

MS Metabolomics Policies and Guidelines for Operation and Sample Preparation/ Quality Control

All projects require submission of a request for project (RFP) application through iLAB and approval for relevance and scientific merit. Before initiating the project and submitting samples, a meeting is required with Principle Investigator requesting the service and Core directors and biostatisticians for design of the project. At that time, investigators will receive additional information on sample preparation and scheduling of the project. At the completion of the project another exit meeting with the Principle Investigator is required to receive the report and explanation of the tier 1 data analysis. Data analysis will not be released without this meeting.

All users of the Core are required to sign up on iLABs for requesting services and providing charge sources for Core charges. Sample submission and preparation for analysis by the Core must be accompanied by a completed sample submission form. BCM investigators may complete and submit the form online ([iLabs](#)) or by email (MetabolomicsCore@bcm.edu) electronic copy and below the email id's of the core directors/staff.

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Rules and requirement for Collection and Submission of Samples

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. Send the completed xl file to core directors.
- ❖ All Samples should 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. Please include all available sample information when you submit the samples, excluding any patient identifiers.

- ❖ In case of submitting samples in person, after submitting the samples please send a follow-up e-mail to core directors and notify the name of the person who received your samples.

Cell line samples:

After growing cells harvest and process as follows:

- ❖ Aspirate the medium and wash the cells in the plate with PBS. Trypsinize cells with Trypsin/EDTA. Add complete medium with serum to inactivate trypsin, transfer the cells into a falcon tube, centrifuge for 5 min at 800 rpm (250xg).
- ❖ Aspirate the supernatant and re-suspend the cell pellet in 1 ml PBS and transfer to a 2 ml conical Eppendorf micro centrifuge tube. Spin and aspirate the supernatant. Repeat PBS wash 2 times. After 3rd wash, re-suspend the pellet in 1 ml PBS and count cells. (All samples should be in equal number.) Centrifuge and aspirate all the supernatant and freeze pellet. Store the solid pellet at -70°C until ready for use.
- ❖ A minimum of 3-5 million cells/pellet is required. We recommend 4-5 biological replicates for each experimental group.
- ❖ During transferred the cells to core, cells should be in dry ice, and make sure all label is clear and readable. For metabolic flux analysis, please contact to Core Directors for further instructions.

Note: Additional details on cell cultures and culture conditions are required. This is critical as it can effect detection of metabolites. This information must be provided on sample submission form.

- ❖ For permanent cell lines provide cell line name, species and tissue source. For primary cell cultures whether it is human patient derived and from what tissue. For primary cultures from animal tissues the species and tissue sources
- ❖ Provide **detailed and accurate** information on culture conditions and treatments.
- ❖ Culture medium, serum and other supplements:
- ❖ Provide information on any drug treatment of cell cultures: Drug name, dosage, and chemical structure. Drugs can potentially interfere with our analysis.
- ❖ Knockdown treatment of cells. (si or shRNA). Documentation of knockdown efficiency is required (protein data).

Tissue samples:

- ❖ Flash freeze tissues and store at -140 °C. A minimum of 100 mg of tissue is required for each sample for each assay.
- ❖ Please weigh each tissue, and make sure the tissue weight (min. 100 mg). During transferred the tissues to core, tissues should be in dry ice, and make sure all label is clear and readable.
- ❖ Core personnel will again weigh each tissue sample provide and perform all subsequent processing of tissue samples.

Note:

When collecting tissues blood/water should be removed as completely as possible prior to freezing. In case of blood, please remove the blood by spinning the blood samples (13,000 rpm; 5 mins). Before frozen the tissue samples use the Kim wipe paper to remove the water droplets before putting in to the tube. Use the high quality Cryo Tubes (Corning Cryogenic Vials; Part number: 430488-2ml).

Serum samples: Volume -500 μ L is preferable, if available, but we can process as little as 100 μ L. Limiting volume could possibly limit the amounts of requested analysis. All blood cells must be removed completely by centrifugation (~13,000 rpm; 15 mins). Collect whole blood in serum separator tubes and follow tube manufacturer's processing instructions. Extract serum, transfer to appropriately labeled tubes (Color less high quality silicon coated Eppendorf) and store at -80°C immediately. Avoid having samples exposed to room temperature for more than 15 minutes. Provide information on the volume and species (human or other) and labeled should be very clean.

Urine samples: A minimum of 500 μ L of serum is required for each sample. Cells must be removed from urine completely by centrifugation (~13,000 rpm; 15 mins). Urine should be frozen and stored at -70C (Color less Silicon coated Eppendorf). Provide information on the volume and species (human or other) and labeled labelling should be legible.

Sample submission and Q/C process: Samples must be prepared by above procedures and parameters to be accepted by the Core for further processing and mass spectrometry analysis. If sample amounts did not meet minimum criteria (ie number of cells, tissue weight, serum/urine volume) or quality (variation in cell numbers between samples, damaged tissues or serum without complete removal of blood cells) the Core reserves the right to reject samples for further analysis. The Core will consider analysis

of rejected samples in cases when they are irreplaceable or too costly to replace. Under these circumstances analysis can only proceed with the understanding and acknowledgment by the Principle Investigator of the risk of failure to obtain quality results and an agreement to pay Core charges regardless of the results.

In special cases with primary cultures from patient material the core will require preliminary samples for a pilot MS analysis in advance of the entire sample set to determine Q/C and to fine-tune parameters. If the pilot analysis fails to detect metabolites accurately the Core will not proceed with the entire set of experimental sample. After pilot samples have passed Q/C the Core will proceed with analysis of the experimental samples.

All samples of an approved project must be collected before submission to the Core and for analysis. The Core will not process partial samples from an experimental protocol. Samples will only be processed all together. After receiving samples that pass Q/C Core personnel will perform all procedures including extractions of samples for different class of metabolites, column chromatography for each class of metabolites, mass spectrometry analysis, Q/C and normalization of mass spectra and tier 1 analysis of data.