BIOLOGICAL INDUSTRIES
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EZ-RNA

Total RNA Isolation Kit

Cat. No.: **20-400-100**
Store at: **2-8°C**

Product Description

EZ-RNA is a complete kit with ready-to-use reagents for the isolation of total RNA from samples of human, animal, plant, yeast, bacterial and viral origin. **EZ-RNA** is an improved version of the Chomczynski and Sacchi method (1), which is based on disruption of cells in guanidine thiocyanate/detergent solution, followed by organic extraction and alcohol precipitation of the RNA, and which allows simultaneous processing of a large number of samples. The resulting RNA is suitable for the isolation of Poly A^+ RNA or for Northern Blotting, Dot Blotting, *in vitro* Translation, Molecular Cloning, RT-PCR and RNase Protection Assays, or other analytical procedures. DNA and proteins can also be recovered from the interphase and the organic phase of the same sample.

Kit Reagents

Cat. No.: 20-400-100A Denaturing Solution, 50ml
Contains: guanidine thiocyanate
Store at: 2-8°C

Cat. No.: 20-400-100B Extraction and Phase Separation Solution, 50ml
Contains: phenol and chloroform
Store at: 2-8°C - *Do not swirl. Make sure there are two phases. Take only from the organic (lower) phase, leaving the upper (aqueous) phase. Do not use if turbid.*

Reagents Required But Not Supplied

RNA	***DNA***	***Proteins***
Isopropanol	Absolute Ethanol	Isopropanol
75% Ethanol	0.1M Sodium Citrate in 10% Ethanol	0.3M Guanidine HCl in 95% Ethanol
DEPC-Treated Water or	75% Ethanol	Absolute Ethanol
0.1mM EDTA	8mM NaOH (fresh preparation)	1% SDS
	1M Hepes, free acid	

Precautions

EZ-RNA contains phenol, which is poisonous, and guanidine thiocyanate, which is an irritant. Therefore, when working with **EZ-RNA**, use gloves and eye protection, avoid contact with skin or clothing, and avoid inhaling vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

1. <u>Protocol for RNA Isolation</u>

1.1 Homogenization

I ***Tissue***

Homogenize samples in the Denaturing Solution (0.5ml/50-100mg tissue) using homogenizer. Sample volume should not exceed 10% of the volume of the Denaturing Solution.

II ***Cells***

<u>Cells grown in monolayer</u> should be lysed directly in a culture dish using 0.5ml Denaturing Solution/10cm^2 of culture dish area. Pass the cell lysate several times through a pipette.

<u>Cells grown in suspension</u> should be first sedimented, then lysed in the Denaturing Solution (0.5ml Denaturing Solution/5-10x10^6 for animal, plant or yeast cells; or 10^7 for bacterial cells) by repeated pipetting.

1.2 Phase Separation

Store the homogenate for 5 minutes at room temperature. Then add 0.5ml Extraction Solution per 0.5ml Denaturing Solution. Shake vigorously for 15 seconds, store at room temperature for 10 minutes and then centrifuge at 12,000g for 15 minutes at 4°C.

* *To increase yield, perform second extraction: Transfer the upper phase and interphase to a fresh tube, add Extraction Solution of the above volume, and repeat centrifugation.*

1.3 RNA Precipitation

Transfer the aqueous colorless (upper) phase to a fresh tube and store the interphase and the organic phase at 4°C for DNA isolation (if desired). Precipitate RNA from the aqueous phase by mixing with 0.5ml isopropanol per 0.5ml Denaturing Solution. Store at room temperature for 10 minutes and then centrifuge at 12,000g for 8 minutes at 4°C.

* *To increase yield, store sample for 30 minutes - overnight at -20°C.*

1.4 LiCl Precipitation (optional)

Polysaccharides and other contaminants may be removed by LiCl precipitation of the RNA. Re-suspend the RNA pellet by mixing with 2.5M LiCl solution. Vortex if necessary. Store at -20°C for at least 30 minutes and then centrifuge at 10,000g for 15 minutes at 4°C.

1.5 RNA Wash

Remove supernatant and wash the RNA pellet (by vortexing) with 1ml 75% ethanol. Then centrifuge at 7,500g for 5 minutes at 4°C. The RNA precipitate can be stored in 75% ethanol at 4°C for one week, or at -20°C for at least one year.

