

Sample Submission Guideline for Mass Spectrometry (Novogene AMEA)

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This sample submission guideline is for customers in the Asia Pacific and Middle East region (AMEA), excluding mainland China. For any questions, please contact your Novogene representative.

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Tissue Weight Reference

Tissue Reference Size	Name	Weight
	Soybean	100 mg/ seed
	Mung bean	50 mg/ seed
	Rice	10 mg/ seed

1. Sample Requirement for Metabolomics Services

Sample Type	Untargeted Metabolomics Plus	Untargeted Metabolomics Pro
Human & Animal tissue	20 mg	40mg
Plant tissue	20 mg	40mg
Plasma/Serum	20 μ L	40 μ L
Feces/Intestinal contents	20 mg	40mg
Saliva	100 μ L	200 μ L
Urine	20 μ L	40 μ L
Amniotic fluid/Bile/Tear, etc.	100 μ L	200 μ L
Supernatant/Fermentation broth	1 mL	2 mL
Microbial thallus	10-50 μ L(pellet)	20-100 μ L(pellet)
Microbial solution	1 mL	2 mL
Culture medium	100 mg	100 mg
Cell*	$\geq 10^6$ (10 μ L pellet)	$\geq 2 \times 10^6$ (20 μ L pellet)

Note:

*Please ensure the cell count can meet the requirement.

*The cell count must be consistent; the bacterial cells are provided as bacterial cell pellets.

2. Sample Preparation for Metabolomics

2.1 Sequencing Human & Animal Tissue Samples

2.1.1 Preparation Steps

Human tissue: For small biopsy samples, quickly rinse with pre-chilled deionized water to remove residual blood. Based on the specific experimental design, dissect the desired region and place it into a 1.5mL centrifuge tube. After labeling, promptly immerse in liquid nitrogen for freezing for at least 15 minutes. Store in a -80°C freezer. Ship with sufficient dry ice.

Animal tissue: If the entire tissue is to be collected, perform perfusion with prechilled deionized water to remove residual blood from the tissue. If only a portion of the tissue is required, rinse the tissue with prechilled deionized water after dissection to remove residual blood. According to the experimental design, dissect the target region and place it into a 1.5 mL centrifuge tube. After labeling, immediately snap-freeze the sample in liquid nitrogen for at least 15 min and then store it at -80°C. Ship the samples on sufficient dry ice.

2.1.2 Notes

1. For biological replicates, try to keep the sampling location as consistent as possible. Flash-freeze the sample with liquid nitrogen for at least 5 min, store at -80°C, and avoid repeated freeze-thaw cycles.
2. Be aware of possible contaminant signals resulting from the collection of tissue samples, such as those from anesthetic, surgical instrument cleaning solutions, or tube-related contaminants.
3. Tissues should be collected on ice to minimize further metabolism. The samples should either be processed immediately upon collection or be snap-frozen and stored at -80°C to avoid any further loss of unstable metabolites.
4. Collect the tissue into a suitable container and then subdivide it into labeled tubes/containers.
5. If the tissue samples are large, it is recommended to cut them into small pieces for freezing and storage. Small pieces of tissue will freeze more rapidly, and separate aliquots will minimize or eliminate freeze-thaw cycles.

Before sending the fecal sample, please confirm whether it has been preserved in a liquid (such as alcohol or buffer solution). If so, please contact the APM in advance to confirm the feasibility of the sample and the subsequent procedures.

2.1.3 References

Want E J, Masson P, Michopoulos F, et al. Global metabolic profiling of animal and human tissues via UPLC-MS[J]. Nature Protocols, 2012, 8(1):17-32.

2.2 Plant Samples

2.2.1 Leaves, Stems, Flowers

2.2.1.1 Preparation Steps

Collect a whole leaf, a stem segment, or a flower. Wrap the sample in aluminum foil, label it, and immediately snap freeze it in liquid nitrogen for at least 15 min. After removal, promptly place the sample into a resealable plastic bag (one bag per group), and include a label inside the bag indicating the sample information. Store the samples at -80°C. Ship the samples on sufficient dry ice.

