


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

Sample Type/Origin	NGS Grade DNA extraction	NGS Grade RNA extraction
Animal Tissue	<p>Option 1: Tissue</p> <ul style="list-style-type: none"> Excise target tissue from animal Rinse tissue with 1X PBS to remove all traces of blood Weight: 50 - 100 mg of sample <p>**Cut and weighed sample must be completed within 3 minutes after tissue removal from animal to ensure the tissue is preserved without degradation.</p> <ul style="list-style-type: none"> Snap freeze sample in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>Option 2: Tissue in fine powder</p> <ul style="list-style-type: none"> Excise target tissue from animal Rinse tissue with 1X PBS to remove all traces of blood Quickly grind tissue into fine powder with liquid nitrogen Weight: 50 - 100 mg of sample No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>Option 3: Tissue in DNA/RNA tissue stabilization solution</p> <ul style="list-style-type: none"> Excise target tissue from animal Cut into slices less than 0.5 cm thickness. Put fresh tissue into screw cap tube (1.5/2.0/15/50 ml). Weight: 50 - 100 mg of sample <p>**Cut and weighed sample must be completed within 3 minutes after tissue removal from animal to ensure the tissue is preserved without degradation.</p> <ul style="list-style-type: none"> 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 sample in -80 °C freezer Shipping condition: Dry ice 	
Cultured Cells	<p>Option 1: Cell pellet</p> <ul style="list-style-type: none"> Cells up to 1 x 10⁷ (1 to 2 quantity of T25 flask, at 70% confluency) Collect pellet from culture, wash cell pellet with PBS and remove PBS completely Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>Option 2: Cell pellet in DNA/RNA stabilization solution</p> <ul style="list-style-type: none"> Cells up to 1 x 10⁷ (1 to 2 quantity of T25 flask, at 70% confluency) Collect pellet from culture, wash cell pellet with PBS and remove 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 sample in -80 °C freezer Shipping condition: Dry ice 	
Animal Tissue/ Cultured Cells for Mitochondria DNA (mtDNA) Extraction	<p>Animal Tissue</p> <ul style="list-style-type: none"> Excise target tissue from animal Rinse tissue with 1X PBS to remove all traces of blood Weight: 50 - 100 mg of sample Snap freeze sample in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>Cell pellet</p> <ul style="list-style-type: none"> Cells up to 1 x 10⁷ (1 to 2 quantity of T25 flask, at 70% confluency) Collect pellet from culture, wash cell pellet with PBS and remove PBS completely Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice 	Not applicable.
Bacteria	<p>Bacterial pellet</p> <ul style="list-style-type: none"> Collect pellet from 10 - 30 ml overnight culture, remove culture medium completely Weight: 200 - 500 mg of sample Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice 	<p>Bacterial pellet</p> <ul style="list-style-type: none"> Dilute overnight culture to 1:50 ratio with culture media, continue grow for 3 - 5 hours **Do not directly use overnight culture. Reduce the dilution factor if slow-growing bacterial is used. Collect the pellet from culture, remove culture medium completely Weight: 200 - 500 mg of sample Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice
Yeast	<p>Yeast pellet</p> <ul style="list-style-type: none"> Collect pellet from culture, remove culture medium completely Weight: 200 - 500 mg of sample Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice 	<p>Yeast pellet</p> <ul style="list-style-type: none"> Dilute overnight culture to 1:50 ratio with culture media, continue grow for 4 - 5 hours **Do not directly use overnight culture. Collect the pellet from culture, remove culture medium completely Weight: 200 - 500 mg of sample Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice

