HiPure Serum/Plasma miRNA Kit

Introduction

The kit offers the unique technology to isolate cell-free total RNA, primarily miRNA and other small RNA from 0.3~0.9ml non-cells liquid samples such as serum, plasma, urine and body fluid. This protocol uses chaotropic lysis followed by selective protein precipitation and silica membrane purification technology, without the need of phenol/chloroform extraction or a time consuming proteinase digest. Purified RNA is ready for applications such as RT-PCR, Northern blot, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation, ect.

Kit Contents

Product	R431401	R431402	R431403
Preparation Times	20	50	250
HiPure Viral Mini Columns	20	50	250
2ml Collection Tubes	40	100	4 x 125
Buffer CFL	10 ml	20 ml	80 ml
Buffer CFP	3 ml	6 ml	30 ml
Buffer MGW1*	15 ml	30 ml	150 ml
Buffer RW2*	10 ml	20 ml	2 x 50 ml
RNase Free Water	5 ml	10 ml	30 ml

Version: 202608

Storage and Stability

The kit components can be stored at room temperature (15–25°C) and are stable for at least 24 months under these conditions.

Materials and Equipment to be Supplied by User

- Dilute Buffer RW2 with 40ml (20 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol and store at room temperature.
- Dilute Buffer MGW1 with 10ml (20 Preps), 30ml (50 Preps) or 125ml (250 Preps)

isopropanol and store at room temperature

- Choice: if customer wants to remove DNA thoroughly, it can proceed with DNase I digestion process and purchase DNase I Set B (Magen Cat# C12138).
- Microcentrifuge capable of at least 13,000 × g

Preparation of serum/plasma from human EDTA blood

1. Centrifuge fresh blood sample for 10 min at 2,000 x g.
2. Transfer serum/plasma without disturbing sedimented cells.
3. Freeze serum/plasma at -20 °C for storage upon RNA isolation.
4. Thaw frozen serum/plasma samples on ice before the protocol.

Protocol

1. **Transfer serum, plasma, urine or other body fluids into a 1.5ml centrifuge tube, centrifuge at 13,000 x g at 4°C for 10 min to remove residual cells, cell debris and particulate matters.**
2. **Transfer 0.3~0.9ml supernatant to a new centrifuge tube, add 1/3 sample volume of Buffer CFL to the sample.** Vortex for 10 sec to mix and incubate at room temperature for 10min.
   - e.g for 0.3ml sample, add 0.1ml Buffer CFL; for 0.9ml sample, add 0.3ml Buffer CFL.
3. **Add 1/10 sample volume of Buffer CFP to the sample and vortex at high speed for over 20 sec. Incubate on ice for 3 min.**
   - e.g for 0.3ml sample, add 30µl Buffer CFP; for 0.9ml sample, add 90µl Buffer CFP.
   - A large amount of precipitate will form after adding Buffer CFP. Vortex thoroughly to disperse the precipitate. This will avoid nucleic acids being entrapped by the precipitate and resulting in reduced RNA yield.
4. **Centrifuge at 13,000 x g for 10 min to pellet the protein.**
5. **Transfer the supernatant to a new centrifuge tube. Add an equal volume of ice-cold isopropanol (containing 2% HAC) to the supernatant.** Mix by inverting the tube 6- 8 times. Then proceed with following Step 6.
   - e.g for 0.43ml supernatant, add 0.43ml isopropanol (containing 2% HAC).
   - Add 20µl HAC (Glacial acetic acid) to each 1ml isopropanol, mix well and incubate on ice before use.

Isolate RNA on Mini Spin Column

6. **Insert a HiPure Viral Mini Column in a 2ml Collection Tube. Transfer the mixture (≤750µl) to the column, centrifuge at 12,000 x g for 1 min.** Discard the filtrate and insert the column back in the collection tube.

7. **Transfer the balance mixture (≤750µl) to the column, centrifuge at 12,000 x g for 1 min.** Discard the filtrate and insert the column back in collection tube.

- Repeat this step until all sample is loaded onto the column.

8. **Add 500µl Buffer MGW1 to the column, Centrifuge at 12,000 × g for 1 min at room temperature.** Discard the filtrate and insert the column back in collection tube.

9. (For choice: remove DNA thoroughly)

9.1 **Prepare DNase I mixture in a new microcentrifuge tube on following table.** This kit not provided, customer can purchase DNase I Set B (Magen Cat# C12138) separately.

Reagent	Volume
DNase Buffer	70 µl
DNase I (10 units/µl())	10 µl

9.2 Apply the DNase I mixture to the centre of the column membrane, and incubate at room temperature (15- 30°C) for 15 min.

10. **Add 500µl Buffer MGW1 to the column, stay at room temperature for 1 min. Centrifuge at 12,000 × g for 1 min.**

11. **Insert the column into a new 2ml Collection Tube, transfer the filtrate back to the column. Centrifuge at 12,000 × g for 1 min.** Discard the filtrate and insert the column back into collection tube.

12. **Add 500µl Buffer RW2 (diluted with ethanol) to the column, centrifuge at 12,000 × g for 1 min.** Discard the filtrate and insert the column back into collection tube.

13. **Repeat Step 12 once.**

14. **Centrifuge the empty Column at 12,000 × g for 3 min to dry the column matrix.**

15. Open the column lid, stay the column at room temperature for 10 min to dry the column

matrix thoroughly.

16. Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 20~50μl RNase Free Water directly to the center of the column membrane. Stay at room temperature for 2 min, and centrifuge at 13,000 × g for 1 min.

17. Store RNA at -20°C or -80°C.

Troubleshooting Guide

1. Clogged HiPure RNA Column

- **Too much starting material:** Too much sample volume increase loading times on column binding. the It is essential to use the correct amount of starting material.
- Precipitate in sample: Make sure not to transfer any precipitate on Step 5 when transferring the supernatant to a new tube.

2. RNA does not perform well (e.g. in RT-PCR)

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding 500ul of Buffer RW2, then centriufge.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and the column is then centrifuged at >12,000 x g for 1min. Dry the column thoroughly before elution step.
- **Too much starting material:** It is recommend to use 300ul sample volume as starting material, this volume is usually sufficient to detect low-abundance miRNA in sample. If using too much sample volume, it may increase the change of co-isolating additional PCR inhibitors with the RNA.

3. DNA contamination in downstream expeiments

- **No DNase enzyme treatment:** Perform the optional DNase I treatment process in Step 9 to remove DNA thoroughly.

4. Low A260/A280 value

- Water was used to dilute RNA for A260/A280 measurement. Use 10 mm Tris·Cl, pH 7.5, not RNAse-free water, to dilute the sample before measuring purity..