2.2.1.2 Notes

1. It is recommended to collect leaf samples at midday when light conditions are optimal.
2. According to the experimental design, maintain sample consistency when collecting samples, especially within the same group of samples (color, degree of aging, leaf vein proportion, lighting, position, etc.).
3. Since marker pen markings on aluminum foil tend to fade, it is recommended to also include a label sheet inside the self-sealing bag.

2.2.2 Roots

2.2.2.1 Preparation Steps

Collect the entire plant root and quickly rinse off the soil with 1× PBS. According to the experimental design, dissect the required root regions and place the samples into centrifuge tubes. Label the samples and immediately snap-freeze them in liquid nitrogen for at least 15 min. Store the samples at -80°C. Ship the samples on sufficient dry ice.

Note:

1. Wash with PBS as quickly as possible.
2. Since root tissues are particularly fragile and will turn mushy when squeezed, it is recommended to store them in centrifuge tubes rather than wrapping them in aluminum foil.

2.2.3 Fruits, Seeds

2.2.3.1 Preparation Steps

1. For juicy and large fruits (e.g., tomatoes, watermelons, apples), cut them into uniform small pieces according to the sample size required for the selected omics technology. Divide the pieces into 1.5 mL centrifuge tubes, label them, and immediately snap freeze in liquid nitrogen for at least 15 min. According to the experimental design, maintain sample consistency when collecting samples, especially within the same group of samples (color, degree of aging, leaf vein proportion, lighting, position, etc.).

2. For small seeds (e.g. Arabidopsis seeds, grain seeds), pool seeds from the same group and divide them into 1.5 mL centrifuge tubes to match the required sample size. Label the tubes and immediately snap freeze in liquid nitrogen for at least 15 min.
3. For whole fruits (e.g., grapes), place the fruit in a 50 mL centrifuge tube or a self-sealing bag. Label the container (including sample information if using a bag) and immediately snap freeze in liquid nitrogen for at least 15 min.

2.2.3.2 Notes

1. Maintaining uniformity in sampling juicy fruits (such as the proportion of tomato skins, flesh, pulp, and seeds) is challenging. It is strongly recommended to grind and freeze-dry the entire fruit, then weigh and package the powder.
2. It is not recommended to wrap it with aluminum foil.

2.2.3.3 Reference

1. De Vos R C, Moco S, Lommen A, et al. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. [J]. Nature Protocols, 2007,2(4):778-791.
2. Kim H K, Choi Y H, Verpoorte R. NMR-based metabolomic analysis of plants[J]. Nature Protocols, 2010,5(3):536

2.3 Plasma/Serum

2.3.1 Preparation Steps for Plasma

It is recommended to collect whole blood using heparin sodium anticoagulant tubes and separate the plasma immediately. Centrifuge the blood at 3,000 rpm for 10 min at 4°C and transfer the plasma (upper layer) into 1.5 mL centrifuge tubes. After labeling, snap-freeze the tubes in liquid nitrogen for at least 15 min and store them at -80°C. Ship the samples on sufficient dry ice.

Note: For multi-omics studies involving proteomics & metabolomics or Olink & metabolomics, samples can be collected following the sample preparation protocols for proteomics or Olink. If EDTA anticoagulant tubes are used, please indicate this clearly.

2.3.2 Preparation Steps for Serum

Collect blood into centrifuge tubes or vacuum blood collection tubes. Allow the samples to clot and separate at 37°C (or at room temperature) for 1 hour. Centrifuge the samples at 3,000 rpm for 5 min and transfer the supernatant to a clean centrifuge tube. Next, centrifuge at 12,000 rpm for 10 min at 4°C (alternatively, a single centrifugation step may be used before sample collection). Transfer the resulting supernatant into 1.5 mL centrifuge tubes, label them clearly, snap-freeze in liquid nitrogen for at least 15 min, and store at -80°C. Ship the samples on sufficient dry ice.

Note: For multi-omics studies involving proteomics & metabolomics or Olink & metabolomics, samples can be collected following the sample preparation protocols for proteomics or Olink.

2.3.3 Notes

1. Note for Sample Preparation

Animal Samples: Ensure animals were fasted for at least 10 hours, whether water deprivation is necessary should be determined according to the experimental design.

