MtoZ Biolabs

# MtoZ Biolabs Sample Submission Guidelines for Metabolomics

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# Contents

# Introduction

In metabolomics analysis, sample quality is the primary factor influencing data reliability and biological interpretability. Metabolites are highly diverse and inherently unstable, making them prone to degradation, transformation, or loss during collection, processing, or transportation. Such changes may obscure true physiological differences or introduce false variations, ultimately affecting pathway analysis and biomarker identification.

Only when samples are collected in a standardized manner, processed promptly, and stored and transported properly can metabolomics data achieve high throughput, high sensitivity, and strong reproducibility. This is especially critical in quantitative comparisons, time-course studies, or multi-omics integrative analyses, where standardized sample handling is essential for minimizing systematic errors and increasing experimental success rates.

To ensure the accuracy and biological interpretability of metabolomics analysis results, a standardized workflow for sample collection, processing, and submission is essential. MtoZ Biolabs offers a comprehensive suite of metabolomics services, including targeted metabolomics, untargeted metabolomics, and lipidomics, utilizing major analytical platforms such as LC-MS/MS, GC-MS, and NMR. To assist clients in efficiently preparing and submitting samples, we have developed this guideline, which provides detailed instructions on sample types, collection, storage and transport conditions, and recommended submission volumes.

## Sample Types and Submission Requirements

## 1. Untargeted Metabolomics

| Sample Type | | LC-Based Untargeted Metabolomics/ Herbal Medicine Untargeted Metabolomics/ Small Molecule Identification | | GC-Based Untargeted Metabolomics | | Untargeted Lipidomics | |
|---|---|---|---|---|---|---|---|
| | | Recom-mended quantity | Mini-mum quantity | Recom mended quantity | Mini-mum quantity | Recom-mended quantity | Mini-mum quantity |
| Animal Tissues (Brain, heart, liver, spleen, lung, kidney, muscle, skin) | | 100mg | 50mg | 30mg | 20mg | 30mg | 20mg |
| Plant Tissues (Leaves and flowers of woody plants, herbaceous plants, algae, ferns, and macrofungi) | | 200mg | 100mg | 200mg | 100mg | 200mg | 100mg |
| Cell Pellet (Adherent/ Suspension cells) | | $1x10^7$ | $5x10^6$ | $1x10^7$ | $5x10^6$ | $1x10^7$ | $5x10^6$ |
| Microbes (Common bacterial or fungal cell pellet) | | 200μL | 100μL | 100μL | 50μL | 100μL | 50μL |
| Body Fluids | Serum/ Plasma | 200μL | 100μL | 10μL | 5μL | 100μL | 50μL |
| | Urine | 2mL | 1mL | 2mL | 1mL | 2mL | 1mL |
| | Cerebros-pinal fluid | 2mL | 1mL | 0.2mL | 0.1mL | 2mL | 1mL |
| | Tears | 2mL | 1mL | 0.5mL | 0.2mL | 2mL | 1mL |

| | | | | | | |
|---|---|---|---|---|---|---|
| Culture Supernatant (Fermentation/cell culture supernatant) | 2mL | 1mL | 20mL | 10mL | 2mL | 1mL |
| Feces/Intestinal Contents | 200mg | 100mg | 20mL | 10mL | 200mg | 100mg |

## 2. Targeted Metabolomics

| Sample Type | | Broad Targeted Metabolomics | |
|---|---|---|---|
| | | Recommended quantity | Minimum quantity |
| Animal Tissues | Common soft tissues (e.g., brain, heart, liver, spleen, lung, kidney, muscle, skin) | 100mg | 50mg |
| Plant Tissues | Common soft plant tissues (e.g., leaves and flowers of woody plants, herbaceous plants, algae, ferns, and macrofungi) | 200mg | 100mg |
| Others | Same as "LC-Based Untargeted Metabolomics" | | |

# Sample Collection, Processing, Storage, and Transportation

To preserve the native state of metabolites within samples, collection procedures must follow the fundamental principles of speed, low temperature, and standardization. It is recommended to collect samples within a unified time window to ensure that experimental and control groups are consistent in sampling conditions, handling procedures, and processing times. Continuous metabolic activity should be avoided throughout the process. Whenever possible, samples should be flash-frozen in liquid nitrogen or immediately transferred for low-temperature centrifugation to minimize biological degradation and external interference, thereby providing a reliable foundation for subsequent metabolomics analysis.

