General Sample Preparation Recommendations for Metabolomic Studies: Quantity, Preparation and Handling

Guidelines for all samples:

- Please notify us of any preservatives or additives that have been applied during the collection process.
- Please use permanent markers (e.g. Sharpie) to directly label tubes since labels may fall off when frozen.
- Please use polypropylene tubes for sending samples.
- Please send an *electronic* document containing sample ID information—preferably in advance of sending the samples. We will gladly supply an Excel template for your use. Send this file to **karibond@umich.edu**.
- Please send samples to: Kari Bonds, 2900 Huron Parkway, Ann Arbor, MI 48105

Blood Plasma and Serum

1. a. **Plasma:** Collect whole blood in tubes (e.g., Vacutainer or Vacuette) containing EDTA (K2, K3, or Na) as anti-coagulant. It is necessary to mix the tube well after collection in the vacutainer, prior to centrifugation. After mixing, centrifuge the samples at ~ 1,000 x g for 10-15 minutes. The clear, upper layer is plasma.
   b. **Serum:** Collect whole blood in serum separator tubes and follow tube manufacturer's processing instructions.
   - Avoid having samples sit at room temperature for more than 15 minutes.

- Immediately aliquot the resulting plasma/serum into chilled, polypropylene tubes. We recommend the following: Sarstedt tubes with O-ring seal, 2 mL (Sarstedt Cat # 72.694.006 or Fisher Cat # 50809242). Eppendorf (Brinkmann), 1.5 mL polypropylene may also be used but are the least preferred (Brinkmann Cat # 022363204 or Fisher Cat # 05-402-25).

2 Volume: 500 µL is preferable if available but we can process as little as 150 µL. The amounts provided must be consistent throughout the supplied samples.

3 Immediately freeze the tubes containing the plasma/serum. Flash freezing in liquid nitrogen is ideal. However, if these resources are not available, immediate placement of tubes in a -80°C freezer is acceptable. Storage should remain at this temperature until shipment.

4 Samples are to be shipped on dry ice. Tubes should be clearly labeled with sample identifiers and include a hard copy of the list of samples to accompany the shipment. Please include all available sample information when shipping samples, excluding any patient identifiers.

5 NB: As a general sample collection guideline, 1.8 ml of whole blood will yield ~1 mL of plasma. When collecting, it is important to treat all of the samples the same to avoid variation. Materials collected for other experimental work and stored at -80°C can be used for metabolomic studies as long as all of the samples were treated the same way during the collection process. **Consistency in sample handling is very important.**

Plasma Anticoagulant Guidelines:

- Best Results: EDTA (K2, K3, or Na)

- Acceptable Results: Citrate
- Least Desirable: Heparin

Urine

1. Collect 500 µl of urine sample in sample vials as described above (step 3 in Blood/Plasma section).
2. Immediately after collection, freeze sample and store as quickly as possible.
3. Ship frozen samples on dry ice.

Tissues

1. For collected solid tissues (e.g. biopsy material), the amount of tissue/sample can vary depending upon study objectives and tissue type—typically, a 100 mg sample will suffice. However, the exact weight of tissue collected is best determined for each study on a case-by-case analysis during experiment design discussions with us.
2. Transfer sample to Wheaton Cryovials, 2.0 mL polypropylene. (Wheaton Cat # 985734 or Fisher Cat # 03-341-18D).
3. Flash-freeze sample (as described above) and store at -80°C until shipped.
4. Ship frozen tissues on dry ice. Where possible, the material should be shipped in tared vials.

Cell Cultures

1. Cultures should be prepared with cells grown in the presence and absence of drug at the concentrations defined in the study design. Harvest the cells after incubation is complete and prepare the shipment as described below. Optionally, save cell media during harvesting for subsequent metabolomic analysis.
2. Each sample should optimally contain 1 x 10^7 cells, preferably 3-5 x 10^6 cells, and at a minimum 1 x 10^6 cells. It is preferable that each vial sent to us contains the same amount of cells. Please notify us if it is difficult to obtain equivalent amounts of cells in each vial.
3. Trypsinize or scrape cells according to established conditions in your laboratory. Gentle trypsinization may result in a more reproducible yield of cells and less cell lysis.
4. Transfer the trypsin-cell suspension to 15 mL polypropylene tubes (Falcon Tube, 15 mL polypropylene or BD-Falcon Cat # 352097 or Fisher Cat # 14-959-70C) or, if a larger volume is needed, a 50 mL polypropylene Falcon Tube may be used (Falcon Cat # 352070 or Fisher Cat # 14-432-22).
5. Spin at about 750-1000 x g for 1-3 minutes to pellet cells, or use other conditions that have been optimized in your laboratory for the specific cell line in question. Avoid spinning conditions that will lyse the cells.
6. Remove the upper trypsin solution.
7. Add PBS and gently re-suspend the pellet
8. Spin and collect the cell pellet.
9. If your process is amenable, repeat steps 7 and 8 two additional times to remove as much residual media as possible.
10. On the final spin, make certain that all solvent is removed to ensure a dry pellet for freezing.

11. Flash-freeze the pellet and store cell pellets at -80°C. Ship on dry ice.