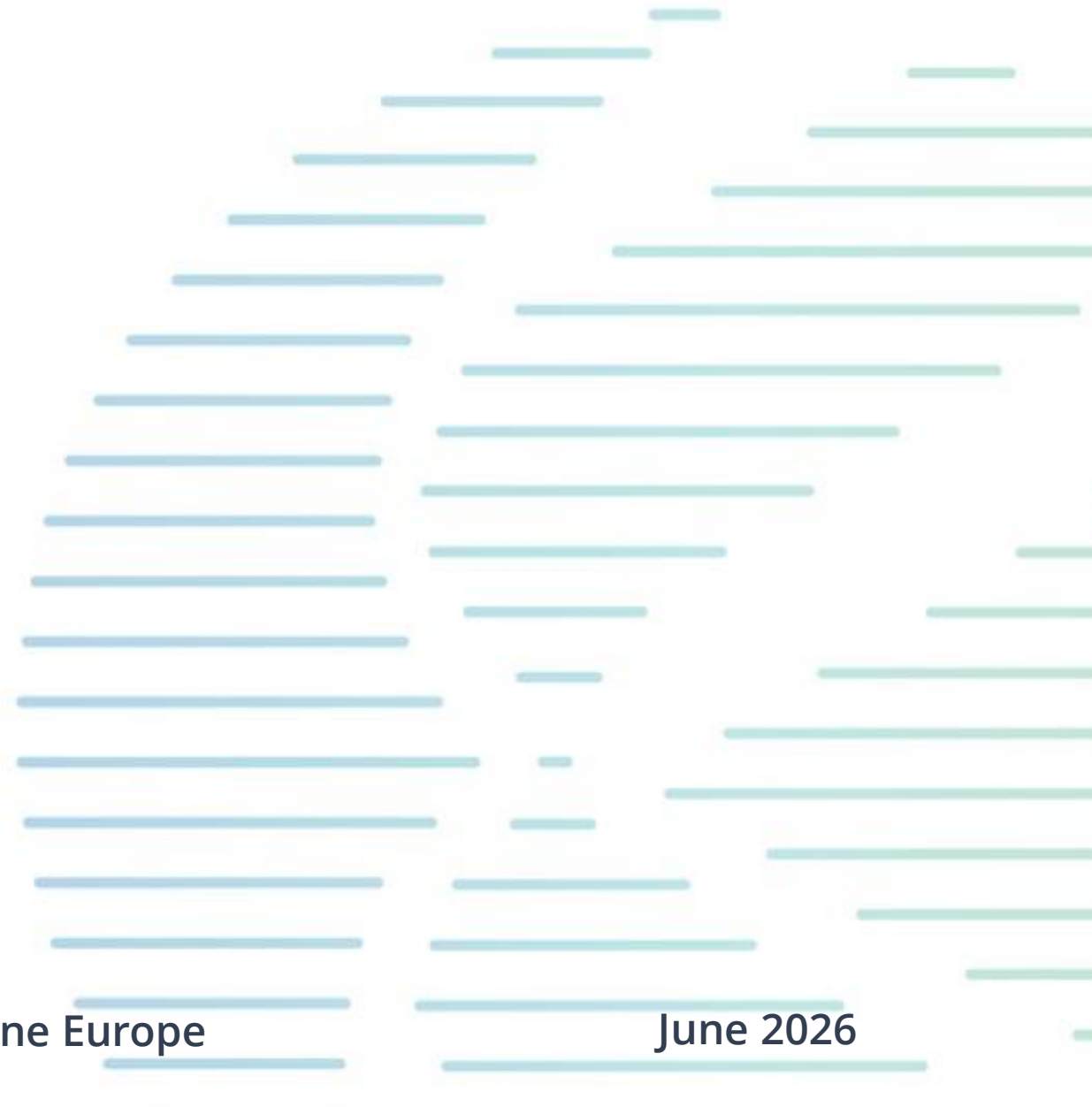
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Sample submission guidelines for Untargeted Metabolomics



Novogene Europe

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Contents

1. GENERAL SUBMISSION GUIDELINES 2
2. SUPPORTED SAMPLE TYPES AND REQUIREMENTS 2
3. SAMPLE PREPARATION GUIDANCE 3
3.1 Human and animal tissue 3
3.2 Plant tissue 3
3.3 Faeces/Intestinal contents 4
3.4 Plasma and serum 5
3.5 Saliva 6
3.6 Urine 6
3.7 Tears 6
3.8 Cell samples 6
4. METABOLITE EXTRACTION METHODS 7

Back to contents

1. General submission guidelines

These guidelines apply to Novogene Europe untargeted metabolomics services.
For sample types or study designs not listed in this document, please contact your local Novogene representative before shipment.

General requirements

- Samples should be stored at −80°C whenever possible.
- Avoid repeated freeze-thaw cycles.
- Ship samples on sufficient dry ice.
- Please submit samples in clearly labelled 1.5mL centrifuge tubes.
- All metabolomics projects should be confirmed before shipment.
- A completed Sample Information Form (SIF) is required before sample shipment.
- The current untargeted metabolomics workflow is optimised for low-salt sample matrices. Samples containing >10 mM total salts, concentrated buffers, fermentation media, microbial culture media, or other high-salt matrices are currently not supported.
- Special sample types may require feasibility confirmation prior to acceptance.

