CHILDREN'S MEDICAL CENTER
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Extraction of Metabolome From Tissue/Organ For HILIC LC-MS

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

*If interested in NAD(P)(H) metabolites, please contact us as the prep will change

1. Freshly collect the tissue/organ into a tube.
2. Snap freeze in liquid nitrogen and store at -80°C until analysis.
3. For extraction of metabolites, section ~20-25 mg of frozen tissue/organ, and record the wet weight.
4. Add 300 uL of acetonitrile/water 80:20 (vol/vol). Note: This volume may depend on tissue type/level of necrosis, etc. Please notify the Core if you optimize this volume!
5. Homogenize the tissue using the blue pestle/manual homogenizer (note: use acetonitrile/water 50:50 (vol/vol) to clean the probe between samples).
6. Subject to three freeze-thaw cycles between liquid nitrogen and 37 °C.
7. Vortex rigorously for 1 min, and then centrifuge at ~20,160 x*g* for 15 min in a refrigerated centrifuge.
8. Transfer the metabolite-containing supernatant into a new tube. Keep the protein pellet to perform protein quantitation, if desired.
9. Perform protein quantitation on the supernatant. **NOTE:** protein measurements will be on residual protein; most protein will crash out during the extraction and pelleting. We recommend running the Pierce BCA kit with the widest range curve. Each extract should be run in triplicate with at least one 10x dilution factor to ensure your samples are in the linear range of the assay.
10. Transfer a volume of at least 100 uL of the supernatant that is equivalent of [0.1mg/mL] of protein (of the supernatant) into a LC-MS sample vial (note: keep samples on ice).
11. Prepare a pooled sample for MS2 data acquisition. From the LC-MS vials in Step 10, take an aliquot of each sample into a new LC-MS vial (with insert) to make the final volume of the pooled sample to be at least 45uL. **NOTE:** If your samples are isotopically labeled, you do NOT need to make a pooled sample, but you WILL need an unlabeled (a 0 time-point) sample.