MagPure Serum miRNA Kit

Introduction

The kit offers the unique feature to isolate total RNA including small RNA and DNA from serum and plasma without the need to resort to the cumbersome phenol/chloroform extraction or a time consuming proteinase digest. RNA purified using the kit is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Principle

This product is based on the purification method of high binding magnetic particles. The sample material is denatured in Lysis Buffer. The protein is then precipitated by Protein Precipitation Solution and pelleted by centrifugation. After adding magnetic particles and binding solution, RNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing solution to remove proteins and impurities, washed with ethanol to remove salts, and finally RNA was eluted by Elution Buffer.

Kit Contents

Product	R662801	R662802	R662803
Preparation Times	48 Preps	96 Preps	5 x 96 Preps
MagPure Particle N	1.7 ml	3.5 ml	17 ml
Buffer CFL	6 ml	12 ml	60 ml
Buffer CPL	1.8 ml	3.5 ml	20 ml
Buffer MGW1*	30 ml	60 ml	250 ml
Buffer MW2*	12 ml	25 ml	100 ml
RNase Free Water	10 ml	20 ml	60 ml

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 18 months under these conditions.

Materials and Equipment to be Supplied by User

- Dilute Buffer MW2 with 48ml (48 Preps), 100ml(96 Preps) or 400ml (5 x 90 Preps) 100% ethanol and store at room temperature
- Dilute Buffer MGW1 with 30ml (48 Preps), 60ml(96 Preps) or 250ml (5 x 90 Preps) (Isopropanol and store at room temperature
- Microcentrifuge capable of at least 12,000 × 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 prior to RNA isolation and centrifuge for 3 min at ⩾ 11,000 x g in order to remove residual cells, cell debris, and particulate matter. Use the supernatant for RNA isolation.

Maunal Protocol

1. Transfer 0.3ml Serum or plasma into 1.5ml microcentrifuge tube.
2. **Add 90µl Buffer CFL to the sample. Mix well and incubate at room temperature for 10min.**

   To process 600 µL or 900 µL sample material, increase volumes for Buffer CFL, CFP, and isopropanol proportionally. Multiple loading steps will be necessary in step6.
3. **Add 30µL Buffer CFP and vortex for 10 s. Incubate for 1 min at room temperature (18–25 °C).** Centrifuge for 3 min at 11,000 x g to pellet the protein.
4. Transfer the supernatant into a new tube.
5. **Add 20µl MagPure Particles N and 400µL isopropanol(2% HAC).**Pipette mix 10 times and then shaking at 700~900rpm for 6 minutes. Place the deep well plate on an Magnet Plate and allow beads to separate for 2 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

   For 1ml Isopropanol, add 20ul Glacial acetic acid, mix well.
6. **Add 500µl Buffer MGW1 and shaking at 900~1200rpm for 1 minute to resuspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant.
7. **Add 500µl Buffer MGW1 and shaking at 900~1200rpm for 1 minute to resuspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant.
8. **Add 500µl Buffer MW2 and shaking for 1 minute to resuspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.
9. Repeat step 8 once.
10. Leave the plate on the magnetic separation device. Wait 1 minute and remove residual liquid with a pipettor. Dry the Mag-Pure Particles for an additional 10 minutes.
11. **Add 50~100µl RNase Free Water to sample and mix by shaking for 5 minutes.** Place the tube to the magnetic rack for 3 minutes.
12. Transfer the purified RNA into a new tube and store at –80°C.

Auto Purify by KingFisher Flex or similar Extractor isolation:

1. Add the Reagents/sample to the well of f 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
Wash Plate 1	500µl Buffer MGW1, Put in 96 magnetic Tip 30µl MagPure RNA Particle N	
Wash Plate 2	500µl Buffer MGW1	
Wash Plate 3	500µl Buffer MW2	
Wash Plate 4	500µl Buffer MW2	
Elution plate	50µl RNase Free Water	

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.
3. Start the protocol with the KingFisher Flex and load the plates.
4. After the run is completed, remove the plates and store the purified total RNA.