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Whole cell lipidome extraction with MBTE (Eggert lab)

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We use this protocol and it's working

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Abstract

Validated lipid extraction protocol from cells using organic solvents.

Sample preparation

1 Perform mycoplasma test (Biovision) on all cell lines before the experiment, as per manufacturer's instructions. This protocol has been validated for HeLa, CaCo, HaCaT and U2OS cell lines.

2 Plate 4×10^5^ cells/well in a 6 well plate, with 4 wells per condition as technical replicates, and grow to 80% confluence for 48 h.

3 After 48 h, incubate cells on ice and wash each well twice with cold Dulbecco's PBS (DPBS).

4 Add 300 µL of freshly prepared 155 mM ammonium bicarbonate (0.61 g in 50 ml DPBS and briefly sonicate).

5 Use a cell scraper to scrape cells in ammonium bicarbonate from the plate surface and use a pipette to transfer the lysate to a 1.5 mL tube.

6 Homogenise each sample by passing it 10 times through a 25 G needle attached to a sterile syringe.

7 Transfer 50 µL of each lysate to another 1.5 mL tube for protein concentration determination using the Pierce™ BCA Protein Assay Kit (Thermo Scientific), as per the manufacturer's instructions.

8 Snap-freeze the remaining cell lysate in liquid nitrogen and store at -80 °C until further processing.

MTBE Extraction

9 To prepare for lipid extraction, clean two glass Wheaton tubes (Fisher Scientific Ltd) per sample by adding 400 µL of LC-MS grade methanol, vortex the tubes briefly and drain. Use Wheaton tube caps with Teflon film (VWR International), resistant to MTBE.

10 Label each Wheaton tube as A and B for every sample including controls. Write the sample name on two sides of the glass tube (preferably on top and bottom on opposite sides, in case the solvent removes one label).

11 Add sample volume equivalent to 60 µg of protein per sample to each corresponding tube A and top up with 155 mM ammonium bicarbonate for a final volume of 200 µL.

12 Add 200 µL of 155 mM ammonium bicarbonate to two extra tubes to be treated as blank extractions.

13 Add 10 µL of SPLASH™ LIPIDOMIX™ Mass Spec Standard (Avanti Research) to each sample.

14 Add 1.5 mL methanol to the 200 µL of sample aliquot. To add methanol, use a P1000 pipette tip twice (750 µL each).

15 Vortex each sample briefly (~4 seconds).

16 Obtain a clean glass pipette and clean the external lower part using HPLC-grade MTBE (Merck) to remove any dust, using a waste MTBE glass container to collect wash MTBE.

17 Using the cleaned glass pipette, add 5 mL of MTBE to each sample.

18 Incubate for 1 h at RT on a digital orbital shaker at 200 rpm in slanting position.

19 Take the samples off the shaker and add 1.25 mL of MS-grade water to each sample, which will generate two-phase separation.

20 Incubate at RT for 10 min and then centrifuge at 1,000 × g at 4°C for 10 min.

21 Using a glass Pasteur pipette, collect the upper phase (organic) from each sample and transfer to the corresponding glass tube labelled B.

22 Using a glass Pasteur pipette, add 2 mL of MTBE/methanol/water (10:3:2.5, v/v/v) to the lower phase in glass tube A of each sample.

23 Incubate at RT for 10 min and then centrifuge at 1,000 × g at 4°C for 10 min.

24 Using a glass Pasteur pipette, collect the upper phase (organic) from each sample and transfer to the corresponding glass tube labelled B.

25 Nitrogen stream evaporate the solvent from the upper phase in tubes labelled B.

26 Store the evaporated samples at -20 °C until resuspension in loading buffer (Isopropanol: Acetonitrile: Water at a ratio of 2:1:1) immediately prior to running samples.

Preparing samples to run in MS

27 Add 100 µl of loading buffer to each dried sample and vortex for 30 seconds.

28 Transfer resuspended sample to a clean 1.5 mL tube and centrifuge at 4 °C for 3 minutes at 13,000 × g.

29 Transfer 90 µl of each sample to HPLC vials with glass inserts.

30 Label each tube with the extraction date and name of the sample.