

RNzol LS: Blood/Liquid Samples Total RNA Extraction Reagent

For laboratory research use only

RP1101	50 ml
RP1102	100 ml

Description:

RNzol LS Reagent is a ready-to-use reagent for the isolation of **total RNA from whole blood and other liquid samples**. 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 LS Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation separates 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 LS 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 LS 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.

WARNING: Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, seek medical advice.

Notes

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

For larger volumes, use glass or polypropylene tubes, and test to be sure that the tubes can withstand $12,000 \times g$ with RNzol LS 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°C overnight, and thoroughly rinse plastic ware with 0.1N NaOH, 1mM EDTA followed by RNase-free water.*

Procedure

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°C, and reagents are at 15 to 30°C.

Reagents required, but not supplied:

1. Chloroform, Isopropyl alcohol
2. 75% Ethanol (in DEPC-treated water)
3. 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

Add 0.75ml RNzol LS Reagent per 0.25ml sample including at least $5 \sim 10 \times 10^6$ cells (such as whole blood, blood serum, blood plasma, cerebrospinal fluid), passing the cell lysate several times through a pipette for sample lysis. For some high-contaminated sample, should add of equal volume water before taking following step. The ratio should always be 3 of the volume of sample and RNzol LS Reagent.

2. Phase Separation

Incubate the homogenized samples for 5 minutes at 15 -30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 0.75 ml of RNzol LS Reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 30°C for 2 to 3 minutes. Centrifuge the samples at no more than 12,000× g for 15 minutes at 2~8°C. Following centrifugation, **RNA remains exclusively in the aqueous phase**. The volume of the aqueous phase is about 70% of the volume of RNzol LS Reagent for initial homogenization.

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 LS Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no **more than 12,000 × g** for 10 minutes at 2 to 8°C. 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, adding at least 1 ml of 75% ethanol per 0.75 ml of RNzol LS 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°C.

5. Redissolve 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°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redissolved in 100% formamide (deionized) and stored at -70°C.

6. RNA Isolation Notes

1. Isolation of RNA from small quantities of samples (1 to 10 mg):

The ratio should always be 3 of the volume of sample and RNzol LS Reagent, If sample is less than 0.25ml, please adding sterile water. 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°C for at least one month. The RNA precipitate (step 4) can be stored in 75% ethanol at 2 to 8°C for at least one week, or at least one year at -5 to -20°C.

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.

Troubleshooting

1. Expected yields of RNA per mg of tissue:

Human or animal blood, 5-20ug

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°C, instead of -60 to -70°C.
- 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 reagent volume.
- b. Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.