Ver. 250301

ODP501

miRNA Isolation Kit

For purification of total RNA, including miRNA, from cells, tissues and animal blood

Origin

ISO 13485:2016 ISO 9001:2015

miRNA Isolation Kit

Kit Contents

Contents	50 Preps
Buffer RW	15mL
Buffer MRD	12mL
RNase-Free ddH_2O	15mL
Buffer MZ	60mL
RNase-Free Columns miRspin set	50 nos
RNase-Free Columns miRelute set	50 nos

Storage

Buffer MZ should be stored at 2-8°C protected from light for 15 months. Other solutions and columns should be stored dry at room temperature (15 -30°C) for 15 months.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Introduction

The kit is a new generation product specially developed for miRNA extraction. It can extract small RNA such as small interfering RNA (siRNA), small nuclear RNA (snRNA) and it also can extract total RNA. The lysis buffer in the kit has been researched and improved for a long time, and it shows super lysis ability and isolation sensitivity that general buffer does not have. The kit utilizes a special silica matrix membrane to enhance adsorption capacity of RNA especially for small RNA (<200 nt) with high purity and quality. This kit could be applied for RNA isolation from various samples (cell, animal tissue, plant tissue, serum, plasma). Each single spin column could handle 30-50mg animal tissue (for RNA enriched tissue like liver, should be less than 30mg), 100mg plant tissue or 1×10^7 cells. RNA of no contamination of DNA and protein could be obtained within 1 hour, and can be used in Northern Blot, Dot Blot, Poly A screening, in vitro translation, RNase protection analysis and molecular cloning,etc.

Important Notes

To avoid RNase contamination, please note that:

1. Change gloves regularly. Bacteria on the skin can result in RNase contamination.
2. Use RNase-Free plastic and tips to avoid cross contamination.
3. RNA can be protected in Buffer MZ. But RNA must be stored or applied in RNase-Free plastic or glassware. To wipe off RNase, the glassware can be heated at 150°C for 4 hours, and plastic can be dipped in 0.5M NaOH for 10 minutes, washed by RNase-Free ddH_2O thoroughly, and sterilized.
4. Use RNase-Free ddH_2O to prepare solution. (Add DEPC to 0.1% final concentration in ddH_2O. Shake solution to mix, and leave overnight at room temperature, autoclave for 15 minutes).

Protocol

Be sure to add ethanol to Buffer RW and Buffer MRD as indicated on the bottle and shake thoroughly.

Protocol I: Purification of miRNA enriched fractions from tissue and cell.

Apply this protocol when there is a high demand for miRNA purity, e.g. miRNA chip and miRNA clone research.

1. Preparation of samples

a. Tissue: Grind tissue in liquid nitrogen. Add 1ml Buffer MZ for per 30-50mg animal tissue (or 100mg plant tissue), homogenize using a tissue homogenizer. Sample volume should not be over 1/10 of Buffer MZ.

b. Monolayer cell: Add 1mL Buffer MZ per unit (10 cm^2). Pipette to mix and ensure that no cell clumps are visible.

Note: Addition volume of Buffer MZ depends on monolayer area, not cell number. If Buffer MZ is not enough, RNA obtained will be contaminated with DNA.

c. Cell suspension: Centrifuge for 5 minutes at 2,100 rpm (400 × g), carefully remove all supernatant and disrupt the cells by adding 1mL Buffer MZ, vortex or pipette to mix. Don't wash cells before adding Buffer MZ, otherwise mRNA will be degraded.

2. Place the tube containing the homogenate at room temperature (15- 30°C) for 5 minutes, to separate nucleic acids and protein.

3. Optional: Centrifuge the lysate at 12,000 rpm (~13,400 × g) for 5 minutes at 4°C to remove any particulate material. Then transfer supernatant to a new tube.

Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. The precipitation after centrifugation contains cell outer membrane, polysaccharide, high molecular mass DNA, while RNA is in the upper supernatant.

