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Lipidomic analysis in HeLa cells

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We use this protocol and it's working

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Abstract

A protocol for the lipidomic analysis in HeLa cells is described.

Troubleshooting

Lipidomic analysis in HeLa cells

1 Collect cells and homogenize cell pellets in 0.1 ml water.

2 To the homogenate, add lipid standards PC25:0, PC43:6, PI25:0, PI31:1, PI43:6, PS25:0, PS31:1, PS37:4 (Avanti Polar Lipids), on the amount of [protein or DNA] in the original sample.

3 Lipid extraction: 700 µl of sample was mixed with 800 µl 1 N HCl:CH3OH 1:8 (v/v), 900 µl CHCl3 and 200 µg/ml of the antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT; Sigma Aldrich).

4 Evaporate the organic fraction using a Savant Speedvac spd111v (Thermo Fisher Scientific) at room temperature.

5 Store the remaining lipid pellet at −20 °C under argon.

6 Just before mass spectrometry, reconstitute lipid pellets in running solution (CH3OH:CHCl3:NH4OH; 90:10:1.25; v/v/v)

7 Analyze p**hospholipid** species by **electrospray ionization** **tandem mass spectrometry** (ESI-MS/MS) on a hybrid triple quadrupole/linear **ion trap** mass spectrometer (4000 QTRAP system; Applied Biosystems SCIEX) equipped with a TriVersa NanoMate (Advion Biosciences) robotic nanosource for automated sample injection and spraying.

8 Perform p**hospholipid** profiling by (positive or negative) precursor ion or neutral loss scanning at a collision energy of 50 eV/45 eV, 35 eV, −35 eV, and −60 eV for precursor 184 (phosphatidylcholine (PC)), neutral loss 87 (phosphatidylserine (PS)) and precursor 241 (phosphatidylinositol (PI)), respectively.

9 Perform phospholipid quantification by **multiple reactions monitoring** (MRM), the transitions being based on the neutral losses or the typical product ions as described in step 8.

10 Relative quantification of phospholipids is obtained as ratio of analytes to their internal standard. The lipid data is normalized to nucleic acid concentration.