miRNA Purification Kit

miRNA extraction kit

Project number: M665531 (50 preps)
Storage conditions: TRIzon Reagent 2-8℃, keep away from light, other components at room temperature (15-30℃).

Product content

individual parts making up a compound	M665531 50 preps
TRIzon 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

Product Introduction

miRNA Extraction Kit is dedicated to the isolation and purification of miRNA from a variety of animal tissues, plant tissues, cells, serum, plasma and other samples, it can also be used to extract other small molecules of RNA less than 200 nt such as siRNA, snRNA, etc, and can also be used for the extraction of total RNA. This product combines phenol/guanidine lysis technology and silicon matrix membrane purification technology. The unique lysate can remove most of the DNA and proteins in cell or tissue samples by organic extraction while effectively inhibiting RNases. For some sensitive downstream experiments, if you need to enrich miRNA, this kit can be applied to enrich miRNA alone. It is suitable for a wide range of samples, and the prepared RNA is of high purity, which can be directly used in sensitive downstream applications, such as Northern Blot analysis, Real-Time PCR, Microarray

Analysis, etc.

Self-contained reagents: chloroform, anhydrous ethanol (freshly opened or for RNA extraction).

Pre-experiment Preparation and Important Notes

1. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.
(2) Glassware should be dry baked at a high temperature of 180 ℃ for 4 hours before use, plasticware can be immersed in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
2. Avoid repeated freezing and thawing of the extracted samples, otherwise the quantity and quality of miRNA extraction will be affected.
3. Anhydrous ethanol should be added to Buffer RWT and Buffer RW2 according to the instructions on the label of the reagent bottle before first use.
4. All centrifugation steps are carried out at room temperature unless otherwise indicated and all steps are performed quickly.

Operation process

Protocol A: miRNA enrichment (can be used directly in sensitive downstream experiments)
1. Sample handling
1a. Tissue: Grind tissue in liquid nitrogen. Add 1 ml of TRIzon Reagent for every 30-50 mg of tissue and mix with shaking. The sample volume should not exceed one tenth of the TRIzon Reagent volume.
1b. Cells in monolayer culture: Aspirate off the culture medium and add TRIzon Reagent, 1 ml of TRIzon Reagent per 10 cm2 (the amount of lysate depends on the area of the culture flask).
1c. Cell suspension: Centrifuge to obtain cell precipitate, discard supernatant. Add 1 ml TRIzon Reagent per 5×106 -1×107 cells (cells do not need to be washed).
1d. Plasma or serum: Take a 200 μl sample of plasma or serum, add 5 times the volume of TRIzon Reagent, and mix with shaking for 30 seconds.
2. Add TRIzon Reagent to the sample and blow the sample several times to fully cleave it. Allow to stand at room temperature for 5 minutes to allow complete separation of the protein-nucleic acid complex.

3. Optional: centrifuge at 12,000 rpm (~13,400 x g) at 4° C for 5 minutes, remove the supernatant and transfer to a new centrifuge tube (supplied) (optional if the sample contains more proteins, fats, polysaccharides, etc.).
4. Add chloroform to the supernatant, 200 μl of chloroform for every 1 ml of TRIzon Reagent, cap the tube, shake vigorously for 15 seconds, and allow to stand at room temperature for 5 minutes.
5. Centrifuge the sample at 12,000 rpm for 15 minutes at 4° C. The sample is divided into three layers: red organic phase, middle layer, and colorless aqueous phase, and the upper layer of the colorless aqueous phase is transferred to a new centrifuge tube (supplied).
6. Add 1/3 times the volume of anhydrous ethanol to the solution obtained in step 5, mix well, and transfer the resulting solution and precipitate to Spin Columns RM in a collection tube. If it is not possible to add all of the solution to the column at one time, make several transfers. centrifuge at 12,000 rpm for 30 seconds, then discard the Spin Columns RM and retain the effluent.
7. Add 2/3 times the volume of anhydrous ethanol to the solution obtained in step 6 and mix well.
8. Transfer the solution obtained in the previous step, together with the precipitate, to the Spin Columns RS in the collection tube. If it is not possible to add all of the solution to the column at one time, make several transfers. centrifuge the column at 12,000 rpm for 30 seconds, pour out the waste liquid from the collection tube, and put the column RS back into the collection tube.
9. Add 700 μl of Buffer RWT to the adsorbent column RS (check that anhydrous 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 adsorbent column RS back into the collection tube.
10. Add 500 μl of Buffer RW2 to the adsorbent column RS (check that anhydrous 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 adsorbent column RS back into the collection tube.
11. Repeat step 10.
12. Centrifuge at 12,000 rpm for 1 minute and pour off the waste liquid from the collection tube. Leave the column RS at room temperature for a few minutes to dry thoroughly. Note: The purpose of this step is to remove any residual ethanol from the sorbent column RS, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
13. Place the adsorption column RS in a new RNase-free centrifuge tube, add 30-50 μl of RNase-Free Water to the middle of the adsorption column, let it stand at room temperature for 1 minute, centrifuge it at 12,000 rpm for 1 minute, and then collect the RNA solution, and store the resulting RNA solution at -70° C to prevent degradation.

Attention:

(1) The volume of RNase-Free Water should not be less than 30 μl, too small a volume affects the recovery.

2) If you want to increase the RNA yield, repeat step 13 with 30-50 μl of fresh RNase-Free Water.
3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column RS and step 13 repeated
Protocol B: Extraction of total RNA (total RNA extracted includes miRNA and other small molecule RNAs <200 nt) 1~5 steps are the same as protocol A.
6. Add 1.25 times the volume of anhydrous ethanol to the solution obtained in step 5 and mix well.
7. Transfer the solution obtained in the previous step, together with the precipitate, to the Spin Columns RM in the collection tube. If it is not possible to add all of the solution to the Spin Columns RM at one time, make several transfers. centrifuge at 12,000 rpm for 30 seconds, pour off the waste liquid from the collection tube, and place the Spin Columns RM back into the collection tube.
8. Add 700 μl Buffer RWT to the adsorbent column RM (check that anhydrous 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 adsorbent column RM back into the collection tube.
9. Add 500 μl of Buffer RW2 to the adsorbent column RM (check that anhydrous 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 adsorbent column RM back into the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm for 1 minute and pour off the waste liquid from the collection tube. Allow the adsorbent column RM to dry thoroughly at room temperature for several minutes.
Note: The purpose of this step is to remove residual ethanol from the adsorption column RM, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
12. Transfer the adsorption column RM into a new RNase-free centrifuge tube, add 30-50 μl of RNase-Free Water to the middle of the adsorption column, let it stand at room temperature for 1 minute, and centrifuge it at 12,000 rpm for 1 minute at room temperature to collect the RNA solution, and store the obtained RNA solution at -70° C to prevent degradation.
Attention:
(1) The volume of RNase-Free Water should not be less than 30 μl, too small a volume affects the recovery.
2) If you want to increase the RNA yield, repeat step 12 with 30-50 μl of fresh RNase-Free Water.
3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column RM and step 12 repeated.