Novogene | Advancing Genomics, Improving Life

Novogene Extraction Service Guidelines

Sample requirements for DNA extraction - below amounts constitute one extraction

Sample Source	Standard Amount	Special Amount for Several Products	Buffer/ Status	Recommended Shipping Method	Notes
Cell pellet	⩾500K Cells	–	Snap frozen	Dry ice	Novogene does not operate a cell counter; please only send the designated amount.
Whole blood	⩾400 ul	⩾2 mL for RRBS	Blood collection tube with EDTA as anticoagulant	Dry ice	1. Every tube should be individually packaged with sufficient dry ice. 2. All tubes should be separated. 3. Any damage or liquid overflow should not be allowed. 4. Seal the tubes well to avoid leakage due to tube damage. 5. Prepare for vibration-absorption. 6. Anticoagulant is required to prevent DNA degradation caused by blood coagulation.
Fresh animal tissue	⩾100 mg	–	Snap frozen	Dry ice	–
Fresh plant tissue	⩾100 mg	–	Snap frozen	Dry ice	–
FFPE slides (for human WGS/ WES/ TRS projects only)	⩾10 slides	–	–	Room temperature/ Blue ice	Thickness: 5~10 um; Area: >1 cm^2
FFPE curls (for human WGS/ WES/ TRS projects only)	2-4 curls	–	–	Room temperature/ Blue ice	Curls: 2-4 curls per eppendorf tube
Saliva (for human/animal projects only)	⩾2 ml	–	Snap frozen	Dry ice	Collected in 10 mL cryogenic storage tube
Buccal Swab (for human/animal projects only)	⩾3 swaps	–	–	Dry ice	Package in buccal swab collection boxes, do not expose to air to avoid contamination. Use plastic containers instead of glass containers to prevent damage caused by glass containers.
Serum/Plasma (for human WGS/ WES/ TRS projects only)	⩾500 ul	–	Snap frozen	Dry ice	–
Stool (for Metagenomics projects only)	⩾100 mg	–	Snap frozen	Dry ice	–
Fungal/Bacteria culture or cell pellet (for microbial WGS and Metagenomics projects only)	⩾500K (cells) or ⩾20 mg (wet weight)	–	Snap frozen	Dry ice	–

Sample requirements for RNA extraction - below amounts constitute one extraction

Sample Source	Standard Amount	Special Amount for Several Products	Buffer/ Status	Recommended Shipping Method	Notes
Cell pellet	⩾ 500K cells	⩾ 1.5 M cells for sRNA-seq/ circRNA-seq; ⩾ 3 M cells for WTS	Snap frozen	Dry ice	Novogene does not operate a cell counter; please only send the designated amount.
Whole blood	⩾400 ul	⩾8 mL for sRNA-seq/ circRNA-seq; ⩾10 mL for WTS	PAXgene Blood Tubes OR Blood collection tube with EDTA as anticoagulant	Dry ice	1. Every tube should be individual-ly packaged with sufficient dry ice. 2. All tubes should be separated. 3. Any damage or liquid overflow should not be allowed. 4. Seal the tubes well to avoid leakage due to tube damage. 5. Prepare for vibration-absorp-tion.
Fresh animal tissue	⩾100 mg	⩾500 mg for sRNA-seq/ circRNA-seq; ⩾800 mg for WTS	Snap frozen	Dry ice	After sample collection, it is recommended to immediately snap-freeze and then cryopreserve the fresh tissues. To limit RNA degradation, NEVER leave fresh tissues at room temperature or wash them even for a short time.
Fresh plant tissue	⩾100 mg	⩾800 mg for sRNA-seq/ circRNA-seq; ⩾1 g for WTS	Snap frozen	Dry ice	
FFPE slides and curls	⩾10 slides; ⩾4 curls	–	–	Room temperature/ Blue Ice	Slide Thickness: 10 um; Area: >1 cm^2 Curls: ⩾4 curls per Eppendorf tube
Stool (for Metatranscriptome projects only)	⩾100 mg	–	Snap frozen	Dry ice	–
Fungal/Bacteria culture or cell pellet (for Prokaryotic RNA & Metatranscriptome projects only)	⩾500K (cells) or ⩾20 mg (wet weight)	–	Snap frozen	Dry ice	–

Notes

1. If the weight of the sample is known, it would be helpful if the client could provide the weight information when submitting the Sample Information Form (SIF).
2. Ideally, samples should be provided in 2 mL centrifuge tubes. For animal tissue, it must be placed on dry ice (-80°C) during shipment. Plant tissue should also be shipped on dry ice if possible.
3. The type, location, and source of the sample must be known and clearly indicated. For example, if it's a mouse kidney tissue or a buccal swab.
4. The client needs to indicate whether it is acceptable to use the entire tissue sample provided. If not, they should specify what they would like to be done with the leftover tissue. We do not offer long-term storage and prefer to ship the unused tissue back to the client upon their request.
5. If possible, please inform us if the sample contains a high polysaccharide and/or high lipid content.
6. RNA extracted from skin tissue typically has a low yield and RIN (RNA Integrity Number).
7. RNA extracted from adipose tissue usually has low yield and quality. Therefore, we recommend that the customer send three times the amount of the recommended sample size for adipose tissue.

