EZ-press RNA Purification Kit

Catalog No.: B0004D

Description

The *EZ*Bioscience® EZ-press RNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of sources, without the need for toxic substances such as phenol or chloroform. The EZ-press RNA Purification Kit can be used with cultured cells and animal tissues. The purified total RNA is suitable for use in a variety of downstream applications, including: RT-PCR, RT-qPCR, Northern blotting, nuclease protection assays, and so on (not suggested for RNA sequencing). Biological samples are first lysed and homogenized in a strong denaturant containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After homogenization, ethanol is added to the sample. The lysate is then passed through RNA binding Spin Column containing a silica-based membrane to which the RNA binds, and the membrane is treated with gDNA Remover to eliminate the DNA bonded on the column. Impurities are effectively removed by subsequent washing. The purified total RNA is then eluted in Elution Buffer and may be used in a variety of downstream applications.

Components

Components	B0004D (100 Preps)
Lysis Buffer	60 ml
Wash Buffer*	13 ml
Elution Buffer	25 ml
gDNA Remover	220 µl
Spin Columns (with Collection Tubes)	100 Preps

*Before using for the first time, add 52 ml 100% ethanol to the Wash Buffer and mix thoroughly.

Storage

Store the gDNA Remover at -20°C. Store other components at room temperature (When using these buffers, be careful to avoid contamination). The Lysis Buffer is suggested to be stored at 4°C, when the room temperature is >25°C, or for long-term storage.

Protocol

The Lysis Buffer should be restored to room temperature before using.

Sample Homogenization

1A. For adherent cells ≤ 3 × 10^6/sample:

a) Remove the growth medium from the cells.

b) Wash cells with appropriate volume of PBS.

c) Add 500 µl of Lysis Buffer. Pipette up and down for 30 times to suspend the cells.

d) Transfer the cell lysate to a new tube and vortex for 10 seconds at high speed to completely lyse the cells.

1B. For cultured suspension cells or adherent cells more than 3 × 10^6/sample:

a) (*For adherent cells only, for suspension cells, start at step b*) Detach cells using the subculturing method routinely employed in your laboratory.

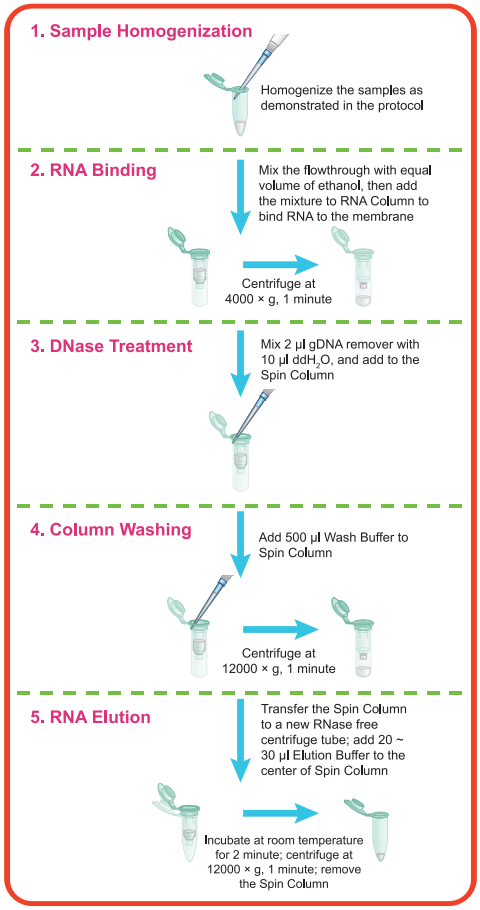
b) Pellet 1 × 10^6 cells in a 1.5 ml centrifuge tube by centrifugation at 500 × g for 3 ~ 5 minutes.

c) Completely remove the supernatant by aspiration.

d) Add 500 µl of Lysis Buffer.

e) Vortex for 10 seconds at high speed to completely lyse the cells.

Experimental Procedure Overview:



1B. For animal tissues (not suggested for tough tissues):

a) Place 1 ~ 100 mg tissue in a 1.5 ml centrifuge tube. Add 300 µl of Lysis Buffer.

b) Homogenize the tissue with a pestle or rotor-stator homogenizer. In case the tissue is larger than 5 mg, transfer a volume of the homogenate that contains 5 mg tissue to a new tube.

c) Add Lysis Buffer to the homogenate to a total volume of 300 µl. Homogenize again, then vortex for 10 seconds at high speed to lyse the tissue completely.

d) Centrifuge at 12000 × g for 2 minutes. Then transfer the supernatant to a new RNase free 1.5 ml centrifuge tube.

RNA Binding

2. Add equal volume of 100% ethanol to each volume of cell or tissue homogenate (There may be precipitates at this step. This is a normal phenomenon).

