Creative Proteomics

Proteomics Sample Submission Guidelines

Thank you for choosing Creative Proteomics. This document is a comprehensive sample submission guide for proteomics services. Ensuring the quality and integrity of your samples is crucial for obtaining accurate and reliable experimental results. This document outlines the best practices for preparing, storing, and transporting various types of samples to maintain their suitability for proteomics analysis. By adhering to these guidelines, you help ensure that your samples yield meaningful data and contribute to the success of your research.

Sampling Principles

Sample Representativeness

The collected samples should undergo strict identification through cytology, histology, pathology, and other relevant assessments, as this directly impacts whether the final experimental results are scientifically meaningful. Therefore, the customer must design a relevant sampling plan and procedures based on the purpose of the experiment.

Sample Accuracy

Normal tissue samples should not contain diseased tissue, and pathological tissue samples should not be contaminated with normal tissue. Whenever possible, efforts should be made to ensure that the samples from both the experimental group and the control group are consistent in terms of collection time, location, and processing conditions. Otherwise, this may affect the reproducibility and reliability of the experimental results. All characteristics of representative samples must be accurately recorded and collected, prepared, stored, and transported as required for the experiment.

Sample Reproducibility

When sampling for biological replicates, efforts should be made to minimize differences between replicates. As far as possible, replicates should be consistent in terms of collection time, location, and processing conditions, as any variations may impact the reproducibility and reliability of the experimental results.

Sample Timeliness

The quality of the sample is the most critical factor affecting experimental results. Therefore, the samples used for research should be collected, stored, transported, and prepared as quickly as possible, minimizing the time from sample collection to the experiment.

Sample Cryopreservation

After the sample is taken, it should be promptly washed, labeled, and immediately flash-frozen in liquid nitrogen for at least 3 hours, then stored in a -80°C freezer or dry ice. The sample should remain at -80°C before experimental procedures to prevent metabolic changes.

Special Treatment of Samples

Special treatments of samples may include salinity treatment, temperature treatment, drug treatment, viral infection, injury, drought stress, and other forms of stress treatment. These treatments may affect the sample quality to varying degrees, often leading to the degradation or reduction of proteins. Therefore, for samples that have undergone special treatment, it is essential to provide detailed notes in the sample information form to maximize the success of extraction and avoid sample waste.

Sample Submission Requirements

Protein Qualitative Sample Recommendations

Type/Detection	Shipping Suggestions
Gel strips, Gel spots	**Send Coomassie Brilliant Blue stained or silver stained dots/strips. Requires clear strips and no degradation.** Note: Coomassie Brilliant Blue stained bands have a higher probability of identifying target proteins than silver stained bands. The silver-stained gel must not contain glutaraldehyde. And ensure that the gel is cut and sent within one week after electrophoresis. Store at 4°C, place in 1.5/2 mL EP tubes, add deionized water to cover gel bands, and ship in ice-packs.
	Protein gel strips identification analysis (including single protein band identification): protein ≥ 20μg, blue or silver staining visible to the naked eye.
	Protein gel spots identification analysis: protein ≥5-10 μg ; blue or silver staining visible to the naked eye.
Interaction Profile (IP/Co-IP/Pulldown)	**Method 1:** SDS-PAGE gel of the protein eluate from IP/Co-IP/Pull-down. Do not run the entire lane. Stop electrophoresis when the sample has run 1-1.5 cm into the separating gel and cut the gel for sending.
	Method 2: Send IP elution. We suggest using SDS loading buffer (~20 μL) for elution step. If using other elution buffer, the components of the solution or detailed sample processing procedures and reagents must be provided.
FFPE	10 slices per section, 1.5×2cm in size, 10 sections in total.

Quantitative Proteomics Sample Recommendations

Sample Type		Label-free/DIA	Size
Animal tissues	Hard tissues (bones, hair)	200mg	300-500mg
	Soft tissues (leaves, flowers of woody plants, herbaceous plants, algae, ferns)	100mg	200mg
Plant tissues	Hard tissues (roots, bark, branches, seeds, etc.)	2g	3-5g
Microbes	Common bacteria, fungal cells (cell pellets)	50μL	100μL
Cells	Suspension/adherent cultured cells (cell count/pellet)	5×10^6	1×10^7
Fluids	Plasma/serum/cerebrospinal fluid (without depletion of high abundance proteins)	20μL	20μL
	Plasma/serum/cerebrospinal fluid (with depletion of high abundance proteins)	50-100μL	100μL
	Follicular fluid	100μL	200μL
	Lymph, synovial fluid, puncture fluid, ascites	3mL	5mL

