ELK Biotechnology

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miRNA Purification Kit

Catalog No.	Specification	Storage/Shelf life
EP028	**50T**	Room temperature/1 year

Storage Conditions: Storage TRIzon Reagent 2-8℃ in the dark， other components at room temperature (15-30℃)Product Content

Component	50T
RIzon Reagent	60 mL
Buffer RWT（concentrate）	15 mL
Buffer RW2（concentrate）	11 mL
RNase-Free Water	10 mL
Spin Columns RM with Collection Tubes	50
Spin Columns RS with Collection Tubes	50
RNase-Free Centrifuge Tubes（1.5 mL）	50

Principle

The miRNA extraction kit is specially used to separate and purify miRNA from various animal tissues, plant tissues, cells, serum, plasma and other samples. It can also extractsiRNA, snRNA and other small molecular RNAsless than 200 ntas well as total RNA. This product combines phenol/guanidine lysis technology and silica matrix membrane purification technology. Its unique lysis solution can effectively inhibit RNases while removing most of the DNA and protein in cells or tissue samples by organic extraction. This product can be directly used for Northern Blot analysis, Real-Time PCR, Microarray Analysis, etc.

Reagents to Be Supplied by User

100% ethanol Chloroform (newly opened or for RNA extraction)

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Important Points Before Starting

1.To prevent RNase contamination, the following aspects should be noted:
1) Use RNase-free plastic products and pipette tips to avoid cross-contamination.
2) Glassware should be dry-baked at 180℃for 4 hours before use, plasticware can be soaked in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and then autoclaved.
3) RNase-free water should be used for the preparation of the solution.
4) Operators wear disposable masks and gloves, and gloves should be changed frequently during the experiment.
2. Avoid repeated freezing and thawing of extracted samples, otherwise the quantity and quality of miRNA extraction will be affected.
3. Add absolute ethanol to Buffer RWT and Buffer RW2 according to the bottle label instructions before first use.
4. All centrifugation steps are performed at room temperature, and all procedures should be performed quickly.

Procedure

Protocol A: miRNA enrichment

1. 1a. Tissue: Grind tissue in liquid nitrogen. Add 1 mL TRIzon Reagent per 30-50 mg of tissue, and shake to mix. The sample volume should not exceed one tenth of the volume of TRIzon Reagent.
1b. Monolayer culture of cells: Aspirate the culture medium, add TRIzon Reagent, and add 1 mL TRIzon Reagent per 10 cm^2 (the amount of lysate depends on the area of the culture flask).
1c. Cell suspension: centrifuge to get the cell pellet, discard the supernatant. Add 1 mL TRIzon Reagent per 5×10^6-1×10^7 cells (cells do not need to be washed).
1d. Plasma or serum: Take 200 µL plasma or serum sample, add 5 times the volume of TRIzon Reagent, shake and mix for 30 seconds.
2. After adding TRIzon Reagent to the sample, pipetting several times to make it fully lysed. Leave at room temperature for 5 minutes to completely separate the protein-nucleic acid complexes.
3. Optional step: Centrifuge at 12,000 rpm (~13,400×g) for 5 minutes at 4 ℃ , take the supernatant and transfer it to a new centrifuge tube (self-provided) (if the sample contains a lot of protein, fat, Page 2 of 5 polysaccharide, etc.,).
4. Chloroform was added to the supernatant, 200 µL chloroform was added for each 1 mL TRIzon Reagent used, the tube was capped, vigorously shaken for 15 seconds, and placed at room temperature for 5 minutes.

