Total RNA Extraction Reagent

Cat. No.: EZB-TZ1

Description

The *EZ*Bioscience® **Total RNA Extraction Reagent** is a ready-to-use reagent, which designs to isolate high quality total RNA from animal tissues, cultured cells, plants, yeast, bacteria and other samples within 1 hour. Total RNA Extraction Reagent is a monophasic solution of phenol, guanidine isothiocyanate and other components, which maintains the integrity of the RNA due to highly effective inhibition of RNase activity during sample homogenization. After homogenizing the sample with Total RNA Extraction Reagent, chloroform is added, and the homogenate is allowed to separate into a clear upper aqueous layer (containing RNA), an interphase, and a red lower organic layer (containing the DNA, polysaccharide, fatty acid, etc). RNA is precipitated from the aqueous layer with isopropanol. The precipitated RNA is washed to remove impurities, and then resuspended for use in downstream applications. Isolated RNA can be used in RT-PCR, Northern Blot analysis, Dot Blot hybridization, poly(A)+ selection, in vitro translation and molecular cloning.

Component

Component	EZB-TZ1	EZB-TZ1-L
Total RNA Extraction Reagent	100 ml	200 ml

Storage

Store the Total RNA Extraction Reagent at 2 ~ 8°C, protect from light.

Preparation before experiment

1) Prepare reagents: chloroform, isopropanol, 75% ethanol (prepared with RNase-free ddH_2O), RNase-free ddH_2O.
2) Prepare RNase-free or sterilized pipette tips (2.5 µl, 20 µl, 200 µl, 1 ml), RNase-free or sterilized 1.5 ml EP tube.
3) Please pre-cool the centrifuge to 4°C in advance.

Experimental precautions

The key of RNA extraction is to prevent RNase contamination. RNA can be degraded not only by endogenous RNase, but also by exogenous RNase. Therefore, protective measures shall be taken from the following aspects:
1) For RNA extraction, please operate in a clean area. During the operation, please wear disposable gloves and masks and avoid speaking. The above measures can prevent RNase pollution in skin and saliva.
2) The reagents used should be special, in order to avoid cross contamination after mixed used.
3) The experimental apparatus used, including pipette tips and centrifugal tube, shall be RNase-free and shall be special.

Protocol

1. Lyse and Homogenize Sample

Lyse and homogenize samples in Total RNA Extraction Reagent according to your starting materials.

Cell grown in monolayer:

a) Remove the culture media and wash the cells with PBS.
b) Add 1 ml of Total RNA Extraction Reagent into the 6-well plate or 35 mm culture plate to lyse the cells.
c) Pipet the lysate up and down for several times to homogenize, or put the culture plate on a shaking table, then pipet it about 10 times to lyse fully.

Note: for cells with firm adherence to the wall, cells can be peeled off with a cell curette.

d) Transfer the cell lysate to a 1.5 ml EP tube, and pipet the lysate up and down for 10 times. Incubate at room temperature for 5 minutes.

Cell grown in monolayer:

a) Pellet the cells by centrifugation at 500 × g for 3 ~ 5 minutes, and discard the supernatant.
b) Wash the cells with PBS.
c) Add 1 ml of Total RNA Extraction Reagent of 5 × 10^6 cells to the pellet.
d) Pipet the lysate up and down for several times to homogenize.

Tissues:

a) Add 1 ml of Total RNA Extraction Reagent per 5 ~ 50 mg of tissue to the sample and homogenize using a homogenizer.

Note: inadequate tissue lysis can affect RNA yield and quality.

b) After homogenization, incubate at room temperature for 5 minutes. Then centrifuge the lysate for 5 minutes at 12,000 × g at 4°C, transfer the clear supernatant to a new tube.

Note: For above samples, if RNA extraction is not continued, the lysate can be frozen at - 80°C.

2. Precipitate the RNA:

1) Add 0.2 ml of chloroform per 1 ml of Total RNA Extraction Reagent, then securely cap the tube and mix by pipetting or hand-shaking. Then

incubate at room temperature for 5 minutes.

2) Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.

