RNA isolation from cells or tissues with TRIZOL

A. Required reagents:

DEPC-treated water (Ambion)

TRIzol Reagent (Invitrogen)

Ice cold PBS

70% ethanol

100% ethanol (kept at -20)

Always use gloves and eye protection. Avoid contact with skin or clothing. Use in a chemical hood. Avoid breathing vapour.

1. Tissues:

Homogenize tissue samples in 1 ml of TRIZOL reagent per 50 to 100 mg of tissue using a glass-Teflon or power homogenizer (e.g. Polytron, Tekmar's TISSUEMIZER). The sample volume should not exceed 10% of the volume of TRIZOL Reagent used for the homogenization.

2. Cells grown in Monolayer:

Rinse cell monolayer with ice cold PBS once. Lyse cells directly in a culture dish by adding 1 ml of TRIZOL Reagent per 3.5 cm diameter dish and scraping with cell scraper. Pass the cell lysate several times through a pipette. Vortex thoroughly. 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. An insufficient amount of TRIZOL Reagent may result in DNA contamination of the isolated RNA.

3. Cells in suspension:

Spin cells for 5 min at 300 X g. Remove media and resuspend cells in ice cold PBS. Pellet cells by spinning at 300 X g for 5 min. Lyse cells with TRIZOL Reagent by repetitive pipetting or by passing through syringe and needle. Use 1 ml of the reagent per 5-10 X 10^6 of animal cells.

Incubate the homogenized sample for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. Centrifuge to remove cell debris. Transfer the supernatant to new tube.

PHASE SEPERATION:

Add 0.2 ml of chloroform per 1 ml of TRIZOL Reagent. Cap sample tubes securely. Vortex samples vigorously for 15 seconds and incubate them at room temperature for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 x g for 15 minutes at 2 to 8 ^{0}C. Following centrifugation, the mixture separates into lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Transfer upper aqueous phase carefully without disturbing the interphase into fresh

tube. Measure the volume of the aqueous phase (The volume of the aqueous phase is about 60% of the volume of TRIZOL Reagent used for homogenization).

RNA PRECIPITATION:

Precipitate the RNA from the aqueous phase by mixing with cold 100% ethanol. Use 2 vol of ethanol per 1 vol of aqueous phase. Incubate samples at -20 ^{o}C for 24 hours or at -80 ^{o}C for 2 hours and centrifuge at not more than 12,000 x g for 10 minutes at 2 to 4 ^{o}C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

RNA WASH:

Remove the supernatant completely. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Mix the samples by vortexing and centrifuge at no more than 7,500 x g for 5 minutes at 2 to 8 ^{o}C. Repeat above washing procedure once. Remove all leftover ethanol.

REDISSOLVING RNA:

Air-dry or vacuum dry RNA pellet for 5-10 minutes. Do not dry the RNA pellet by centrifuge under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A_{260}/A_{280} ratio < 1.6. Dissolve RNA in DEPC-treated water by passing solution a few times through a pipette tip.