Metabolite extraction for HepG2 cells

1. Culture medium of HepG2 cells in 100-mm dish was aspirated and cells were washed twice by 5% mannitol solution (10 mL first and then 2 mL).

2. The cells were then treated with 800 μL of methanol and left at rest for 30 s in order to inactivate enzymes.

3. The cell extract was then treated with 550 μL of Milli-Q water containing internal standards (H3304-1002, Human Metabolome Technologies (HMT), Tsuruoka, Yamagata, Japan) and left at rest for another 30s.

4. The extract was obtained and centrifuged at 2,300 ×*g* and 4ºC for 5 min and then 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*, 4°C, 120 min).

5. The filtrate was centrifugally concentrated and re-suspended in 50 μL of Milli-Q water for metabolome analysis at HMT.