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Product Information

RNAzol RT Reagent

Catalog Number	Packaging Size
FP314	100 mL

Storage upon receipt:
- RT
- Protect from light

Product Description

RNAzol RT Reagent is a ready-to-use reagent, designed to isolate high quality total RNA and small RNA from samples of human, animal, plant, bacterial and viral origin. **RNAzol RT Reagent** is a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of a variety of RNA species of large or small molecular size. **RNAzol RT Reagent** maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. **RNAzol RT Reagent** allows for simultaneous processing of a large number of samples.

RNAzol RT Reagent separates RNA from other molecules in a single-step based on the interaction of phenol and guanidine with cellular components. No chloroform-induced phase separation is necessary to obtain pure RNA. A biological sample is homogenized or lysed in RNAzol RT Reagent. DNA, proteins, polysaccharides and other molecules are precipitated from the homogenate/lysate by the addition of water and removed by centrifugation. The pure RNA is isolated from the resulting supernatant by alcohol precipitation, followed by washing and solubilization.

- The isolation procedure can be completed in less than one hour. The isolated RNA is ready for use in RT-PCR, qRT-PCR, microarrays, poly A+ selection, northern blotting, RNase protection assay and other molecular biology applications.
- RNAzol RT isolates large RNA and small RNA in separate fractions. Alternatively, total RNA containing all classes of RNA in a single fraction can be isolated. In addition, RNAzol RT allows for the sequential isolation of RNA and DNA.

Required materials not supplied

- Isopropanol
- 75% Ethanol
- RNase-free water
- microcentrifuge tubes
- Centrifuge capable of reaching 12,000 × g

PROTOCOL FOR ISOLATION OF LARGE RNA AND SMALL RNA FRACTIONS

This protocol yields a large RNA fraction containing RNA > 150 - 200 bases, and a small RNA fraction containing RNA < 150 - 200 bases.

1. HOMOGENIZATION.

- **Tissues:** Add 1 mL of RNAzol RT Reagent per 10–100 mg of tissue to the sample and homogenize using a homogenizer.
- **Cell grown in monolayer:** Remove growth media; Add 1 mL of RNAzol RT Reagent per 3.5 cm culture dish (10 cm^2) to lyse the cells; Pipet the lysate up and down several times to homogenize.
- **Cells grown in suspension:** Pellet the cells by centrifugation and discard the supernatant; Add 1 mL of RNAzol RT Reagent per 10^7 cells to the pellet; Pipet the lysate up and down several times to homogenize.
- **Liquid Samples:** Homogenize/lyse liquid samples using 1 mL of RNAzol RT Reagent per 0.4 mL of a liquid sample. For processing a small volume sample, mix the sample with 1 mL of RNAzol RT Reagent and supplement the mixture with water to approach the sample+ water volume of 0.4 mL.
- **Samples With High Fat Content:** After complete homogenization, centrifuge high-fat samples at 12,000 g for 5 minutes. Excess lipid forms a layer at the top of the tube. Transfer the clear supernatant to a new tube.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

Add to the homogenate/lysate 0.4 mL of water per 1 mL of RNAzol RT Reagent used for homogenization. Shake the resulting mixture vigorously for 15 seconds and store for 5 - 15 minutes. Samples with 100 mg tissue/ml RNAzol RT Reagent require a 15 minute storage at room temperature. Centrifuge the sample at 12,000 g for 15 minutes. Following centrifugation, DNA, proteins and most polysaccharides form a semisolid pellet at the bottom of the tube. The RNA remains soluble in the supernatant.

3A. PRECIPITATION OF LARGE RNA FRACTION.

Transfer 1 mL of the supernatant (~75% of total supernatant volume) to a new tube, leaving a layer of the supernatant above the DNA/protein pellet. Precipitate RNA by mixing the transferred 1 mL of supernatant with 0.4 mL of 75% ethanol (v/v). Store sample for 10 minutes and centrifuge at 12,000 g for 8 minutes. RNA precipitate forms a white pellet at the bottom of the tube. Transfer the supernatant to a new tube and store it at 4 °C or at -20 °C for isolation of small RNA.

3B. PRECIPITATION OF SMALL RNA FRACTION.

Mix 0.8 volumes of isopropanol with the supernatant obtained after precipitation of large RNA in Step 3A. Store the sample for 30 minutes at room temperature or 4 °C and sediment the precipitated RNA at 12,000 g for 15 minutes at 4 - 28 °C. The RNA precipitate forms a white pellet at the bottom of a tube. The sample may be stored at 4 °C to potentially increase yield of some microRNAs.

