METABOLON®

SAMPLE PREPARATION
AND SHIPPING GUIDELINES

THESE GUIDELINES PROVIDE STANDARD SAMPLE PREPARATION AND SHIPPING PROCEDURES FOR MOST METABOLON STUDIES (METABOLOMIC AND LIPIDOMIC ANALYSES).

GENERAL SAMPLE GUIDELINES

Collect

- **Consistency in sample handling is very important!** Minimize operational variability wherever possible (e.g. collection technique, time of sampling, time to freezer, etc.). Materials collected for other experimental work and stored at -80°C may be suitable for metabolomic studies, depending on the collection procedure and consistency of treatment across all samples.
- Please notify your Metabolon Study Director of any preservatives that will been used in the collection process.
- Please use polypropylene tubes with external threading (2.0 mL cryovials or 1.5 mL Eppendorf) for sample storage/shipment.
- **For studies combining metabolomics with complex lipid analysis**, be sure to collect **two aliquots** of the recommended volume/amount required for each sample.

Label

- All tubes must be clearly labeled with unique sample identifiers. Please use permanent markers (e.g. Sharpie) to directly label tubes while warm; cold tubes will not hold the ink. Note that stick-on labels tend to fall off when frozen, so unless specialized freezer-safe labels are available, direct marking is preferred.
- Samples may be labeled with 2D barcodes, but please also include an identifier on the tube that can be read by eye.

Freeze

- Flash-freezing of samples in liquid nitrogen is ideal and recommended across all sample types. If these resources are not available, immediate placement of tubes in a -80°C freezer or dry ice/ethanol bath is acceptable. Note, however, that ethanol may wash off labeling.

Ship

- Your Metabolon representative will provide a sample manifest template that will need to be completed for each shipment. **Please include a hard copy of the completed manifest with your sample shipment.**
- Prior to shipping the samples, please send an electronic copy of the completed sample manifest in Excel format to: ***samplemanager@metabolon.com***. **Please include your assigned project code in the subject line.**
- The completed manifest:
  - ✓ **Must** include the project code on the cover page **and** in the subject line of email for the electronic copy
  - ✓ **Must** include all available sample information for each sample shipped, including any preservatives used in the collection/storage process
  - ✓ **Must NOT** include personally identifying information for human samples

Ship samples overnight on 8-12 kg of dry ice** to:

Metabolon Sample Acceptance
617 Davis Drive, Suite 400
Durham, NC 27713
Phone: +1.919.572.1711

**8 kg is the recommended minimum amount of dry ice for domestic shipping.
12 kg is the recommended minimum amount of dry ice for international shipping.

Remember to include a hard copy of the completed sample manifest with your sample shipment!

SAMPLE-SPECIFIC GUIDELINES

The sample guidelines below outline optimal sample amounts for any metabolomics or lipidomics analysis conducted by Metabolon. A lower sample volume may be acceptable depending on the nature of your study. Contact your Metabolon representative if you are considering a lower sample volume/amount.

Please remember that for combined metabolomics/lipidomics studies, two aliquots of the recommended volume are required for each sample.

Blood Samples

1. Volumes of 100-200 µL are preferable. Sample collection should be conducted in the following manner:
   - **Whole blood:** Whole blood must be prevented from coagulating, preferably by the addition of EDTA.
   - **Serum:** Collect whole blood in serum separator tubes and follow tube manufacturer's processing instructions.
   - **Plasma:** Collect whole blood in tubes (e.g. Vacutainer or Vacuette) containing anti-coagulant and follow tube manufacturer's processing instructions.

     Plasma Anticoagulant Guidelines:
     - Best Results: EDTA (K2, K3, Na; avoid Li).
     - For Metabolomics studies, avoid Citrate.
     - For Lipidomics studies, avoid Heparin.
     - **For all studies, never include multiple anticoagulant sample types in the same experiment.**
2. Immediately aliquot the collected plasma/serum/whole blood into chilled, polypropylene tubes and flash-freeze. Store samples at -80°C until shipment.

Urine Samples

1. Collect 100-200 µL of urine sample in polypropylene tubes and flash-freeze immediately. Store samples at -80°C until shipment.

Fecal Samples

1. 100mg (wet weight) of fecal material is preferable for analysis but 50 mg is acceptable.
2. Collect material, lyophilize if possible and transfer to a polypropylene tube for immediate freezing and storage at -80°C until shipped.

Tissue Samples

1. For collected solid tissues (e.g. biopsy material), the amount of tissue/sample can vary depending upon study objectives and tissue type. 100 mg is recommended, but smaller amounts may suffice. Contact your Metabolon representative for sample sizes below 50 mg.
2. Transfer sample to polypropylene tubes and flash-freeze immediately. Store samples at -80°C until shipment.

Cerebrospinal Fluid (CSF)

1. Collect 100-200 µL of CSF sample in polypropylene tubes and flash-freeze immediately. Store samples at -80°C until shipment.

Cell Culture Samples (Eukaryotic and Bacterial)

1. In general, a 100 µL packed cell pellet is preferable for analysis, but a 50 µL packed pellet is acceptable. For all lipidomics studies, an accurate cell count for each sample must be provided.
2. Cultures should be prepared with cells grown under various conditions as defined in the study design. (Note that if the study conditions lead to large differences in cell volume between treatment groups, this may affect results). Harvest the cells and prepare for shipment as described below. Optionally, save 200 µL cell culture media and several aliquots of fresh media [spin to pellet residual cells and debris and collect top layer (supernatant)] during harvesting for metabolomic analysis.
3. Ideally, each vial sent to Metabolon contains the same number of viable cells, but in any case the cell counts for each vial should be defined. For non-attached cells, go to step 5.
4. For attached cells, trypsinize or scrape cells according to established conditions in your laboratory. Gentle trypsinization may result in a more reproducible yield of cells and reduced cell lysis.
5. Transfer the cell suspension to 15 mL polypropylene tubes or, if a larger volume is needed, a 50 mL polypropylene Falcon tube may be used.
6. Spin at approximately 750-1000 x g for 1-3 minutes to pellet cells, or use other conditions that have been optimized in your laboratory. Avoid spinning conditions that will lyse the cells. Remove and discard all but ~0.75 mL of the supernatant.
7. Gently re-suspend and transfer cells to pre-labeled 2.0 mL polypropylene tubes.
8. Spin (750-1000 x g for 1-3 minutes) and carefully remove all supernatant. If possible, use narrow-bore (gel-loading or small volume) micropipette tips to minimize buffer residual and ensure a dry pellet for freezing.
9. Flash-freeze sample (as described above) and store at -80°C until shipment.

Plant Samples

1. Preferably, grind fresh plant material to a powder with a mortar and pestle under liquid nitrogen. The powdered material should be lyophilized if possible. Transfer the powder to a polypropylene tube for shipping.
2. Each powdered sample should contain approximately 50 mg or more (dry weight), or 100 mg or more (fresh weight) of plant material. Store at -80°C until shipped.

For other sample types or questions, please consult with your Metabolon representative. If you have an assigned project code, please include it in the subject line of any email correspondence.

METABOLON®
Where **knowing** comes to **life**™

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