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miRNA Extraction Kit Instructions

Composition

miRNA Extraction Kit Cat. No.	5 Preps 5007005	50 Preps 5007050
Filter Columns	5	50
Spin Columns	5	50
Buffer TL	6 ml	55 ml
Buffer EX	1.2 ml	12 ml
Buffer WA (concentrate)	1.5 ml	10 ml
Buffer WBR (concentrate)	1 ml	6 ml
RNase-free Water	1.5 ml	2 ml×2
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Storage

Buffer TL should be stored at 2~8℃, other reagents and articles if stored at room temperature (0~30℃), can maintain no significant change in performance within 2 years; If the product is stored at 2~8℃, the validity period can be extended to more than 2 years.

Technical Support

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Introduction

This product is suitable for the extraction and purification of miRNA from a variety of samples from different sources. The kit uses column purification technology to obtain high-purity small RNA (<200 nt) with high efficiency, which is suitable for molecular biology experiments such as Northern Blot, Dot Blot, polyA screening, in vitro translation, RNase protection and molecular cloning.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tube (RNase-free 1.5 ml centrifuge tube must be used).
3. Pipette and tips (To avoid RNase contamination, please choose RNase-free tip with filter).
4. Syringes with 21–25-gauge needles.
5. Latex gloves, protective supplies such as disposable masks, and paper towels.
6. Microcentrifuge(s) (with rotors for 1.5 ml and 2 ml Collection Tubes).
7. Vortexer.
8. A lab that does not use RNase.

Pre-use preparation

1. If the centrifuge has a refrigeration function, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".
3. Because RNases are present in saliva and skin, latex gloves and a mask are required for the entire process of RNA extraction.

Protocol

1. Treatment for different sources samples:

Human or animal tissue:

Grind about 300-500 mg of tissue in a mortar with liquid nitrogen to a powder, weigh 50-100 mg of human or animal tissue in a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, and add 1 ml Buffer TL. Repeat 8-10 aspirations with a syringe containing a 21–25-gauge needle (taking care to keep the needle below the liquid level to reduce foam production) to proceed to step 2.

* Try to add Buffer TL before the tissue powder melts to reduce the RNA degradation due to RNases that are intrinsic to the tissue.
* If the tissue sample is small (≤100 mg) and not easily dispersed, grind the tissue into a powder by adding liquid nitrogen in the mortar, then directly add 1 ml Buffer TL to the mortar, and continue grinding for about 30 sec to lyse the tissue, then transfer the homogenate into a 1.5ml centrifuge tube. Repeat 8 -10 aspirations with a syringe containing a 21–25-gauge needle (taking care to keep the needle below the liquid level to reduce foam formation) to proceed to step 2.

Cultured animal cells:

Adherent cultured cells: Add 1 ml Buffer TL to every 10 cm^2 cultured cells (for example, a culture dish with a diameter of 3.5cm cells, directly add 1 ml Buffer TL after the medium is discarded), do not discard the tip, pipette the cells with the tip several times to lyse the cells, transfer the homogenate to a 1.5 ml centrifuge tube. Proceed to the operation in step 2.

Suspension cultured cells: Centrifuge 5~10×10^6 cells with 1.5 ml centrifuge tube, add 100 µl PBS solution, vortex until all cells are suspended, add 1 ml Buffer TL, do not discard the tip, directly pipette the cells with the tip several times to lyse the cells. Proceed to the operation in step 2.

Plant tissue/plant cell/yeast/bacteria:

Grind about 300~500 mg of sample to powder in the mortar with liquid nitrogen, then weigh about 100 mg powdered tissue to a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 1 ml Buffer TL, do not discard the tip, pipette the sample several times with the tip to lyse it. Proceed to the operation in step 2.

2. ***Optional steps:*** **If the sample contains a lot of protein, fat, polysaccharide or extracellular substances (muscle, plant nodule part, etc.) can be centrifuged at 12000×g for 5 min to take the supernatant. The precipitates obtained by centrifugation include the outer cell membrane, polysaccharide, high molecular weight DNA, and the supernatant contains RNA. When dealing with adipose tissue, a large amount of grease in the upper layer should be removed. The clarified solution after centrifugation should be taken to the next step.**
3. **Add 200 µl Buffer EX, close the lid, shake vigorously for 15 sec, and centrifuge 12000× g for 15 min.**
4. **Transfer 500 µl supernatant to a clean 1.5 ml centrifugal tube, add 215 µl absolute ethanol, do not discard the tip, directly pipette three times and mix well, transfer all the mixed liquid to a filter column, close the lid, and centrifuge at 12000 rpm for 30 sec.**
5. **Discard the filter column, add 780 µl absolute ethanol to the filtrate, do not discard the tip, directly pipette three times and mix well, transfer 730 µl mixture to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, centrifuge at 12000 rpm for 30 sec.**
6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer all the remaining liquid in Step 5 into the Spin Column, close the lid, and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded. To avoid contamination of the centrifuge by the filtrate adhering to the mouth of the centrifuge tube, the 2 ml Collection Tube can be inverted and slapped once on a paper towel.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to the Buffer WA.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to the Buffer WBR.

9. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the effect of the subsequent experiment may be affected because the purified nucleic acid is mixed with ethanol.

10. **Discard 2 ml Collection Tube, place the Spin Column in an RNase-free 1.5 ml centrifuge tube, add 30-50 µl RNase-free Water into the Spin Column, close the lid, incubate at room temperature for 1 min and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cover, change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the tube cover falling off.

11. **Discard the Spin Column, and the eluted RNA can be immediately used in various molecular biology experiments or stored below -70℃ for later use.**