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Fungi	<p>Fungal pellet</p> <ul style="list-style-type: none"> Collect pellet from culture, remove culture medium completely Weight: 0.5 - 1 g of sample Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 - 5 Store sample in -80 °C freezer Shipping condition: Dry ice 	
Algae	<p>Microalgae pellet</p> <ul style="list-style-type: none"> Collect pellet from culture, remove culture medium completely Weight: 0.5 - 1 g of sample Snap freeze pellet in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice 	
Blood	<p>A) Genomic DNA extraction from blood</p> <p>Option 1: Whole Blood, *Malaysia Customer only</p> <ul style="list-style-type: none"> Volume: 3 ml freshly collected blood in EDTA Tube Blood samples to be submitted immediately after withdrawal. Results will not be guaranteed for blood samples that are more than 3 days old No. of replicates: 3 Shipping condition: <ul style="list-style-type: none"> i) 4°C or Blue ice (reach Apical Scientific within 3 days) ii) Dry ice (reach Apical Scientific > 3 days) <p>Option 2: Buffy Coat</p> <ul style="list-style-type: none"> Collect up to 3 ml blood in EDTA tube, centrifuge the blood with 2,500 x g for 10 minutes using swing bucket centrifuge Remove the plasma with 1 ml syringe, 21G needle Collect the buffy coat in 1.5/2 ml tube No. of replicates: 3 Store buffy coat in -80 °C freezer Shipping condition: Dry ice <p>Option 3: White Blood Cell pellet</p> <ul style="list-style-type: none"> Volume: up to 3 ml freshly collected blood in EDTA Tube Pretreat the whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-100ml), follow Appendix Protocol below for pretreatment. No. of replicates: 3 Store the treated dry WBC pellet in -80 °C freezer. Alternatively, the WBC pellet can be stored in RNAlater solution. Shipping condition: Dry ice <p>Appendix Protocol: Pretreatment of whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-100ml)</p> <ol style="list-style-type: none"> Transfer 1 mL whole blood into a new 2 mL microcentrifuge tube. Add 1 mL RBC Lysis Buffer (not provided). Invert the tube 10 times. Centrifuge at 700 x g for 5 minutes at room temperature. Carefully remove 1 mL top layer of the supernatant by pipetting. Note: Do not remove the middle and bottom layer which is the white blood cells and red blood cells respectively. Add 1 mL RBC Lysis Buffer (not provided). Resuspend the pellet by pipetting 4 – 5 times. Centrifuge at 700 x g for 5 minutes. Carefully remove 1 mL supernatant by aspirate from top via pipetting. Leave the remaining supernatant and cell pellet in the tube. Repeat Step iv. Remove supernatant completely. [For preservation] Resuspend cell pellet in 1 mL RNAlater Solution (not provided). <p>B) For cell-free DNA (cfDNA) extraction from blood plasma, please contact us to enquire.</p>	<p>Total RNA extraction from blood</p> <p>Option 1: Whole Blood in Tempus™ Blood RNA Tube</p> <ul style="list-style-type: none"> Collect up to 3 ml blood in Tempus™ Blood RNA Tube Shake vigorously for 10 seconds to mix sample with stabilizing reagent in the Tempus Tube No. of replicates: 2 Store sample in 4 °C fridge (up to 7 days) or -20°C/-80°C freezer (> 7 days). Shipping condition: <ul style="list-style-type: none"> i) 4°C or Blue ice (reach Apical Scientific within 5 days) ii) Dry ice (reach Apical Scientific > 5 days), avoid direct contact of sample with dry ice!! <p>Option 2: White Blood Cell pellet</p> <ul style="list-style-type: none"> Collect up to 3 ml blood in EDTA tube Must preprocess the blood sample within the same day of sample collection. Pretreat the whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-100ml), follow Appendix Protocol below for pretreatment. No. of replicates: 3 Store the treated dry WBC pellet in -80 °C freezer. Alternatively, the WBC pellet can be stored in RNAlater solution. Shipping condition: Dry ice <p>Appendix Protocol: Pretreatment of whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-100ml)</p> <ol style="list-style-type: none"> Transfer 3 mL whole blood into a 15 mL centrifuge tube. Add 3 mL RBC Lysis Buffer (not provided). Invert the tube 10 times. Centrifuge at 700 x g for 5 minutes at room temperature. Carefully remove 2 mL top layer of the supernatant by pipetting. Note: Do not remove the middle and bottom layer which is the white blood cells and red blood cells respectively. Add 3 mL RBC Lysis Buffer (not provided). Resuspend the pellet by pipetting 4 – 5 times. Centrifuge at 700 x g for 5 minutes. Carefully remove 3 mL supernatant by aspirate from top via pipetting. Leave the remaining supernatant and cell pellet in the tube. Repeat Step iv. Remove supernatant completely. [For preservation] Resuspend cell pellet in 1 mL RNAlater Solution (not provided). <p>Not applicable.</p>
Insect	<p>Option 1: Insect</p> <ul style="list-style-type: none"> Immerse the live insect in absolute ethanol until the insect stops moving Blot dry the insect Weight: 50 - 100 mg of sample Snap freeze insect in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>Option 2: Insect in fine powder</p> <ul style="list-style-type: none"> Immerse the live insect in absolute ethanol until the insect stops moving Blot dry the insect Quickly grind the insect into fine powder with liquid nitrogen Weight: 50 - 100 mg of sample No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>Option 3: Insect in DNA/RNA tissue stabilization solution</p> <ul style="list-style-type: none"> Immerse the live insect in absolute ethanol until the insect stops moving Blot dry the insect Put fresh insect into 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 used</u>, following <u>the ratio</u> of stabilization reagent volume to sample size to ensure effective sample preservation. No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice 	

