



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**

1. Collect 10 μ L of plasma into a tube pre-added with 990 μ L of ice-cold 80% **acetonitrile**/water (vol/vol).
2. Vortex rigorously for 1 min, and then centrifuge at $\sim 20,160$ xg for 15 min in a refrigerated centrifuge.
3. Transfer the metabolite-containing supernatant to a new Eppendorf tube.
4. Vortex rigorously for 1 min, and then centrifuge at $\sim 20,160$ xg for 15 min in a refrigerated centrifuge. **NOTE:** This second spin down is CRITICAL to preventing instrument contamination.
5. Prepare pooled sample(s) for each biological condition for MS2 data acquisition. Take an aliquot of each sample into a new Eppendorf tube to make the final volume of the pooled sample to be at least 45 μ L. **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.
6. Transfer the metabolite-containing supernatant to an LC-MS vial (with insert) and cap. **NOTE:** The Core will provide all LC-MS vials.