Clinical Samples: Maintain a light diet for 3 days before sampling. Fast from food and water after 8:00 PM in the evening before sampling. The sample processing process should be as consistent as possible, including sample placement time, centrifugation time, etc.

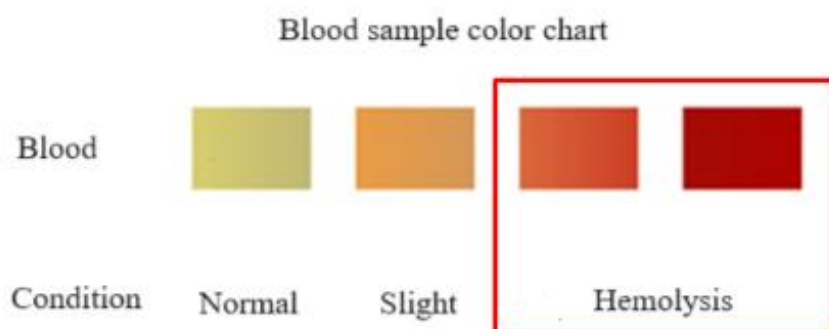
2. Blood Collection Tube Selection

Plasma: It is recommended to use heparin sodium anticoagulant tube (green cap). EDTA anticoagulant (purple cap) can interfere with metabolites and cause matrix effects. However, the interference of EDTA anticoagulant can be eliminated by desalinization. If using EDTA tubes, please note this in advance.

Serum: Please use a vacuum blood collection tube (red cap) without anticoagulant during blood collection.

3. It is recommended to collect as many samples as possible and aliquot them into 1.5 mL centrifuge tubes for freezing. Be sure to avoid repeated freeze-thaw cycles. Serum reference yield: 30% to 50% (e.g., 1 mL of whole blood can yield approximately 0.3 to 0.5 mL of serum). Plasma reference yield: approximately 50% (e.g., 1 mL of whole blood can yield approximately 0.5 mL of plasma).

4. If hemolysis is observed, please refer to the color chart below. Samples corresponding to the last two-color levels indicate severe hemolysis and are not suitable for metabolomics analysis; recollection is required.



5. If alcohol is used to disinfect the puncture site before blood collection, wipe the area dry and wait until the alcohol has completely evaporated before collecting the sample.

2.3.4 References

1. BarriT, Dragsted L O. UPLC-ESI-QTOF/MS and multivariate data analysis for blood plasma and serum metabolomics: effect of experimental artefacts and anticoagulant[J]. *Analytica Chimica Acta*, 2013, 768(1):118-128.
2. Zhao X, Su H, Chen H, et al. Integrated serum pharmacochimistry and network pharmacology to explore the mechanism of Yi-Shan-Hong formula in alleviating chronic liver injury[J]. *Phytomedicine*. 2024, 128: 155439.

2.4 Feces/Intestinal Contents

2.4.1 Preparation Steps

After collecting fresh fecal or intestinal content samples, aliquot them into centrifuge tubes, then immediately snap freeze in liquid nitrogen for 15 minutes. Label the samples clearly and store them at -80 °C. Ship the samples on sufficient dry ice.

2.4.2 Notes

Due to the high abundance of microorganisms in fecal/intestinal content samples and their rapid metabolic activity, repeated freeze-thaw cycles can significantly affect metabolite levels. It is recommended to aliquot samples according to the required volume immediately after collection and store them frozen.

2.4.3 References

Wu J, An Y, Yao J, et al. An optimized sample preparation method for NMR-based faecal metabonomic analysis[J]. Analyst, 2010, 135(5):1023-30.

2.5 Saliva

2.5.1 Preparation Steps

Sample collection should be performed between 9:30 AM and 11:30 AM. Rinse the mouth with saline and expectorate saliva into a sterile sputum cup (keep the cup on ice). Avoid expectorating sputum. Collect an adequate volume of saliva in a short period. Centrifuge the samples at 2,000 × g for 10 min at 4°C. Transfer the supernatant and, if desired, filter through a 0.22 µm membrane for sterilization. Aliquot the filtrate into 2–3 tubes, snap-freeze in liquid nitrogen for at least 15 min, and store at -80°C. Transport the samples on sufficient dry ice.

