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# 🌐 Lipidomic analysis in HeLa cells for HexCer levels

Forked from Lipidomic analysis in HeLa cells

Rosanne Wouters[1,2], Igor Beletchi[1], Chris Van den Haute[3,4,2], Veerle Baekelandt[3,2], Shaun Martin[1], jan eggermont[1], Peter Vangheluwe[1,2]

[1]Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD;
[3]Research Group for Neurobiology and Gene Therapy, Department of Neurosciences, KU Leuven;
[4]Leuven Viral Vector Core, KU Leuven

👤 **Rania Abou El Asrar**
KU Leuven

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**Protocol status:** 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 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).

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.