Genorise Tissue RNA Extraction Kit 50

Genorise Tissue RNA Extraction Kit 50 is to isolate RNA from animal tissue and for 50 applications. This kit can significantly improve the quantity and quality of RNA and is much cheaper than the similar products ($59/50 applications).

Materials provided:
35 ml of Genorise Reagent A
0.5 ml of Reagent C
0.5 ml of Reagent D
50 ml of RNA water

Materials required but not provided
Water-saturated phenol
Chloroform
Diethyl pyrocarbonate (DEPC)
2-mercaptoethanol
Isopropanol (2-propanol)/Ethanol

Protocol

1. Take 50-100 mg of fresh tissue and quickly minced on ice and transfer to a 1.7ml microtube containing 0.7 ml of Genorise Reagent B.
2. Sonicate at power 3 for 5 sec to completely homogenize the tissue, incubate for 15 min on ice.
3. Add 0.7 ml water-saturated phenol to the tube, vortex 15sec, and add 0.3 ml chloroform-isoamyl alcohol, vortex 15sec.
4. Incubate on ice for 15min and centrifuge at no more than 12,000 x g at 4°C for 15 min.
5. Transfer the supernatant (0.7 ml) containing RNA to a new tube and 0.7 ml of isopropanol and incubate at room temperature for 10 min to precipitate RNA.
6. Centrifuge at no more than 12,000 x g at 4°C for 10 min.
7. Wash the RNA pellet with 1ml of 75% ethanol at no more than 7,500 x g at 4°C for 5 min and repeat the wash once.
8. Discard the supernatant, remove the residue liquid by a pipette and air dry for 5 min.
9. Add 30 µl of RNA water to completely dissolve the RNA pellet by a pipette or brief vortex.
10. Read at A_{260} and A_{280} and run RNA electrophoresis to estimate RNA quality and quantity. Store the RNA samples at -80°C.

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Reagent preparations

Genorise Reagent B: Add 0.25 ml of 2-mercaptoethanol to 35 ml of Genorise Reagent A. and it can be stored at room temperature at least 1 month.

Water-saturated phenol: Dissolve 5 g phenol crystal in 5 ml of RNA water at 60°C. Aspirate the most upper water phase, add 0.05 ml Reagent C and 0.05 ml Reagent D, mix well, store at 4°C and use it within a month.

RNA Water: Prepare 0.02% DEPC water, stir in the hood overnight, and autoclave at 120°C for 30 min. Prepare 75% ethanol in RNA water

Additional Protocols

Quantitate RNA

Quantitate RNA by diluting 5 µl in 0.195 ml of 1 mM Na_2HPO4 solution (pH >7.5) and reading the A_{260} and A_{280}. The ratio of the A_{260} and A_{280} should be >1.8. Do not use distilled water since it has pH <6.

RNA Electrophoresis to evaluate the RNA quality

Reagent preparation

10 x MOP Running Buffer: 0.4 M MOP, 0.1 M Sodium Acetate, 0.01 M EDTA

1 x Formaldehyde Gel Running Buffer: 1 x MOP Running Buffer, 0.61% formaldehyde

RNA Sample Loading Buffer with Ethidium Bromide: 62.5% Formamide, 11.33% Formaldehyde, 1.25 x MOPS running buffer, 50 ug/ml Bromophenol blue (BPB), 50 ug/ml Xylene Cyano (XC), 50 ug/ml Ethidium Bomide, 10% glycerol

1% agarose/formaldehyde gel

1g agarose in 87 ml H_2O, heat 1 min in microwave to completely melt the agarose, add 10 ml 10 x MOP running buffer and 3 ml of 12.3 M formaldehyde, mix, cool down and pour to the gel tray.

Add sufficient 1 x Formaldehyde gel running buffer to cover the gel

Prepare sample and run gel

1. Mix 5 µl RNA with 15 µl RNA sample buffer
2. Denature at 65ºC for 10 min
3. Chill on ice at least 5 min
4. Run the gel at 10 V/cm until the bromphenol blue dye migrate one-half length of the gel.

Visualization of RNA under UV light: Photograph the RNA gel

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Technical notes

1. Water-saturated phenol should be used within a month otherwise RNA isolation may fail.
2. Tissue lysis should be complete.
3. Air drying of RNA pellet should not exceed 5 min.
4. RNA yield is usually 50 μg to 300 μg per 50 mg tissue depending on the type of the tissue and species. Normal $A_{260/280}$ is approximately 2.0. If less or more tissues are used, the reagents should be reduced or increased accordingly.