# 1. Sample Collection and Shipping Requirements

(1) Maintain the required storage temperature throughout collection and shipping. For multi-omics analysis, it is advised to aliquot the sample during collection to avoid repeated freeze–thaw cycles.
(2) Use ultrapure water for all steps involving water during sample collection. For procedures requiring organic solvents, reagents should be of chromatographic grade or higher.
(3) Use high-quality centrifuge tubes for sample handling. After filling, seal the tubes securely with sufficient sealing film to prevent leakage or contamination (recommended brands: Eppendorf, Axygen, Corning, etc.).
(4) To avoid issues with instrument or software recognition, sample names should be in alphabetic characters only. If symbols are necessary, use underscores "_" instead of special characters.
(5) Label samples using permanent markers in multiple locations. Wrap tubes with shock-absorbing material to prevent damage during low-temperature transportation.
(6) Use double-layered foam boxes for shipping. Include sufficient dry ice: 5 kg per day in summer and 4 kg per day in winter. Prepare the amount of dry ice based on expected shipping duration.

# 2. Sample Collection, Processing, Storage, and Transportation by Sample Type

## 2.1 Animal Tissue Samples

### 2.1.1 Common Animal Tissues (e.g., brain, heart, liver, spleen, kidney, lung)

(1) Precisely excise the target tissue, promptly remove fat and connective tissue or any non-research-relevant components, and divide the sample into 20–50 mg pieces (approximately the size of a soybean);
(2) Quickly rinse the sample in saline or PBS to remove blood and surface contaminants;
(3) Use tweezers to transfer the sample into a pre-labeled centrifuge tube, flash-freeze in liquid nitrogen for 10 minutes, and store at -80°C;
(4) Ship on dry ice and avoid repeated freeze-thaw cycles.
Note: Tumor tissues should be sectioned based on pathological guidance; adjacent non-tumor tissues should be collected at least 2 cm away from the lesion.

## 2.2 Plant Tissue Samples

### 2.2.1 Common Plant Tissues (e.g., leaves, flowers, ferns)

(1) Harvest the specific part of the plant, remove non-target tissues, rinse off soil and visible impurities, and gently blot dry with lint-free paper;

(2) Wrap the sample in aluminum foil or place it in a centrifuge tube, label properly, flash-freeze in liquid nitrogen for 10 minutes, and store at -80°C;

(3) Ship on dry ice and avoid repeated freeze-thaw cycles.

### 2.2.2 Algae

(1) Apply appropriate centrifugal force according to the algal type to separate algae while maintaining cell integrity. Wash 2–3 times with PBS, flash-freeze in liquid nitrogen for 10 minutes, and store at -80°C;

(2) Ship on dry ice and avoid repeated freeze-thaw cycles.

## 2.3 Cell Samples

### 2.3.1 Suspension Cells

(1) Centrifuge at 1,000 g for 10 minutes to collect cells; discard supernatant.

(2) Wash with cold PBS 2–3 times. Centrifuge and discard the supernatant. Collect pellet into a 1.5 mL centrifuge tube.

(3) Flash-freeze in liquid nitrogen for 10 minutes. Store at –80°C.

(4) Ship on dry ice. Avoid freeze-thaw cycles.

### 2.3.2 Adherent Cells

(1) Discard the culture medium, invert dish onto absorbent paper to remove liquid.

(2) Add pre-chilled PBS (4°C), gently shake horizontally for 1 minute to wash the cells, and discard the PBS.

(Repeat steps (1) and (2) twice to discard the culture medium)

(3) Digest cells with trypsin on ice. Resuspend in pre-chilled PBS.

(4) Collect cell suspension into 15 mL tubes, centrifuge at 1,000 g for 10 minutes at 4°C. Discard PBS.

(5) Resuspend in 1 mL PBS, transfer to a new 1.5 mL tubes, centrifuge, discard PBS, and retain pellet. Flash-freeze 10 minutes in liquid nitrogen, store at –80°C.

(6) Ship on dry ice. Avoid freeze-thaw cycles.

## 2.4 Liquid Samples

### 2.4.1 Serum

(1) Collect blood using vacuum tubes without anticoagulant (red cap recommended) or clean tubes.

(2) Gently invert 8–10 times, rest at 4°C for 30 minutes.

(3) Centrifuge at 1,000 g for 10 minutes at 4°C.

(4) Transfer yellow supernatant (serum) to clean tube, mix, and spin briefly.

