Universal microRNA Purification Kit

Catalog No.: EZB-miRN1

Description

The **EZBioscience® Universal microRNA Purification Kit** integrates phenol/guanidine-based sample lysis and silica-membrane purification of high-quality 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 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 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, RNA Sequencing, RNA chip and so on.

Components

Components	EZB-miRN1 (50 Preps)
Lysis Buffer	30 ml
Buffer A	22 ml
Wash Buffer 1*	8 ml
Wash Buffer 2*	8 ml
Elution Buffer	25 ml
Spin Columns (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 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 Homogenization

The suggested tissue weight according to tissue types are as following:

Tissue types	Liver	Tumor, embryos, heart, kidney, spleen, pancreas, lung, eye	Muscle, skin, vessel
Weight (mg)	5~ 10	5~ 50	20~ 50

1A. For animal tissues:

a1. Place 1 ~ 50 mg tissue in a 1.5 ml centrifuge tube. Add 500 µl of Lysis Buffer. Homogenize the tissue with a pestle or rotor-stator homogenizer.

a2. Incubate at room temperature for 5 minutes.

1B. For cells samples:

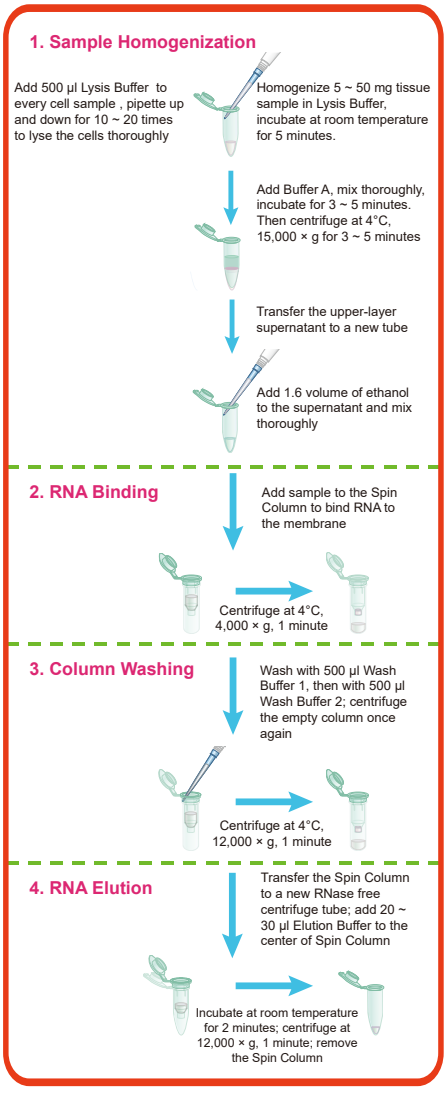
b1. For adherent cells ≤ 3×10^6/samples: remove the growth medium from the cells, wash cells with PBS, add 500 µl of Lysis Buffer, pipette up and down for 10 ~ 20 times to lyse cells, then transfer the cell lysate to a 1.5 EP tube and vortex for 10 seconds at high speed to completely lyse the cells.

b2. For cultured suspension cells: Pellet 3×10^6 cells in a 1.5 ml centrifuge tube by centrifugation at 500 × g for 3 ~ 5 minutes, remove the supernatant by aspiration, then add 500 µl of Lysis Buffer, pipette up and down for 10 ~ 20 times to lyse cells

2. Add 200 µl Buffer A to the tissue or cell lysate and shake it vigorously for 15 s. Incubate at room temperature for 3 ~ 5 minutes.

3. 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).

Experimental Procedure Overview



RNA Binding

4. Add 1.6 volume of 100% ethanol to each volume of the supernatant, mix thoroughly by pipetting. Then transfer the sample to the Spin Column. Centrifuge at 4,000 × g, 4°C for 1 minute. Pour off the liquid.

Column Washing

5. 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.
6. Add 500 µl of Wash Buffer 2 to the Spin Column. Centrifuge at 12,000 × g, 4°C for 1 minute.
7. Pour off the liquid and eliminate the residual liquid using towel paper. Place the empty column back on the collection tube and Centrifuge at 12,000 × g, 4°C for 1 minute.
8. 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

9. 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.
10. Centrifuge at 12,000 × g, 4°C for 1 minute (transfer the eluate back to the column, incubate for 5 minutes and centrifuge once more will get more microRNA).
11. 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

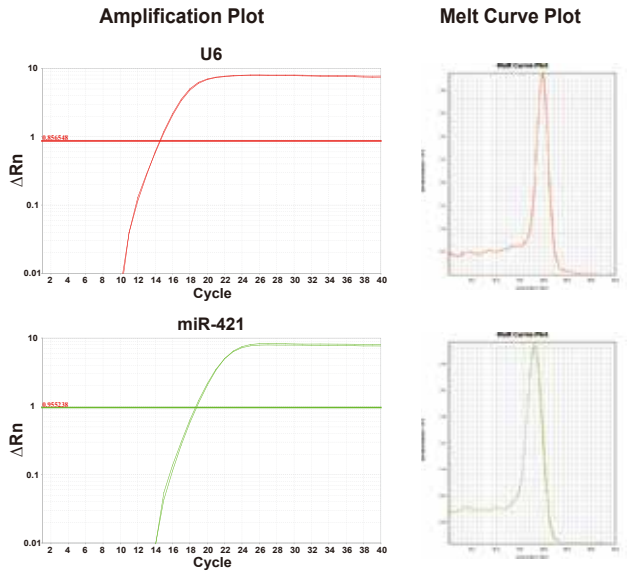


Figure 1. microRNA is isolated using *EZ*Bioscience® Universal microRNA Purification Kit. As shown in the following figure, the amplification curve, melt curve and Ct value of these microRNAs were very good, indicating that the expression of microRNA can be well detected using this kit.

Trouble shooting

The quantity of microRNA 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 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 microRNA purification by this Kit, **it must be manipulated at room temperature (unless specially required) but not on ice** (until the microRNA 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 32 ml 100% ethanol to each bottle of the Wash Buffer** and mix thoroughly.
3. The tissue pieces must be weighed before microRNA extraction. The weight of tissue should be less than 50 mg/sample. For tissues rich in microRNA (such as liver, spleen and thymus), the amount of tissue should be reduced to no more than 10 mg/sample.
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 1.6 volume of ethanol to the supernatant** 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 microRNA.**