EZ-press RNA Purification Kit PLUS

Catalog No.: B0004-plus

Description

The *EZ*Bioscience® EZ-press RNA Purification Kit PLUS provides a simple, reliable, and rapid method for isolating high-quality RNA from a wide variety of sources, without the need for toxic substances such as phenol or chloroform. The kit can be used with animal cells. 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, the lysate is passed through the DNA Removing Spin Column which traps the DNA on the column, while RNA can pass through the column and retain in the flow-through. Ethanol is added to the flow-through, creating conditions that promote selective binding of RNA to the silica membrane. Then the sample is applied to a Spin Column for RNA, where the RNA binds to the membrane. Contaminants are efficiently washed away by subsequent step of washing. The high-quality RNA is then eluted in Elution Buffer and may be used in a variety of downstream applications, including: RT-PCR, RT-qPCR, RNA-seq, Northern blotting and so on.

Components

Components	B0004-plus (100 Preps)
Lysis Buffer	55 ml
Wash Buffer*	13 ml
Elution Buffer	25 ml
DNA Removing Spin Columns (with Collection Tubes)	100 Preps
Spin Columns for RNA (with Collection Tubes)	100 Preps

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

Storage

Store all components at room temperature (When using these buffers, be careful to avoid of contamination).

Protocol

Sample Homogenization

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

a) Completely aspirate the cell-culture medium, then wash cells with appropriate volume of PBS.

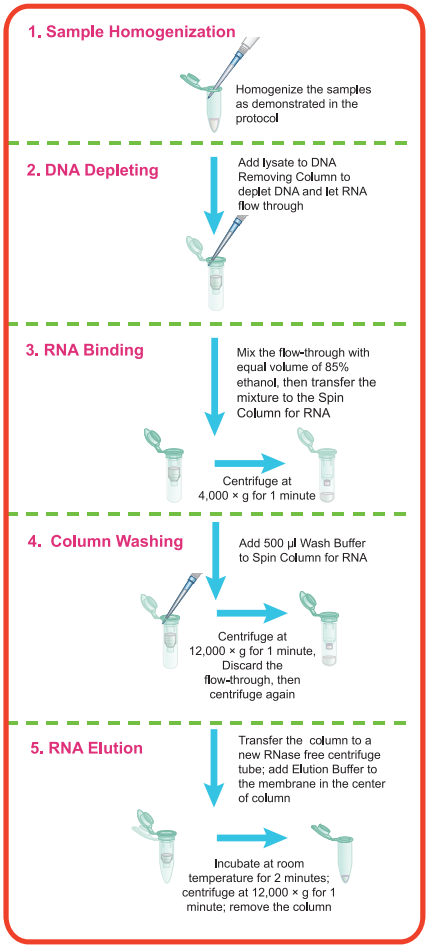
b) Add 500 µl Lysis Buffer to the cell-culture dish, place it on a shaker and shake for 1 ~ 2 minutes (120 rpm~150 rpm) at room temperature, or pipette up and down for 30 times to lyse the cells.

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

(For adherent cells only, for suspension cells, start at step b)

a) Detach cells using the sub-culturing method routinely employed in your laboratory. Pellet 1 × 10^6 cells in a 1.5 ml centrifuge tube by centrifugation at 500 × g for 3 ~ 5 minutes.

Experimental Procedure Overview:



b) Pellet 1 × 10^6 ~ 3 × 10^6 cells (For immune cells such as T and cells, it is recommended to take 2 × 10^6 ~ 1 × 10^7 cells) in a 1.5 ml centrifuge tube by centrifugation at 500 × g for 3 ~ 5 minutes. Completely remove the cell-culture medium by aspiration.

c) Wash cells with appropriate volume of PBS, then centrifuge at 500 × g for 3 ~ 5 minutes. Completely aspirate the PBS.

d) Add 500 µl of Lysis Buffer to the tube, pipette up and down for 15 times or vortex for 10 seconds at high speed to lyse the cells.

DNA Depleting

2. Transfer the homogenized lysate of step 1 to the DNA Removing Spin Column. Centrifuge at 4,000 × g for 1 minute. Discard the column, and save the flow-through.