2.5.2 Notes

1. Refrain from smoking, drinking, and eating for at least 1 hour before sampling. Do not brush your teeth prior to sampling.
2. It is recommended to collect as many samples as possible and pack them in 1.5 mL centrifuge tubes for freezing. Avoid repeated freeze-thaw cycles.

2.5.3 References

Bertram, H. C., Eggers, N., & Eller, N. Potential of Human Saliva for Nuclear Magnetic Resonance-Based Metabolomics and for Health-Related Biomarker Identification[J]. Analytical Chemistry, 2009, 81(21), 9188–9193.

2.6 Urine

2.6.1 Preparation Steps

Midstream urine (clinical) or 1 hour morning urine (animal) was centrifuged at 3000 rpm, 4°C for 10 minutes. Clear urine was collected and divided into centrifuge tubes. After labeling, freeze in liquid nitrogen for 15 minutes and store at -80°C. Use sufficient dry ice during transport.

2.6.2 Notes

1. If the output of animal's 1-hour urine is insufficient, it can be collected multiple times. For 24-hour urine collection, please use a metabolic cage equipped with a low-temperature device and add sodium azide (0.05-0.1% w/v).
2. Sodium azide is a preservative and bactericide, but it is toxic. Please handle with extreme caution.

2.6.3 References

Want E J, Wilson I D, Gika H, et al. Global metabolic profiling procedures for urine using UPLC-MS.[J]. Nature Protocols, 2010, 5(6):1005-1018.

2.7 Tears

2.7.1 Preparation Steps

Collect samples between 9:00 AM and 12:00 PM. Ask the subject to look upward. Using a prepared Schirmer strip, insert the curved end into the conjunctival sac at the outer one-third of the eyelid, leaving the other end hanging outside the eye. Instruct the subject to gently close their eyes. Allow the strip to remain in place for 5 minutes. After removal, place the strips into a 1.5 mL centrifuge tube, snap-freeze in liquid nitrogen for at least 15 min, and store at -80°C. Ship the samples on sufficient dry ice.

2.7.2 Notes

Collect no more than one tear sample per eye per day.

2.7.3 References

1. Pieragostino D, D'Alessandro M, di Ioia M, et al. Unraveling the molecular repertoire of tears as a source of biomarkers: beyond ocular diseases[J]. Proteomics–Clinical Applications, 2015, 9(1-2): 169-186.
2. Chen L, Zhou L, Chan E C Y, et al. Characterization of the human tear metabolome by LC-MS/MS[J]. Journal of proteome research, 2011, 10(10): 4876-4882.

2.8 Cerebrospinal Fluid (Other Body Fluids May Follow the Same Procedure)

2.8.1 Preparation Steps

Collect cerebrospinal fluid using polypropylene (EP) tubes. It is recommended to perform centrifugation within 2 hours at 2000 × g for 10 minutes at 4°C. Transfer the supernatant into centrifuge tubes, aliquot, and label clearly. Then snap-freeze in liquid nitrogen for 15 minutes and store at -80°C. Ship the samples on sufficient dry ice.

2.8.2 Notes

After sample collection, centrifuge as soon as possible to minimize blood contamination and avoid repeated freeze-thaw cycles.

2.8.3 References

Lehmann R. From bedside to bench-practical considerations to avoid pre-analytical pitfalls and assess sample quality for high-resolution metabolomics and lipidomics analyses of body fluids[J]. Anal Bioanal Chem. 2021;413(22):5567-5585

2.9 Microbial Samples

2.9.1 Preparation Steps for Microbial Thallus

Collect microbial thallus using centrifugation (ensuring consistent volume after centrifugation), rinse 2-3 times quickly with pre-chilled PBS, and centrifuge for 5 minutes at 4°C, 5000 rpm after each wash. Completely discard the supernatant, collect the bacterial cells in a 1.5 mL centrifuge tube (cryogenic vials), and immediately freeze in liquid nitrogen for 15 minutes and store at -80°C. Use sufficient dry ice during transport.