5. Centrifuge at 12,000 rpm at 4 ℃ for 15 minutes. The sample is divided into three layers: red organic phase, middle layer, and colorless aqueous phase. Transfer the upper colorless aqueous phase to a new centrifuge tube (provided by yourself).
6. Add 1/3 volume of anhydrous ethanol to the solution obtained in step 5, mix well, and transfer the obtained solution together with the precipitate into the Spin Columns RM that has been loaded into the collection tube. If the whole solution cannot be added to the adsorption column at one time, please transfer it in several times. Centrifuge at 12,000 rpm for 30 seconds, discard the adsorption column RM after centrifugation, and keep the effluent.
7. Add 2/3 volume of absolute ethanol to the solution obtained in step 6 and mix well.
8 . The solution obtained in the previous step and the precipitate were transferred to the adsorption column RS that had been loaded into the collection tube. If the whole solution cannot be added to the adsorption column at one time, please transfer it in several times. Centrifuge at 12,000 rpm for 30 seconds, discard the waste liquid in the collection tube, and put the adsorption column RS back into the collection tube.
9. Add 700 μL Buffer RWT to the adsorption column RS (check whether absolute ethanol is added before use), centrifuge at 12,000 rpm for 30 seconds, pour off the waste liquid in the collection tube, and put the adsorption column RS back into the collection tube.
10. Add 500 μL Buffer RW2 to the adsorption column RS (check whether absolute ethanol is added before use), centrifuge at 12,000 rpm for 30 seconds, pour off the waste liquid in the collection tube, and put the adsorption column RS back into the collection tube.
Repeat step 10.
11. Centrifuge at 12,000 rpm for 1 minute and discard the waste in the collection tube.
12. Leave the adsorption column RS at room temperature for a few minutes to dry thoroughly.
Note: The purpose of this step is to remove the residual ethanol in the adsorption column RS, and the residual ethanol will affect the subsequent enzymatic reactions (enzymatic digestion, PCR, etc.).
Put the adsorption column RS in a new RNase-free centrifuge tube, add 30-50 μL
13. RNase-Free Water to the middle of the adsorption column, leave it at room temperature for 1 minute, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, and get the RNA The solution was stored at -70℃ to prevent degradation.

Note:

1) The volume of RNase-Free Water should not be less than 30 μl, and the recovery rate will be affected if the volume is too small.

2) To increase the yield of RNA, repeat step 13 with 30-50 µL of new RNase-Free Water.
3) If you want to increase the RNA concentration, you can re-add the obtained solution to the adsorption column RS, and repeat step 13.

Protocol B: Extraction of total RNA (extracted total RNA includes miRNA and other small RNAs <200 nt)

Steps 1-5 are the same as protocol A.
6. Add 1.25 times the volume of absolute ethanol to the solution obtained in step 5, and mix well.
7. The solution obtained in the previous step and the precipitate were transferred to the adsorption column (Spin Columns RM) which had been loaded into the collection tube. If the whole solution cannot be added to the adsorption column RM at one time, please transfer it in several times. Centrifuge at 12,000 rpm for 30 seconds, discard the waste liquid in the collection tube, and put the adsorption column RM back into the collection tube.
8. Add 700 µL Buffer RWT to the adsorption column RM (check whether absolute ethanol is added before use), centrifuge at 12,000 rpm for 30 seconds, pour off the waste liquid in the collection tube, and put the adsorption column RM back into the collection tube.
9. Add 500 µL Buffer RW2 to the adsorption column RM (check whether absolute ethanol is added before use), centrifuge at 12,000 rpm for 30 seconds, pour off the waste liquid in the collection tube, and put the adsorption column RM back into the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm for 1 minute and discard the waste in the collection tube.The adsorption column RM was left at room temperature for several minutes to dry thoroughly. Note: The purpose of this step is to remove the residual ethanol in the adsorption column RM, and the residual ethanol will affect the subsequent enzymatic reactions (enzyme digestion, PCR, etc.). Page 4 of 5
12. Transfer the adsorption column RM to a new RNase-free centrifuge tube, add 30-50 µL RNase-Free Water to the middle of the adsorption column, place it at room temperature for 1 minute, and centrifuge it at 12,000 rpm for 1 minute at room temperature to collect the RNA solution. The solution was stored at -70 ℃ to prevent degradation.

Note:

1) The volume of RNase-Free Water should not be less than 30 µL, and the recovery rate will be affected if the volume is too small.

2) To increase the yield of RNA, repeat step 12 with 30-50 μL of new RNase-Free Water.
3) If you want to increase the RNA concentration, you can re-add the obtained solution to the adsorption column RM, and repeat step 12.