

Sample Submission Guidelines for NGS-Grade Nucleic Acid Extraction: NGS-1000 series

Sample Type/ Origin	NGS-Grade DNA extraction	NGS-Grade RNA extraction
Animal Tissue	Option 1: Tissue • Excise the target tissue from the animal • Rinse the tissue with 1X PBS to remove all traces of blood • Weight: 50–100 mg of sample **Cutting and weighing of the sample must be completed within 3 minutes of removing the tissue from the animal to ensure that the tissue is preserved without degradation. • Snap-freeze the sample in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Option 2: Tissue ground into a fine powder • Excise the target tissue from the animal • Rinse the tissue with 1X PBS to remove all traces of blood • Quickly grind the tissue into a fine powder with liquid nitrogen • Weight: 50–100 mg of sample • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Option 3: Tissue in DNA/ RNA tissue stabilization solution • Excise the target tissue from the animal • Cut the tissue into slices less than 0.5 cm thick. • Place the fresh tissue in a screw-cap tube (1.5/ 2.0/ 15/ 50 mL). • Weight: 50–100 mg of sample **Cutting and weighing of the sample must be completed within 3 minutes of removing the tissue from the animal to ensure that the tissue is preserved without degradation. • Preserve the sample according to the guidelines of the stabilization reagent used, following the ratio of stabilization reagent volume to sample size to ensure effective sample preservation. • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	
Cultured Cells	Option 1: Cell pellet • Up to 1 × 10⁷ cells (from 1–2 T25 flasks at 70% confluency) • Collect the pellet from the culture, wash the cell pellet with PBS and remove the PBS completely • Snap-freeze the pellet in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Option 2: Cell pellet in DNA/ RNA stabilization solution • Up to 1 × 10⁷ cells (from 1–2 T25 flasks at 70% confluency) • Collect the pellet from the culture, wash the cell pellet with PBS and remove the PBS completely • Preserve the sample according to the guidelines of the stabilization reagent used, following the ratio of stabilization reagent volume to sample size to ensure effective sample preservation. • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	
Mammalian Tissue/ Cultured Cells for Mitochondrial DNA (mtDNA) Extraction	Animal Tissue • Excise the target tissue from the animal • Rinse the tissue with 1X PBS to remove all traces of blood • Weight: 50–100 mg of sample • Snap-freeze the sample in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Cell pellet • Up to 1 × 10⁷ cells (from 1–2 T25 flasks at 70% confluency) • Collect the pellet from the culture, wash the cell pellet with PBS and remove the PBS completely • Snap-freeze the pellet in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	Not applicable.
Bacteria	Bacterial pellet • Dilute the overnight culture at a ratio of 1:50 with culture medium and continue growing the culture for 3–5 hours **Do not use the overnight culture directly. Reduce the dilution factor if slow-growing bacteria are used. • Collect the pellet from the culture and remove the culture medium completely • Weight: 200–500 mg of sample • Snap-freeze the pellet in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	
Yeast	Yeast pellet • Dilute the overnight culture at a ratio of 1:50 with culture medium and continue growing the culture for 4–5 hours **Do not use the overnight culture directly. • Collect the pellet from the culture and remove the culture medium completely • Weight: 200–500 mg of sample • Snap-freeze the pellet in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	