3. Invert the centrifuge tube for several times or pipette up and down to disperse the precipitates, and transfer the sample to the Spin Column. Centrifuge at 4000 × g for 1 minute (for tissue sample, 12000 × g is recommended).

DNase Treatment

4. Mix 2 µl gDNA Remover with 10 µl ddH_2O, and add to the center of the column. Incubate at room temperature for 5 minutes to degrade the residual DNA.

Column Washing

5. Add 500 µl of Wash Buffer to the column. Centrifuge at 12000 × g for 1 minute (be careful to avoid contacting the bottom of the column with the liquid when taking out of the column. **Optional operation:** Pour off the liquid in the collection tube and centrifuge the empty column with the collection tube once more).
6. Don't need to pour off the liquid, directly transfer the column to an RNase free 1.5 ml centrifuge tube, open the lid and keep in the air for 2 minutes.

RNA Elution

7. Add 20 ~ 30 µl of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes.
8. Centrifuge at 12000 × g for 1 minute (transfer the eluate back to the column, incubate for 5 minutes and centrifuge once more will get more RNA).
9. Discard the column，determine the RNA concentration, do the following experiment with the purified RNA, or store the RNA at -80°C until needed.

Representative Results

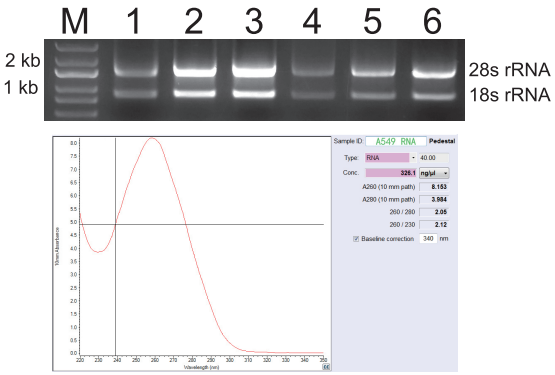
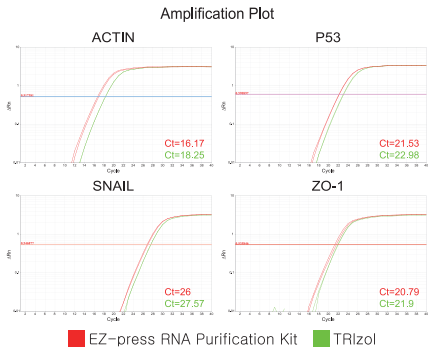


Figure 1. RNA isolated from different amounts of A549 cells using ***EZ*Bioscience**® EZ-press RNA Purification Kit and TRIzol. M: 250bp DNA Ladder; Lanes 1 ~ 3: EZ-press RNA Purification Kit; Lanes 4 ~ 6: TRIzol; 1: 2 × 10^5 cells; 2: 4 × 10^5 cells; 3: 6 × 10^5 cells; 4: 2 × 10^5 cells; 5: 4 × 10^5 cells; 6: 6 × 10^5 cells. These results showed that high-purity RNA can be isolated from the same number of cells by ***EZ*Bioscience**® EZ-press RNA Purification Kit, better than TRIzol.



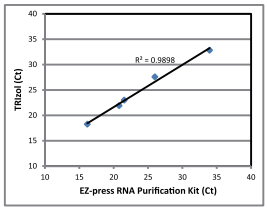


Figure 2. Detection of mRNA level of 4 genes (ACTIN, P53, SNAIL and ZO-1) in A549 cells by RT-qPCR (the RNA was purified by EZ-press RNA Purification Kit and TRIzol method respectively). Figure 2. Shows that, the Ct values of these genes in the RNA sample purified by EZ-press RNA Purification Kit are highly correlated with the RNA purified by TRIzol method (R^2=0.9898).

The data from Figure 1. and Figure 2. indicate that, EZ-press RNA Purification Kit can completely replace the TRIzol method in RNA purification.

Trouble shooting

1. 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. When the sample is very little, heating the Elution Buffer to 60°C and then performing the elution step can improve the yield of RNA.

d. 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 but not on ice** (until the RNA is eluted after the centrifuge), to avoid 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 the Wash Buffer** and mix thoroughly.

3) The cells or tissues lysates should be mixed adequately by using vortex. Then **add equal volume of ethanol** to each samples and mix thoroughly, then transfer them to the column.

4) After washing the sample, the column should be taken out carefully to avoid of contacting the bottom of column with liquid.

5) When doing the step of RNA binding and RNA elution, after centrifuge, **transfer the liquid back to the column and repeat the centrifuge once again will get more RNA.**

2. Can this Kit be used for **microRNA purification?**

Yes, this kit can be used for microRNA purification. You should add 1.6 volume of 100% ethanol to each volume of cell lysate. But Universal microRNA purification kit (Cat. No.: EZB-miRN1) is more suitable for microRNA purification.