MagPure Serum/Plasma miRNA Kit

Introduction

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

This kit is available for manual and automated extraction systems.

Kit Contents

Product	R662800	R662801	R662802	R662803
Preparation Times	20 Preps	48 Preps	96 Preps	5x96 Preps
MagPure Particle N	1.1 ml	2.5 ml	3.5 ml	17 ml
Buffer CFL	3 ml	10 ml	20 ml	60 ml
Buffer CFP	1.8 ml	2.5 ml	5 ml	20 ml
Buffer MGW1*	30 ml	30 ml	60 ml	270 ml
Buffer MW2*	10 ml	25 ml	50 ml	3 x 50 ml
RNase Free Water	10 ml	20 ml	30 ml	60 ml

Version: 202608

Storage and Stability

MagPure Particle N should be stored at 2–8°C upon arrival. However, short-term storage (up to 8 weeks) at room temperature (15–25°C) does not affect their performance. The remaining 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 MWV2 with 40ml (20 Preps), 100ml (48 Preps), 200ml(96 Preps) or 3 x 200ml (5 x 90 Preps) 100% ethanol and store at room temperature.
- Dilute Buffer MGW1 with 30ml (20 Preps), 30ml (48 Preps), 60ml(96 Preps) or 270ml (5 x 90 Preps) Isopropanol and store at room temperature.
- Vortex MagPure Particles N to mix thoroughly before use.
- Microcentrifuge capable of at least 13,000 × g

Preparation of plasma from human EDTA blood

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

Sample Preparation Protocol

1. Transfer serum, plasma, urine or other body fluids into 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 300µl supernatant into a new1.5ml microcentrifuge tube. add 100µl Buffer CFL to the sample.** Vortex for 10 sec to mix and incubate at room temperature for 10min.
3. **Add 30µl Buffer CFP to the sample and vortex at high speed for over 20 sec. Incubate on ice for 3 min.**

- 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 400µl supernatant into a new centrifuge tube. **Add 30µl MagPure Particles N and 400µl isopropanol(2% HAC)**. Incubate at room temperature with shaking for 10 min. Place the tube to the magnetic rack for 2 minutes, until the MagPure Particles N has formed a tight pellet, then remove all the liquid carefully.

- For 400µl Isopropanol, add 20µl Glacial acetic acid and mix well.

6. **Add 500µl Buffer MGW1 and vortex for 15 seconds to resuspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove all the liquid carefully.

7. **Repeat Step 6 once.**

8. **Add 500µl Buffer MW2 and vortex for 15 seconds to resuspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove all the liquid carefully.

9. **Repeat Step 8 once.**

10. Spin shortly to collect liquid on tube. Place the tube to the magnetic rack for 1 minute, then remove all the liquid carefully. Dry the particles on air for 10 min.

11. **Add 50µl RNase Free Water to sample and mix by shaking for 5~6 min.** Place the tube to the magnetic rack for 5 min.

12. Transfer the liquid containing purified RNA into a new tube and store at -20°C or -80°C.

Auto Purify by KingFisher Flex or other extraction system:

Follow Step 1-4 process in Sample Preparation Protocol.

5. Add the Reagents/sample to the deep well plate according to the table below.

Name of the Plate	Pre-loaded reagents	Addition before use
Sample plate	400µl Isopropanol 30µl MagPure Particles N	400µl supernatant (from step 4)
Wash Plate 1	500µl Buffer MGW1, Put in 96 magnetic Tip	
Wash Plate 2	500µl Buffer MGW1	
Wash Plate 3	500µl Buffer MW2	
Wash Plate 4	500µl Buffer MW2	
Elution plate	50µl RNase Free Water	

6. Place a 96 tip comb for deep well magnets on Wash Plate 1.

7. Start the protocol with the KingFisher Flex and load the plates.

8. After the run is completed, remove the plates and store the purified total RNA at -20°C or -80°C.