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