1. HOMOGENIZATION

1-1.Homogenize tissue samples in 1 ml of TRIZOL Reagent per 50-100 mg of tissue using a glass-Teflon or Polytron power homogenizer. The sample volume should not exceed 10% of the volume of TRIZOL Reagent used for homogenization.

1-2. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 X g for 10 minutes at 2 to 8∞C.

2. PHASE SEPARATION

2-1. Incubate the homogenized samples for 5 minutes at 15 to 30∞C to permit the complete dissociation of nucleoprotein complexes.

2-2. Add 0.2 ml of chloroform per 1 ml of TRIZOL Reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30∞C for 2 to 3 minutes. Option: consider Eppendorf PLG.

2-3. Centrifuge the samples at no more than 12,000 X g for 15 minutes at 2 to 8∞C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIZOL Reagent used for homogenization.

3. RNA PRECIPITATION

3-1. Transfer the aqueous phase to a fresh tube and precipitate the RNA from the aqueous phase by adding to the aqueous phase 0.25 ml of isopropanol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml of TRIZOL Reagent used for the homogenization.

3-2. Mix the resulting solution, centrifuge at 12,000 X g for 15 minutes at 2 to 8∞C and proceed with the RNA isolation protocol.

4. RNA WASH

4-1. Decant the supernatant and drain the sample on several layers of sterile KimWipes for approximately 5 minutes.

4-2. Wash the RNA pellet with ice-cold 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL Reagent used for the initial homogenization.

4-3. Mix the sample by vortexing and centrifuge at 10,000X g for 10 minutes at 2 to 8∞C.

4-4. Repeat step 4-3 one more time.

4-5. Decant the supernatant and drain the tube by inversion on several layers of sterile KimWipes for approximately 5 minutes.

5. REDISSOLVING THE RNA

5-1. Resuspend the RNA pellet in DEPC water and proceed on to quantitation of total RNA.

6. QUANTIFYING TOTAL RNA

6-1. Remove a 10 µl aliquot to a 1.5 ml microfuge tube containing 490 µl of TE.

6-2. Read A260 and A280. Ensure the spectrophotometer is blanked with TE prior to reading absorption.

6-3. Calculate the amount of RNA recovered. Remember one OD260 unit equals 40 µg RNA.