EZ-press microRNA Purification Kit PLUS

Catalog No.: B0005-plus

Description

The EZBioscience® EZ-press microRNA Purification Kit PLUS provides a simple, reliable, and rapid method for isolating high-quality miRNA (total RNA including miRNA, mRNA, lncRNA, CircRNA) from a wide variety of sources, without the need for toxic substances such as phenol or chloroform. This Kit can be used with cultured cells and part of animal tissues. The purified miRNA is suitable for use in a variety of downstream applications, including: RT-PCR, RT-qPCR, Northern blotting, nuclease protection assays, RNA sequencing and so on. Biological samples are first lysed and homogenized in a strong denaturant containing buffer, which immediately inactivates RNases to ensure isolation of intact miRNA. After homogenization, the lysate is passed through the DNA Removing Spin Column which traps the DNA on the column, while miRNA can pass through the column with the flow-through. Then ethanol is added to the miRNA containing flow-through. The mixture is then passed through the RNA binding Spin Column to which the miRNA binds. Impurities are effectively removed by subsequent DNase treatment and washing. The purified miRNA is then eluted in Elution Buffer and may be used in a variety of downstream applications.

Components

Components	B0005-plus (50 Preps)
Lysis Buffer	30 ml
Wash Buffer*	8 ml
Elution Buffer	25 ml
gDNA Remover	110 μl
DNA Removing Spin Columns (with Collection Tubes)	50 Preps
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.

Storage

Store the gDNA Remover at -20°C. Store other 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) Remove the growth medium from the cells.

b) Wash cells with appropriate volume of PBS.

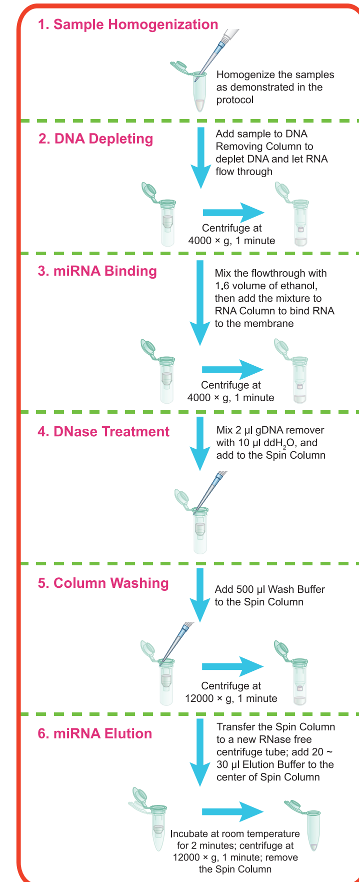
c) Add 500 μl of Lysis Buffer. Pipette up and down for 10 times to suspend the cells.

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:

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

Experimental Procedure Overview:



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.

b) Completely remove the supernatant by aspiration.

c) Add 500 μl of Lysis Buffer.

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

1C. For animal tissues:

(For most tissue miRNA purification, **EZB-RN4** Kit is suggested.)

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. If the tissue is *less than 5 mg*, go the **step c** directly. In case the tissue is *larger than 5 mg*, transfer a volume of the homogenate that contains 5 mg tissue to a new tube. Add Lysis Buffer to the homogenate to a total volume of 300 μl. Homogenize again.

c) Put the homogenized lysates at room temperature for 5 minutes. 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.

DNA Depleting

2. Transfer the homogenized lysate of step 1 to the DNA Removing Spin Column.

3. Centrifuge at 4000 × g for 1 minute. Transfer the miRNA containing flow-through to a new RNase free 1.5 ml centrifuge tube for the isolation of miRNA.

RNA Binding

4. Add 1.6 volume of ethanol to each volume of the flow-through (See step 3. There may be precipitates at this step. This is a normal phenomenon).
5. Pipette up and down for several times to disperse the precipitates, and transfer the sample to the RNA Spin Column. Centrifuge at 4000 × g for 1 minute.

DNase Treatment

6. 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.

RNA Column Washing

7. Add 500 µl of Wash Buffer to the RNA column. Centrifuge at 12000 × g for 1 minute (be careful to avoid of 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).
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 µl of Elution Buffer to the center of the RNA column and incubate at room temperature for 2 minutes.
10. Centrifuge at 12000 × g for 1 minute.
11. 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

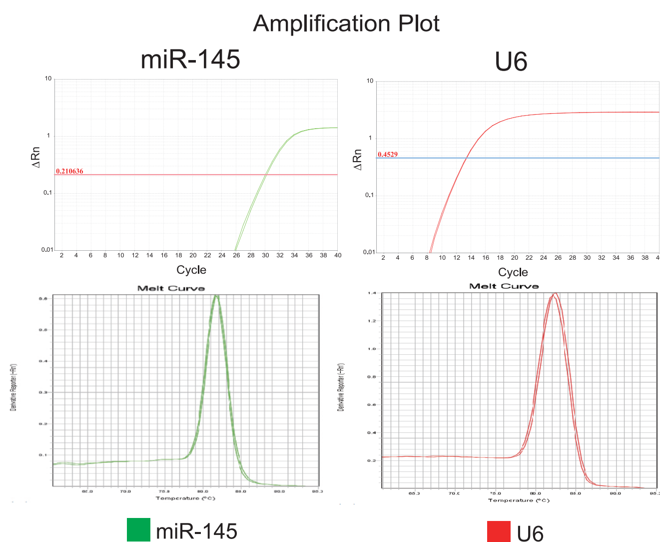


Figure 1. Detection of microRNA level in A549 cells by RT-qPCR (the microRNA was purified by *EZ*Bioscience® EZ-press microRNA Purification Kit PLUS). These results showed that both the miR-145 and U6 can be amplified efficiently and specially.

Trouble shooting

1) The quantity of miRNA 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 miRNA 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 miRNA 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 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 the Wash Buffer** and mix thoroughly.

3. To get better results, **wash the cells once with PBS** before cell lysing is recommended.

4. **The cells or tissues lysates should be mixed adequately by using vortex**. Then transfer the homogenized lysate of step 1 to the DNA Removing Spin Column and centrifuge.

5. Then **Transfer the RNA containing flow-through to a new RNase free 1.5 ml centrifuge tube** for the isolation of RNA. Add 1.6 volume of ethanol to each volume of the **flow-through** and mix thoroughly, and then add to the RNA column.

6. The volume of Elution Buffer can be adjusted between 20 µl to 30 µl, according to the miRNA quantity predicted.

2) The OD260/OD280 or OD260/OD230 value of total RNA purified by this Kit is too small (<1.8), or too large (>2.3).

a. **Examine whether the sample is lysed efficiently.** Put the homogenized lysates at room temperature for 5 minutes, then vortex for 10 seconds, and then centrifuge at 8000 × g for 1 minute to precipitate the water-insoluble (Optional). Then transfer the lysate of step 1 to the DNA Removing Spin Column and centrifuge.

b. Wash the RNA column for one more time using the wash buffer.