2. Supported sample types and requirements

Sample type	Minimum amount	Recommended amount
Human / animal tissue	26 mg	100 mg
Plant tissue	26 mg	100 mg
Faeces / intestinal contents	26 mg	100 mg
Soil	1.3 g	2 g
Plasma / serum	26 μL	100 μL
Urine	26 μL	100 μL
Biological fluids (saliva, tears, amniotic fluid, bile and similar low-salt matrices)	130 μL	200 μL
Liquid culture medium, supernatant, non-fermented liquids…	1.3 mL	2 mL
Cell pellets	≥10^6 cells	1×10^7 cells
Solid culture medium	130 mg	200 mg

Notes

- Please ensure the cell count can meet the requirement.
- Recommended amounts are suggested to improve analytical robustness and allow potential repeat analysis.
- For cell culture supernatants, the matrix must be low-salt and non-microbial. Medium composition may be requested before acceptance.

Back to contents

3. Sample preparation guidance

3.1 Human and animal tissue

Human tissue

When collecting samples from human patients, generally only a small biopsy is obtained. It is recommended to snap-freeze these samples as soon as possible.

To minimize tissue contamination, as much blood as possible should be removed from the samples. This can be achieved by rinsing with deionized water.

Animal tissue

For large tissue samples, it is advisable to subdivide and aliquot them to minimize freeze-thaw cycles. In the case of rodents, tissue removal should be performed after exsanguination to minimize the presence of blood in the tissue.

1. Select specific regions according to the experimental design and place them into centrifuge tubes.
2. Properly label the tubes, then quickly freeze them in liquid nitrogen for at least 15 minutes.
3. Store the samples at −80°C and ship them with sufficient dry ice.

Key considerations

- Keep sampling locations as consistent as possible between biological replicates.
- Avoid contamination from anaesthetics, cleaning reagents, or collection materials.
- Collect tissues on ice whenever possible to minimise further metabolism.
- If tissue samples are large, divide them into smaller aliquots before freezing.
- Minimise freeze-thaw cycles.

3.2 Plant tissue

Leaves, stems, flowers

1. Collect the whole leaf, stem, or flower and wrap it in aluminum foil.
2. Label the wrapped samples and quickly freeze it in liquid nitrogen for at least 15 minutes.
3. Transfer the samples into centrifuge tubes.
4. Store the samples at −80°C.
5. Use sufficient dry ice during transport.

Key considerations

- Collect samples under consistent lighting and developmental conditions.
- Maintain consistency within the same sample group.
- Include an internal label sheet, as pen markings on aluminium foil may fade.

Roots

1. Collect the entire plant root and quickly rinse off soil using 1× PBS.
2. According to the experimental design, collect the required root regions and place them into centrifuge tubes.
3. Label the samples and immediately freeze them in liquid nitrogen for at least 15 minutes.
4. Store at −80°C and ship with sufficient dry ice.

Key considerations

- Wash roots quickly to minimise metabolic changes.
- Root tissues are fragile and should preferably be stored in centrifuge tubes rather than wrapped in foil.

Fruits and seeds

1. For large fruits (e.g., tomatoes, watermelons, apples), cut samples into uniform pieces and transfer into 1.5 mL centrifuge tubes.
2. For seeds, mix samples within the same group before aliquoting into tubes.
3. For whole fruits, place samples into centrifuge tubes.
4. Freeze samples in liquid nitrogen for at least 15 minutes.
5. Store at −80°C and ship with sufficient dry ice.

Key considerations

- Maintaining consistency between fruit tissues such as peel, pulp, and seeds.
- Freeze-drying and powder homogenisation are recommended for large juicy fruits.
- Wrapping juicy fruit samples in aluminum foil is not recommended.

3.3 Faeces/Intestinal contents

1. Collect fresh feces or intestinal contents.
2. Freeze samples in liquid nitrogen for at least 15 minutes immediately after collection.
3. Store at −80°C and ship with sufficient dry ice.

Key considerations

- Microbial metabolism remains highly active after collection.
- Avoid repeated freeze-thaw cycles.
- Aliquot samples before freezing whenever possible.