2.9.2 Preparation Steps for Microbial Liquid Culture Medium

After mixing the cultured microbial liquid, take more than 1 mL of culture, and centrifuge at 3000 rpm, 4 °C for 10 minutes. Collect all the supernatant and immediately freeze in liquid nitrogen for 15 minutes. Store at -80°C. Use sufficient dry ice during transport.

2.9.3 Preparation Steps for Solid Culture Medium

Cut the culture medium into square pieces with a blade. Immediately freeze the pieces in liquid nitrogen for 15 minutes, and store at -80°C. Use sufficient dry ice during transport.

2.9.4 Notes

1. Microbial metabolism is very fast, so all steps need to be completed as soon as possible.
2. The number of bacterial cells should be consistent among different samples.
3. Generally, an OD600 between 0.6 and 0.8 indicates that the bacteria are in logarithmic growth phase, with an empirical concentration of 108 cells/mL, which needs to be confirmed based on specific circumstances.

2.9.5 References

1. Winder C L, Dunn W B, Schuler S, et al. Global metabolic profiling of Escherichia coli cultures: an evaluation of methods for quenching and extraction of intracellular metabolites[J]. Analytical Chemistry, 2008, 80(8):2939-48.
2. MarcinowskaR, TryggJ, Wolf-WatzH,etal. Optimization of a sample preparation method for the metabolomic analysis of clinically relevant bacteria [J].Journalof Microbiological Methods,2011,87(1):24-31.
3. Bertrand S., Azzollini A., SchumppO., et al. multi-well fungal co-culture for de novo metabolite-induction in time-series studies based on untargeted metabolomics[J]. Mol. BioSyst. 2014; 10:2289–2298.

2.10 Cell Samples

2.10.1 Preparation Steps for Cells

1. Suspension Cell Samples:
Collect suspension cells by centrifugation. Quickly wash 2–3 times with pre-chilled PBS. Centrifuge at $1000 \times g$ for 1 minute at 4°C . Discard the supernatant and collect the cells (cell pellet) into a 1.5 mL centrifuge tube. Rapidly freeze in liquid nitrogen for 15 minutes, then store at -80°C . Ship with sufficient dry ice.
2. Adherent Cell Samples:
Remove the old medium from the cultured cells and wash the cells 2–3 times with prechilled PBS. Discard the supernatant and then add 0.5 mL of trypsin for digestion. After digestion, wash the cells 2–3 times with prechilled PBS to quench trypsin activity. Transfer the cells into a 1.5 mL centrifuge tube and centrifuge at $1,000 \times g$ for 1 min at 4°C . Discard the supernatant and resuspend the cells in a 1.5 mL centrifuge tube. Snap-freeze the cells in liquid nitrogen for at least 15 min, store at -80°C , and transport on sufficient dry ice. (For setting up replicate wells, perform trypsin digestion for cell counting.)

Note: Cell count results for each sample are necessary.

2.10.2 Preparation Steps for Cell Culture Medium

Remove the old culture medium from the cultured cells, wash 2–3 times with PBS, then add serum-free medium and continue culturing for a specified period. Based on the experimental design or cell status, collect more than 1 mL of the medium from adherent cells at the appropriate time. Centrifuge at $1000 \times g$ for 1 minute at 4°C , collect the entire supernatant, rapidly freeze in liquid nitrogen for 15 minutes, and store in a -80°C freezer. Ship with sufficient dry ice.

2.10.3 Preparation Steps for Cell Culture Supernatant

The old culture medium was removed from the cells, and the cells were washed 2–3 times with PBS. Serum-free medium was then added, and the cells were incubated for a specified period. At the appropriate time point, based on the experimental design, the culture medium was collected and centrifuged at $1,000 \times g$ for 10 min at 4°C to remove cells. The supernatant was then centrifuged again at $10,000 \times g$ for 10 min at 4°C to remove cell debris. The resulting supernatant was aliquoted into 1.5 mL centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C .

2.10.4 Notes

1. Try to ensure that the number of cells collected for each sample should be as consistent as possible, and the entire process should be completed as quickly as possible.
2. The type of culture medium affects the types of metabolites, and it is necessary to ensure that the type of culture medium is consistent during the cell culture process.
3. To study extracellular metabolism, please use serum-free culture medium while ensuring cell viability.