(5) Aliquot 100–200 µL per tube. Flash-freeze in liquid nitrogen for 10 minutes, store at –80°C.

(6) Ship on dry ice. Avoid freeze-thaw cycles.

### 2.4.2 Plasma

(1) Collect blood in anticoagulant tubes (EDTA, purple cap recommended) or clean tubes.

(2) Invert gently 8–10 times.

(3) Centrifuge at 1,000 g for 10 minutes at 4°C.

(4) Transfer yellow supernatant (plasma) to clean tube, mix, and spin briefly.

(5) Aliquot 100–200 µL per tube. Flash-freeze in liquid nitrogen, store at –80°C.

(6) Ship on dry ice. Avoid freeze-thaw cycles.

Note: Handle gently to avoid hemolysis. Samples should appear clear yellow; pink or red indicates hemolysis and may impact results.

### 2.4.3 Urine

(1) Collect midstream first-morning urine. Store temporarily at 4°C (not exceeding 8 hours). Avoid bacterial contamination.

(2) Centrifuge at 1,000 g for 15 minutes at 4°C to remove debris.

(3) Transfer supernatant to new tube, aliquot 1 mL per tube. Snap-freeze in liquid nitrogen, store at –80°C.

(4) Ship on dry ice. Avoid freeze-thaw cycles.

### 2.4.4 Cerebrospinal Fluid

(1) Collect using clinical procedures.

(2) Centrifuge at 1,000–2,000 g for 5 minutes at 4°C.

(3) (Optional) Filter supernatant with 0.22 µm membrane.

(4) Aliquot 100 µL per tube, flash-freeze in liquid nitrogen for 10 minutes, store at –80°C.

### 2.4.5 Tears

(1) Use capillary pipettes for collection. Centrifuge at 8,000–14,000 g for 5 minutes at 4°C.

(2) Transfer supernatant to tubes, flash-freeze in liquid nitrogen for 10 minutes, store at –80°C.

(3) Ship on dry ice. Avoid freeze-thaw cycles.

### 2.4.6 Culture Supernatant

(1) Remove media, collect cells following cell protocols.

(2) Replace with serum-free medium and continue culture for 24–48 hours based on growth rate.

(3) Centrifuge at 300 g for 10 minutes at 4°C.

(4) Transfer supernatant to a 15mL tube, centrifuge again at 3,000g for 15 minutes to remove cell debris.

(5) Transfer clarified supernatant to clean tubes, aliquot 5–10 mL per tube, flash-freeze in liquid nitrogen for 10 minutes, store at –80°C.

(6) Ship on dry ice. Avoid freeze-thaw cycles.

Note: Serum-containing medium may introduce high-abundance proteins, reducing protein identification.

## 2.5 Microbes

(1) Centrifuge at 5,000 g for 15 minutes at 4°C, discard supernatant.

(2) Resuspend the pellet in pre-chilled PBS, centrifuge and discard supernatant. Repeat 3 times.

(3) Transfer pellet to clean tube, aliquot 50–100 µL per tube. Flash-freeze in liquid nitrogen for 10 minutes, store at –80°C.

(4) Ship on dry ice. Avoid freeze-thaw cycles.

## 2.6 Feces and Intestinal Contents

### 2.6.1 Feces

(1) After defecation, use a sampling spoon to collect the mid-portion of the feces and transfer it to a labeled centrifuge tube;

(2) Aliquot the sample as needed, 100—500 mg per tube, flash-freeze in liquid nitrogen for 10 minutes, store at -80°C;

(3) Ship on dry ice. Avoid freeze-thaw cycles.

### 2.6.2 Intestinal Contents

(1) Excise the entire intestine, isolate the target intestinal segment, and cut it open longitudinally to expose the contents;

(2) Use a sterile sampling tool to collect intestinal contents into labeled centrifuge tubes (try to sample from deeper regions while avoiding mucosal lining and blood contaminants);

(3) Aliquot 100—500 mg per tube, flash-freeze in liquid nitrogen for 10 minutes, store at -80°C;
(4) Ship on dry ice. Avoid freeze-thaw cycles.

For sample types not covered in this guideline, or if you have any questions regarding sample preparation, handling, packaging, or transportation, please feel free to contact us. MtoZ Biolabs has an experienced metabolomics support team ready to provide one-on-one guidance on sample pre-processing and technical consultation. We are committed to ensuring your samples enter the analytical workflow in optimal condition, enabling efficient project execution and reliable data output.