Metabolomics Sample Submission Guidelines

This document serves as the sample submission guidelines for Creative Proteomics's metabolomics services. Accurate and representative sample preparation is crucial to ensure the scientific validity and reliability of experimental results. Proper handling, storage, and transportation of samples are essential to maintain their integrity and avoid contamination or degradation.

Our guidelines cover a range of sample types including tissues, cells, fluids, and environmental samples, each with specific requirements for preparation and submission. Adhering to these protocols will help in achieving accurate and reproducible results.

General Sample Preparation Guidelines

Representativeness: The representativeness of the sample is crucial for the scientific validity of the experimental results. Researchers should carefully design their sampling plans based on the experiment's objectives to ensure the samples accurately reflect the target conditions.

Accuracy: It is essential to accurately record all characteristics of representative samples. Samples should be collected, prepared, stored, and transported promptly and according to specified conditions (e.g., low temperatures) to maintain their integrity.

Timeliness: Sample quality is a critical factor influencing experimental outcomes. Therefore, minimize the time from sample collection to experimentation by ensuring rapid processing throughout collection, preparation, storage, and transportation stages.

Low Temperature: After collection, samples should be immediately flash-frozen in liquid nitrogen and subsequently stored at -80°C to prevent any degradation.

Precautions for Metabolomic Sample Preparation

1) As a general rule, we do not accept original experimental samples that are infectious or pathogenic. If a sample contains pathogenic microorganisms or infectious lesions, it must be thoroughly inactivated before extraction and submission. Please clearly indicate this on both the sample registration form and the sample label. Sales staff must verify with the technical team whether the project can be accepted before proceeding.
2) LC-MS does not accept oil samples (these are only accepted on the GC-MS platform).
3) If conditions permit, it is recommended to prepare an additional sample as a backup.
4) During sample collection, label and immediately freeze the samples in liquid nitrogen, store them at -80°C for long-term storage, and transport with dry ice to avoid repeated freeze-thaw cycles.
5) Biological replicates must be clearly labeled, preferably using -1, -2, -3, etc. For all sample group numbers, use letters and numbers (e.g., CK-1, Control-1).
6) If the sample contains toxic, polymer, surfactant or corrosive substances, this must be declared in advance and noted on the sample registration form and sample label.
7) For hard samples like wood and horn, customers should grind them before sending.
8) For large samples like soil, mix thoroughly and weigh an appropriate amount according to the sample preparation guidelines before sending. Avoid sending excessive amounts.
9) Some samples may expand in volume after freezing, which can lead to container rupture. Use sufficient dry ice for long-distance shipping.
10) Do not use aluminum foil or glass containers; use centrifuge tubes with screw caps for sealing.
11) Extracted samples (or samples containing liquids) should specify the solvent, components, or directly indicate the pre-treatment protocol (or process).
12) If customers have special pre-treatment requirements, communicate before starting the project, note it on the sample registration form, and provide methods or references, if possible, to avoid affecting

experimental results.

13) For targeted metabolomics involving method development, a pre-experiment sample must be provided, with a quantity 3-5 times that of the standard sample amount for method optimization. To avoid repeated freeze-thaw cycles, pre-package the samples.

14) For targeted detection, some metabolites may be present in very low concentrations. To obtain more accurate quantitative results, send as much of the sample as possible. For plant hormone targeted experiments, it is best to send more than 2g.

15) One cell sample can be used for both GCMS and LCMS projects. It is recommended that the number of cells in a single sample be greater than 10^7.

16) For other special samples not listed above, confirm the sample preparation plan with technical staff.

Metabolomic Sample Quantity Requirements

Sample Type	Untargeted Metabolomics	Targeted Metabolomics	Lipidomics	Metabolic Flux
Animal Tissue	100-200 mg	100-200 mg	100-200 mg	
Plant Tissue	100-200 mg	100-200 mg	100-200 mg	
Plasma/Serum	>100 μL	>100 μL	>100 μL	
Urine	200-500 μL	200-500 μL	200-500 μL	
Saliva, Amniotic fluid, Bile, Tears, etc.	>200 μL	>200 μL	>200 μL	
Cells	$>1*10^7$	$>1*10^7$	$>1*10^7$	$>1*10^7$
Culture Supernatant	>2 mL	>2 mL	>2 mL	
Wastewater/Culture Medium	>2 mL	>2 mL	>2 mL	
Microbial Culture	>2 mL	>2 mL	>2 mL	
Feces/Intestinal Contents	100-200 mg	100-200 mg	100-200 mg	
Soil Sample	>1 g	>1 g	>1 g	
Swab	2		2	

Metabolomic Sample Processing Methods

Tissue Sample

Animal Tissue Samples

- For whole tissue samples, use perfusion with pre-chilled deionized water to remove residual blood from the tissue.
- For partial tissue samples, rinse the tissue with pre-chilled deionized water after homogenization to remove blood residues.