2.10.5 References

1. Sellick C A, Hansen R, Stephens G M, et al. Metabolite extraction from suspension cultured mammalian cells for global metabolite profiling[J]. Nature Protocol, 2011, 6(8):1241-9.
2. Yuan M, Breitkopf S B, Yang X, et al. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue[J]. Nature Protocols, 2012, 7(5):872-81.

3. Metabolites Extraction Method for Untargeted Metabolomics

3.1 Tissue Sample

Tissues (20 mg for Plus and 40mg for Pro) were individually grounded in liquid nitrogen and the homogenates were resuspended with prechilled 90% methanol followed by thorough vortexing. The samples were incubated on ice for 5 min and then centrifuged at 15,000 rpm, 4°C for 20 min. An aliquot of the supernatant was diluted with LC-MS grade water to a final concentration of 53% methanol. The samples were then transferred to a fresh Eppendorf tube and then were centrifuged at 15000 rpm, 4°C for 20 min. Finally, the supernatants were subjected to LC-MS/MS analysis [1].

3.2 Liquid Sample

The samples were placed in the EP tubes and resuspended with prechilled methanol by well vortex. Then the samples were incubated on ice for 5 min and centrifuged at 15,000 rpm, 4°C for 20 min. Some of supernatant was diluted to final concentration containing 53% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then were centrifuged at 15000 rpm, 4°C for 20 min. Finally, the supernatant was injected into the LC-MS/MS system analysis [2-3].

3.3 Cell or Bacteria Sample

The samples were placed in Eppendorf tubes and resuspended in prechilled 80% methanol, followed by thorough vortexing. The samples were then thawed on ice and vortexed for 30 s. After sonication in a water bath for 15 min, the samples were centrifuged at 15,000 rpm at 4°C for 20 min. The supernatant was freeze-dried and reconstituted in 10% methanol. Finally, the solution was injected into the LC-MS/MS system for analysis. [4-5].

3.4 Cell or Bacteria Culture Medium Sample

The samples (1 mL for Plus and 2 mL for Pro) were freeze-dried and resuspended in prechilled 80% methanol, followed by thorough vortexing. The samples were then incubated on ice for 5 min and centrifuged at 15,000 rpm at 4°C for 20 min. An aliquot of the supernatant was diluted with LC-MS grade water to a final methanol concentration of 53%. The samples were subsequently transferred to fresh Eppendorf tubes and centrifuged again at 15,000 rpm at 4°C for 20 min. Finally, the supernatant was injected into the LC-MS/MS system for analysis.

3.5 Reference

1. Want E J, Masson P, Michopoulos F , et al. Global metabolic profiling of animal and human tissues via UPLC-MS[J]. Nature Protocols, 2012, 8(1):17-32.
2. Want E J, O'Maille G, Smith C A , et al. Solvent-Dependent Metabolite Distribution, Clustering, and Protein Extraction for Serum Profiling with Mass Spectrometry[J]. Analytical Chemistry, 2006, 78(3):743-752.
3. BarriT, Dragsted L O. UPLC-ESI-QTOF/MS and multivariate data analysis for blood plasma and serum metabolomics: effect of experimental artefacts and anticoagulant[J]. Analytica Chimica Acta, 2013, 768(1):118-128.
4. Sellick C A, Hansen R, Stephens G M, et al. Metabolite extraction from suspension cultured mammalian cells for global metabolite profiling[J]. Nature Protocol, 2011, 6(8):1241-9.
5. Yuan M, Breitkopf S B, Yang X, et al. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue[J]. Nature Protocols, 2012, 7(5):872.