RNA Binding

3. Add 1 volume of 85% ethanol to the flow-through, and mix well by pipetting. Transfer the mixture to the Spin Column for RNA. Centrifuge at 4,000 × g for 1 minute (**Note:** If liquid remains in the column after centrifugation, centrifuge the Spin Column for RNA once more at 12,000 × g for 1 minute). Discard the flow-through.

Column Washing

4. Add 500 µl Wash Buffer (add ethanol as indicated) to the Spin Column for RNA. Centrifuge at 12,000 × g for 1 minute to wash the membrane of the Spin Column for RNA. Discard the flow-through (**Note:** carefully remove the column from the collection tube so that the column does not contact the flow-through).
5. Optional: Centrifuge the Spin Column for RNA once more at 12,000 × g for 1 minute.
6. No need to discard the flow-through, directly discard the collection tube and transfer the column to an RNase free 1.5 ml centrifuge tube, then open the lid and keep in the air for 2 minutes.

RNA Elution

7. Add 30 ~ 50 µl of Elution Buffer to the membrane in the center of the column, and incubate at room temperature for 2 minutes (**Note:** If expecting higher yields of RNA, preheat the Elution Buffer to 95°C before elution).
8. Centrifuge at 12,000 × g for 1 minute to elute the RNA (**Optional:** If expecting higher yields of RNA, transfer the eluate back to the column, incubate at room temperature for 3 minutes and centrifuge at 12,000 × g for 1 minute. If preheated Elution Buffer is used for elution, this optional operation is not required).
9. Discard the column, the RNA obtained is quickly transferred to ice, and the concentration is determined after thoroughly mixing, then do the following experiment with the purified RNA, or store the RNA at -80°C until needed.

Representative Results

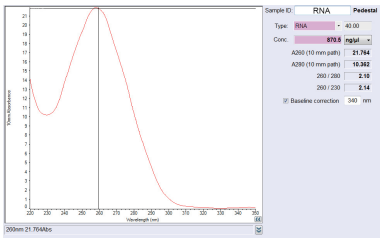


Figure 1. RNA isolated from A549 cells using ***EZ*Bioscience®** EZ-press RNA Purification Kit PLUS was detected with Nanodrop (eluted in 30 µl of Elution Buffer). The result showed that high quality RNA can be isolated by ***EZ*Bioscience®** EZ-press RNA Purification Kit PLUS.

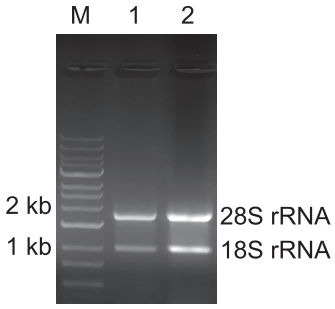


Figure 2. RNA isolated from A549 cells using ***EZ*Bioscience®** EZ-press RNA Purification Kit PLUS and TRIzol (eluted in 30 µl of Elution Buffer each, 5 µl RNA of each is loaded for electrophoresis). M: 250bp DNA Ladder; Lane 1: TRIzol; Lane 2: ***EZ*Bioscience®** EZ-press RNA Purification Kit PLUS. The result showed that high quality RNA can be isolated by ***EZ*Bioscience®** EZ-press RNA Purification Kit PLUS.

Trouble shooting

The yield of RNA purified by this Kit is too low, or OD260/280 or OD260/230 is less than 1.8, or the qPCR results is not so good, such as 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 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 of 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 but not on ice** (until the RNA is eluted after centrifugation), to avoid of blocking the membrane of the Spin Column for RNA 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 should be fully lysed by shaking on a shaker or pipetting up and down for 30 times.

4. Transfer cell lysate to the DNA Removing Spin Column and centrifuge. Then DNA is bound to the membrane of the column and RNA is retained in the flow-through.

5. The flow-through should be added with equal volume of 85% ethanol and mix thoroughly before applied to the Spin Column for RNA.

6. When doing the step of RNA elution, preheating the Elution Buffer to 95°C before elution, or **transferring the liquid back to the column after centrifugation and repeat the centrifugation, which will get more RNA.**