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UPLC-MS/MS procedures of lipidomics for plasma

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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)/H_2O (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