4. Sample Requirement for Quantitative Proteomics Services

Sample Type		Deep/Rapid DIA Proteomics
Animal tissue	General tissues (brain, heart, liver, spleen, lung, kidney, muscle, etc.)	10 mg
Plant Tissues	Tough tissue (hair, cartilage, etc.)	200 mg
	Soft tissues (leaves, flowers, roots of woody or herbaceous plants, algae, ferns, etc.)	100 mg
	Tough tissue (root, branch, etc.)	1 g
	Fruit pulp	2 g
	Seeds	100 mg
	Pollen	50 µL
Microorganisms	Common bacteria	30 mg or 30 µL pellet
	Fungal mycelium	50 mg or 50 µL pellet
Cells	Suspension/ Adherent cultured cells	2×10 ⁶ (10 µL cell pellet)
Body Fluids	Serum/Plasma (neat, no pretreatment)	5 µL
	Serum/Plasma (nanoparticle-based pretreatment) *	110 µL
	Cerebrospinal fluid/Semen/Follicular fluid/Saliva/Tear	500 µL
	Urine	10 mL
	Breast milk	10 µL
FFPE	Area: 0.5 cm × 0.5 cm; Thickness: 10 µm	2 slices ***

Note:

- * It is recommended to use fresh plasma/serum samples collected within three months to prevent hemolysis. For this pretreatment step, 110 µL of sample is suggested as the optimal volume. If the sample volume is less than 50 µL, the laboratory will notify you of the potential risks.
- ** For protein solution, the recommended protein concentration is ≥ 0.5 µg/µL.
- *** Recommended: 2 slices; Minimum: 1 slice.

5. Sample Preparation for Quantitative Proteomics Services

5.1 Animal Tissue Samples

5.1.1 General animal tissues (brain, heart, liver, spleen, lung, kidney, muscle, etc.)

1. Extract the desired tissue and rinse the sample with pre-chilled saline or PBS to remove remaining blood.
2. Cut the samples into 0.5 cm width or ~100 mg pieces and put them into tubes. Label the tubes clearly with sample name, species, and tissue type.
3. Flash freeze the samples with liquid nitrogen for at least 5 minutes, store at -80 °C, avoid repeated freeze-thaw cycles, and ship the samples with sufficient dry ice.

5.2 Plant Samples

5.2.1 Soft Tissues & Fruits & Seeds

1. Extract the desired tissues and remove non-target tissues. Rinse the sample with pre-chilled saline or PBS to remove debris.
2. Put the samples into tubes. Label the tubes clearly with sample name, species, and tissue type.
3. Flash freeze the samples with liquid nitrogen for at least 5 minutes, store at -80 °C, avoid repeated freeze-thaw cycles, and ship the samples with sufficient dry ice

5.2.2 Pollen

1. Collect pollen during the flowering period. Examine the collected samples under a microscope to remove impurities, then transfer them to tubes.
2. Label the tubes clearly and flash freeze in liquid nitrogen, store at -80 °C, and ship with sufficient dry ice.

5.2.3 Algae

1. Apply an appropriate centrifugation force based on the type of algae to separate the algae while ensuring cell integrity.
2. Wash with PBS 2-3 times, freeze the tubes with liquid nitrogen, store at -80 °C, and ship the samples with sufficient dry ice.

5.3 Cells

5.3.1 Suspended Cells

1. Centrifuge at 400-1000 g for 5-10 minutes to collect suspended cells, discard the supernatant.
2. Wash the cells 2-3 times with pre-chilled PBS. After each wash, centrifuge and discard the supernatant.
3. Transfer the collected cells into a suitable tube. It is recommended to prepare at least 2-3 tubes per sample, with each tube containing more than 10 μ L of cell pellets.
4. Record the cell volume, flash freeze the tubes in liquid nitrogen, store at -80 °C, and ship the samples with sufficient dry ice.

5.3.2 Adherent Cells

1. Discard the culture medium, wash the adherent cells 3 times with 10 mL PBS.
2. Place the dish on ice, digest with trypsin, and then add PBS to suspend the cells
3. Collect the cells into a 15 mL centrifuge tube, centrifuge at 1000 g for 5-10 minutes at 4 °C and discard the PBS.
4. Resuspend the cells in 1 mL PBS and transfer to a new 1.5 mL centrifuge tube; centrifuge to remove PBS. It is recommended to prepare at least 2-3 tubes per sample, with each tube containing more than 10 μ L of cell pellets.
5. Record the cell volume, freeze the tubes in liquid nitrogen, store at -80 °C, and ship the samples with sufficient dry ice

