- 96-well plate polypropylene
- 1.5ml amber glass vial
- 150 ul glass vial inserts
- 2ml round bottom Eppendorf polypropylene tube with cap

Chemicals

- Water
- Acetonitrile
- Methanol
- MTBE
- HILIC iSTDs

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1	Cells should arrive pelleted and frozen from collaborator
2	Using wide-mouth pipette wash pellet with 500uL 150 mM ammonium acetate (25x aspirations)
3	Centrifuge @ 200 g for 5 min
4	Remove supernatant
5	Repeat steps 2-4 a second time
6	Add 4-6 non-sterile 2.3mm stainless steel beads to the tube.
7	Dispense 225 ul of ice-cold Methanol at -20 deg C containing 1.5% iSTD "SPLASH" mix into extraction tube 1.
8	Seal the sample tube and place it in the bead beater at an amplitude 20 Hz for 15 minutes to homogenize the sample & extract the metabolites.
9	Close the tube.
10	Vortex extraction tube 1 for 10 seconds.
⋈ proto	Open extraction tube 1 and dispense 750ul of ice-cold MTBE.

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12	Close extraction tube 1 and vortex for 10s.
13	Shake extraction tube 1 for 6 min at 4 deg C.
14	Open extraction tube 1 and dispense 188 ul of LC-MS grade water containing the 18 HILIC iSTDs at 1/3rd of their normal concentration as defined in the HILIC extraction SOP except CUDA.
15	Close extraction tube 1 and vortex it for 20 seconds
16	Centrifuge extraction tube 1 for 2 min at 14,000xg.
17	Open extraction tube 1 and transfer 700ul of the upper phase into a single 700ul tubetwo new separate tubes(350ul/ tube); henceforth referred to as (Lipidomics tube 1) and (Lipidomics tube 2).
18	Transfer 250ul of the bottom phase from extraction tube 1 into a new single-tube hereafter referred to as (Hilic tube 1).
19	Centrivap Lipidomics tube 1, and Hilic tube 1 at room temperature until dry.
20	Reconstitute Linidomics tube 1 in 120 ul of Methanol containing CLIDA istd and close the tube

21 Perform lipid cleanup on HILIC tube 1: Add 500uL of 1:1 ACN:Water and vortex for 20s

22	Centrifuge at 14,000 g for 2 min.
23	Transfer supernatant to a new tube and dry down
24	Reconstitute HILIC tube 1 in 60ul of ACN:H2O (4:1 v/v) containing CUDA and close the tube.
25	Sonicate Lipidomics tube 1 and Hilic tube 1 at room temperature for 5 min.
26	Centrifuge Lipidomics tube 1 and Hilic tube 1 at 14,000xg for 2 min.
27	Open lipidomics tube 1, transfer equal fractions of the extract into two separate amber glass vials with micro-inserts, hereafter referred to as (lipidomics vial 1 pos) and (lipidomics vial 1 neg), and close the vials.a
28	Open Hilic tube 1 and transfer the extract into an amber glass vial with a micro-insert, hereafter referred to as (Hilic vial) and close the vial.
29	Analyze the samples via UHPLC-Orbitrap as follows.