

胍盐法 Total RNA 提取*

Materials

Denaturing solution

2 M sodium acetate, pH 4

Water-saturated phenol 49:1 (v/v) chloroform/isoamyl alcohol or bromochloropropane

100% isopropanol

75% ethanol (prepared with DEPC-treated water)

DEPC-treated water or freshly deionized formamide

Glass Teflon homogenizer

5-ml polypropylene centrifuge tube

Sorvall SS-34 rotor (or equivalent)

CAUTION: Phenol is a poison and causes burns. When handling phenol, use gloves and eye protection.

NOTE: Carry out all steps at room temperature unless otherwise stated.

Homogenize cells

1a. For tissue: Add 1 ml denaturing solution per 100 mg tissue and homogenize with a few strokes in a glass Teflon homogenizer.

1b. For cultured cells: Either centrifuge suspension cells and discard supernatant, or remove the culture medium from cells grown in monolayer cultures. Add 1 ml denaturing solution per 10^7 cells and pass the lysate through a pipet seven to ten times.

Do not wash cells with saline. Cells grown in monolayer cultures can be lysed directly in the culture dish or flask.

The procedure can be carried out in sterile, disposable, round-bottom polypropylene tubes with caps; no additional treatment of the tubes is necessary. Before using, test if the tubes can withstand centrifugation at $10,000 \times g$ with the mixture of denaturing solution and phenol/chloroform.

2. Transfer the homogenate into a 5-ml polypropylene tube. Add 0.1 ml of 2 M sodium acetate, pH 4, and mix thoroughly by inversion. Add 1 ml water-saturated phenol mix thoroughly, and add 0.2 ml of 49:1 chloroform/isoamyl alcohol or bromochloropropane. Mix thoroughly and incubate the suspension 15 min at 0° to 4°C. Make sure that caps are tightly closed when mixing. The volumes used are per 1 ml denaturing solution.

Bromochloropropane is less toxic than chloroform and its use for phase separation decreases possibility of contaminating RNA with DNA (Chomczynski and Mackey, 1995).

3. Centrifuge 20 min at $10,000 \times g$ (9000 rpm in SS-34 rotor), 4°C. Transfer the upper aqueous phase to a clean tube.

The upper aqueous phase contains the RNA, whereas the DNA and proteins are in the interphase and lower organic phase. The volume of the aqueous phase is ~ 1 ml, equal to the initial volume of denaturing solution.

Isolate RNA

4. Precipitate the RNA by adding 1 ml (1 vol) of 100% isopropanol. Incubate the samples 30 min at -20°C . Centrifuge 10 min at $10,000 \times g$, 4°C, and discard

supernatant.

For isolation of RNA from tissues with a high glycogen content (e.g., liver), a modification of the single-step method is recommended to diminish glycogen contamination (Puissant and Houdebine, 1990). Following this isopropanol precipitation, wash out glycogen from the RNA pellet by vortexing in 4 M LiCl. Sediment the insoluble RNA 10 min at $5000 \times g$.

Dissolve the pellet in denaturing solution and follow the remainder of the protocol.

5. Dissolve the RNA pellet in 0.3 ml denaturing solution and transfer into a 1.5-ml microcentrifuge tube.

6. Precipitate the RNA with 0.3 ml (1 vol) of 100% isopropanol for 30 min at -20°C . Centrifuge 10 min at $10,000 \times g$, 4°C , and discard supernatant.

7. Resuspend the RNA pellet in 75% ethanol, vortex, and incubate 10 to 15 min at room temperature to dissolve residual amounts of guanidine contaminating the pellet.

8. Centrifuge 5 min at $10,000 \times g$, 4°C , and discard supernatant. Dry the RNA pellet in a vacuum for 5 min.

Do not let the RNA pellet dry completely, as this greatly decreases its solubility. Avoid drying the pellet by centrifugation under vacuum. Drying is not necessary for solubilization of RNA in formamide.

9. Dissolve the RNA pellet in 100 to 200 μl DEPC-treated water or freshly deionized formamide by passing the solution a few times through a pipe tip. Incubate 10 to 15 min at 55° to 60°C . Store RNA dissolved in water at -70°C and RNA dissolved in formamide at either -20° or -70°C .

RNA dissolved in formamide is protected from degradation by RNase and can be used directly for formaldehyde-agarose gel electrophoresis in northern blotting (Chomczynski, 1992). However, before use in RT-PCR, RNA should be precipitated from formamide by adding 4 vol ethanol and centrifuging 5 min at $10,000 \times g$.

Quantitate RNA

10. Quantitate RNA by diluting 5 μl in 1 ml alkaline water and reading the A_{260} and A_{280} .

Water used for spectrophotometric measurement of RNA should have $\text{pH} > 7.5$. Acidic pH affects the UV absorption spectrum of RNA and significantly decreases its A_{260}/A_{280} ratio (Willfinger et al., 1996). Typically, distilled water has $\text{pH} < 6$. Adjust water to a slightly alkaline pH by adding concentrated Na_2HPO_4 solution to a final concentration of 1 mM

REAGENTS AND SOLUTIONS**Denaturing solution**

Stock solution: Mix 293 ml water, 17.6 ml of 0.75 M sodium citrate, $\text{pH} 7.0$, and 26.4 ml of 10% (w/v) *N*-lauroylsarcosine (Sarkosyl). Add 250 g guanidine thiocyanate and stir at 60° to 65°C to dissolve. Store up to 3 months at room temperature.

Working solution: Add 0.35 ml 2-mercaptoethanol (2-ME) to 50 ml of stock solution. Store up to 1 month at room temperature.

Final concentrations are 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% Sarkosyl, and 0.1 M

2-ME.

Sodium acetate, 2 M

Add 16.42 g sodium acetate (anhydrous) to 40 ml water and 35 ml glacial acetic acid. Adjust solution to pH 4 with glacial acetic acid and dilute to 100 ml final with water (solution is 2 M with respect to sodium ions). Store up to 1 year at room temperature.

Water-saturated phenol

Dissolve 100 g phenol crystals in water at 60° to 65°C. Aspirate the upper water phase and store up to 1 month at 4°C.

Do not use buffered phenol in place of water-saturated phenol.

Formamide

Prepare freshly deionized formamide by stirring with 1 g AG 501-X8 ion-exchange resin (Bio-Rad) per 10 ml formamide for 30 min and filter at room temperature. Alternatively, use a commercially available stabilized, ultrapure formamide (Formazol, Molecular Research Center).

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