GSI Cell RNA Isolation (250)

GSI Cell RNA Isolation (250) kit is to isolate total RNA from cells and is for 250 applications. This kit can significantly improve quality and quantity of RNA and is much cheaper than the similar products ($313/250 applications).

Materials provided:
250 ml of GSI RNA Reagent
50 ml of RNA water
5 ml RNA sample loading buffer

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

Protocol

1. Take $5x10^6$-$1x10^7$ cells to a 1.7ml microtube and remove the medium by brief centrifugation, and add 1 ml of GSI RNA Reagent.
2. Completely homogenize the cells by a pipette, incubate for 15 min on ice.
3. Add 0.2 ml chloroform, vortex 15sec and incubate on ice for 15 min.
4. Centrifuge at no more than 12,000g at 4°C for 15min.
5. Transfer the supernatant (0.5 ml) containing RNA to a new tube and add 0.5ml of isopropanol and incubate at room temperature for 10min to precipitate RNA.
6. Centrifuge at no more than 12,000g at 4°C for 10min.
7. Wash the RNA pellet with 1ml of 75% ethanol at no more than 7500g at 4°C for 5min and repeat the wash once.
8. Discard the supernatant, remove the residue liquid by a pipette and air dry for 5min.
9. Add 30µl of RNA water to completely dissolve the RNA pellet by a pipette or brief vortex, and combine the aliquotes.
10. Read OD260/280 and conduct RNA electrophoresis to estimate RNA quality and quantity, or measure RNA quantity and quality by 2100 Bioanalyzer (Agilent). 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 30min.
Prepare 75% ethanol in RNA water

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Additional Protocols

Quantitate RNA

Quantitate RNA by diluting 5ul in 0.195ml alkaline water (pH >7.5) and reading the A_{260} and A_{280}. The ratio of the A_{260} and A_{280} should be >1.8.

Distilled water has pH <6, adjust water to a slightly alkaline pH by adding concentrated Na_2HPO4 solution to a final concentration of 1 mM.

RNA Electrophoresis to evaluate the RNA quality

Reagent preparation

10 x MOPs Running Buffer: 0.4 M MOPs, 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 H2O, 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, 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. Cell lysis should be complete.
2. Air drying of RNA pellet should not exceed 5 min.
3. RNA yield is usually 2 µg to 8 µg per 5 million cells. Normal $A_{260/280}$ is approximately 2.0. If less or more cells are used, the reagents should be reduced or increased accordingly.