

# Universal microRNA Purification Kit

## Catalog No.: EZB-miRN1

### Description

The **EZBioscience**® Universal microRNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality microRNA from animal tissues and cells, providing high sample processing capability to deal with difficult specimens. The purified microRNA is suitable for use in a variety of downstream applications, including: RT-qPCR, RNA sequencing, RNA chip and so on. Tissue and cells samples are first lysed and homogenized in a strong denaturant and phenol containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After homogenization, Buffer A is mixed with the tissue/cell lysate and incubated for 3 minutes. And then the mixture is centrifuged for phase separation; the upper-layer supernatant is collected and mixed with ethanol. The liquid is then passed through the RNA binding Spin Column containing a silica-based membrane to which the RNA binds. Impurities are effectively removed by subsequent washing. The purified microRNA is then eluted with Elution Buffer and may be used in a variety of downstream applications.

### Components

Components	EZB-miRN1 (50 Preps)
Lysis Buffer	30 ml
Buffer A	6 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, brain, eye	Muscle, skin, vessel	Adipose tissue
Weight (mg)	5~ 10	5~ 50	20~ 50	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.

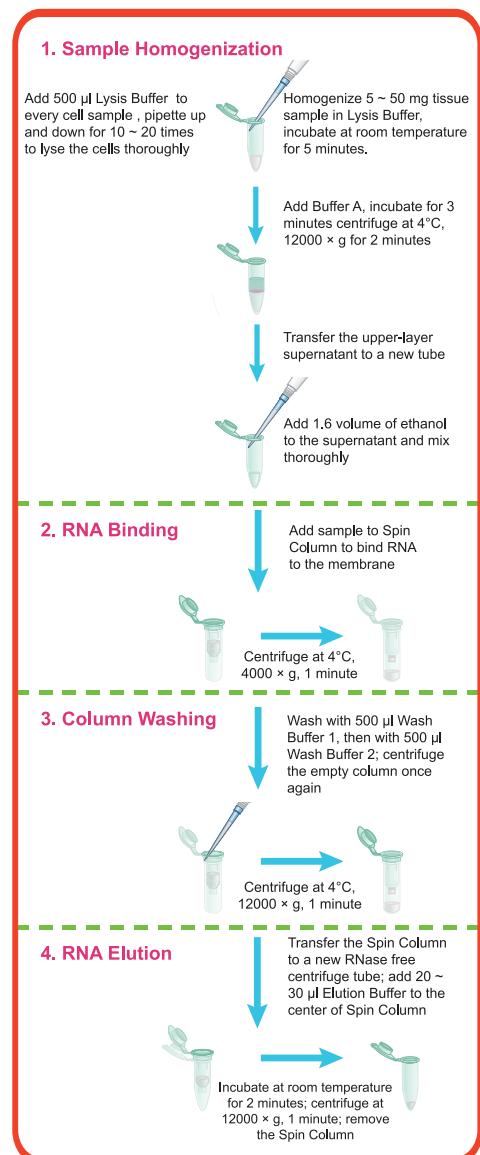
#### 1B. For cells samples:

- b1. For adherent cells  $\leq 3 \times 10^6$ /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  $3 \times 10^6$  cells in a 1.5 ml centrifuge tube by centrifugation at  $500 \times g$  for 3 minute, Completely 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 100 µl Buffer A to the tissue lysate, mix by pipette and hand-shaking. Incubate at room temperature for 3 minutes.
3. Centrifuge at  $12000 \times g$ , 4°C for 2 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).

### Experimental Procedure Overview



## RNA Binding

4. Add **1.6 volume of 100% ethanol** to each volume of supernatant. 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  $4000 \times g$ ,  $4^{\circ}\text{C}$  for 1 minute. Pour off the liquid.

## Column Washing

5. Add 500  $\mu\text{l}$  of Wash Buffer 1 to the column. Centrifuge at  $12000 \times g$ ,  $4^{\circ}\text{C}$  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  $\mu\text{l}$  of Wash Buffer 2 to the column. Centrifuge at  $12000 \times g$ ,  $4^{\circ}\text{C}$  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  $12000 \times g$ ,  $4^{\circ}\text{C}$  for 1 minute.
8. 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

9. Add 20 ~ 30  $\mu\text{l}$  of Elution Buffer **to the center of the column** and incubate at room temperature for 2 minutes.
10. Centrifuge at  $12000 \times g$ ,  $4^{\circ}\text{C}$  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 column, determine the microRNA concentration, do the following experiment with the purified microRNA, or store the microRNA at  $-80^{\circ}\text{C}$  until needed.

## Representative Results

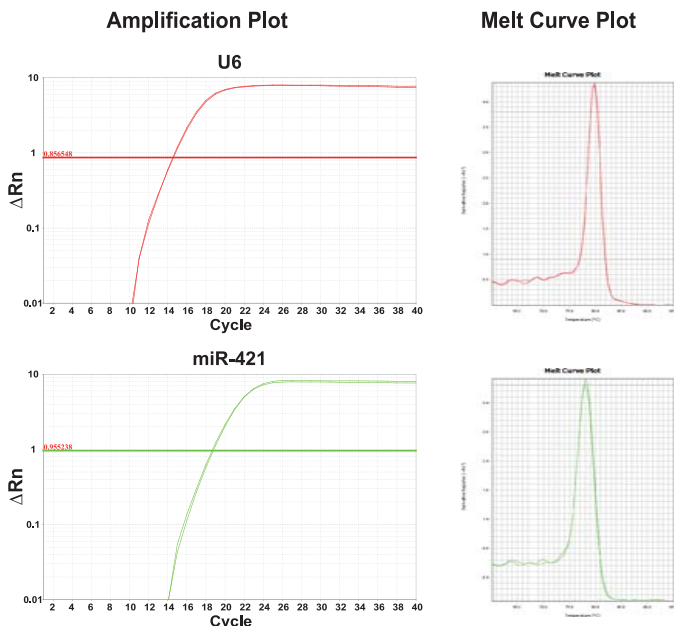


Figure 1. microRNA is isolated using **EZBioscience®** Universal microRNA Purification Kit. As shown in the following figure, the amplification curve, melt curve and Ct value of these microRNAs were very good, indicating that the expression of microRNAs can be well detected using this kit.

## 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  $-20^{\circ}\text{C}$ , 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.**