	Saliva/tears/milk	1 mL	3-5mL
	Culture supernatant (serum-free medium cannot be used)	10mL	20mL
Others	Pure protein (best buffer is 8MUrea)	150μg	300μg
FFPE	Each slice: 10μm thickness, 1.5×2cm area	10 slices	15-20 slices

Quantitative Proteomics for Trace Samples

Sample Type		DIA
Animal tissues	General tissues (brain, heart, liver, spleen, lung, kidney, muscle, etc.)	30-50mg
	Suspension/adherent cultured cells (cell count/pellet)	200-5000
Cells	Plasma/serum/cerebrospinal fluid (without depletion of high abundance proteins)	10μL
Fluids	Plasma/serum/cerebrospinal fluid (with depletion of high abundance proteins)	20μL
	Follicular fluid	20μL
	Lymph, synovial fluid, puncture fluid, ascites	1mL
	Saliva/tears	500μL
	Culture supernatant (serum-free medium cannot be used)	5mL

Exosome Proteomics Sample Recommendations

Sample Source	Exosomes	Serum/Plasma	Cell Culture Supernatant	Urine	Saliva	Nasal Mucus	Cerebrospinal Fluid	Amniotic Fluid
Exosome particles	1010 particles per sample							
Recommended Optimized Volume	100μL	5mL	50mL	100mL	50mL	50mL	20mL	50mL

Proteomics Sample Preparation, Storage, and Transportation Methods

Animal Tissues

Routine Animal Tissues (Heart, Liver, Spleen, Kidney, Lung, etc.)

- After accurately extracting the desired tissue, immediately remove any non-research-related tissues and cut the sample into small pieces.
- Quickly rinse the sample in saline or PBS to remove blood and debris.
- Using tweezers, place the sample in liquid nitrogen for rapid freezing for 5-10 minutes.
- Transfer the sample into cryovials or centrifuge tubes, and store at -80°C.
- Fill out the sample registration form, including the sample name, species, tissue type, identification number, collection date, and sample processing details.
- Ship with dry ice to maintain low temperatures, avoiding repeated freeze-thaw cycles.

Animal Hair

- Rinse the sample with an appropriate amount of 2% SDS and 50 mM sodium phosphate buffer (pH 7.8) to remove contaminants.
- Dry the sample, store at -80°C, and ship with dry ice.

Hard Tissues, Such as Animal Cartilage

- Wash with PBS.
- Rapidly freeze the sample with liquid nitrogen, store at -80°C, and ship with dry ice.

Plant Tissues

Plant Leaves, Flowers, Fruits, Seeds, Roots, Bark, Ferns, etc.

- Collect specific tissues based on the research objective, remove non-target tissues and impurities, wipe dry with dust-free paper, and wrap in aluminum foil or place in centrifuge tubes.
- Label the specific tissue parts, then flash freeze in liquid nitrogen for more than 5 minutes, store at -80°C, and ship with dry ice, avoiding repeated freeze-thaw cycles.

Pollen

- Collect pollen during the flowering period, check under a microscope to remove impurities, transfer to centrifuge tubes, flash freeze in liquid nitrogen, store at -80°C, and ship with dry ice.

Algae

- Apply appropriate centrifugation force according to the type of algae to separate them while ensuring cell integrity, wash 2-3 times with PBS, flash freeze in liquid nitrogen, store at -80°C, and ship with dry ice.

Liquid Samples

Serum

- Collect blood using a vacuum blood collection tube (without anticoagulant, red cap).

- Gently invert the tube 5-6 times to mix.
- Let the sample sit at 4°C for 15-30 minutes, then centrifuge at 1600g for 10 minutes.
- Use a pipette to transfer the pale yellow serum to a centrifuge tube, add protease inhibitor (generally at a 1:50 ratio; follow specific instructions if applicable), mix well, and briefly centrifuge.
- Flash freeze in liquid nitrogen, store at -80°C for short-term storage, and ship with dry ice.

Note:

- *Hemolysis must be avoided during sample preparation.*
- Serum yield is approximately 30%-50% (e.g., *1 mL of whole blood typically yields 0.3-0.5 mL of serum).*

Plasma

- Collect blood using an EDTA anticoagulant tube (purple cap) for blood routine tests (do not use tubes with heparin anticoagulant).
- Gently invert the tube 8-10 times to mix, then immediately centrifuge at 1600g at 4°C for 10 minutes.
- Use a pipette to transfer the plasma to a centrifuge tube, add protease inhibitor, mix well, and briefly centrifuge.
- Flash freeze in liquid nitrogen, store at -80°C for short-term storage, and ship with dry ice.