3.4 Plasma and serum

Plasma

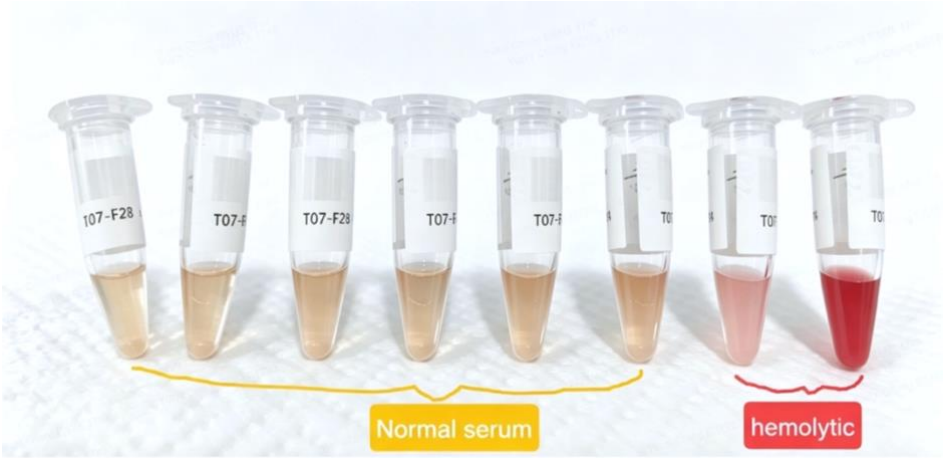
1. Use sodium heparin anticoagulant tubes for blood collection whenever possible.
2. Centrifuge at 3000 rpm at 4°C for 10 minutes.
3. Transfer the plasma layer into labelled 1.5 mL centrifuge tubes.
4. Freeze samples in liquid nitrogen for at least 15 minutes.
5. Store at −80°C and ship with sufficient dry ice.

Serum

1. Collect blood using tubes without anticoagulant.
2. Allow samples to clot at room temperature for approximately 1 hour.
3. Centrifuge at 3000 rpm for 5 minutes.
4. Transfer the supernatant into clean centrifuge tubes.
5. Freeze samples in liquid nitrogen for at least 15 minutes.
6. Store at −80°C and ship with sufficient dry ice.

Key considerations

- Keep sample handling and centrifugation conditions consistent.
- Avoid haemolysis whenever possible.
- Sodium heparin tubes are recommended for plasma collection.
- Aliquot samples to minimise freeze-thaw cycles.
- Severe haemolysis may affect metabolomics analysis quality. In such cases, the haemolytic sample needs to be recollected:



Back to contents

3.5 Saliva

1. Sample collection is recommended between 9:30 AM and 11:30 AM.
2. Rinse the mouth with saline before collection.
3. Collect saliva into sterile tubes while keeping samples on ice.
4. Centrifuge at 4°C and 2000 g for 10 minutes.
5. Collect the supernatant and optionally filter through a 0.22 μm membrane.
6. Aliquot into tubes, freeze in liquid nitrogen for at least 15 minutes, and store at −80°C.

Key considerations

- Avoid smoking, eating, or drinking for at least 1 hour before sampling.
- Do not brush teeth prior to collection.
- Aliquot samples to minimise freeze-thaw cycles.

3.6 Urine

1. Collect midstream urine samples.
2. Centrifuge at 3000 rpm at 4°C for 10 minutes.
3. Transfer clear urine into centrifuge tubes.
4. Freeze samples in liquid nitrogen for at least 15 minutes.
5. Store at −80°C and ship with sufficient dry ice.

Key considerations

- For low-volume animal urine collection, multiple collections may be combined.

3.7 Tears

1. Collect samples between 9:00 AM and 12:00 PM.
2. Use Schirmer strips for tear collection.
3. Place strips into labelled 1.5 mL centrifuge tubes.
4. Freeze in liquid nitrogen for at least 15 minutes.
5. Store at −80°C and ship with sufficient dry ice.

Key considerations

- For low-volume animal urine collection, multiple collections may be combined.

3.8 Cell samples

This section applies to non-microbial cell samples only, such as mammalian, animal or plant-derived cell pellets.

Suspension cells

1. Collect cells by centrifugation.
2. Wash pellets 2–3 times using pre-chilled PBS or another compatible low-salt wash buffer.

3. Remove the supernatant completely.
4. Transfer pellets into cryogenic tubes.
5. Freeze in liquid nitrogen for at least 15 minutes.
6. Store at −80°C and ship with sufficient dry ice

Adherent cells

1. Remove the culture medium.
2. Wash cells 2–3 times using pre-chilled PBS or another compatible low-salt wash buffer.
3. Digest cells using trypsin where appropriate.
4. Wash again using pre-chilled PBS.
5. Centrifuge and collect the cell pellet.
6. Freeze in liquid nitrogen for at least 15 minutes.
7. Store at −80°C and ship with sufficient dry ice.

Cell culture supernatant / extracellular metabolite studies

1. Use non-microbial, low-salt culture systems only.
2. Remove cells by centrifugation.
3. Collect the clarified supernatant.
4. Aliquot and freeze in liquid nitrogen or at -80°C as quickly as possible.
5. Store at -80°C and ship with sufficient dry ice.

Key considerations

- Complete the entire workflow as quickly as possible.
- Maintain consistent cell numbers and culture conditions across all samples.
- Serum-free medium is recommended for extracellular metabolite studies.
- Samples may require desalting or additional sample cleanup prior to LC-MS analysis

4. Metabolite extraction methods

Metabolite extraction workflows are performed according to Novogene validated untargeted metabolomics protocols.

Extraction procedures may vary depending on sample type and analytical platform requirements.

Client-side desalting or extraction is not recommended unless explicitly agreed in writing before shipment.