



## **Extraction of Metabolome From Tissue/Organ**

*\*For batches of samples larger than 50, please contact our Metabolomics Facility regarding experimental design*

1. Freshly collect the tissue/organ into a tube.
2. Snap freeze in liquid nitrogen and store at  $-80^{\circ}\text{C}$  until analysis.
3. For extraction of metabolites, section  $\sim 50\text{-}100$  mg of frozen tissue/organ, and record the wet weight (note: keep the sample weight consistent across different samples).
4. Add 1 mL of methanol/water 80:20 (vol/vol).
5. Homogenize the tissue using a homogenizer or ultrasonicator (note: use methanol/water 50:50 (vol/vol) to clean the probe between samples).
6. Vortex the homogenate and transfer 200  $\mu\text{L}$  into a tube pre-added with 800  $\mu\text{L}$  of ice-cold methanol/water 80% (vol/vol).
7. Vortex rigorously for 1 min, and then centrifuge at  $\sim 20,160$   $xg$  for 15 min in a refrigerated centrifuge.
8. Transfer the metabolite-containing supernatant into a new tube.
9. Keep the protein pellet to perform protein quantitation using BCA assay if desired.
10. Use a SpeedVac to dry the tube containing the supernatant to a pellet using no heat. Apply a breathable membrane to each tube to prevent cross-contamination.
11. Ship dried metabolite pellet to us on dry ice (note: the dried metabolite pellet can be kept at  $-80^{\circ}\text{C}$  for 1-2 months).