1.6 RNA Solubilization

Remove the ethanol wash and air-dry the RNA pellet for 5 minutes. Do not let the RNA pellet dry completely. Dissolve the RNA in 100µl of DEPC-treated water with 0.1mM EDTA, or in 0.5% SDS solution (prepared with DEPC-treated water) by incubating for 10-15 minutes at 55°C.

* ***<u>Important</u>: for best results in RT-PCR, dissolve the RNA in DEPC-treated water <u>without</u> EDTA (heat if necessary).***

The final preparation of total RNA will be free of DNA and proteins, and will have a 260/280 O.D. ratio of 1.6 to 1.9.

2. Protocol for DNA Isolation

2.1 DNA Precipitation

Carefully remove the remaining upper aqueous phase and discard. Add 0.3ml of absolute ethanol per 0.5ml of Denaturing Solution, and mix by inversion. Store at room temperature for 3 minutes and then centrifuge at 2000g for 5 minutes at 4°C. Remove the phenol-ethanol supernatant and store at 4°C for protein isolation (if desired).

2.2 DNA Wash

Wash the DNA pellet twice in a solution containing 0.1M Sodium Citrate in 10% ethanol. Use 1ml of solution per 0.5ml Denaturing Solution. Store at room temperature for 30 minutes with occasional mixing, and then centrifuge at 2,000g for 5 minutes at 4°C. Dissolve the DNA pellet in 75% ethanol (1.5-2ml per 0.5ml Denaturing Solution). Store at room temperature for 10-20 minutes with occasional mixing, and then centrifuge at 2000g for 5 minutes at 4°C.

2.3 DNA Solubilization

Remove the ethanol wash and air-dry for 5 minutes. Dissolve the DNA in 8mM NaOH by careful pipetting. Add 0.3-0.6ml 8mM NaOH to DNA isolated from 50mg of tissue or 10^7 cells. To remove any insoluble material, centrifuge at 12,000g for 10 minutes and transfer the supernatant to a new tube. Samples can be stored at 4°C overnight. For prolonged storage, adjust sample to pH 7-8 (with 1M Hepes, free acid) and adjust the EDTA concentration to 1mM.

2.4 pH Adjustment of DNA Samples Dissolved in 8mM NaOH

For 1ml of 8mM NaOH, use the following amounts of 1M Hepes, free acid:

Final pH	1M Hepes (µl)
7.0	42
7.2	30
7.5	18
7.8	13.5
8.0	11.5
8.4	9.5

2.5 Amplification of DNA by PCR

Following solubilization in 8mM NaOH, adjust the pH of the DNA sample to 8.4 with 1M Hepes, free acid. Add 0.1-1.0µg of the DNA sample to a PCR reaction mixture and perform the standard PCR protocol.

2.6 Digestion of DNA by Restriction Endonucleases

Adjust the pH of the DNA solution to a required value using 1M Hepes, free acid (see table). Use 3-5 units of enzymes per microgram of DNA. Use the conditions recommended by the enzyme manufacturer.

3. Protocol for Protein Isolation

3.1 Protein Precipitation

Precipitate proteins from the phenol-ethanol supernatant (step 2.1) with 1.5ml isopropanol per 0.5ml of Denaturing Solution used for the initial homogenization. Store samples for 10 minutes at room temperature and then centrifuge at 12,000g for 10 minutes at 4°C.

3.2 Protein Wash

Remove the supernatant and wash the pellet 3 times with 0.3M guanidine HCl in 95% ethanol. Use 2ml of wash solution per 0.5ml of Denaturing Solution for each wash. Store samples in wash solution for 20 minutes at room temperature. Centrifuge at 7,500g for 5 minutes at 4°C. After the final wash, add 2ml of absolute ethanol and vortex the protein pellet. Store for 20 minutes at room temperature and then centrifuge at 7500g for 5 minutes at 4°C.

3.3 Protein Solubilization

Air dry the protein pellet for 10 minutes. Dissolve the pellet in 1% SDS solution by pipetting. Complete solubilization of the protein pellet may require incubation at 50°C. Remove any insoluble material by centrifugation at 10,000g for 10 minutes at 4°C and transfer the supernatant to a new tube. The proteins may be used immediately for Western Blotting or stored at -20°C.

Reference

(1) Chomczynski, P. and Sacchi, N., *Anal. Biochem.,* 162:156-159 (1987)