4. Add 200μL chloroform to the supernatant and cap it securely. Shake the tube vigorously for 15 seconds, place the tube at room temperature (15-30°C) for 5 minutes.
5. Centrifuge for 15 minutes at 12,000 rpm (~13,400 × g) at 4°C. After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower organic phase. The volume of aqueous phase is around 50% of Buffer MZ added. Transfer the aqueous phase to a new tube.
6. Add 0.43 volume of ethanol (96-100%) (e.g. add 215μL ethanol (96- 100%) to 500μL transferred liquid) and mix thoroughly by pipetting up and down several times (A precipitate may form after addition of ethanol, but this will not affect the procedure). Transfer the obtained liquid, include any precipitate that may have formed into Column miRspin, centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds at room temperature. If the volume exceeds 700μL, centrifuge successive aliquots in the same column. Discard the Column miRspin after centrifugation, and keep the flow-through.
7. Add 0.75 volume of ethanol (96-100%) (e.g. add 525μL ethanol (96-100%) to 700μL flow-through) and mix thoroughly by pipetting up and down several times (A precipitate may form after addition of ethanol, but this will not affect the procedure). Transfer the obtained liquid, include any precipitate that may have formed into a Column miRelute, centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds at room temperature . If the volume exceeds 700μL, centrifuge successive aliquots in the same column. Discard the flow-through, and keep the Column miRelute.
8. Add 500μL Buffer MRD **(ensure that ethanol (96-100%) has been added)** to the Column miRelute. Close the lid gently and incubate 2 minutes at room temperature. Then centrifuge for 30 seconds at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
9. Add 500μL Buffer RW **(ensure that ethanol (96-100%) has been added)** to the Column miRelute. Close the lid gently and incubate 2 minutes at room temperature. Then centrifuge for 30 seconds at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
10. Repeat step 9.
11. Place the Column miRelute into a new 2 mL collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, and discard the flow-through.
    Note: Perform this step to eliminate any possible carryover of ethanol. After centrifugation, place the Column miRelute at clean bench for a while, to completely dry the column membrane. Residual ethanol will have negative influence on following RT-PCR experiment.
12. Transfer the Column miRelute into a new 1.5mL RNase-Free Centrifuge Tube, add 15-30μL RNase-Free ddH_2O directly onto the membrane and incubate at room temperature for 2 minutes. Close the lid gently and centrifuge for 2 minutes at 12,000 rpm (~13,400 × g) to elute the RNA.

Note: The volume of elution buffer should not be less than 15μL. Small elution volume may have a negative effect on RNA yield. Store RNA solution at -70°C. To obtain a higher total RNA yield, please repeat step 12.

Protocol II: Purification of total RNA from tissue and cells

Apply this protocol when there is no high demand for miRNA purity, e.g. miRNA RT-PCR, and miRNA Northern blot.

1. Preparation of samples (refer to step 1 of Protocol I: Purification of miRNA enriched fractions from tissue and cell).
2. Refer to step 2 of Protocol I: Purification of miRNA enriched fractions from tissue and cell.
3. Refer to step 3 of Protocol I: Purification of miRNA enriched fractions from tissue and cell.
4. Refer to step 4 of Protocol I: Purification of miRNA enriched fractions from tissue and cell.
5. Refer to step 5 of Protocol I: Purification of miRNA enriched fractions from tissue and cell.
6. Add 1.5 volume of Ethanol (96-100%) (e.g. add 750μL Ethanol (96-100%) to 500μL transferred liquid) and mix thoroughly by pipetting up and down several times (A precipitate may form after addition of ethanol, but this will not affect the procedure). Transfer the obtained liquid, include any precipitate that may have formed into a Column miRspin, centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds at room temperature (15-30°C). If the volume exceeds 700μL, centrifuge successive aliquots in the same column. Discard the flow-through, and keep the Column miRspin after centrifugation.
7. Add 500μL Buffer MRD (ensure that ethanol (96-100%) has been added) to the Column miRspin. Close the lid gently and incubate at room temperature for 2 minutes. Then centrifuge for 30 seconds at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
8. Add 500μL Buffer RW (ensure that ethanol (96-100%) has been added) to the Column miRspin. Close the lid gently and incubate at room temperature for 2 minutes. Then centrifuge for 30 seconds at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
9. Repeat step 8.
10. Place the Column miRspin into a new 2mL collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, and discard the flow-through.