8. The client must provide information on whether the sample is in a stabilizing solution and specify the type of solution used. If RNAlater is used, the recommended ratio between RNAlater and tissue is 3:1. The tissue should be completely submerged in RNAlater, with no air left within the tube. The RNAlater-tissue mix should be snap-frozen and cryopreserved before shipping with dry ice.
9. For cell samples, it is advisable to collect cells in the logarithmic growth phase and freeze them directly in trizol. Please avoid using RNAlater for cell samples. Acceptable solutions for cell samples include PBS/TBS, Serum (FBS, NCS), Cell media (Optimem, DMEM), RLT lysis buffer, and Cryopreserve reagents.
10. All tissues must be free of any pathogenic agents that could contaminate the samples. Acceptable buffer options for tissues include no buffer, NFW (nuclease-free water), RNALater, or Trizol.
11. Please ensure that there are no repeated freeze-thaw cycles during sample storage and transportation to maintain sample integrity.
12. The above sample amounts do include redundancy for additional processing if needed. Our lab technicians will at random excise enough tissue to complete one round of extractions. Processing all tissue at your request may result in additional charges. If you want the remaining tissues returned, please notify your Project Coordinator immediately after the extraction is finished.

DNA Extractions

Plant tissue samples

- Freshly collected tissues are preferred. Select tissues with relatively high nucleic acid content, such as young parts of plants.
- If cryopreservation is needed, it is recommended to freeze samples with liquid nitrogen immediately after separation. Prior to freezing, wipe collected samples with sterile water or 75% ethanol.
- Absorbent paper can be used to blot the sample surface. Cut sample into small pieces of about 100mg and freeze immediately with liquid nitrogen.
- Place frozen sample in pre-chilled cryopreservation tubes, 15 mL/50 mL centrifuge tubes or Ziplock bags, and store at -80°C.
- When collecting samples, it is recommended that the samples should be stored in separate tubes according to the amount of primary extraction.

Animal tissue samples

- Freshly collected samples for DNA extraction are preferred. It is recommended to select tissues with high content of nucleic acid (such as animal liver or other tissues).
- It is recommended to rinse collected samples with cold saline to remove blood and contaminants. Unwanted tissues (such as connective tissue, hair) should be removed.
- Divide the samples into small pieces of about 50 mg (the smaller the tissue is, the better preservation effect it has). Cut tissues into smaller pieces on ice, to improve preservation.
- If cryopreservation is needed, the fresh samples collected from animals should be frozen immediately with liquid nitrogen. Samples are to be put into 1.5 mL or 2.0 mL precooled EP or cryopreservation tube with screw cap, sealed with sealing film and stored at-80°C. Please avoid overfilling the container to prevent cracking and sample contamination during freezing.
- If nucleic acid stabilizer is used for preservation of tissue samples, please operate strictly in accordance with the requirements of the reagent specification. Do keep tissue block within the size range required by the reagent, to ensure samples can be fully penetrated by the reagent to avoid degradation.

Microbial samples

- Use an alcohol-sterilized shove to dig and collect 5~20 cm depth of soil, remove visible roots and filter the soil with 2 mm sieve. Each sample is collected from three different sampling points and pooled together.
- Samples are collected in sterile centrifuge tubes and placed below 0°C, for transportation back to the laboratory for DNA extraction. If the extraction cannot be carried out immediately, the soil samples should be frozen in the freezer at -80°C or -20°C.
- It is strongly recommended to use 5 mL EP or 15 mL/50 mL centrifuge tube for transporting soil samples. Seal tubes with parafilm. Note that the use of self-sealing bags has great risk of sample cross contamination.
- Aliquot samples into various tubes to avoid sample degradation due to repeated freeze-thawing.

Stool samples

- For detailed steps, please check: Kyle G. Bowel care. Part 3--obtaining a stool sample[J]. Nursing times, 2007, 103(44):24-25. The specific steps are as follows:
  - Prepare feces container, wash hands, and put on gloves.
  - Collect the sample with a spoon (1 g can be collected and packed separately in each tube) and put into 5 mL EP or 15 mL, 50 mL centrifugal tube. After collecting, screw the cap tightly and seal with parafilm. Keep the sample in an anaerobic environment, if possible, with an anaerobic bag.
  - Discard the feces container and gloves and store the samples at-80°C to avoid repeated freezing and thawing. If fecal samples are too large or cannot be collected immediately, the collection shall be completed within 4 hours at the latest.
- Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

Water samples

- Filter collected water through filter membranes. According to the turbidity of the water samples, filter membranes with corresponding pore sizes can be selected. For turbid water, a 0.45 μm pore size filter membrane can first be used to remove suspended particles. Filter membranes with small pore sizes (0.22 μm) are then selected to filter sediment-free water samples. Filter 10 L of clear water to obtain microbial-enriched filter membrane.
- After filtering, the microbial enriched filter membrane can be installed in a 50 mL centrifugal tube (the area of the filter membrane should not be too large, take the part with a diameter of 3~4 cm rich in microorganisms) and storage.

