ABCEPTA CUSTOM SERVICES:

RNA Extract Protocol

Solutions

- TRIZOL Reagent
- Chloroform
- Isopropyl alcohol
- 75% Ethanol (in DEPC-treated water)
- RNase-free water

Procedures

1. HOMOGENIZATION

a. *Tissues*

Homogenize tissue samples in 1 mL of TRIZOL Reagent per 50-100 mg of tissue using a glass-Teflon or power homogenizer.

b. *Adherent cells*

Lyse cells directly in a culture dish by adding 1 mL of TRIZOL Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRIZOL Reagent added is based on the area of the culture dish (1 mL per 10 cm^2) and not on the number of cells present.

Note: An insufficient amount of TRIZOL Reagent may result in contamination of the isolated RNA with DNA.

c. *Suspension cells*

Pellet cells by centrifugation. Lyse cells in TRIZOL Reagent by repetitive pipetting. Use 1 mL of the reagent per 5-10 $\times 10^6$ of animal, plant or yeast cells, or per 1 $\times 10^7$ bacterial cells.

2. PHASE SEPARATION

1) Incubate the homogenized samples for 5 minutes at room temperature (RT) to permit the complete dissociation of nucleoprotein complexes.

2) Add 0.2 mL of chloroform per 1 mL of TRIZOL Reagent. Shake tubes strongly by hand for 15 seconds and incubate them at RT for 2 to 3 minutes.

3) Centrifuge the samples at no more than 12,000 $\times g$ for 15 minutes at 4 ℃. (The mixture separates into a lower phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains in the aqueous phase.)

3. RNA PRECIPITATION

4) Transfer the aqueous phase to a fresh tube.

5) Precipitate the RNA by mixing with isopropyl alcohol (0.5 mL of isopropyl alcohol per 1 mL of TRIZOL Reagent).

6) Incubate samples at RT for 10 minutes

7) Centrifuge at no more than 12,000 $\times g$ for 10 minutes at 4 ℃. The RNA precipitate will form a gel-like pellet on the side and bottom of the tube.

4. WASH RNA

8) Remove the supernatant. Add at least 1 mL of 75% ethanol per 1 mL of TRIZOL Reagent to

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wash the RNA pellet.

9) Mix the sample by vortexing and centrifuge at no more than 7,500 ×*g* for 5 minutes at 4 ℃.

10) Stored with ethanol at -80 ℃,or redissolved for using.

5. REDISSOLVING RNA

11) Remove the supernatant and briefly dry the RNA pellet. Do not let the RNA pellet dry completely as this will greatly decrease its solubility.

12) Dissolve RNA in RNase-free water by flipping the tube a few times, and incubating for 10 minutes at 55 to 60 ℃.

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