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Plant	<p>Fresh leaf/needle/stem/root, prefer young leaf/needle</p> <ul style="list-style-type: none"> Rinse with clean water to remove dirt attached to the plant sample Cut sample into small pieces (< 0.5 cm), keep in 50 ml centrifuge tube Weight: <ul style="list-style-type: none"> i) 2 - 5 g wet weight OR ii) 2 - 5 g liquid nitrogen ground fine powder **Cut and weighed sample must be completed within 3 minutes after tissue removal from plant to ensure the tissue is preserved without degradation. Snap freeze in liquid nitrogen bath for 10 minutes No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p><!-- Avoid pooling different plant parts/organs into the same tube, as lysis procedure is different for each part.</p>	
Plant for Chloroplast DNA (cpDNA) Extraction	<p>Fresh green leaves without brown spots and holes</p> <ul style="list-style-type: none"> Weight: Harvest ≥ 30 g of fresh green leaves Wrap leaves with aluminium foil No. of replicates: 3 Storage/Shipping condition: 4°C or Blue ice in dark condition <p><!-- The sample must reach Apical Scientific within 48 - 72 hours upon sample collection. Suggest to prearrange the best day for sample submission with our Customer Care team.</p> <p>Example of fresh leaf</p> 	Not applicable.
Soil/Sludge	<p>For Amplicon Sequencing application</p> <ul style="list-style-type: none"> Weight: 0.5 - 1 g of sample in screw cap tube No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice <p>For Shotgun Metagenomics application, we recommend to collect soil sample in soil preservation solution, e.g. Qiagen LifeGuard Soil Preservation solution.</p> <ul style="list-style-type: none"> Collect soil sample in soil preservation solution, e.g. Qiagen LifeGuard Soil Preservation solution. Weigh 2 g of soil sample in 15 ml screw cap tube Add 5 ml of soil preservation solution Vortex or invert tube by hand until the entire soil sample and preservation solution are mixed well. Excess preservation solution should be sitting on top of the soil sample. No. of replicates: 3 Store sample in 4°C for overnight, transfer to -20°C freezer the next day. Shipping condition: Dry ice <p>*Depends on soil types, additional sample replicates of 4 to 8 may be required in order to get sufficient DNA amount for downstream applications.</p>	<ul style="list-style-type: none"> Collect soil sample in soil preservation solution, e.g. Qiagen LifeGuard Soil Preservation solution. Weigh 2 g of soil sample in 15 ml screw cap tube Add 5 ml of soil preservation solution Vortex or invert tube by hand until the entire soil sample and preservation solution are mixed well. Excess preservation solution should be sitting on top of the soil sample. No. of replicates: 3 Store sample in 4°C for overnight, transfer to -20°C freezer the next day. Shipping condition: Dry ice <p>*Depends on soil types, additional sample replicates of 4 to 8 may be required in order to get sufficient RNA amount for downstream applications.</p>
Stool	<ul style="list-style-type: none"> Weight: 200 - 500 mg of sample in screw cap tube Label sample with printed labelling No. of replicates: 3 Store sample in -80 °C freezer Shipping condition: Dry ice 	
Manure	<ul style="list-style-type: none"> Weight: 200 - 500 mg of sample in screw cap tube Label sample with printed labelling Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice 	Not available.
Water	<p>Option 1: Filtered content on 0.22 µm membrane filter</p> <ul style="list-style-type: none"> Prefilter water sample with larger pore size cell strainer (e.g. 100 µm) to remove large size debris Continue vacuum filtration of 300 - 500 ml* water sample with 0.22 µm pore size, 25/47 mm membrane filter *If the sample has low turbidity (contained less filtered content), please continue to filter to reach volume of 1.5 L in total Use a sterile scalpel blade to remove the membrane filter Insert the membrane filter into a new tube by rolling the membrane using sterile forceps, with the side containing trapped sample facing inward of the tube No. of replicates: 3 Store membrane filter in -80 °C freezer Shipping condition: Dry ice <p>Option 2: Water sample</p> <ul style="list-style-type: none"> Volume: 500 ml** **If the sample has low turbidity, please submit at least triplicates, i.e. 500 ml x 3 = 1.5 L Store sample in dark in 4°C in a screw cap bottle Shipping condition: 4°C or Blue ice 	Not available.