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Fungi	Fungal pellet • Collect the pellet from the culture and remove the culture medium completely • Weight: 0.5–1 g of sample • Snap-freeze the pellet in a liquid nitrogen bath for 10 minutes • No. of replicates: 5 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Fruiting body • Rinse with clean water to remove dirt attached to the sample • Cut the sample into small pieces (< 0.5 cm) and place them in a 50 mL centrifuge tube • Weight: 2–5 g wet weight **Cutting and weighing of the sample must be completed within 3 minutes of tissue removal to ensure that the tissue is preserved without degradation. • Snap-freeze the sample in a liquid nitrogen bath for 10 minutes • No. of replicates: 5 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	
Algae	Microalgae pellet • Collect the pellet from the culture and remove the culture medium completely • Weight: 0.5–1 g of sample • Snap-freeze the pellet in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	
Blood	A) Genomic DNA extraction from blood Option 1: Whole Blood, *for customers in Malaysia only • Collect up to 3 mL of fresh blood in an EDTA tube • Submit blood samples immediately after collection. • Results will not be guaranteed for blood samples that are more than 3 days old • No. of replicates: 3 • Shipping condition: i) 4 °C or blue ice (arrival at Apical Scientific within 3 days) ii) Dry ice (arrival at Apical Scientific after 3 days) Option 2: Buffy Coat • Collect up to 3 mL of blood in an EDTA tube. Centrifuge the blood at 2,500 × g for 10 minutes using a swinging-bucket centrifuge • Remove the plasma using a 1 mL syringe fitted with a 20G needle • Collect the buffy coat in a 1.5 or 2 mL tube • No. of replicates: 3 • Store the buffy coat in a –80 °C freezer • Shipping condition: Dry ice Option 3: White Blood Cell pellet • Collect up to 3 mL of fresh blood in an EDTA tube • Pretreat the whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-220ml). Refer to "Appendix Protocol I" below for the pretreatment procedure. • No. of replicates: 3 • Store the treated dry WBC pellet in a –80 °C freezer. Alternatively, the WBC pellet can be stored in RNAlater solution. • Shipping condition: Dry ice Appendix Protocol I: Pretreatment of whole blood samples with RBC lysis buffer (1st BASE, K.BUF-9101-220ml) 1. Transfer 1 mL of whole blood into a new 2 mL microcentrifuge tube. 2. Add 1 mL of RBC Lysis Buffer (not provided). Invert the tube 10 times. Centrifuge at 700 x g for 5 minutes at room temperature. 3. Remove 1 mL of supernatant (top layer) by pipetting. Note: Do not remove the middle and bottom layers, which contain white blood cells and red blood cells, respectively. 4. Add 1 mL of RBC Lysis Buffer (not provided). Resuspend the pellet by pipetting 4–5 times. Centrifuge at 700 x g for 5 minutes. 5. Carefully remove 1 mL of supernatant by pipetting from the top. Leave the remaining supernatant and cell pellet in the tube. 6. Repeat Step 4. Remove the supernatant completely. [For preservation] Resuspend the cell pellet in 1 mL of RNAlater solution (not provided). B) For cell-free DNA (cfDNA) extraction from blood plasma, please contact us to enquire.	Total RNA extraction from blood Option 1: Whole Blood in Tempus™ Blood RNA Tube • Collect up to 3 mL of blood in a Tempus™ Blood RNA Tube • Shake vigorously for 10 seconds to mix the sample with the stabilizing reagent in the Tempus Tube • No. of replicates: 2 • Store the sample in a refrigerator at 4 °C (up to 7 days) or in a freezer at –20 °C/ –80 °C (> 7 days). • Shipping condition: i) 4 °C or blue ice (arrival at Apical Scientific within 5 days) ii) Dry ice (arrival at Apical Scientific after 5 days), avoid direct contact between the sample and dry ice. Option 2: White Blood Cell pellet • Collect up to 3 mL of blood in an EDTA tube • Preprocess the blood sample on the day of collection. • Pretreat the whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-220ml). Refer to "Appendix Protocol II" below for the pretreatment procedure. • No. of replicates: 3 • Store the treated dry WBC pellet in a –80 °C freezer. Alternatively, the WBC pellet can be stored in RNAlater solution. • Shipping condition: Dry ice Appendix Protocol II: Pretreatment of whole blood samples with RBC lysis buffer (1st BASE, K.BUF-9101-220ml) 1. Transfer 3 mL of whole blood into a new 15 mL centrifuge tube. 2. Add 3 mL of RBC Lysis Buffer (not provided). Invert the tube 10 times. Centrifuge at 700 x g for 5 minutes at room temperature. 3. Remove 2 mL of supernatant (top layer) by pipetting. Note: Do not remove the middle and bottom layers, which contain white blood cells and red blood cells, respectively. 4. Add 3 mL of RBC Lysis Buffer (not provided). Resuspend the pellet by pipetting 4–5 times. Centrifuge at 700 x g for 5 minutes. 5. Carefully remove 3 mL of supernatant by pipetting from the top. Leave the remaining supernatant and cell pellet in the tube. 6. Repeat Step 4. Remove the supernatant completely. [For preservation] Resuspend the cell pellet in 1 mL of RNAlater solution (not provided). Not applicable.
Insect	Option 1: Insect • Immerse the live insect in absolute ethanol until the insect stops moving • Blot the insect dry • Weight: 50–100 mg of sample • Snap-freeze the insect in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Option 2: Insect ground into a fine powder • Immerse the live insect in absolute ethanol until the insect stops moving • Blot the insect dry • Quickly grind the insect into a fine powder with liquid nitrogen • Weight: 50–100 mg of sample • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Option 3: Insect in DNA/RNA tissue stabilization solution • Immerse the live insect in absolute ethanol until the insect stops moving • Blot the insect dry • Place the fresh insect in a screw-cap tube (1.5/ 2.0/ 15/ 50 mL). • Weight: 50–100 mg of sample • Preserve the sample according to <u>the guidelines of the stabilization reagent</u> used, following <u>the ratio</u> of stabilization reagent volume to sample size to ensure effective sample preservation. • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	

