Jan 11, 2022

Integrated Extraction for Cells

Juan Sanchez[1]

[1]CZ Biohub

Juan Sanchez: Stanford

dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1

Juan Sanchez

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

DOI

dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1

Juan Sanchez 2022. Integrated Extraction for Cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1

protocol ,

Jan 11, 2022

Jan 11, 2022

56768

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

:

Citation: Juan Sanchez Integrated Extraction for Cells https://dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1

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.

Open extraction tube 1 and dispense 750ul of ice-cold MTBE.

Citation: Juan Sanchez Integrated Extraction for Cells https://dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1

11

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 Lipidomics tube 1 in 120 ul of Methanol containing CUDA istd and close the tube.

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

Citation: Juan Sanchez Integrated Extraction for Cells https://dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1

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.

Citation: Juan Sanchez Integrated Extraction for Cells https://dx.doi.org/10.17504/protocols.io.kqdg3p5bql25/v1