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Food	<p>Food (Solid)</p> <ul style="list-style-type: none"> Weight: 10 g sample in screw cap tube/container/zip lock bag No. of replicates: 2 Shipping condition: follow the food storage condition, as long as the food is not rotten during shipment a) If it was stored in 4°C, send in blue ice b) If it was stored in freezer, send in dry ice <p>Food (Liquid)</p> <ul style="list-style-type: none"> Weight: 20 - 50 ml sample in screw cap tube No. of replicates: 2 Shipping condition: follow the food storage condition, as long as the food is not rotten during shipment a) If it was stored in 4°C, send in blue ice b) If it was stored in freezer, send in dry ice 	Not available.
Swab sample (Buccal swab/ environmental swab)	<p>Option 1:</p> <ul style="list-style-type: none"> Collect sample using commercial swab sample collection kit Follow collection kit guidelines to preserve the swab sample No. of replicates: 3 Storage condition: Follow the guidelines from the commercial kit Shipping condition: Follow the guidelines from the commercial kit <p>Option 2:</p> <ul style="list-style-type: none"> Collect sample using sterile swab (Optional) Store swab in 1X PBS No. of replicates: 3 Storage condition: -80°C freezer Shipping condition: Dry ice 	<ul style="list-style-type: none"> Collect sample using commercial swab sample collection kit (for RNA application) Follow collection kit guidelines to preserve the swab sample No. of replicates: 3 Storage condition: Follow the guidelines from the commercial kit Shipping condition: Follow the guidelines from the commercial kit
Saliva	<p>Option 1:</p> <ul style="list-style-type: none"> Suggest to follow commercially available saliva collection kit guidelines 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 kit recommendation <p>Option 2:</p> <ul style="list-style-type: none"> Collect 2 ml saliva in a new 50 ml centrifuge tube Add 10 ml 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. No. of replicates: 2 Store dry pellet in -80 °C freezer Shipping condition: Dry ice 	<p>Option 1:</p> <ul style="list-style-type: none"> Suggest to follow commercially available saliva collection kit guidelines 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 kit recommendation <p>Option 2:</p> <ul style="list-style-type: none"> Collect 2 ml fresh saliva in a 50 ml centrifuge tube No. of replicates: 2 Add 2 ml RNAlater solution. Vortex vigorously to mix thoroughly. Incubate sample at 4°C fridge for overnight. Store in -80°C freezer the next day. Shipping condition: Dry ice
Others	Please enquire.	

How to snap freeze sample?

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