CHILDREN'S MEDICAL CENTER
RESEARCH INSTITUTE
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Extraction of Metabolome From Plasma

**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

*If these are **material-limiting** samples, please contact us as the prep will change

1. Collect 10 µL of plasma into a tube pre-added with 990 µL of ice-cold 80% **methanol**/water (vol/vol).
2. Vortex rigorously for 1 min, and then centrifuge at ~20,160 x*g* for 15 min in a refrigerated centrifuge.
3. Transfer the metabolite-containing supernatant to a new Eppendorf tube.
4. Dry sample in SpeedVac.
5. Resuspend sample in 1 mL of 80% **acetonitrile**/water (vol/vol).
6. Vortex rigorously for 1 min, and then centrifuge at ~20,160 x*g* for 15 min in a refrigerated centrifuge. **NOTE:** This second spin down is CRITICAL to preventing instrument contamination.
7. Transfer the metabolite-containing supernatant to an LC vial labeled as "100x Dilution."
8. Keep the protein pellet (from Step 3) to perform protein quantitation using BCA assay if desired.
9. Prepare a pooled sample for MS2 data acquisition. From the LC-MS vials in Step 7, 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.