BioTeke Corporation

RNzol: Total RNA Extraction Reagent

Cat.No: RP1001； RP1002； RP1003

DESCRIPTION

RNzol Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi. During sample homogenization or lysis, RNzol Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Add chloroform, and centrifuge to separate the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation.

Total RNA isolated by RNzol Reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly(A)+ selection, in vitro translation, RNase protection assay, and molecular cloning. For use in the polymerase chain reaction (PCR), treatment of the isolated RNA with amplification grade DNase I is recommended when the two primers lie within a single exon.

RNzol Reagent facilitates isolation of a variety of RNA species of large or small molecular size. For example, RNA isolated from rat liver, electrophoresed on an agarose gel, and stained with ethidium bromide, shows discrete bands of high molecular weight RNA between 7 kb and 15 kb in size, (composed of mRNA's and hnRNA's) two predominant ribosomal RNA bands at ~5 kb (28S) and at ~2 kb (18S), and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). The isolated RNA has an A260/A280 ratio ≥1.8 when diluted into TE.

Caution: When working with RNzol LS Reagent use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical

fume hood. Avoid breathing vapor. Unless otherwise stated, the procedure is carried out at 15 to 30℃, and reagents are at 15 to 30℃.

Ⅱ. NOTES

Use of disposable tubes made of clear polypropylene is recommended when working with less than 2ml volumes of RNzol Reagent.

For larger volumes, use glass or polypropylene tubes, and test to be sure that the tubes can withstand 12,000 ×g with RNzol Reagent and chloroform. Do not use tubes that leak or crack.

Carefully equilibrate the weights of the tubes prior to centrifugation.

Glass tubes must be sealed with parafilm topped with a layer of foil, and polypropylene tubes must be capped before centrifugation.

Due to the prevalence of RNases, wear gloves at all times and change them whenever may have been contacted, please follow standard laboratory procedures of "Molecular Clone" rules.

* Wear gloves in whole process. There are many bacteria on the skin of our hands, which will be the main source of RNase affecting RNA extracting.

* Whenever possible, sterile disposable plasticware should be used for handling RNA, avoiding contaminating by public equipments

* Treat non-disposable glassware and plasticware before use to ensure that it is RNase-free. Bake glassware at 200℃ overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA followed by RNase-free water.

III. PROCEDURE

Caution: When working with RNzol Reagent use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor. Unless otherwise stated, the procedure is carried out at 15 to

30 ℃, and reagents are at 15 to 30 ℃.

Reagents required, but not supplied:

- Chloroform, Isopropyl alcohol
- 75% Ethanol (in DEPC-treated water)
- RNase-free water or 0.5% SDS solution (To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water).

1. HOMOGENIZATION:

a. Tissues

Please homogenize tissue in an appropriate volume of Buffer RNzol (50-100mg/mL) until no visible tissue. Pay attention to the volume of sample should not beyond 1/10 total volume of Buffer RNzol.

b. Cells Grown in Monolayer

You can directly append an appropriate volume Buffer RNzol to the culture plate for dissolve cell, and transfer dissolution by pipetting. The volume of Buffer RL is decided by the area of culture plate, about 10cm^2 per 1ml. Once appending not enough Buffer RNzol, it's possible to contaminated genomic DNA.

c. Cells Grown in Suspension

Pellet cells by centrifugation. Lyse cells in RNzol Reagent by repetitive pipetting. Use 0.75 ml of the reagent per 5-10 $\times 10^6$ of animal, plant or yeast cells, or per 1 $\times 10^7$ bacterial cells. Washing cells before addition of RNzol 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.

OPTIONAL: 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 × g for 10 minutes at 2 to 8 ℃. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant 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.

2. PHASE SEPARATION

Incubate the homogenized samples for 5 minutes at 15-30 ℃ to permit the complete dissociation of nuclear protein complexes. Add 0.2 ml of chloroform per 1 ml of RNzol Reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 30 ℃ for 2 to 3 minutes. Centrifuge the samples at no more than 12,000× g for 15 minutes at 2~8 ℃. Following centrifugation, RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 70% of the volume of RNzol Reagent

3. 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 isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 0.75 ml of RNzol Reagent used for the initial homogenization. Incubate samples at 15 to 30 ℃ for 10 minutes and centrifuge at no more than 12,000 × 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.

4. RNA WASH

Remove the supernatant. Wash the RNA pellet once with 75% ethanol; add at least 1

ml of

75% ethanol per 1 ml of RNzol Reagent used for the initial homogenization, mix the sample by vortexing and centrifuge at no more than 7,500 × g for 5 minutes at 2 to 8 ℃.

5. REDISSOLVING THE RNA

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10minutes). 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 ℃. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redissolved in 100% formamide (deionized) and stored at -70 ℃.

6. RNA ISOLATION NOTES:

1. Isolation of RNA from small quantities of tissue (1 to 10 mg) or Cell (10^2~10^4) Samples: add 800 ul RNzol. Following sample lysis, add chloroform and proceed with the phase separation as described in step 2. Prior to precipitating the RNA with isopropyl alcohol, add 5-10 μg RNase-free glycogen as carrier to the aqueous phase. To reduce viscosity, shear the genomic DNA with 2 passes through a 26 gauge needle prior to chloroform addition. The glycogen remains in the aqueous phase and is co-precipitated with the RNA. It does not inhibit first-strand synthesis at concentrations up to 4 mg/ml and does not inhibit PCR.
2. After homogenization and before addition of chloroform, samples can be stored at -60 to -70 ℃for at least one month. The RNA precipitate (step 4) can be stored in 75%ethanol at 2 to 8 ℃ for at least one week, or at least one year at –5 to -20 ℃.

3. Table-top centrifuges that can attain a maximum of 2,600 × g are suitable for use in these protocols if the centrifugation time is increased to 30-60 minutes in steps 2 and 3.

Ⅳ. TROUBLESHOOTING

1. Expected yields of RNA per mg of tissue or 1 × 10^6 cultured cells:

Liver and spleen, 6-10 μg

Kidney, 3-4 μg

Skeletal muscles and brain, 1-1.5 μg

Placenta, 1-4 μg

Epithelial cells (1 ×10^6 cultured cells), 8-15 μg

Fibroblasts, (1 ×10^6 cultured cells) 5-7 μg

2. Low yield

a. Incomplete homogenization or lysis of samples.

b. Final RNA pellet incompletely redissolved.

3. A260/A280 ratio <1.65

a. RNA sample was diluted in water instead of TE prior to spectrophotometric analysis. Low

ionic strength and low pH solutions increase absorbance at 280 nm.

b. Sample homogenized in too small a reagent volume.

c. Following homogenization, samples were not stored at room temperature for 5 minutes.

d. The aqueous phase was contaminated with the phenol phase.

e. Incomplete dissolution of the final RNA pellet.

4. RNA degradation

a. Tissues were not immediately processed or frozen after removal from the animal.

b. Samples used for isolation, or the isolated RNA preparations were stored at -5

to-20 ℃, instead of -60 to -70 ℃.

c. Cells were dispersed by trypsin digestion.

d. Aqueous solutions or tubes were not RNase-free.

e. Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

5. DNA contamination

a. Sample homogenized in too small a reagent volume.

b. Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers,

or alkaline solution.

6. Proteoglycan and polysaccharide contamination

The following modification of the RNA precipitation (step 3) removes these contaminating compounds from the isolated RNA. Add 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 RNzol Reagent used for the homogenization. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form. A combination of the modified precipitation with an additional centrifugation of the initial homogenate (note 2, RNA isolation protocol) is required to isolate pure RNA from plant material containing a very high level of polysaccharides.