3) Transfer the upper-layer supernatant to a new RNase-free 1.5 ml centrifuge tube.

Note: do not transfer the middle or bottom layer.

4) Add 1 ml of isopropanol to the aqueous phase, per 1 ml of Total RNA Extraction Reagent used for lysis.

5) Invert the centrifuge tube for several times or pipette up and down for 10 times to mix thoroughly. Then incubate at room temperature for 10 minutes.

6) Centrifuge for 10 minutes at 12,000 × g at 4°C. Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.

3. Wash the RNA:

1) Discard the supernatant with a micropipettor.

2) Resuspend the pellet in 1 ml of 75% ethanol per 1 ml of Total RNA Extraction Reagent used for lysis.

3) Invert the centrifuge tube for several times to wash the RNA pellet thoroughly, then centrifuge for 5 minutes at 7,500 × g at 4°C.

4) Discard the supernatant with a micropipettor.

4. Solubilize the RNA:

1) Open the centrifuge tube, dry the RNA pellet at room temperature in a clean environment for a few minutes (Do not dry the pellet by vacuum centrifuge, and do not dry the RNA pellet excessively, to ensure total solubilization of the RNA).

2) After the RNA pellet is dried, resuspend the pellet in 20 ~ 50 µl RNase-free ddH_2O by pipetting up and down for several times. After the pellet is completely dissolved, take a small amount of it for detection, and store the rest at - 80°C.

Detect the RNA

1. Purity and concentration detection

1) The purity of the RNA can be detected by NanoDrop™ Spectophotometer. The ratio of OD260/280 is between 1.8 and 2.2, indicating that RNA is considered pure.

2) The RNA concentration can be measured by spectrophotometer, RNA concentration (ng/µl) = OD260 × dilution × 40; or by Agilent 2100 biological analyzer or qubit.

2. Integrity detection

Transfer 0.5 ~ 1 µg RNA to a new centrifuge tube, then add 8 µl of RNase-free ddH_2O and 1 µl of 10x DNA loading buffer, mixed thoroughly. Then perform the agarose gel electrophoresis to examine the RNA Integrity and purity. Or use the Agilent 2100/4200 Bioanalyzer to determine the RIN value of RNA.

For intact RNA, there may be two kinds of ribosomal RNA (eukaryotic cells: 28S and 18S), and the band brightness of 28S/18S is about 2:1. However, if the ribosomal RNA bands are diffused, the RNA may have been degraded.

Troubleshooting

Problems	Possible cause(s)	Suggestion(s)
The sample is degraded.	Samples were not immediately extracted or frozen after collection.	Samples must be extracted or frozen immediately after collection.
	Samples were stored at the incorrect temperature.	Store samples at –80°C or liquid nitrogen.
A lower yield than expected is observed.	The samples were incompletely lysed or homogenized.	Decrease the amount of starting material.
		Increase the volume of Total RNA Extraction Reagent appropriately.
		For tissue samples, the tissues should cut into small pieces and then homogenized; For cell samples, it is necessary to pipet the cell with a pipette several times after adding the Total RNA Extraction Reagent.
	The pellet was incompletely solubilized.	Pipetting the sample for several times.
		Do not dry the pellet by vacuum centrifuge and do not dry the RNA pellet excessively.
The RNA purity is not good.	The samples were incompletely lysed or homogenized.	Reduce the initial amount of samples.
		Increase the volume of Total RNA Extraction Reagent appropriately.
		For tissue samples, the tissues should cut into small pieces and then homogenized; For cell samples, it is necessary to pipet the cell with a pipette several times after adding the Total RNA Extraction Reagent.
	When transferring the upper-layer supernatant, the middle and lower layers were accidentally aspirated.	When transferring the supernatant, you can transfer it with a small pipette tip. Do not insert the pipette tip too deep into the liquid surface.
The RNA is degraded.	Sample is not fresh.	Fresh tissue or cell shall be used for RNA extraction, or tissue and cell shall be frozen rapidly with liquid nitrogen and stored at - 80°C.
	The instruments used to extract RNA were not sterilized.	When extracting RNA, all pipette tips, centrifuges and reagents should be enzyme free or sterilized.