GSI Tissue RNA Isolation (500)

GSI Tissue RNA Isolation kit (500) is to isolate RNA from tissue and for 500 applications. This kit can significantly improve the quantity and quality of RNA and is much cheaper than the similar products ($563/500 applications).

Materials provided:
500ml of GSI RNA Reagent
200ml of RNA water
5ml RNA sample loading buffer

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

Protocol

1. Take 50-100 mg of fresh tissue and quickly minced on ice and transfer to a 1.7 ml microtube containing 1 ml of GSI RNA Reagent.
2. Sonicate at power 3 for 5 sec to completely homogenize the tissue, incubate for 15 min on ice.
3. Add 0.2 ml chloroform, vortex 15sec. and incubate on ice for 15min.
4. Centrifuge at no more than 12,000 x g at 4°C for 15min.
5. Transfer the supernatant (0.5 ml) containing RNA to a new tube and 0.5 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 7,500g 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 5min.
9. Add 50 µl of RNA water to completely dissolve the RNA pellet by a pipette or brief vortex, and combine the aliquotes.
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.

Reagent preparations

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

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Additional Protocols

Quantitate RNA

Quantitate RNA by diluting 5ul in 0.195ml 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

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

Technical notes

1. Tissue lysis should be complete and sonication should not exceed 5 sec. homogenize the tissue completely if lysis is not complete by sonication.
2. Air drying of RNA pellet should not exceed 5 min.
3. RNA yield is usually 50 µg to 300 µg per 50mg 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.