Exosome RNA Purification Kit

Catalog No.: EZB-exo-RN1

Description

The ***EZ*Bioscience**® Exosome RNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA (including miRNA, mRNA, lncRNA and circRNA, etc.) from exosomes, providing high sample processing capability. Exosomes can be derived from ultracentrifugation or isolated by exosome isolation kit, such as ***EZ*Bioscience**® Exosome Isolation Kit (from plasma/serum, Cat. No.: EZB-exo1) /Exosome Isolation Kit (from cell culture medium, Cat. No.: EZB-exo2). Exosome samples are first lysed in a strong denaturant and phenol containing buffer, which immediately inactivates RNases to ensure isolation of intact total RNA. After addition of Buffer A, 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 RNA is suitable for use in a variety of downstream applications, including: RT-PCR, RT-qPCR, Northern blotting, RNA sequencing, RNA Chip, and so on.

Components

Components	EZB-exo-RN1 (50 Preps)
Lysis Buffer	30 ml
Buffer A	12 ml
Supplemental Reagent	550 µl
Wash Buffer 1*	8 ml
Wash Buffer 2*	8 ml
Elution Buffer	25 ml
Spin Columns for RNA (with Collection Tubes)	50 Preps

*: Before using for the first time, add 32 ml of 100% ethanol to the Wash Buffer 1, 32 ml of 100% ethanol to the Wash Buffer 2.

Storage

Store the Lysis Buffer and Buffer A at 2 ~ 8°C, protect from light. Store the Supplemental Reagent at -20°C. Store other components at room temperature (When using these buffers, be careful to avoid of contamination). Divide the Elution Buffer into small aliquots upon reception is suggested.

Protocol

Sample Lysis

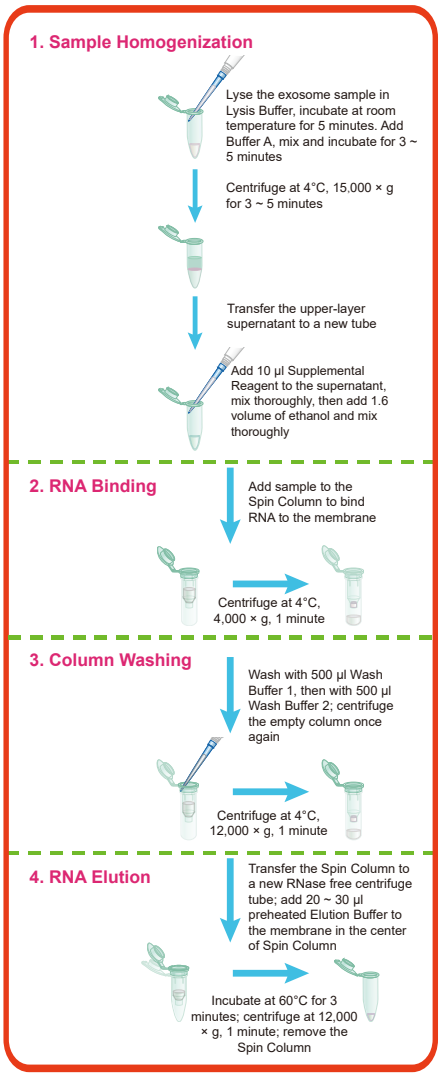
The suggested exosomes according to sample types:

1. Place 10 ~ 100 µl exosomes in a 1.5 ml centrifuge tube. Add 500 µl of Lysis Buffer. (For exosome purification, more than 0.5 ml of serum/plasma, or more than 10 ml of cell culture medium is recommended).
2. Lyse the exosomes by pipette up and down for 10 ~ 20 times. Incubate at room temperature for 5 minutes.
3. Add 150 µl Buffer A to the exosome lysate, shake it vigorously for 15 s to mix thoroughly. Incubate at room temperature for 3 ~ 5 minutes.
4. Centrifuge at 15,000 × g, 4°C for 3 ~ 5 minutes. Then the mixture separates into three phases. Transfer the upper-layer supernatant (about 300 µl) to a new RNase free 1.5 ml centrifuge tube (be careful to avoid disturbing the middle or bottom layer).

RNA Binding

5. Add 10 µl Supplemental Reagent to each volume of supernatant, mix thoroughly, then add 1.6 volume of 100% ethanol to each volume of the above solution (supernatant + Supplemental Reagent) for microRNA included total RNA purification.
6. Invert the centrifuge tube for several times or pipette up and down for 10 times to mix thoroughly, and transfer the sample to the Spin Column. Centrifuge at 4,000 × g, 4°C for 1 minute. Pour off the liquid.

Experimental Procedure Overview



Column Washing

7. 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 of contacting the bottom of the column with the liquid when taking out of the column). Pour off the liquid.
8. Add 500 µl of Wash Buffer 2 to the Spin Column. Centrifuge at 12,000 × g, 4°C for 1 minute.
9. Pour off the liquid and eliminate the residual liquid using towel paper. Place the Spin Column back on the collection tube and Centrifuge at 12,000 × g, 4°C for 1 minute.
10. 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

11. Preheat the Elution Buffer to 60°C, add 20 ~ 30 µl of Elution Buffer to the membrane in the center of the Spin Column, and then place the EP tube with the spin column in a water bath or metal bath at 60°C for 3 minutes to dissolve the RNA.
12. 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).
13. 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

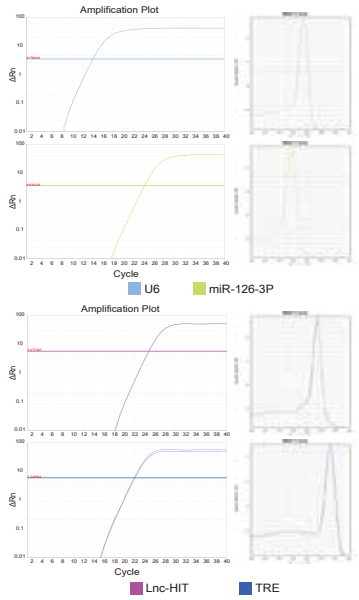


Figure 1. microRNA and lncRNA isolated from exosomes derived from the culture medium of 293T cells using were detected. These results showed that, this *EZ*Bioscience® Exosome RNA Purification Kit can be used for microRNA and lncRNA included total RNA purification and achieved good performance.

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 Elution Buffer into 3 ~ 4 parts, each part in a 1.5 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 4°C (unless specially required)**.
2. Before using this Kit for the first time, **add 32 ml 100% ethanol to each bottle of the Wash Buffer** and mix thoroughly.
3. For exosome RNA purification, the exosomes should be isolated from more than 0.5 ml of serum/plasma, or more than 10 ml of cell culture medium.
4. Transfer the upper-layer supernatant to a new tube after phase separation. **Avoid transferring any of the middle or bottom layer into the pipette when removing the upper-layer**.
5. **Add 10 µl Supplemental Reagent to the supernatant, mix thoroughly, then add 1.6 volume of ethanol** and mix thoroughly, then load onto the column.
6. After each column washing, the column should be taken out carefully to avoid of contacting the bottom of column with liquid.
7. The column must be dried in air for 2 minutes after the centrifugation of empty column.
8. When doing the step of RNA binding and RNA elution, **load the flow-through of centrifugation to the column and repeat the centrifugation once again will get more RNA.**