

General Sample Collection 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.
- Use permanent markers (e.g. Sharpie) to directly label tubes since labels may fall off when frozen.
- Send an *electronic* document containing sample ID information—preferably in advance of sending the samples. We will supply an Excel template for your use. Send the completed file to **karibond@umich.edu**.
- Ship samples to: Ms. Kari Bonds, University of Michigan, Brehm 5458, 1000 Wall Street, Ann Arbor, MI 48105-5714.

Blood Plasma and Serum

1. **Plasma:** Collect whole blood in tubes containing EDTA (K2, K3, or Na) as anti-coagulant. Extract plasma, cool or freeze immediately, and transfer to appropriately labeled tubes. Freeze as soon as practical, and store frozen. Frozen samples being stored longer than 1 week should be stored at -80°C before shipment on dry ice.
Serum: Collect whole blood in serum separator tubes and follow tube manufacturer's processing instructions. Extract serum, transfer to appropriately labeled tubes, and store at -80°C immediately. Avoid having samples exposed to room temperature for more than 15 minutes.
2. Volume - 500 µL is preferable, if available, but we can process as little as 100 µL. Limiting volume could possibly limit the amounts of requested analysis.
3. Samples are to be shipped frozen and 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.
4. 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.

Urine

1. Collect a minimum of 5 mL whole urine (first catch preferred).
2. Immediately after collection, place sample at -80°C.
3. Samples are to be shipped frozen and 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.

Saliva

Collect saliva samples in a non-stimulated fashion. Dietary restrictions are not necessary, but food intake should be documented. Instruct research subjects to rinse their mouths vigorously three times

with at least 30 mL of water before saliva collection, without brushing their teeth or using any mouthwashes. Five minutes after the water rinse, collect about 3 ml of saliva in a clean plastic container by spitting. Freeze samples as soon as possible and maintain at -20°C for up to one week. Samples stored for more than one week before analysis should be stored at -70°C .

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.
2. Flash-freeze sample immediately after sectioning and store at -80°C until shipped.
3. Samples are to be shipped frozen and 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.

Fecal/intestinal samples

1. Collect and weigh 50-100mg of fecal, cecal, or chyme samples in a 1.5ml eppendorf tube with sample identification information.
2. Immediately store samples frozen at -80°C
3. Samples should be shipped frozen on dry ice. Please include a hard copy of sample list with all available information in the package.

Cultured Cells-see the recommended general procedure below.

1. Cultures should be prepared with cells grown as defined per your individual study design.
2. Each sample should optimally contain 1×10^7 cells, preferably $3-5 \times 10^6$ cells, and at a minimum 1×10^6 cells. (Cell size will heavily impact this request, smaller the cell size the higher the cell count should be to optimize preparation and requested analysis.) No cell count will be performed on individual samples within our preparation method hence it is preferable that each plate sent to us contains the same amount of cells.
3. Remove media as quickly as possible and freeze immediately if destined for metabolomics analyses. Most attached cells may be washed for less than 5-10 seconds in distilled water or isotonic buffer. See the recommended general procedure below for more details.
4. Flash-freeze the plate using liquid nitrogen if possible and store at -80°C . Samples are to be shipped frozen on dry ice. Plates should be wrapped in aluminum foil before storage and shipment. Samples 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

Recommended general procedure for cell treatment for metabolomic analysis

1. Cell growth and experimental manipulation:
 - Plate cells as needed for experiment. Can use individual 100 mm or 60 mm plates. Multi-well plates (e.g., 6-well, 12-well, or 24-well plates) are not recommended since they cannot be quenched (with liquid nitrogen) individually. For untargeted metabolomics analyses, a minimum of 3 replicate plates per group are required; 5 or more are recommended.

- When ready, perform any desired manipulations of experimental factors according to your preferred protocol. If duration of treatment is critical, then stagger the start time for different plates, to allow time to perform the rinse/quench procedure individually on each plate (see section 2)
- Once all experimental manipulations are complete and all needed media for extracellular metabolomics has been harvested, proceed to section 2 for intracellular metabolomics.

2 Rinse and quench (preparation for intracellular metabolomics):

- The following steps are easiest carried out outside of a cell hood (the short duration of time ensures that contamination from the non-sterile environment will not significantly affect the cells' metabolome) and close to the incubator.
- Assemble all needed supplies:
 - o vacuum aspirator, preferably with tip held by a clamp to allow hands free use,
 - o liquid nitrogen in a small hand-held dewar,
 - o dry ice in a cooler (preferably with a box to contain sample plates)
 - o Deionized water (rinse buffer) in dispenser-top bottle
 - Generally, our experience is that reasonably robust adherent cells are not harmed by very brief exposure to deionized water. If 5-10 second exposure to deionized water is intolerable for your cells, as an alternative, 150 mM ammonium acetate in water can be used. This salt dissolves to give a solution with a near-neutral pH and is "gentler" on cells than pure water, but it is more mass spectrometer compatible than inorganic saline solutions.
 - o Dispenser-top bottle pre-set at the appropriate volume (~4mL for 60 mm plates, ~10mL for 100 mm plates), or un-capped tubes filled with the appropriate volume of rinse solution for rapid dispensing of rinse solution.
- a) Remove one (or a few) plates from incubator. Working one plate at a time, thoroughly aspirate media.

Do the following 3 steps (b-f) very rapidly, so that the cells are exposed to water or rinse buffer for no more than approx. 5 seconds:

- b) Add deionized water rinse buffer to plate (either dispense using dispenser top bottle or dump from tube with pre-measured volume). Use a volume somewhat greater than the volume of media which was in the plate.
- c) Aspirate all rinse buffer from plate.
- d) Set plate on bench top and pour liquid nitrogen directly onto surface of open plate to rapidly freeze cells.
- e) Once most vigorous bubbling stops but while some liquid nitrogen remains at bottom of wells/plate, transfer plate to cooler with dry ice. Repeat the last 5 steps until all plates are quenched.
- f) Cover all plates and store at -80°C until ready for shipment. This is the preferred procedure to collect and ship samples (keep hard frozen, -80°C or on dry ice, at all times during transport).

Alternatively, pre-extracted samples can be sent. Please contact us for further details on extraction procedure if desired. See the reference below (Lorenz et al.) for more details.

Suspended Cell Cultures (in development-call for current details)

Bacteria and Yeast (in development-call for current details)

References

1) Lorenz MA, Burant CF, Kennedy RT. Reducing Time and Increasing Sensitivity in Sample Preparation for Adherent Mammalian Cell Metabolomics Anal Chem. 2011 May 1;83(9):3406-14