INSTRUCTIONS FOR RNA ISOLATION:
CAUTION: When working with TRIZOL Reagent use gloves and eye protection (shield,safety goggles).
Avoid contact with skin or clothing. Use in a chemical fume hood.
Avoid breathing vapor.
Note: Unless otherwise stated, the procedure is carried out at 15 to 30℃, and reagents are at 15 to 30℃.

1. HOMOGENIZATION (see notes 1-3)

a. *Tissues*
Homogenize tissue samples in **1 mL of TRIZOL Reagent per 50-100 mg** of tissue
using a glass-Teflon® or power homogenizer (Polytron, or Tekmar's TISSUMIZER® or equivalent). The sample volume should not exceed 10% of the volume of TRIZOL Reagent used for homogenization.

b. *Cells Grown in Monolayer*
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 cm2) and not on the number of cells present. An insufficient amount of TRIZOL Reagent may result in contamination of the isolated RNA with DNA.

c. *Cells Grown in Suspension*
Pellet cells by centrifugation. Lyse cells in TRIZOL Reagent by repetitive pipetting. Use **1 mL of the reagent per 5-10 x 106 of animal, plant or yeast cells, or per 1 x 107 bacterial cells**. Washing cells before addition of TRIZOL Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. Optional Step

An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 x g for 10 minutes at 2 to 8℃. The resulting pellet contains extracellul ar membranes, polysaccharides, and high molecular weight DNA, while the supernate contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

3. PHASE SEPARATION

Incubate the homogenized samples for 5 minutes at 15 to 30℃ to permit the complete dissociation of nucleoprotein complexes. 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℃ for 2 to 3 minutes.

Centrifuge the samples at no more than 12,000 x g for 15 minutes at 2 to 8℃. 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.

4. RNA PRECIPITATION

Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the **aqueous** phase by mixing with Isopropanol. Use 0.5 mL of Isopropanol per 1 mL of TRIZOL Reagent used for the initial homogenization. Incubate samples at 15 to 30℃ for 10 minutes and centrifuge at no more than 12,000 x g for 10 minutes at 2 to 8℃.
The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

5. RNA WASH

Remove the supernate. Wash the RNA pellet once with 75% ethanol, adding at least 1mL of 75% ethanol per 1 mL of TRIZOL Reagent used for the initial homogenization.
Mix the sample by vortexing and centrifuge at no more than 7,500 x g for 5 minutes at 2 to 8℃.

6. REDISSOLVING THE RNA

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes).
Do not dry the RNA by centrifugation 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 A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60℃.