Extraction Methods

1.1 RNA Extraction

1) Tissue Homogenization Transfer an appropriate amount of tissue to a 1.5 mL centrifuge tube containing 1 mL of pre-chilled TRIzol reagent after thorough grinding in liquid nitrogen. Vortex vigorously for complete lysis and incubate at room temperature for 5 minutes. For tissues that are difficult to extract, the incubation time may be extended accordingly.

2) Phase Separation Following centrifugation at 13,000 g for 5 minutes at 4°C, carefully transfer the supernatant to a fresh centrifuge tube. Add pre-chilled chloroform at a ratio of 0.2 mL chloroform per 1 mL TRIzol (200 μL), vortex thoroughly, and incubate at room temperature for 5 minutes.

3) Aqueous Phase Collection After centrifugation at 13,000 g for 15 minutes at 4°C, carefully aspirate the upper aqueous phase (400 μL) to a new centrifuge tube, avoiding contact with the intermediate protein layer. Add an equal volume of pre-chilled isopropanol and incubate at room temperature for 10 minutes.

4) RNA Precipitation Following centrifugation at 13,000 g for 10 minutes at 4°C, discard the supernatant. A small white pellet should be visible at the bottom of the tube (which may not be visible to the naked eye when RNA yield is very low). Add 1 mL of pre-chilled 75% ethanol to resuspend the pellet.

5) Pellet Washing and Drying After centrifugation at 12,000 g for 5 minutes at 4°C, carefully remove the supernatant using a 1000 μL pipette with 10 μL tips. Briefly centrifuge the tube for a few seconds to collect any residual liquid on the tube walls, then remove all remaining liquid using 10 μL pipette tips. Air-dry the pellet at room temperature for 3-5 minutes. Note: Avoid over-drying as this may impair RNA solubility and potentially cause degradation.

6) RNA Resuspension Dissolve the RNA pellet in 20-50 μL of sterile 0.1% DEPC-treated water (volume adjusted according to the expected RNA yield).

1.2 DNA Extraction

1) Sample Preparation Add 200 μL Buffer TL to a 1.5 mL centrifuge tube. Transfer the ground tissue sample (approximately 30 mg) to the tube and mix thoroughly.

2) Protease Digestion Add 25 μL protease OB and mix well. Incubate at 55°C for 1-3 hours until the solution becomes clear, with thorough mixing every 20 minutes during incubation.

3) Supernatant Collection Centrifuge at 10,000 g for 5 minutes and carefully transfer the supernatant to a fresh 1.5 mL centrifuge tube.

4) RNA Digestion Add 2.5 μL RNase A, mix thoroughly, and incubate at room temperature for 10 minutes.

5) Lysis Buffer Treatment Add 220 μL Buffer BL, mix well, and incubate at 70°C for 10 minutes.

6) Ethanol Precipitation Add 200 μL absolute ethanol and mix thoroughly.

7) Column Binding Transfer the entire mixture to an adsorption column placed in a 2 mL collection tube. Centrifuge at 8,000 g for 1 minute, then discard both the collection tube and flow-through.

8) First Wash Place the adsorption column in a new collection tube and add 500 μL Buffer HBC. Centrifuge at 8,000 g for 1 minute, then discard the collection tube and flow-through.

9) Second Wash Transfer the adsorption column to a new collection tube and add 700 μL DNA Wash Buffer (ensure the specified volume of absolute ethanol has been added). Centrifuge at 8,000 g for 1 minute, discard the flow-through, and place the column back in the collection tube.

10) Third Wash Repeat step 9.

11) Column Drying and Elution Centrifuge empty at 13,000 g for 2 minutes. Discard the collection tube and transfer the adsorption column to a new 1.5 mL centrifuge tube (note: tube caps may become detached during high-speed centrifugation). Add 100 μL pre-warmed Elution Buffer (65°C), incubate for 5 minutes, then centrifuge at 10,000 g for 1 minute.