Tissue RNA Purification Kit PLUS

Catalog No.: EZB-RN001-plus

Description

The *EZ*Bioscience® Tissue RNA Purification Kit PLUS integrates phenol/guanidine-based sample lysis and silica-membrane purification of high-quality total RNA from animal tissues and cells. Samples are first lysed and homogenized in Lysis Buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is collected, and ethanol is added to provide appropriate binding conditions. The sample is then applied to the RNA Spin Column, where the total RNA binds to the membrane, and contaminants are effectively washed away. Finally, high-quality RNA is eluted in Elution Buffer. The purified total RNA is suitable for use in a variety of downstream applications, including: RT-PCR, RT-qPCR, RNA-seq, Northern blotting, Poly A+ RNA selection, Microarray analysis, and so on.

Components

Components	EZB-RN001-plus (100 Preps)
Lysis Buffer	55 ml
Wash Buffer 1*	13 ml
Wash Buffer 2*	13 ml
Elution Buffer	25 ml
Spin Columns (with Collection Tubes)	100 Preps

*Before using for the first time, add 52 ml of 100% ethanol to the Wash Buffer 1 and Wash Buffer 2 separately.

Storage

Store the Lysis Buffer at 2 ~ 8°C, protected from light. Store other components at room temperature (when using these buffers, be careful to avoid contamination). Dividing the Elution Buffer into small aliquots upon reception is suggested.

Protocol

Sample Homogenization

The suggested tissue weight according to tissue types:

Tissue types	Tumor, embryos, heart, kidney, spleen, pancreas, lung, eye	Muscle, skin, vessel
Weight (mg)	5~ 50	20~ 50

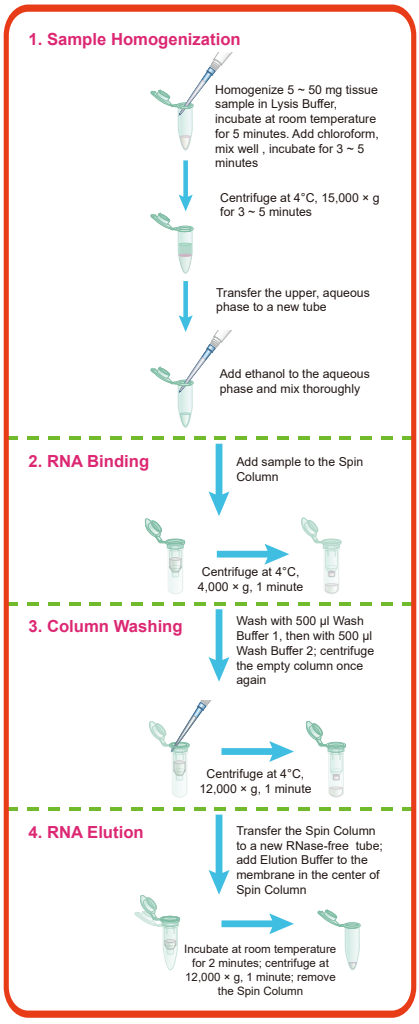
1. Place 5 ~ 50 mg tissue in a 1.5 ml centrifuge tube. Add 500 µl of Lysis Buffer.

   Note: If the samples are cells: Pellet 3 × 10^5 ~ 3 × 10^6 cells and wash with appropriate volume of PBS, then add 500 µl of Lysis Buffer and pipette up and down for 20 times to lyse cells thoroughly. After fully lysed, proceed directly to step 3.
2. Homogenize the tissue with a pestle or rotor-stator homogenizer. Incubate at room temperature for 5 minutes.
3. Add 150 µl chloroform to the tissue lysate and shake it vigorously for 15 s. Incubate at room temperature for 3 ~ 5 minutes.
4. Centrifuge at 15,000 × g, 4°C for 3 ~ 5 minutes. Then the sample separates into three phases. Transfer the upper, aqueous phase (Note: The volume of the aqueous phase is approximately 200 µl) to a new RNase-free 1.5 ml centrifuge tube (be careful to avoid pipetting the interphase and lower phase).

RNA Binding

5. Add 1 volume of 100% ethanol for mRNA and lncRNA purification (If extracting circRNA, add 1.6 volume of 100% ethanol), mix thoroughly by pipetting. Then transfer the sample to the Spin Column. Centrifuge at 4,000 × g, 4°C for 1 minute. Discard the flow-through.