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Plant	Fresh leaves, needles, stems or roots; young leaves or needles are preferred • Rinse with clean water to remove dirt attached to the plant sample • Cut the sample into small pieces (< 0.5 cm) and place them in a 50 mL centrifuge tube • Weight: i) 2–5 g wet weight OR ii) 2–5 g of tissue ground into a fine powder using liquid nitrogen **Cutting and weighing of the sample must be completed within 3 minutes of removing the tissue from the plant to ensure that the tissue is preserved without degradation. • Snap-freeze the sample in a liquid nitrogen bath for 10 minutes • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice <I> Avoid pooling different plant parts/ organs into the same tube, as the lysis procedure differs for each part.	
Plant for Chloroplast DNA (cpDNA) Extraction	Fresh green leaves without brown spots or holes • Weight: Harvest ≥ 30 g of fresh green leaves • Wrap the leaves in aluminium foil • No. of replicates: 3 • Storage/ Shipping condition: 4 °C or blue ice in the dark <I> The sample must reach Apical Scientific within 48–72 hours of sample collection. We recommend arranging a suitable day for sample submission with our Customer Care team in advance. Example of fresh leaf 	Not applicable.
Soil/ Sludge	For amplicon sequencing applications • Weight: 0.5–1 g of sample in a screw-cap tube • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice For shotgun metagenomics applications, we recommend collecting soil samples in a preservation solution. • Collect soil samples in a commercially available preservation solution. Follow the kit instructions for sample collection, preservation, storage, and shipment. • Weight: 1–2 g of sample • No. of replicates: 3 • Shipping condition: Dry ice *Depending on the soil type, 4–8 additional sample replicates may be required to obtain sufficient DNA for downstream applications.	• Collect soil samples in a commercially available preservation solution. Follow the kit instructions for sample collection, preservation, storage, and shipment. • Weight: 1–2 g of sample • No. of replicates: 3 • Shipping condition: Dry ice *Depending on the soil type, 4–8 additional sample replicates may be required to obtain sufficient RNA for downstream applications.
Stool	Option 1: • Weight: 200–500 mg of sample in a screw-cap container/ tube • Label the sample with a printed label • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice Option 2: • We recommend following the guidelines provided with a commercially available stool collection kit for sample collection, preservation, storage and shipping. For example, DNA Genotek, OMNIgene•GUT DNA and RNA. • No. of replicates: 2 • Storage/ Shipping condition: Dry ice	
Manure/ Biofilm mat	• Weight: 0.5–1 g of sample in a screw-cap container/ tube • Label the sample with a printed label • No. of replicates: 3 • Store the sample in a –80 °C freezer • Shipping condition: Dry ice	Not applicable.
Water	Option 1: Filtered material on 0.22 µm membrane filter • Prefilter the water sample using a cell strainer with a larger pore size (e.g., 100 µm) to remove large debris. • Continue filtering the water sample (200–500 mL*) using vacuum filtration with a 0.22 µm pore size membrane filter (25 or 47 mm). *If the sample has low turbidity (i.e. little material is retained on the membrane), continue filtration until a total volume of up to 1.5 L has been filtered. • Use a sterile scalpel blade to remove the membrane filter from the filtration unit. • Insert the membrane filter into a new sterile tube by rolling the membrane using sterile forceps, ensuring that the sample-retaining side of the membrane is facing inward. • No. of replicates: 3 • Store the membrane filter in a –80 °C freezer • Shipping condition: Dry ice Option 2: Water sample • Volume: 500 mL** **If the sample has low turbidity, please submit at least three replicates, i.e. 500 mL x 3 = 1.5 L • Store the sample in a screw-cap bottle in the dark at 4 °C • Shipping condition: 4 °C or blue ice	Not applicable.

