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Lipidomic analysis in HeLa cells for HexCer levels

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We use this protocol and it's working

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Abstract

A protocol for the lipidomics analysis in HeLa cells for HexCer levels is described.

Troubleshooting

Lipidomics of Hexosylceramide in HeLa cells

- 1 Collect cells and homogenize cell pellets in 1 ml PBS (with calcium and magnesium).
- 2 To the homogenate, add sphingolipid internal standards.
- 3 Precipitate the protein with methanol and collect the supernatant.
- 4 Adjust the supernatant solution to 1:1 (v/v) methanol: water.
- 5 Lipid extraction: 700 μ l of sample was mixed with 800 μ l 1 N HCl:CH₃OH 1:8 (v/v), 900 μ l CHCl₃ and 200 μ g/ml of the antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT; Sigma Aldrich).
- 6 Evaporate the organic fraction using a Savant Speedvac spd111v (Thermo Fisher Scientific) at room temperature.
- 7 Store the remaining lipid pellet at -20 °C under argon.
- 8 Just before mass spectrometry, reconstitute lipid pellets in running solution (CH₃OH:CHCl₃:NH₄OH; 90:10:1.25; v/v/v)
- 9 Analyze **phospholipid** 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.
- 10 Perform **phospholipid** 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.
- 11 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.

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