4. RNA WASHES.

Wash the RNA twice by mixing the large RNA pellet (Step 3A) with 75% ethanol (v/v) or the small RNA pellet (Step 3B) with 70% isopropanol (v/v). Centrifuge the pellets at 4,000 - 8,000 g for 1 - 3 minutes. When performing the isolation in 1.5 mL microcentrifuge tubes, use 0.4 - 0.6 mL of the alcohol solution. For samples processed in larger tubes use 0.5 mL of alcohol solution per 1 mL of the supernatant used for precipitation. Remove the alcohol solution using a micropipette.

5. RNA SOLUBILIZATION.

a. Resuspend the RNA pellet in 20–50 µL of RNase-free water by pipetting up and down.

b. Incubate in a water bath or heat block set at 50-55°C for 5-10 minutes.

6. DETERMINE the RNA YIELD by OD MEASUREMENT

a. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm.

b. Calculate the RNA concentration using the formula A260 × dilution × 40 = µg RNA/mL.

c. Calculate the A260/A280 ratio. A ratio of ~2 is considered pure.

PROTOCOL FOR ISOLATION OF TOTAL RNA

This protocol yields all classes of RNA in one fraction containing: large nuclear RNA, rRNA, mRNA, small RNA and microRNA.

1. HOMOGENIZATION.

- **Tissues:** Add 1 mL of RNAzol RT Reagent per 10–100 mg of tissue to the sample and homogenize using a homogenizer.
- **Cell grown in monolayer:** Remove growth media; Add 1 mL of RNAzol RT Reagent per 3.5 cm culture dish (10 cm^2) to lyse the cells; Pipet the lysate up and down several times to homogenize.
- **Cells grown in suspension:** Pellet the cells by centrifugation and discard the supernatant; Add 1 mL of RNAzol RT Reagent per 10^7 cells to the pellet; Pipet the lysate up and down several times to homogenize.
- **Liquid Samples:** Homogenize/lyse liquid samples using 1 mL of RNAzol RT Reagent per 0.4 mL of a liquid sample. For processing a small volume sample, mix the sample with 1 mL of RNAzol RT Reagent and supplement the mixture with water to approach the sample+ water volume of 0.4 mL.
- **Samples With High Fat Content:** After complete homogenization, centrifuge high-fat samples at 12,000 g for 5 minutes. Excess lipid forms a layer at the top of the tube. Transfer the clear supernatant to a new tube.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

Add to the homogenate/lysate 0.4 mL of water per 1 mL of RNAzol RT Reagent used for homogenization. Shake the resulting mixture vigorously for 15 seconds and store for 5 - 15 minutes. Samples with 100 mg tissue/ml RNAzol RT Reagent require a 15 minute storage at room temperature. Centrifuge the sample at 12,000 g for 15 minutes. Following centrifugation, DNA, proteins and most polysaccharides form a semisolid pellet at the bottom of the tube. The RNA remains soluble in the supernatant.

3. PRECIPITATION OF TOTAL RNA.

Transfer 1 mL of the supernatant (~75% of total supernatant volume) to a new tube, leaving a layer of the supernatant above the DNA/protein pellet. Precipitate RNA by mixing the transferred 1 mL of supernatant with 1 mL of isopropanol (v/v). Store sample for 10 minutes and centrifuge at 12,000 g for 10 minutes. RNA precipitate forms a white pellet at the bottom of the tube. Remove the supernatant.

4. RNA WASHES.

Wash the RNA twice by mixing the RNA pellet with 75% ethanol (v/v). Centrifuge the pellets at 4,000 - 8,000 g for 1 - 3 minutes. When performing the isolation in 1.5 mL microcentrifuge tubes, use 0.4 - 0.6 mL of the alcohol solution. For samples processed in larger tubes use 0.5 mL of alcohol solution per 1 mL of the supernatant used for precipitation. Remove the alcohol solution using a micropipette.

5. RNA SOLUBILIZATION.

a. Resuspend the RNA pellet in 20–50 µL of RNase-free water by pipetting up and down.

b. Incubate in a water bath or heat block set at 50-55°C for 5-10 minutes.

6. DETERMINE the RNA YIELD by OD MEASUREMENT

a. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm.

b. Calculate the RNA concentration using the formula A260 × dilution × 40 = µg RNA/mL.

c. Calculate the A260/A280 ratio. A ratio of ~2 is considered pure.

Typical RNA (A260/280 of >1.8) yields from various starting materials

Starting material	Quantity	RNA yield
Liver	100 mg	600-800 µg
Kidney	100 mg	300-400 µg
Spleen	100 mg	300-400 µg
Muscle	100 mg	50-150 µg
Lung	100 mg	60-170 µg
Placenta	100 mg	100-300 µg
Epithelial cells	1×10^7 cells	50-100 µg
Fibroblasts	1×10^7 cells	40-60 µg