5.4 Body Fluids

5.4.1 Serum/Plasma

a) Serum

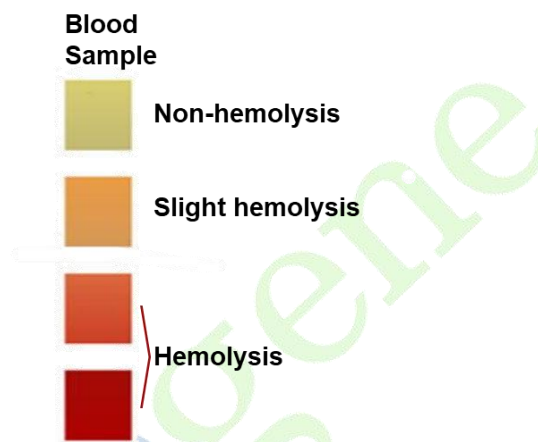
1. Collect blood using a vacuum blood collection tube (without anticoagulant, red cap).
2. Gently invert the tube 5-6 times to mix.
3. Let the sample sit for 15-30 minutes, then centrifuge at 800 g for 10 minutes.
4. Transfer the serum to a suitable tube using a pipette, add protease inhibitor (if applicable), and mix well.
5. Flash freeze in liquid nitrogen, store at -80 °C for short-term storage, and ship with dry ice.

b) Plasma

1. Collect blood using an EDTA anticoagulant tube (purple cap; do not use tubes with heparin anticoagulant).
2. Gently invert the tube 8-10 times to mix, then immediately centrifuge at 800 g at 4 °C for 10 minutes.
3. Transfer the plasma to a suitable tube using a pipette, add protease inhibitor (if applicable), and mix well.
4. Flash freeze in liquid nitrogen, store at -80 °C for short-term storage, and ship the samples with sufficient dry ice.

Notes:

1. *Hemolysis must be avoided during sample preparation. There are some potential reasons for hemolysis: a) the iodine or ethanol solution used for disinfection before venipuncture is not completely dry; b) excessive damage on the venous vessel during the blood collection process; c) excessive mixing intensity; d) insufficient mixing with the anticoagulant; e) unsuitable temperature of storage and transport; f) intravenous infusion is being administered to the same arm used for venipuncture; g) blood is collected immediately after withdrawing the butterfly needle; h) undergoing anti-inflammatory medication treatment (such as aspirin), etc.
2. Recommended solutions for avoiding hemolysis: a) have a trained phlebotomist perform the blood collection; b) gently mix the sample; c) shake up and down more than ten times; d) avoid venipuncture when the object is on special medications.
3. Serum yield is approximately 30~50% (e.g., 0.3-0.5 mL of plasma yield from 1 mL of whole blood typically). Plasma yield is approximately 50% (e.g., 0.5 mL of serum yield from 1 mL of whole blood typically).



5.4.2 Cerebrospinal Fluid

1. Collect the fluids and place it on ice. Avoid blood contamination when collecting samples.
2. Centrifuge at 2000 g, 4 °C for 10-15 minutes to remove cell and cell debris.
3. Collect the supernatant, flash freeze in liquid nitrogen, store at -80 °C, and ship the samples with sufficient dry ice.

5.4.3 Saliva

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

5.4.4 Tears

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

5.4.5 Urine

1. Collect mid/late-morning urine, store temporarily at 4 °C (not exceeding 8 hours), and avoid bacterial contamination. Control diet before collection.
2. Centrifuge the urine at 3000 g 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 the samples with sufficient dry ice.

5.4.6 Breast Milk

Collect milk into centrifuge tubes, freeze flash in liquid nitrogen, store at -80 °C, and ship the samples with sufficient dry ice.

5.5 Microorganisms

1. Collect microbial cells using centrifugation, quickly wash 2-3 times with pre-chilled PBS to avoid contamination from culture medium proteins.
2. Centrifuge the pellets at 5000 g at 4 °C for 5 min after each wash procedure. Repeat the wash process 2-3 times.
3. Transfer to tubes, record the microbial volume, freeze in liquid nitrogen, store at -80 °C, and ship the samples with sufficient dry ice.

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[www.novogene.com/amea-en/
marketing_amea@novogeneait.sg](http://www.novogene.com/amea-en/marketing_amea@novogeneait.sg)

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