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Lipidomic analysis in WM115 cells

Forked from 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 WM115 cells

Troubleshooting

Lipidomic analysis in WM115 cells

1 Collect cells and homogenize cell pellets in 0.7 ml water with a handheld sonicator.

2 To the homogenate, add 0.8 ml HCl(1 M):CH3OH 1:8 (v/v), 0.9 ml CHCl3, 0.2 mg/ml of the antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT; Sigma Aldrich), 3 µl of SPLASH ® LIPIDOMIX® Mass Spec Standard (Cat No: 330707, Avanti Polar Lipids), 3 µl of Ceramides and 3 µl of Hexosylceramides internal Standards (#5040167 and #5040398, AB SCIEX).

3 After vortexing and centrifugation, collect the lower organic fraction.

4 Evaporate the lower 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 the lipid pellets in 100% ethanol.

7 Analyze lipid species by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) on a Nexera X2 UHPLC system (Shimadzu) coupled with hybrid triple quadrupole/linear ion trap mass spectrometer (6500+ QTRAP system; AB SCIEX).

8 Perform chromatographic separation on a XBridge amide column (150 mm × 4.6 mm, 3.5 µm; Waters) maintained at 35 °C using mobile phase A [1 mM ammonium acetate in water-acetonitrile 5:95 (v/v)] and mobile phase B [1 mM ammonium acetate in water-acetonitrile 50:50 (v/v)] in the following gradient: (0–6 min: 0 % B → 6 % B; 6–10 min: 6 % B → 25 % B; 10–11 min: 25 % B → 98 % B; 11–13 min: 98 % B → 100 % B; 13–19 min: 100 % B; 19–24 min: 0 % B) at a flow rate of 0.7 ml/min which was increased to 1.5 ml/min from 13 min onwards.

9 Measure phosphtidylserine, phosphtidylcholine and phosphtidylinositol in negative ion mode by fatty acyl fragment ions.

10 Perform phospholipid quantification by **multiple reactions monitoring** (MRM), the transitions being based on the neutral losses or the typical product ions.

11 The following instrument parameters are used: Curtain Gas = 35 psi; Collision Gas = 8 a.u. (medium); IonSpray Voltage = 5500 V and −4500 V; Temperature = 550 °C; Ion Source Gas 1 = 50 psi; Ion Source Gas 2 = 60 psi; Declustering Potential = 60 V and −80 V; Entrance Potential = 10 V and −10 V; Collision Cell Exit Potential = 15 V and −15 V.

12 The following fatty acyl moieties were taken into account for the lipidomic analysis: 14:0, 14:1, 16:0, 16:1, 16:2, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 20:5, 22:0, 22:1, 22:2, 22:4, 22:5 and 22:6. Data Analysis:

13 Perform peak integration with the MultiQuant™ software version 3.0.3. Lipid species signals were corrected for isotopic contributions (calculated with Python Molmass 2023.8.30;DOI:https://doi.org/10.5281/zenodo.7135495) and were quantified based on internal standard signals and adheres to the guidelines of the Lipidomics Standards Initiative (LSI) (level 2 type quantification as defined by the LSI).