Metabolite extraction for the liver of ZDF rats

1. Approximately 50 mg of frozen tissue was plunged into 1,500 μL of 50% acetonitrile/Milli-Q water containing internal standards (H3304-1002, Human Metabolome Technologies (HMT), Tsuruoka, Yamagata, Japan) at 0ºC.
2. The tissue was homogenized thrice at 1,500 rpm for 120 s using a tissue homogenizer (Micro Smash MS100R, Tomy Digital Biology Co., Ltd., Tokyo, Japan) and then the homogenate was centrifuged at 2,300 ×g and 4ºC for 5 min.
3. Subsequently, 800 μL of upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter (UltrafreeMC-PLHCC, HMT) to remove macromolecules (9,100 ×g, 4ºC, 120 min).
4. The filtrate was centrifugally concentrated and re-suspended in 50 μL of Milli-Q water for CE-TOFMS analysis at HMT.