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. DP501

Kit Contents

Contents	DP501 50 preps
Buffer MZ	60 ml
Buffer RW	15 ml
Buffer MRD	12 ml
RNase-Free ddH_2O	15 ml
RNase-Free Spin Columns miRspin	50
RNase-Free Spin Columns miRelute	50
RNase-Free Centrifuge Tubes 1.5 ml	50
RNase-Free Collection Tubes 2 ml	2×50
Handbook	1

Storage

Buffer MZ should be stored at 2-8°C protected from light. The miRcute miRNA Isolation Kit should be stored dry at room temperature (15-25°C).

Introduction

The miRcute miRNA Isolation Kit is designed for isolation and purification of miRNA, small interfering RNA (siRNA), small nuclear RNA (snRNA), and total RNA. Lysis buffer in the kit is optimized to show super lysis ability and isolation sensitivity. The kit utilizes a silica-based system to enrich small RNA from various sample sources, especially for small RNA of <200 nt. 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 used in Northern Blot, Dot Blot, Poly A screening, *in vitro* translation, RNase protection analysis and also molecular cloning.

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 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 min).

Protocol

Ensure ethanol (96-100%) has been added to Buffer RW and Buffer MRD with appropriate volume as indicated on the bottle and shake thoroughly.

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

Apply this protocol when there is high demand of 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 every 30-50 mg animal tissue (or 100 mg plant tissue), homogenize minced 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 cm^2). Pipet 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 by aspiration and disrupt the cells by adding 1 ml Buffer MZ, vortex or pipet to mix. Don't wash cells before adding Buffer MZ, otherwise mRNA will be degraded.

2. Place the tube containing the homogenate on the benchtop at room temperature (15-25°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, RNA is in the upper supernatant.

4. Add 200 µl chloroform to the supernatant and cap it securely.

Shake the tube vigorously for 15 s, put the tube containing the homogenate on the bench top at room temperature (15-25°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 Spin Column miRspin, centrifuge at 12,000 rpm (~13,400 × g) for 30 s at room temperature (15-25°C). If the volume exceeds 700 µl, centrifuge successive aliquots in the same column. Discard the Spin Column miRspin after centrifugation, and keep the flow-through.
7. Add 0.75 volume of Ethanol (96-100%) (e.g. add 525 µl Ethanol (96-100%) to 700 µl 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 Spin Column miRelute, centrifuge at 12,000 rpm (~13,400 × g) for 30 s at room temperature (15-25°C). If the volume exceeds 700 µl, centrifuge successive aliquots in the same column. Discard the flow-through, and keep the Spin Column miRelute.
8. Add 500 µl Buffer MRD to the Spin Column miRelute (**ensure**

that Ethanol (96-100%) has been added). Close the lid gently and incubate 2 min at room temperature (15-25°C). Then centrifuge for 30 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.

9. Add 600 µl Buffer RW to the Spin Column miRelute (**ensure that Ethanol (96-100%) has been added**). Close the lid gently and incubate 2 min at room temperature (15-25°C). Then centrifuge for 30 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
10. Repeat step 9.
11. Place the Spin Column miRelute into a new 2 ml collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and discard the flow-through.

    Note: Perform this step to eliminate any possible carryover of Buffer RW. After centrifugation place the Spin Column miRelute at clean bench for a while, to completely dry the column membrane. Residual Buffer RW will have negative influence on following RT experiment.
12. Transfer the miRelute column into a new 1.5 ml RNase-Free Centrifuge Tube, add 15-30 µl RNase-Free ddH_2O directly onto the miRelute column membrane and incubate 2 min at room temperature (15-25°C). 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 15 µl. Small elution volume may have a negative effect on RNA yield. Store RNA solution at -70°C. To obtain a higher total RNA concentration, please repeat step 12.

Protocol B: Purification of total RNA from tissue and cells

(Obtained total RNA includes small RNA like miRNA). Apply this protocol when there is not high demand of miRNA purity, e.g. miRNA RT-PCR, and miRNA Northen blot research.

