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WORKS FOR ME 1

Untargeted lipidomics analysis for Golgi immunopurification (Golgi-IP)

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ABSTRACT

The Golgi apparatus functions as a central hub in the cell that processes, packages, and distributes proteins. Despite its critical cellular function, there has been challenges to quantitatively assess Golgi metabolite profiles. To overcome this hurdle, we developed a rapid harvesting and purification method using immunoprecipitation (GolgiIP). This protocol provides details for analyzing GolgiIP lipidomics samples using liquid chromatography mass spectrometry (LC-MS) for nonpolar lipid profiling.

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MATERIALS TEXT

Reagents

- Optima LC/MS water (Fisher, cat. no. W6-4)
- Optima LC/MS acetonitrile (Fisher, cat. no. A955-4)
- Optima LC/MS 2-propanol (Fisher, cat. no. A461-500)
- Ammonium formate
- Formic acid
- EASYIC^{TM}

Equipment

- ID-X Orbitrap Tribrid Mass Spectrometer (Thermo Fisher Scientific) with a heated electrospray ionization (HESI) probe
- Ascentis Express C18 150 x 2.1 mm column (Millipore Sigma 53825-U)
- 5 x 2.1 mm guard (Sigma-Aldrich 53500-U)

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

LC/MS lipidomics settings

1 Set an ID-X tribrid mass spectrometer (Thermo Fisher Scientific) with a heated electrospray ionization (HESI) probe, for initial nonpolar lipid profiling.

Prepare an Ascentis Express C18 150 x 2.1 mm column (Millipore Sigma 53825-U) coupled with a 5 x 2.1 mm guard (Sigma-Aldrich 53500-U), to carry out C18-based lipid separation prior to mass spectrometry. Use EASYIC^{TM} for internal calibration.

2 For C18-based lipid separation, use [M] 10 millimolar (mM) ammonium formate and [M] 0.1 % (v/v) formic acid dissolved in [M] 60 % (v/v) LC/MS grade water and [M] 40 % (v/v) LC/MS grade acetonitrile for Buffer A, and [M] 10 millimolar (mM) ammonium formate and [M] 0.1 % (v/v) formic acid dissolved in [M] 90 % (v/v) LC/MS grade 2-propanol and [M] 10 % (v/v) LC/MS grade acetonitrile for Buffer B.

3 Set the chromatographic gradient flow rate to 0.26 mL/min.

Use Orbitrap resolution 120,000 for MS1 and 30,000 for MS2, RF lens at 40%, AGC target $4x10^5$ for MS1 and $5x10^4$for MS2, and maximum injection time 50 ms for MS1 and 54 ms for MS2. Set positive ion voltage to 3250 V, negative ion voltage to 3000 V, ion transfer tube temperature to 300 °C, and vaporizer temperature to

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375 °C . Set sheath gas flow to 40 units, auxiliary gas flow to 10 units, and sweep gas flow to 1 unit.

Operate the mass spectrometer in full-scan mode with data-dependent tandem mass spectrometry (ddMS2) at *m/z* 250-1500, with cycle time of 1.5 sec, microscans of 1 unit, isolation window of *m/z* 1, intensity threshold of $1x10^4$, and dynamic exclusion time of 2.5 sec. For HCD fragmentation, use step-wise collision energies of 15%, 25%, and 35%.

4 Perform the elution with a gradient of 40 minutes: from 0–1.5 min isocratically elute at 32% B; froming 1.5-4min linearly increase to 45% B; from 4-5min linearly incrase to 52% B; from 5-8 min linearly increase to 58% B; from 8-11min linearly increase to 66% B; from 11-14min linearly increase to 70%; from 14-18min linearly increase to 75%; from 18-21min linearly increase to 97% B; from 21-35min hold at 97% B; from 35-35.1min linearly decrease to 32% B; and from 35.1-40min hold at 32%min.

Untargeted lipidomics workflow

5 LipidSearch and Compound Discoverer (Thermo Fisher Scientific) were used for unbiased differential analysis. Lipid annotation was acquired from LipidSearch with the precursor tolerance at 5 ppm and product tolerance at 8 ppm.

6 The mass list from LipidSearch is then exported and used in Compound Discoverer for improved alignment and quantitation. Mass tolerance, 10 ppm; minimum and maximum precursor mass, 0-5,000 Da; retention time limit, 0.1-30 min; Peak filter signal to noise ratio, 1.5; retention time alignment maximum shift, 1 min; minimum peak intensity, 10,000; compound detection signal to noise ratio, 3. Isotope and adduct settings were kept at default values. Gap filling and background filtering were performed by default settings. The MassList Search was customized with 5 ppm mass tolerance and 1 minute retention time tolerance. Area normalization was performed by constant median after blank exclusion.

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