Human Tissue Samples

- For small biopsy samples, quickly rinse with pre-chilled deionized water to remove blood residues.
- Collect specific tissue sections according to the experimental design, and place 100-200 mg/sample into centrifuge tubes.
- After labeling, rapidly freeze the samples in liquid nitrogen for at least 15 minutes.
- Store at -80°C. Ship with sufficient dry ice.

Notes:

- *For biological replicates, try to collect samples from the same anatomical location.*
- *During tissue collection and handling, avoid contamination from anesthesia, Eppendorf tubes, collection instruments, etc.*
- *Collect samples quickly and handle them on ice to minimize time stored at room temperature.*
- *Transfer samples to storage promptly to avoid repeated freeze-thaw cycles. For small tissue samples from individual experimental animals, such as zebrafish brain tissue or mouse hippocampus, please consult with the division.*

Cell Sample

Suspension Cells

Collect suspension cells by centrifugation. Wash cells 2-3 times with pre-cooled PBS, centrifuge at 1000g for 1 min at 4°C, and discard the supernatant. Collect the cell pellet (50 μL) in a 1.5 mL centrifuge tube. Quick-freeze in liquid nitrogen for 15 min and store at -80°C. Ship on dry ice.

Adherent Cells (untargeted metabolomics)

Remove the old culture medium and wash cells 2-3 times with pre-cooled PBS. Add 0.5 mL trypsin to digest cells. After digestion, wash cells 2-3 times with pre-cooled PBS to terminate trypsin activity. Transfer cells to a 1.5 mL centrifuge tube. Centrifuge at 1000g for 1 min at 4°C, and discard the supernatant. Collect the cell pellet in a 1.5 mL centrifuge tube. Quick-freeze in liquid nitrogen for 15 min and store at -80°C. Ship on dry ice. (Set up replicates for cell counting using the trypsinization method.)

Adherent Cells (targeted metabolomics)

Remove the old culture medium and wash cells 2-3 times with pre-cooled PBS. Add a small amount of PBS and scrape cells with a cell scraper into a 1.5 mL centrifuge tube. Centrifuge at 1000g for 1 min at 4°C, and

discard the supernatant. Collect the cell pellet in a 1.5 mL centrifuge tube. Quick-freeze in liquid nitrogen for 15 min and store at -80°C. Ship on dry ice. (Set up replicates for cell counting using the trypsinization method.)

Cell Culture Medium (for extracellular metabolite analysis)

Remove the old culture medium and wash cells 2-3 times with PBS. Add serum-free medium and continue culturing for a period of time. Based on the experimental design or cell status, collect more than 5 mL of culture medium from adherent cells. Centrifuge at 1000g for 1 min at 4°C. Collect the entire supernatant, quick-freeze in liquid nitrogen for 15 min, and store at -80°C. Ship on dry ice.

Cell Culture Supernatant

Remove the old culture medium and wash cells 2-3 times with PBS. Add serum-free medium and continue culturing for a period of time. Collect the cell supernatant at the appropriate time according to the experimental design or cell culture status. Centrifuge at 1000g for 10 min at 4°C to remove cells. Centrifuge the supernatant at 10000g for 10 min at 4°C to remove cell debris. Transfer the supernatant to 1.5 mL centrifuge tubes, quick-freeze in liquid nitrogen, and store at -80°C.

Note:

- *Ensure that the number of cells collected for each sample is consistent. The entire process should be completed as quickly as possible.*
- *The type of culture medium affects the types of metabolites. Ensure that the same type of culture medium is used throughout the cell culture.*
- *For extracellular metabolite analysis, use serum-free medium to ensure cell viability.*
- *Label each sample properly.*
- *After washing adherent cells with PBS, remove the supernatant as completely as possible to ensure that the volume is consistent for each sample.*

Cell Sample for Metabolic Flux Analysis

Suspension Cells

1) Count cells using a hemocytometer or a comparable system, then transfer the cell suspension from the culture dish to a centrifuge tube using a pipette (in a dry ice or dry ice/ethanol bath).
2) Centrifuge at 1000g for 2 min at 0°C, and remove the supernatant using a pipette or micropipette.
3) Add 1 mL of ice-cold PBS buffer to the centrifuge tube and wash cells (1-3 times). Centrifuge at 1000g for 2 min at 0°C, and remove the wash solution using a pipette or micropipette.
4) Store the sample at -80°C and ship on dry ice.