Strain Bacteria Culture samples

- For bacterial samples, it is recommended to collect bacteria during the phase of logarithmic growth.
- Collect the strains by centrifugation at low speed. Discard supernatant. Wash bacterial pellet with sterile water or PBS buffer (1~3 times). Store sample in 1.5/2 mL EP tube. Seal with sealing film, and store it at-8°C.
- Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

Blood samples

- Collect whole blood samples with EDTA anticoagulation blood collection tubes (Avoid heparin anticoagulant).
- Blood samples should not be subjected to repeated freeze-thaw cycles. Fresh blood can be placed at 4°C for 12~24 hours. However, the frozen samples must be stored at low temperature.

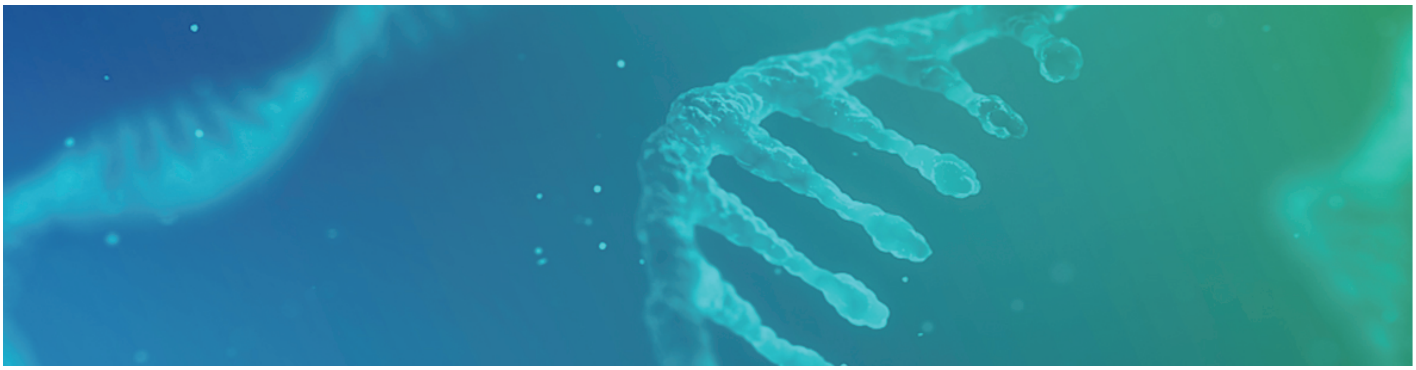
RNA Extractions

Plant tissue samples

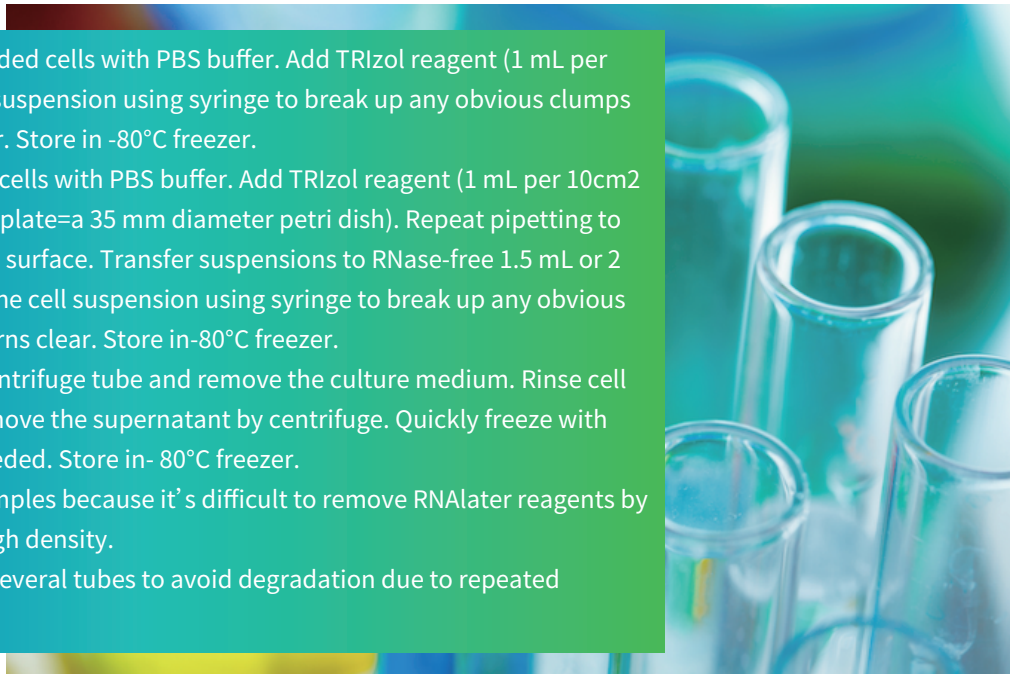
- Freshly collected tissues are preferred. Tissues with relatively high nucleic acid content are advised to be selected, such as young parts of plants.
- It is recommended to quickly wipe, or rinse freshly collected plant sample with RNase free water, and blot dry with absorbent paper.
- Quickly cut tissues into smaller pieces (~100 mg) on ice. To ensure RNA preservation, it is recommended to complete this step within 3 minutes after tissue separation.
- Freeze sample with liquid nitrogen and store in a pre-chilled RNase-free 1.5 mL/2.0 mL EP tubes or freezing tubes with a screw cap. Seal tubes with parafilm and stored at -80°C immediately.
- Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