Experimental Procedure Overview



Column Washing

6. Add 500 µl of Wash Buffer 1 to the Spin Column. Centrifuge at 12,000 × g, 4°C for 1 minute (be careful to avoid contacting the bottom of the Spin Column with the liquid when taking out of the column). Discard the flow-through.
7. Add 500 µl of Wash Buffer 2 to the Spin Column. Centrifuge at 12,000 × g, 4°C for 1 minute.
8. Discard the flow-through, place the Spin Column back on the collection tube and centrifuge at 12,000 × g, 4°C for 1 minute.
9. Discard the collection tube with the flow-through and place the Spin Column to a new RNase-free 1.5 ml centrifuge tube, open the lid and keep in the air for 2 minutes.

RNA Elution

10. Add 20 ~ 30 µl of Elution Buffer to the membrane in the center of the Spin Column and incubate at room temperature for 2 minutes.
11. Centrifuge at 12,000 × g, 4°C for 1 minute (transfer the eluate back to the Spin Column, incubate for 5 minutes and centrifuge once more will get more RNA).
12. Discard the Spin Column, determine the RNA concentration after mix well, then do the following experiment with the purified RNA, or store the RNA at -80°C until needed.

Representative Results

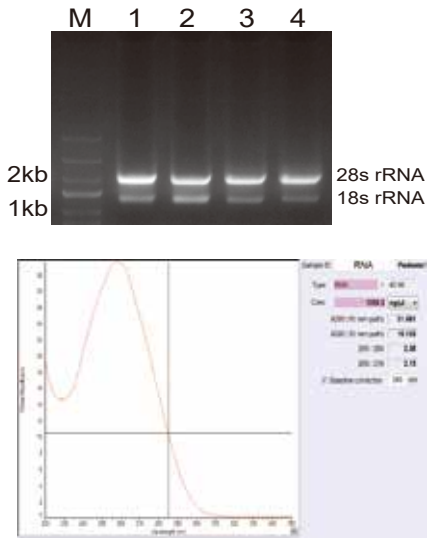


Figure 1. RNA isolated from 30 mg mouse muscle using ***EZ*Bioscience**® Tissue RNA Purification Kit PLUS and TRIzol (eluted in 30 µl of Elution Buffer each, 5 µl RNA of each is loaded for electrophoresis). M: 250bp DNA Ladder; Lanes 1 and 2: ***EZ*Bioscience**® Tissue RNA Purification Kit PLUS; Lanes 3 and 4: TRIzol; These results showed that more RNA can be isolated from the same amount of tissue by ***EZ*Bioscience**® Tissue RNA Purification Kit PLUS than TRIzol.

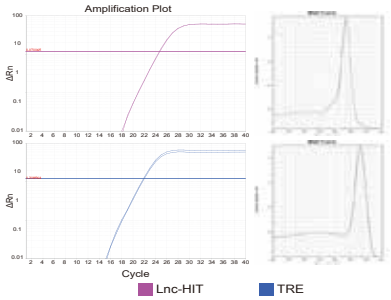


Figure 2. lncRNA isolated from 293T cells are detected. These results showed that, this ***EZ*Bioscience**® Tissue RNA Purification Kit PLUS can be used for lncRNA purification and achieved good performance.

Trouble shooting

The quantity of RNA purified by this Kit is too small, or the qPCR results is not so good, the Ct value is too large, or some specific genes can not be amplified normally.

Suggestions:

a. **Examine whether any of the reagents in the Kit is contaminated**: use a new Kit as positive control to confirm whether the reagents are contaminated (If the reagents are contaminated, the results between these two will be different, obviously). Therefore, **it is recommended to divide the reagents into 3 ~ 4 parts, each part in a 15 ml/50 ml centrifuge tube.** Be careful when taking the reagents out of the bottles to avoid contamination.

b. The gene specific primers for qPCR should be divided to aliquots and stored at -20°C, to decrease the possibility of degradation or contamination.

c. **Examine whether the experiment is carried out correctly.**

e.g.:

1. During the whole process of RNA purification by this Kit, **it must be manipulated at room temperature (unless specially required) but not on ice** (until the RNA is eluted after the centrifuge), to avoid of blocking the membrane in the Spin Column by the water-insoluble substances formed during ice cooling.
2. Before using this Kit for the first time, **add 52 ml 100% ethanol to each bottle of the Wash Buffer** and mix thoroughly.
3. The tissues must be weighed before RNA extraction. The weight of tissue should be less than 50 mg/sample. For tissues high RNA content (such as liver, spleen and thymus), the amount of tissue should be reduced to no more than 10 mg/sample.
4. Transfer the upper, aqueous phase to a new tube after phase separation, avoid pipetting the interphase and lower phase.
5. The Spin Column must be dried in air for 2 minutes after the centrifugation of empty column.
6. In order to increase the RNA yield, please preheat the Elution Buffer to 95°C and then perform elution, or repeat the elution by adding the RNA solution back to the column membrane, let it stand for 5 minutes and then centrifuge.