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Food	Food • Weight: Solid food: 10 g sample in a screw-cap tube/ container/ zip-lock bag Liquid food: 20–50 mL sample in a screw-cap tube • No. of replicates: 2 • Shipping condition: Follow the recommended food storage conditions and ensure that the food sample does not spoil or rot during shipment. a) If the sample was stored at 4 °C, ship it with blue ice b) If the sample was stored in a freezer, ship it on dry ice	Not applicable.
Swab sample (Buccal swab/ Environmental swab)	Option 1: • Collect the sample using a commercial swab sample collection kit • Follow the collection kit guidelines to preserve the swab sample • No. of replicates: 3 • Storage/ Shipping condition: Follow the guidelines from the commercial kit Option 2: • Collect the sample using a sterile swab • (Optional) Store the swab in 1X PBS • No. of replicates: 3 • Storage condition: -80 °C freezer • Shipping condition: Dry ice	• Collect the sample using a commercial swab sample collection kit (for RNA applications) • Follow the collection kit guidelines to preserve the swab sample • No. of replicates: 3 • Storage/ Shipping condition: Follow the guidelines from the commercial kit
Saliva	Option 1: • We recommend following the guidelines provided with a commercially available saliva collection kit for sample collection, preservation, storage and shipping. - For example, DNA Genotek, OMNigene•SALIVA DNA and RNA device. • No. of replicates: 2 • Storage/ Shipping condition: Follow the kit manufacturer’s recommendations Option 2: • Collect 2 mL of saliva in a new 50 mL centrifuge tube • No. of replicates: 2 • Add 10 mL of 1X PBS. • Shake vigorously to mix for at least 20 seconds. • Immediately centrifuge at 2,000 x g for 5 minutes at room temperature to pellet cells. • Decant the supernatant without delay. • Store the dry pellet in a –80 °C freezer • Shipping condition: Dry ice	Option 1: • We recommend following the guidelines provided with a commercially available saliva collection kit for sample collection, preservation, storage and shipping. - For example, DNA Genotek, OMNigene•SALIVA DNA and RNA device. • No. of replicates: 2 • Storage/ Shipping condition: Follow the kit manufacturer’s recommendations Option 2: • Collect 2 mL of fresh saliva in a 50 mL centrifuge tube • No. of replicates: 2 • Add 2 mL of RNAlater solution. • Vortex vigorously to mix thoroughly. • Incubate the sample overnight in a refrigerator at 4 °C. • Store the sample in a –80 °C freezer the next day. • Shipping condition: Dry ice
Others	Please enquire.	

How to snap-freeze a sample

- Place the sample in a sterile, empty tube (1.5/ 2 mL tube with a safety-lock cap or 15/ 50 mL screw-cap centrifuge tube)
- Close the tube
- Immediately submerge the tube in a liquid nitrogen bath
- The sample should be frozen for at least 10 minutes
- Store the sample in a –80 °C freezer