Adherent Cells:

1) Remove the culture medium from the culture plate (e.g., 6-well plate, 6 cm or 10 cm dish) and place on ice. Collect the culture medium and store at -80°C or in liquid nitrogen for extracellular metabolite analysis.
2) Add 1 mL of ice-cold PBS buffer to each well of the culture plate (assuming a 6-well plate) and wash cells (1-3 times). Discard the wash solution and keep the plate on ice.
3) Cover cells with liquid nitrogen to quickly stop cellular activity (optional but most effective).

4) Add 400 µL of pre-cooled methanol (after liquid nitrogen has evaporated) and gently vortex to ensure all cells are covered with methanol. Cap the tube and store at -80°C for at least 30 minutes.

5) Add 100 µL of pre-cooled ultrapure water and mix gently.

6) Use an appropriate cell scraper to scrape cells, ensuring that >90% of cells are detached from the culture material and suspended in the methanol-water solution. Collect cells and solvent in a suitable, well-sealed screw-cap centrifuge tube or cryogenic vial.

7) Add 200 µL of pre-cooled 80% methanol solution to wash the cell residue and combine the wash solution with the previously collected sample. Store the sample at -80°C and ship on dry ice.

Note:

If the sample collection method differs from the above, please attach the sample collection method along with the samples. Additionally, please provide information on labeled substrates and labeling types when submitting samples.

Plasma and Serum Samples

Plasma Samples

Collect whole blood in a sodium heparin anticoagulant tube. Centrifuge at 3000 rpm for 10 minutes at 4°C as soon as possible. Transfer 0.2 mL of the upper plasma layer to a 1.5 mL centrifuge tube. Label the tubes, quick-freeze in liquid nitrogen for 15 minutes, and store at -80°C. Ship on dry ice.

Serum Samples

Collect blood in a serum collection tube. Allow the blood to clot at 37°C (or room temperature) for 1 hour. Centrifuge at 3000 rpm for 5 minutes. Transfer the supernatant to a clean centrifuge tube. Centrifuge again at 12000 rpm for 10 minutes at 4°C (or complete the process with only one centrifugation). Transfer 0.2 mL of the supernatant to a 1.5 mL centrifuge tube. Label the tubes, quick-freeze in liquid nitrogen for 15 minutes, and store at -80°C. Ship on dry ice.

Note:

- *For animal samples, fast the animals for at least 10 hours before sampling. For clinical samples, maintain a light diet for 3 days prior to sampling and fast for 8 hours before sampling. Ensure consistency in sample handling, including sample storage time and centrifugation time.*
- *If using alcohol for skin disinfection, wipe the area dry and allow the alcohol to evaporate completely before sampling.*
- *Tube selection: For plasma, sodium heparin anticoagulant tubes (green cap) are recommended. Sodium citrate anticoagulant (blue or black cap) and EDTA anticoagulant (purple cap) may interfere with metabolites and cause matrix effects, but the interference from EDTA anticoagulant can be eliminated by desalting the sample. Please note if using EDTA anticoagulant tubes. For serum, use serum collection tubes without anticoagulant (red cap).*
- *It is recommended to collect as many aliquot samples as possible and store them in 1.5 mL centrifuge tubes. Avoid repeated freeze-thaw cycles.*
- *Reference yield for serum: 30%-50% (e.g., 1 mL of whole blood yields approximately 0.3-0.5 mL of serum). Reference yield for plasma: ≈50% (e.g., 1 mL of whole blood yields approximately 0.5 mL of plasma).*

Saliva Sample

Collect saliva between 9:30 AM and 11:30 AM. After rinsing the mouth with saline, spit the saliva into a sterile sputum cup (kept on ice). Avoid coughing. Collect a sufficient amount of sample within a short period of time. Centrifuge at 4°C, 2000g for 10 min. Collect the supernatant and filter through a 0.22 μm filter (optional). Aliquot into 2-3 tubes, quick-freeze in liquid nitrogen for 15 min, and store at -80°C. Ship on dry ice.

Notes:

- *Fast, do not smoke or drink for at least 1 hour before sampling. Avoid brushing your teeth before sampling.*
- *It is recommended to collect as much aliquot sample as possible and aliquot into 1.5 mL centrifuge tubes. Avoid repeated freeze-thaw cycles.*

Urine Sample

Collect midstream urine in the morning (for clinical samples) or 1-hour urine in the morning (for animal samples). Centrifuge at 3000 rpm for 10 minutes at 4°C. Transfer 500 μL of the clear supernatant to a centrifuge tube. Label the tubes, quick-freeze in liquid nitrogen for 15 minutes, and store at -80°C. Ship on dry ice.