Note: Perform this step to eliminate any possible carryover of ethanol. After centrifugation, place the Column miRspin at clean bench for a while, to completely dry the column membrane. Residual ethanol will have negative influence on following RT-PCR experiment.

11. Transfer the Column miRspin to a new 1.5 ml RNase-Free Centrifuge Tube, add 30-100 μl RNase-Free ddH_2O directly onto the membrane and incubate 2 min at room temperature. Close the lid gently and centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute the RNA.

Note: The volume of elution buffer should not be less than 30μL. Small elution volume may have a negative effect on RNA yield. Store RNA solution at -70°C. To obtain a higher total RNA yield, please repeat step 11.

Protocol III: Purification of miRNA enriched fractions from whole blood, serum or plasma.

1. Preparation of samples
   Add equal volume of Buffer MZ to 200μL whole blood, serum or plasma, vortex 30 seconds to mix thoroughly.
2. Place the tube containing the homogenate at room temperature (15- 30°C) for 5 minutes, to separate nucleic acids and protein.
3. Centrifuge the lysate at 12,000 rpm (~13,400 × g) for 10 minutes at room temperature (15-30°C) to remove any particulate material. Then transfer supernatant to a new RNase-Free centrifuge tube.
4. Add 200μL chloroform to the supernatant and cap it securely. Shake the tube vigorously for 15 seconds, incubate the tube containing the homogenate at room temperature for 5 minutes.
5. Centrifuge for 15 minutes at 12,000 rpm (~13,400 × g) at 4°C. After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower yellow organic phase. Transfer the aqueous phase to a new tube.
6. Add 1/3 volume of Ethanol (96-100%) (e.g. add 100μL ethanol (96- 100%) to 300μL transferred liquid) and mix thoroughly by pipetting up and down several times (A precipitate may form after addition of ethanol, but this will not affect the procedure). Transfer the obtained liquid, include any precipitate that may have formed into a Column miRspin, incubate at room temperature for 2 minutes, then centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds. Discard the Column miRspin after centrifugation, and keep the flow-through.
7. Add 2/3 volume of ethanol (96-100%) (e.g. add 200μL ethanol (96-100%) to 300μL flow-through) and mix thoroughly (A precipitate may form after addition of ethanol, but this will not affect the procedure). Transfer the obtained liquid, include any precipitate that may have formed into a Column miRelute, incubate at room temperature for 2 minutes, centrifuge at 12,000 rpm (~13,400 × g) for 30 seconds. Discard the flow-through, and keep the Column miRelute.
8. Add 500μL Buffer MRD (ensure that ethanol (96-100%) has been added) to the Column miRelute. Close the lid gently and incubate for 2 minutes at room temperature. Then centrifuge for 30 seconds at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
9. Add 600μL Buffer RW (ensure that ethanol (96-100%) has been added) to the Spin Column miRelute. Close the lid gently and incubate for 2 minutes at room temperature. Then centrifuge for 30 seconds at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
10. Repeat step 9.
11. Place the Column miRelute into a 2mL collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 1 minute, and discard the flow-through.

Note: Perform this step to eliminate any possible carryover of ethanol. After centrifugation place the Column miRelute at clean bench for a while, to completely dry the column membrane. Residual ethanol will have negative influence on following RT experiment.

12. Transfer the Column miRelute to a new 1.5mL RNase-Free Centrifuge Tube, add 15-30μL RNase-Free ddH_2O directly onto the membrane and incubate 2 minutes at room temperature. Close the lid gently and centrifuge for 2 minutes at 12,000 rpm (~13,400 × g) to elute the RNA.

Note: The volume of elution buffer should not be less than 15μL. Small elution volume may have a negative effect on RNA yield. Please store RNA solution at -70°C. To obtain a higher total RNA yield, please repeat step 12; or increase sample volume and Buffer MZ and chloroform volume in proportion.