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UPLC-MS/MS procedures of lipidomics for plasma

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Troubleshooting

- 1 Left samples at -20°C for 30 min and thawed at 4°C until no ice was observed in the tubes
- 2 For each sample, take 40 µL plasma in a new 96 well using multichannel adjustable spacer manual pipette, and then add 120 µL precooled isopropanol (IPA) in each well
- 3 Vortexing the 96 well for 1 min and incubated for 10min in room temperature, the mixture was stored overnight in refrigerator at -20°C to improve protein precipitation
- 4 Centrifuged the samples for 20 min at 14,000 g
- 5 Remove the supernatant to a new 96 well, and further diluted with IPA/acetonitrile (ACN)/H₂O (2:1:1 v:v:v)
- 6 Equal amount of all samples were pooled as QC sample for LC-MS system conditioning and quality control process
- 7 Equilibrate the CSH column with 99% Phase B, set the flow rate at 0.4 mL/min. The initial elution was started from 40% B and was immediately increased by a linear gradient to 43% B for the first 2 min, followed by an increase to 50% B within 0.1 min. Over the next 3.9 min, the gradient was increased to 54% B, and the amount of B was increased to 70% during next 0.1 min. In the final part of the gradient, B was increased to 99% and maintained for 1.9 min. Finally, B was returned to 40% over the next 0.1 min and equilibrated for 1.9 min for the next injection
- 8 Using the sodium formate solution for mass calibration and Leucine enkephalin (MW=555.62) was applied as a lock mass for accurate mass measurements
- 9 Both positive and negative modes were performed and operated in Centroid MS^E mode with an acquisition time of 0.2 s per scan. Scan range was set at 50–1,800 Da. The capillary was set at 0.25 kV and 2 kV in positive ion mode and negative ion mode, respectively. And sampling cone voltages were set 40 V in two modes. The source temperature was set to 120°C. The desolvation temperature and gas flow were 500°C and 800 L/h
- 10 Run 10 QC samples to evaluate the LC-MS system and the run samples interspersed with QC in positive mode, then run all sample in negative mode



- 11 Check the reproducibility of QC samples and then analysis the data by Progenesis QI and metaX