Notes:

- *If the 1-hour urine volume is insufficient for animal samples, collect multiple times. For 24-hour urine collection, use a metabolic cage with a cooling device and add sodium azide (0.05-0.1% w/v).*
- *Sodium azide is a toxic preservative. Handle with extreme care.*

Tear Fluid Sample

Collect tear fluid between 9:00 AM and 12:00 PM. Ask the subject to look up and place the curved end of a pre-prepared Schirmer strip into the conjunctival sac at the outer 1/3 of the eyelid, with the other end hanging outside the eye. Ask the subject to gently close their eye. Collect for 5 minutes. Place the removed strip into a 1.5 mL centrifuge tube and quickly freeze in liquid nitrogen for 15 minutes. Store at -80°C until shipment. Ship on dry ice.

Note: *Collect no more than one tear sample per eye per day.*

Fecal/Intestinal Content Sample

Collect fresh fecal or intestinal content samples. Aliquot 200 mg of sample per tube and immediately freeze in liquid nitrogen for 15 minutes. Label the tubes and store at -80°C. Ship on dry ice.

Note:

Due to the high abundance of microorganisms in feces/intestinal contents and their rapid metabolic activity, repeated freeze-thaw cycles can significantly impact metabolite levels. It is recommended to aliquot samples into 200 mg per sample after collection and freeze immediately.

Plant Sample

Leaves, Stems, and Flowers

Take a whole leaf/stem segment/flower, wrap it in tin foil, label it, and quickly freeze in liquid nitrogen for at

least 15 minutes. Transfer the sample to a self-sealing bag (one bag per group), place a label with sample information inside the bag, and quickly store at -80°C. Ship on dry ice.

Notes:

- *Collect leaf samples preferably at noon when sunlight is abundant.*
- *Maintain sample consistency when collecting samples according to the experimental design, especially for samples within the same group (e.g., color, senescence, vein proportion, light exposure, location).*
- *Since marker pen marks on tin foil can easily smudge, it is recommended to also place a label with sample information inside the self-sealing bag.*

Roots

1) Take the entire root system of the plant and quickly rinse with 1×PBS to remove soil.
2) Collect specific root sections according to the experimental design. Dissect approximately 500 mg of tissue per sample and place in a centrifuge tube.
3) Label the samples and quickly freeze in liquid nitrogen for at least 15 minutes.
4) Store at -80°C. Ship on dry ice.

Notes:

- *Rinse with PBS as quickly as possible.*
- *Since root tissues are particularly fragile and can become mushy when squeezed, it is recommended to store them in centrifuge tubes instead of wrapping them in tin foil.*

Fruits and Seeds

For juicy and large fruits (e.g., tomatoes, watermelons, apples), cut them into "uniform" small pieces (≈250 mg/sample) using a sharp knife, and place them in 1.5 mL centrifuge tubes. Label the tubes and quickly freeze in liquid nitrogen for at least 15 minutes.
For small, granular seeds (e.g., Arabidopsis seeds, cereal grains), mix the seeds from the same group and then aliquot (≈250 mg/sample) into 1.5 mL centrifuge tubes. Label the tubes and quickly freeze in liquid nitrogen for at least 15 minutes.
For samples requiring extraction of the entire fruit (e.g., whole grapes), place the fruit in a 50 mL centrifuge tube or self-sealing bag, label it (and place a label with sample information inside the self-sealing bag), and quickly freeze in liquid nitrogen for at least 15 minutes.

Notes:

- *It is difficult to maintain uniformity when sampling juicy fruits (e.g., tomatoes, skin, flesh, pulp, seeds). It is strongly recommended to grind and lyophilize the entire fruit, and then weigh and aliquot the powder.*
- *It is not recommended to wrap in tin foil.*

Swab Sample

Throat Swab

Illuminate the open mouth of the patient with bright light from above the examiner's shoulder. Have the patient take a deep breath and say "ah," using a tongue depressor to gently press down the tongue. Then, use a clean, pre-weighed throat swab to swab both sides of the tonsils and the posterior pharyngeal wall. Place the swab head into a centrifuge tube, discard the tail, and tighten the lid.