Animal tissue samples

- Using freshly collected samples for RNA extraction is preferred. For sample collection, it is recommended to select tissues with high content of nucleic acid (such as animal liver or other tissues).
- It is recommended to rinse freshly extracted tissue samples from living animals immediately, with precooled RNase-free water. Ensure removal of blood, unwanted tissues (such as hair and connective tissue) and other contaminants.
- Quickly cut tissues into smaller pieces (50~100 mg) on ice and freeze using liquid nitrogen. Store sample in pre-chilled RNase-free 1.5 mL/2.0 mL EP tubes or freezing tubes with a screw cap. Seal tubes with sealing film and stored at-80°C immediately. To ensure RNA preservation, it is recommended to complete this step within 3 minutes upon tissue removal from living body. Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.
- If using commercial RNA protection reagent (such as RNAlater), please follow manufacturer recommendation.



Cells



- Cell suspensions: Rinse suspended cells with PBS buffer. Add TRIzol reagent (1 mL per 5×106 cells). Aspirate the cell suspension using syringe to break up any obvious clumps until the suspension turns clear. Store in -80°C freezer.
- Adherent cells: Rinse adherent cells with PBS buffer. Add TRIzol reagent (1 mL per 10cm2 of culture area=a well of 6-well plate=a 35 mm diameter petri dish). Repeat pipetting to ensure TRIzol to contact all cell surface. Transfer suspensions to RNase-free 1.5 mL or 2 mL centrifuge tubes. Aspirate the cell suspension using syringe to break up any obvious clumps until the suspension turns clear. Store in-80°C freezer.
- Collect cell samples into the centrifuge tube and remove the culture medium. Rinse cell pellets with PBS buffer and remove the supernatant by centrifuge. Quickly freeze with liquid nitrogen. No lysate is needed. Store in- 80°C freezer.
- Do not use RNAlater for cell samples because it's difficult to remove RNAlater reagents by centrifuge as RNAlater has a high density.
- Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.

Bacterial Culture Samples

- For bacterial samples, it is recommended to collect bacteria during the phase of logarithmic growth.
- Collect the strains by centrifugation at low speed and discard supernatant. Wash pellet with sterile water or PBS buffer (1~3 times). Store sample in pre-chilled RNase-free 1.5 mL/2.0 mL EP tubes or freezing tubes with a screw cap. Seal tubes with sealing film and stored at -80°C immediately. Aliquot and store sample into several tubes to avoid degradation due to repeated freezing and thawing.
- It's recommended not to send samples utilizing TRIzol lysate because TRIzol method fails to the extraction of nucleic acid from some of bacteria.
- It is not recommended to store samples with low bulk density in RNAlater and other tissue with RNA protection reagents. Because the density of RNAlater is a little high which increase the difficulty of collection by centrifugation for extraction.

Blood samples

- Sample preparation by TRIzol method
  - Add 6 mL TRIzol and 2 mL fresh blood (TRIzol: blood=3: 1) together in a 15 mL tube. Repeat pipetting to ensure TRIzol to contact all cell surface.
  - Intensely shake and mix the sample for one to two minutes until the floc is completely dissolved.
  - Incubate at room temperature for 5 minutes.
  - Aliquot and store sample into 4 cryovials (2 mL). Seal with film and store at-80°C.

  Note: RNAlater is not recommended.
- BD PAXgene Blood RNA Tube: Collect blood - Product name: BD 762165 PAXgene Blood RNA Tube
- Please refer to the manufacture's introduction for more detailed information.