

miRcute miRNA Isolation Kit

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

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miRcute miRNA Isolation Kit

(Spin Column)

Cat. no. GDP501

Kit Contents

Contents		GDP501 (50 preps)
GDP501H	Buffer RW	12 ml
	Buffer MRD	12 ml
	RNase-Free ddH ₂ O	15 ml
	RNase-Free Columns miRspin set	50
	RNase-Free Columns miRelute set	50
	RNase-Free Centrifuge Tubes 1.5 ml	50
	Handbook	1
GRK149	Buffer MZ	60 ml

Note: GDP501H, GRK149 are shipped and packaged separately

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.

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 30-50 mg animal tissue (for RNA enriched tissue like liver, should be less than 30 mg), 100 mg 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 0.5 M NaOH for 10 min, washed by RNase-Free ddH₂O thoroughly, and sterilized.
4. Use RNase-Free ddH₂O to prepare solution. (Add DEPC to 0.1% final concentration in ddH₂O. Shake solution to mix, and leave overnight at room temperature, autoclave for 15 min).

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 30-50 mg animal tissue (or 100 mg plant tissue), homogenize using a tissue homogenizer. Sample volume should not be over 1/10 of Buffer MZ.
- b. Monolayer cell: Add 1 ml Buffer MZ per unit (10 cm²). 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 min at 2,100 rpm (400 × g), carefully remove all supernatant and disrupt the cells by adding 1 ml Buffer MZ, vortex or pipett 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 min, to separate nucleic acids and protein.
3. **Optional:** Centrifuge the lysate at 12,000 rpm (~13,400 × g) for 5 min 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 200 µl chloroform to the supernatant and cap it securely. Shake the tube vigorously for 15 sec, place the tube at room temperature (15-30°C) for 5 min.
5. Centrifuge for 15 min 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 215 µl Ethanol (96-100%) to 500 µl 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 sec at room

temperature. If the volume exceeds 700 μ l, 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 525 μ l Ethanol (96-100%) to 700 μ l 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 (\sim 13,400 \times g) for 30 sec at room temperature . If the volume exceeds 700 μ l, centrifuge successive aliquots in the same column. Discard the flow-through, and keep the Column miRelute.
8. Add 500 μ l Buffer MRD (**ensure that ethanol (96-100%) has been added**) to the Column miRelute . Close the lid gently and incubate 2 min at room temperature. Then centrifuge for 30 sec at 12,000 rpm (\sim 13,400 \times g) to wash the column. Discard the flow-through.
9. Add 500 μ l Buffer RW (**ensure that ethanol (96-100%) has been added**) to the Column miRelute . Close the lid gently and incubate 2 min at room temperature. Then centrifuge for 30 sec at 12,000 rpm (\sim 13,400 \times 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 (\sim 13,400 \times g) for 1 min, 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 1.5 ml RNase-Free Centrifuge Tube, add 15-30 μ l RNase-Free ddH₂O directly onto the membrane and incubate at room temperature for 2 min. Close the lid gently and centrifuge for 2 min at 12,000 rpm (\sim 13,400 \times g) to elute the RNA.

Note: The volume of elution buffer should not be less than 15 μ l. 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 750 μ l Ethanol (96-100%) to 500 μ l 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 ($\sim 13,400 \times g$) for 30 sec at room temperature (15-30°C). If the volume exceeds 700 μ l, centrifuge successive aliquots in the same column. Discard the flow-through, and keep the Column miRspin after centrifugation.
7. Add 500 μ l 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 min. Then centrifuge for 30 sec at 12,000 rpm ($\sim 13,400 \times g$) to wash the column. Discard the flow-through.
8. Add 500 μ l 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 min. Then centrifuge for 30 sec at 12,000 rpm ($\sim 13,400 \times g$) to wash the column. Discard the flow-through.
9. Repeat step 8.
10. Place the Column miRspin into a new 2 ml collection tube, centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 1 min, 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 μ l RNase-Free ddH₂O directly onto the membrane and incubate 2 min at room temperature. Close the lid gently and centrifuge for 2 min at 12,000 rpm (\sim 13,400 \times g) to elute the RNA.

Note: The volume of elution buffer should not be less than 30 μ l. 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 200 μ l whole blood, serum or plasma, vortex 30 sec to mix thoroughly.

2. Place the tube containing the homogenate at room temperature (15-30°C) for 5 min, to separate nucleic acids and protein.
3. Centrifuge the lysate at 12,000 rpm (\sim 13,400 \times g) for 10 min at room temperature (15-30°C) to remove any particulate material. Then transfer supernatant to a new RNase-Free centrifuge tube.
4. Add 200 μ l chloroform to the supernatant and cap it securely. Shake the tube vigorously for 15 sec, incubate the tube containing the homogenate at room temperature for 5 min.
5. Centrifuge for 15 min at 12,000 rpm (\sim 13,400 \times 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 100 μ l ethanol (96-100%) to 300 μ l 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 min, then centrifuge at 12,000 rpm (\sim 13,400 \times g) for 30 sec. Discard the Column miRspin after centrifugation, and keep the flow-through.
7. Add 2/3 volume of ethanol (96-100%) (e.g. add 200 μ l ethanol (96-100%) to 300 μ l 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 min, centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 30 sec. Discard the flow-through, and keep the Column miRelute.

8. Add 500 μ l Buffer MRD (**ensure that ethanol (96-100%) has been added**) to the Column miRelute . Close the lid gently and incubate for 2 min at room temperature. Then centrifuge for 30 sec at 12,000 rpm ($\sim 13,400 \times g$) to wash the column. Discard the flow-through.
9. Add 600 μ l Buffer RW (**ensure that ethanol (96-100%) has been added**) to the Spin Column miRelute . Close the lid gently and incubate for 2 min at room temperature. Then centrifuge for 30 sec at 12,000 rpm ($\sim 13,400 \times g$) to wash the column. Discard the flow-through.
10. Repeat step 9.
11. Place the Column miRelute into a 2 ml collection tube, centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 1 min, 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 1.5 ml RNase-Free Centrifuge Tube, add 15-30 μ l RNase-Free ddH₂O directly onto the membrane and incubate 2 min at room temperature. Close the lid gently and centrifuge for 2 min at 12,000 rpm ($\sim 13,400 \times g$) to elute the RNA.

Note: The volume of elution buffer should not be less than 15 μ l. 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.