1. Preparation of samples (refer to step 1 of Protocol A: Purification of miRNA enriched fractions from tissue and cell).
2. Refer to step 2 of Protocol A: Purification of miRNA enriched fractions from tissue and cell.
3. Refer to step 3 of Protocol A: Purification of miRNA enriched fractions from tissue and cell.
4. Refer to step 4 of Protocol A: Purification of miRNA enriched fractions from tissue and cell.
5. Refer to step 5 of Protocol A: Purification of miRNA enriched fractions from tissue and cell.
6. Add 1.5 volume of Ethanol (96-100%) (e.g. add 750 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 Spin Column miRspin, centrifuge at 12,000 rpm (~13,400 × g) for 30 s at room temperature (15-25°C). If the volume exceeds 700 µl, centrifuge successive aliquots in the same column. Discard the flow-through, and keep the Spin Column miRspin after centrifugation.
7. Add 500 µl Buffer MRD to the Spin Column miRspin (**ensure that Ethanol (96-100%) has been added**). Close the lid gently and incubate 2 min at room temperature (15-25°C). Then centrifuge for 30 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
8. Add 600 µl Buffer RW to the Spin Column miRspin (**ensure that**

Ethanol (96-100%) has been added). Close the lid gently and incubate 2 min at room temperature (15-25°C). Then centrifuge for 30 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.

9. Repeat step 8.
10. Place the Spin Column miRspin into a new 2 ml collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and discard the flow-through.

    Note: Perform this step to eliminate any possible carryover of Buffer RW. After centrifugation place the Spin Column miRelute at clean bench for a while, to completely dry the column membrane. Residual Buffer RW will have negative influence on following RT experiment.
11. Transfer the Spin Column miRspin to a new 1.5 ml RNase-Free Centrifuge Tube, add 30-100 µl RNase-Free ddH_2O directly onto the miRspin column membrane and incubate 2 min at room temperature (15-25°C). 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 30 µl. Small elution volume may have a negative effect on RNA yield. Store RNA solution at -70°C. To obtain a higher total RNA concentration, please repeat step 11.

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

1. Preparation of samples

   Add equal volume of Buffer MZ to whole blood, serum or plasma, vortex 30 s to mix thoroughly.

   Note: The starting volume of sample should be at least 200 µl, otherwise RNA yield will be low.

2. Place the tube containing the homogenate on the benchtop at room temperature (15-25°C) for 5 min, to separate nucleic acids and protein.
3. Centrifuge the lysate at 12,000 rpm (~13,400 × g) for 10 min at room temperature (15-25°C) to remove any particulate material. Then transfer supernatant to a new RNase-Free centrifuge tube.
4. Add 200 µl chloroform to the supernatant and cap it securely. Shake the tube vigorously for 15 s, incubate the tube containing the homogenate on the bench top at room temperature (15-25°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 yellow organic phase. Transfer the aqueous phase to a new tube.
6. Add 1/3 volume of Ethanol (96-100%) (e.g. add 100 µl Ethanol (96-100%) to 300 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 Spin Column miRspin, incubate at room temperature (15-25°C) for 2 min, then centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the Spin Column miRspin after centrifugation, and keep the flow-through.
7. Add 2/3 volume of Ethanol (96-100%) (e.g. add 200 µl Ethanol (96-100%) to 300 µl 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 Spin

Column miRelute, incubate at room temperature (15-25°C) for 2 min, centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through, and keep the Spin Column miRelute.

8. Add 500 µl Buffer MRD to the Spin Column miRelute (**ensure that Ethanol (96-100%) has been added**). Close the lid gently and incubate 2 min at room temperature (15-25°C). Then centrifuge for 30 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
9. Add 600 µl Buffer RW to the Spin Column miRelute (**ensure that Ethanol (96-100%) has been added**). Close the lid gently and incubate 2 min at room temperature (15-25°C). Then centrifuge for 30 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
10. Repeat step 9.
11. Place the Spin Column miRelute into a 2 ml collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and discard the flow-through.

    Note: Perform this step to eliminate any possible carryover of Buffer RW. After centrifugation place the Spin Column miRelute at clean bench for a while, to completely dry the column membrane. Residual Buffer RW will have negative influence on following RT experiment.
12. Transfer the miRelute column to a new 1.5 ml RNase-Free Centrifuge Tube, add 15-30 µl RNase-Free ddH_2O directly onto the miRelute column membrane and incubate 2 min at room temperature (15-25°C). 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 15 µl. Small elution volume may have a negative effect on RNA yield. Please store RNA solution at -70°C. To obtain a higher total RNA concentration, please repeat step 12; or

increase sample volume and Buffer MZ and chloroform volume in proportion.

Ordering Information

Product	Size	Cat. no.
miRcute miRNA First-strand cDNA Synthesis Kit	25 preps	KR201-01
	50 preps	KR201-02
miRcute miRNA qPCR Detection Kit (SYBR Green)	20 µl × 125 rxn	FP401-01