



Redzol (a reagent for total RNA isolation)

User's Instruction

WARNING: Toxic in contact with skin and if swallowed. Cause burns. After contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, seek medical advice (show label where possible).

Storage Conditions

Redzol has demonstrated stability of 12 months when stored at RT. However, we recommend storage at 2~8°C for optimal performance.

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 0.1% (v/v). Let it stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water.

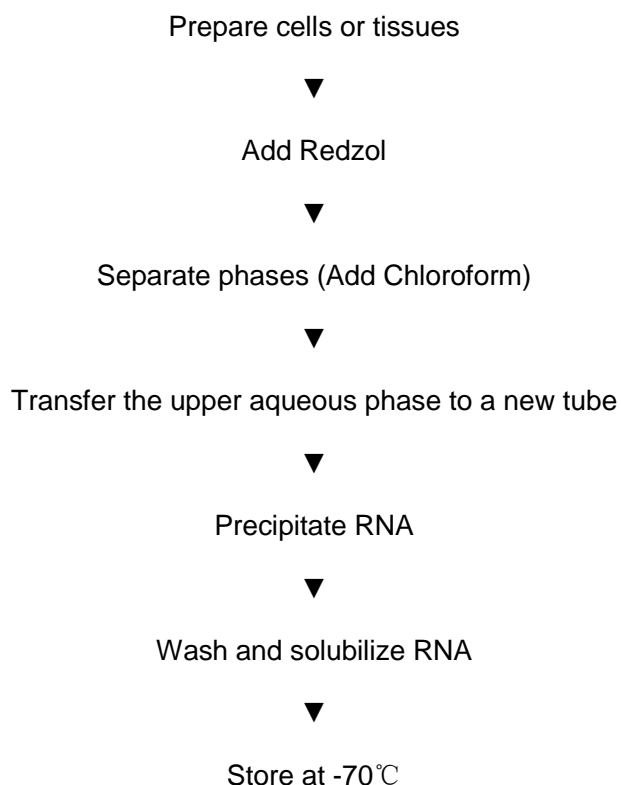
Precautions for Preventing RNase Contamination

RNase can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.

- Always wear disposable gloves. Skin often contains bacteria that can contaminate RNA preparation and be a source of RNase. Practice good microbiological technique to prevent microbial contamination.
- Use sterile, disposable plasticware and automatic pipettors reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any non-disposable items (such as automatic pipettors) can be rich sources of RNases.
- In the presence of Redzol reagent, RNA is protected from RNase contamination. Downstream sample handling requires the non-disposable glassware or plasticware to be RNase-free. Glass items can be baked at 150°C for 4 hours, and plastic items can be soaked for 10 minutes in 0.5 M NaOH, then rinsed thoroughly with water, and autoclaved.



Instructions for RNA Isolation



CAUTION: When working with Redzol Reagent, use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Conduct the procedures in a chemical fume hood. Avoid breathing vapor.

Note: Unless otherwise stated, the procedure is carried out at 15~30°C, and reagents are stored at 15~30°C.

1. HOMGENIZATION

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

b. Cells Grown in Monolayer Lyse cells directly in culture dish by adding 1 ml of Redzol reagent to a 3.5 cm diameter dish, and pipetting the cell lysate several times. The amount of Redzol reagent added is based on the area of the culture dish (1 ml per 10 cm²) but not on the number of cells present. An insufficient amount of Redzol Reagent may result in contamination of the isolated RNA with DNA.

c. Cells Grown in Suspension Pellet cells by centrifugation. Lyse cells in Redzol reagent by pipetting. Use 1 ml of Redzol for 5~10×10⁶ of animal, plant or yeast cells, or for 1×10⁷ bacterial cells. Washing cells before adding Redzol reagent should be avoided as this may increase the possibility of mRNA degradation. To lyse some yeast and bacterial cells may require the use of a homogenizer.



2. 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 materials from the homogenate by centrifugation at 12,000 rpm for 10 minutes at 2~8°C. 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 below.

3. PHASE SEPARATION

Incubate the homogenized samples for 5 minutes at 15~30°C to allow the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of Redzol reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15~30°C for 2~3 minutes. Centrifuge the samples at no more than 12,000 rpm for 15 minutes at 2~8°C. Following centrifugation, the mixture separates into a lower phenol-chloroform phase, a cloudy white 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 Redzol 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 isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of Redzol reagent used for the initial homogenization. Incubate samples at 15~30°C for 10 minutes and centrifuge at no more than 12,000 rpm for 10 minutes at 2~8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet at the side and bottom of the tube.

5. RNA WASH

Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of Redzol reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500 rpm for 5 minutes at 2~8°C.

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/A280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by pipetting the solution a few times, and incubate for 10 minutes at 55~60°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.

Troubleshooting Guide



- **Expected yield of RNA per mg of tissues 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	5~8 μg	Fibroblasts	5~7 μg

- **Low yield**

Incomplete homogenization or lysis of samples.

Final RNA pellet incompletely redissolved.

- **A260/A280 ratio <1.65**

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. Insufficient amount of Redzol was used in sample homogenization. Following homogenization, samples were not stored at room temperature for 5 minutes. The aqueous phase was contaminated with the phenol phase. Incomplete dissolution of the final RNA pellet.

- **RNA degradation**

Tissues were not immediately processed or frozen after removal from animals. Samples used for isolation, or the isolated RNA preparations were stored at $-5 \sim -20^\circ\text{C}$, instead of $-60 \sim -70^\circ\text{C}$. Cells were dispersed by trypsin digestion. Aqueous solutions or tubes were not RNase-free. Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

- **DNA contamination**

Sample homogenized in too less volume of Redzol.

Sample used for the isolation contained organic solvents (e.g. ethanol, DMSO), strong buffers, or alkaline solution.

- **Proteoglycan and polysaccharide contamination**

The following modification of the RNA precipitation removes these contamination compounds from the isolated RNA. Add to the aqueous phase 0.25 ml of isopropyl alcohol 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 Redzol 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 solution. A combination of the modified precipitation step with an additional centrifugation of the initial homogenate is required to isolate pure RNA from plant materials containing a very high level of polysaccharides.