Note:

- *If plasma samples need to remove high-abundance proteins, use EDTA anticoagulant only; heparin cannot be used.*
- *Plasma yield is approximately 50% (e.g., 1 mL of whole blood typically yields 0.5 mL of plasma).*

Milk and Ascitic Fluid

Collect milk or ascitic fluid into centrifuge tubes, flash freeze in liquid nitrogen, store at -80°C, and ship with dry ice.

Saliva

- Fast for more than 2 hours and collect saliva between 9-12 am. Centrifuge at 1000g-2000g for 5 minutes.
- Collect the supernatant, flash freeze in liquid nitrogen, store at -80°C, and ship with dry ice.

Tears

- Use a capillary micro-pipette to collect the sample, then centrifuge at 8000-14000g at 4°C for 5 minutes.
- Collect the supernatant, flash freeze in liquid nitrogen, store at -80°C, and ship with dry ice.

Sputum

- Collect sputum early in the morning when the volume is high and bacterial content is significant. Rinse the mouth with a mouthwash, followed by cool boiled water or saline to remove oral bacteria. Take a deep breath and cough up 1-2 mouthfuls of sputum into a sterile wide-mouth container. For minimal sputum, nebulize with 45°C 10% sodium chloride solution to induce sputum production.
- Liquefaction method: Use a syringe to aspirate and expel the sputum to mix it well. Flash freeze in liquid nitrogen, store at -80°C for short-term storage, and ship with dry ice.

Note: *Fast for 12 hours before sample collection.*

Cerebrospinal Fluid, Lymph Fluid, Joint Fluid, and Puncture Fluid

- Centrifuge at 1000g-2000g for 5 minutes.
- Collect the supernatant, flash freeze in liquid nitrogen, store at -80°C, and ship with dry ice.

Urine

- Collect "fresh" mid/late-morning urine from the subject, store temporarily at 4°C (not exceeding 8 hours), and avoid bacterial contamination. Control diet before collection.
- Centrifuge the urine at 3000g for 15 minutes at 4°C to remove cells or cell debris. Flash freeze for 15 minutes in liquid nitrogen, store at -80°C, and ship with ample dry ice.

Note: *It is recommended to initiate the project within one month of urine collection, as long-term storage may affect exosome morphology.*

Cells

Suspended Cells

- Centrifuge at 400-1000g for 5-10 minutes to collect suspended cells, discard the supernatant.
- Wash the cells 2-3 times with pre-chilled PBS, centrifuge, and discard the supernatant. Collect the cells into a 1.5 mL centrifuge tube.
- It is recommended to have at least 2-3 tubes per sample, with each tube containing more than 50 μL of cell pellets. Record the cell volume, flash freeze in liquid nitrogen, and store at -80°C.

Adherent Cells

- Remove the culture medium, wash the culture dish 3 times with 10 mL PBS, then invert the dish to remove excess liquid.
- Place the dish on ice, digest with trypsin, and then add PBS to suspend the cells.
- Collect the cells into a 15 mL centrifuge tube, centrifuge at 1000g for 5-10 minutes at 4°C, and discard the PBS.
- Resuspend the cells in 1 mL PBS and transfer to a new 1.5 mL centrifuge tube; centrifuge to remove PBS and ship cell pellets with dry ice.

Microbes

- Collect microbial cells using centrifugation, quickly wash 2-3 times with pre-chilled PBS to avoid contamination from culture medium proteins.
- Transfer to cryovials, record the microbial volume, flash freeze in liquid nitrogen, and store at -80°C.

Soil

Sampling Principles

Sampling should follow a specific route and adhere to the principles of “random,” “equal quantity,” and “multiple-point mixing.” Avoid non-research areas such as weeds, stones, and other non-soil debris. Minimize differences within the same sampling unit and maximize differences between different sampling units.

Sampling Method

At the designated sampling points, use a small soil scoop to remove the top 3 mm of soil, then cut soil to a depth of 30 cm at an angle. Place the collected soil into a sterile bag. Quickly store the soil in ice, mix samples from various points thoroughly, and pack the required amount into bags for transport. Avoid including stones, gravel, and plant residues.

Sample Processing

Freeze-dry the soil, then sieve through a 2 mm diameter sieve. Store at -80°C and ship with ample dry ice for low-temperature transport.

For any sample types not covered in this guide or if you have specific requirements for your proteomics project, please contact our sales team for further consultation and assistance. Your satisfaction and the success of your research are our top priorities.