TIANGEN®

miRcute Serum/ Plasma miRNA Isolation Kit

High-efficiency miRNA extraction kit specifically targeting serum/plasma samples

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This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

miRcute Serum/Plasma miRNA Isolation Kit

(Spin Column)
Cat. no. GDP503

Kit Contents

	Contents	GDP503 (50 preps)
GDP503H	Buffer RW	12 ml
	Buffer MRD	24 ml
	RNase-Free ddH_2O	15 ml
	RNase-Free Columns miRelute set	50
	RNase-Free Centrifuge Tubes 1.5 ml	50
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GRK188	Buffer MZA	50 ml

Note: GDP503H, GRK188 are shipped and packaged separately

Storage

Buffer MZA should be stored at 2-8°C protected from light for 18 months. The miRcute Serum/plasma miRNA Isolation Kit should be stored dry at room temperature(15-30°C) for 15 months.

Introduction

The miRcute Serum/Plasma miRNA Isolation Kit is designed for isolation and purification of miRNA from serum/plasma. The optimized lysis buffer in the kit has super lysis ability and isolation sensitivity. The kit utilizes a silica-based system to adsorb RNA, especially small RNA (<200 nt). miRNA with high purity and quality with no contamination of DNA and protein could be obtained within 1 hour. The purified miRNA 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, for 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 MZA. The following steps after lysis should be performed 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, and washed by RNase-Free ddH_2O thoroughly, then send for autoclaving.
4. Use RNase-Free ddH_2O to prepare the solutions. (Add DEPC to 0.1% final concentration in ddH_2O. Shake to mix, and leave overnight at room temperature, then 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.

1. Preparation of samples: Add 900 μl Buffer MZA to 200 μl serum or plasma, vortex 30 s to mix thoroughly, and then invert up and down to mix. If the External Control (Cat.no. GCR100-01) is applied, please add in the External Control (for 1 μM, add in 1 μl) after the complete homogenization and before the inverting mixing.

   Note: The starting volume of sample should be no more than 200 μl, otherwise RNA yield and purity will be low. The volume of Buffer MZA must strictly in accordance with the volume in the instructions. If the amount of Buffer MZA is small, the phase separation will not be complete, which will cause low yield and purity of RNA.

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

3. 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 for 5 min.
4. 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 and proceed to the next step.
5. Add 2× the volume of ethanol (96-100%) to the aqueous phase solution (e.g. add 1 ml ethanol (96-100%) to 500 µl aqueous phase solution) and mix thoroughly by pipetting up and down several times (precipitate may form after addition of ethanol, but this will not affect the procedure). Transfer the obtained liquidand any precipitate that may have formed into a RNase-Free Column miRelute, incubate at room temperature for 2 min, then centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow through after centrifugation, and keep the Column miRelute.
6. Add 700 µl 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 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow through.
7. Add 500 µl 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 s at 12,000 rpm (~13,400 × g) to wash the column. Discard the flow-through.
8. Repeat step 7.
9. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min at room temperature, and discard the 2 ml collection tube.

   Note: This step is to eliminate any possible carry-over of Buffer RW. After centrifugation. place the 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.
10. Transfer the Column miRelute 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. 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 10; or increase sample volume and Buffer MZA and chloroform volume in proportion.