Preparation of metabolite extracts from medium (for metabolic footprinting)

Remember: you will need 3 extra samples with ‘fresh’ unspent medium (without cells), which has been incubated for the same length of time as your other experimental medium samples from wells containing cells.

1. Centrifuge media samples for 5 min at 300 x g at 4 °C to remove any cells.
2. Collect the supernatant in a new centrifuge tube on ice.
3. Centrifuge the medium at 16,000xg for 5 min at 4 °C to remove any cell debris.
   If desired, transfer the content to a new centrifuge tube and store at -80 °C.
4. Prepare Eppendorf tubes with 500 µl ice-cold 80% MeOH/20% water and place on ice.
5. Add 20 µl of clarified medium from step 3.
6. Vortex for 30 sec.
7. Incubate for 1 hr at -80 °C.
8. Place on ice to warm up. Vortex briefly.
9. Spin for 10 min at 16,000 xg at 4 °C.
10. Transfer 450 ul of the supernatant into a glass vial (or new Eppendorf tube, if using a speed vac).
11. Dry in a Genevac evaporator or in a speed vac without applying heat. Choose an appropriate drying time and remove the samples promptly when the program is finished.
12. Store the dried extracts at -80 °C.

Useful general reference:
Lu et al, 2018: Metabolite Measurement: Pitfalls to Avoid and Practices to Follow
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5734093/

Equipment and reagents needed:
glass vials: 13-622-351 Fisher Scientific Chromacol™ GOLD-Grade Inert Glass Vials;
Thermo 2SVWGK

caps: 03-379-123 Fisher Scientific 9 mm autosampler vial screw thread caps (PTFE,Silicone)
MeOH: A456-1 Fisher Scientific Methanol (Optima* LC/MS)
H2O: W5-1 Fisher Scientific Water, Glass Bottle; 1L