Nasal Swab

Allow bright light to illuminate the nasal cavity. Gently insert a clean, pre-weighed nasopharyngeal swab into the nasal passage at the palatine region, leave it in place for a moment, then slowly rotate and withdraw it. Use a second swab to sample the other nostril. Place the swab head into a centrifuge tube, discard the tail, and tighten the lid.

Dental Plaque

Do not brush teeth the night before sampling; collect the sample the following morning before eating. First, rinse the collection area with sterile saline, and use a cotton roll to dry the area. Then, depending on the experimental purpose, use collection swabs to gather dental plaque from the tongue surface, lip surface, and carious damage areas of the teeth. It is recommended that the same person perform all plaque collection operations and that each sample be collected using a single swab by scraping back and forth 3-4 times at the same site. After collection, place the swab in a centrifuge tube for 15 minutes. Store at -80°C until shipment, and transport the sample on dry ice.

Note:

- *Weigh the swabs before and after sampling to determine the sample amount.*
- *During throat sampling, the swab should not come into contact with the oral or tongue mucosa.*
- *It is best to perform sampling within 72 hours of onset, preferably in the morning.*

Microbial Sample

Microbial Cell Pellet

Use centrifugation to collect the microbial cells (ensure that the volume of the cell pellet is consistent after centrifugation). Wash quickly 2-3 times with pre-cooled PBS. After each wash, centrifuge at 5000 rpm for 5 minutes at 4°C. Discard the supernatant completely and collect the microbial cells in a 1.5 mL centrifuge tube (or cryovial). Rapidly freeze in liquid nitrogen for 15 minutes, store at -80°C, and transport on dry ice.

Microbial Culture Medium (for studying extracellular metabolism)

Mix the cultured microbial liquid thoroughly, then take more than 5 mL of the culture liquid. Centrifuge at 3000 rpm for 10 minutes at 4°C, collect the supernatant, rapidly freeze in liquid nitrogen for 15 minutes, and store at -80°C. Transport on dry ice.

Microbial Solid Culture Medium

Cut the culture medium into square pieces with a blade, rapidly freeze in liquid nitrogen for 15 minutes, and store at -80°C. Transport on dry ice.

Note:

- *Microbial metabolism is very fast, so all steps should be completed as quickly as possible.*
- *Ensure the cell numbers between different samples are consistent.*
- *Generally, an OD600 between 0.6 and 0.8 indicates that the bacteria are in the logarithmic growth phase, with an approximate concentration of 10^8/mL. Verify based on specific conditions.*

Soil Sample

Collect soil samples and place them in centrifuge tubes. Freeze-dry to remove excess moisture. Sieve the soil using a 2mm sieve with medium-speed vibration for 2 minutes, and collect the sieved soil. Transfer 1g

of the collected soil into a 1.5 mL centrifuge tube. Quick-freeze in liquid nitrogen for 15 minutes and store at -80°C. Ship on dry ice.

Note:

Soil sieves and collection containers should be wiped with 70% ethanol and ensured to be dry before use.

Root Exudate Sample

Laboratory-grown Plants: Select healthy seedlings and rinse the roots with deionized water to remove residual soil. Culture the seedlings in a nutrient solution for 1-2 months. Select well-growing plants and remove them. Rinse the roots with deionized water 2-3 times to remove residual nutrient solution. Then, culture the plants in a suitable amount of deionized water for 24 hours (keeping the roots in the dark). Mix the hydroponic solutions from 3 or more individual plants, filter, and lyophilize. Store at -80°C.

Field Crops: Select healthy individual plants from the field. Dig out the entire root system and cover the roots with a bag, then bury it back in the original location for 72 hours. Remove the pre-treated plant, rinse the roots thoroughly with deionized water, and wrap the roots in moist filter paper. Place the wrapped plant in a 50 mL centrifuge tube, seal the tube, and bury it back in the original location. After 24 hours, cut off the root section covered by the filter paper, temporarily store it in an ice box, and transfer it to the laboratory. Add 100 mL of deionized water and shake for 30 minutes. Finally, filter the liquid and concentrate it by evaporation. Store the final liquid or dry powder at -80°C.

Recommended Biological Replicates

Sample Type	Recommended Number of Biological Replicates
Microorganisms and Plants	≥ 6-8
Model Organisms	≥ 8-10
Clinical Samples	≥ 30

Notes:

- *Under permissive conditions, it is recommended to prepare an extra sample as a backup;*
- *For projects with less than 10 samples, the laboratory does not perform quality control by default. If necessary, please note this when placing the order.*

For sample types not listed in this guide or for any specific inquiries regarding sample preparation, please contact our sales team. Our technical experts will assist in confirming the appropriate preparation procedures to meet your experimental needs.