MagPure Blood RNA Kit

Introduction

This product supplies a simple and rapid extraction of total RNA from Blood, buffy coat, bone marrow, Cell suspension and other body fluids. The kit is based on superparamagnetic particles purification technology, no phenol-chloroform extraction or alcohol precipitation. Purified RNA is ready for downstream applications such as RT-PCR, virus RNA testing and so on.

Principle

The Kit can be used for both manual extraction process and automatic nucleic acid extraction machines. This Kits is suitable for extracting RNA from $\leqslant 5x10^6$ cells suspension, 200~300 µl Whole Blood, 200µl buffy coat, 100µl bone marrow.This product is based on the purification method of high binding magnetic particles. The sample is lysed and digested under the action of lysate and Protease. RNA/DNA is released into the lysate. After adding magnetic particles and binding solution, DNA/RNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were digested using DNase and washed with washing buffer to remove proteins and impurities, washed with ethanol to remove salts, and finally RNA was eluted by Elution Buffer.

Kit Contents

Cat.No.	R661101	R661102	R661103
Purification times	48 Preps	96 Preps	480 Preps
MagPure RNA Particles	1.7 ml	3.5 ml	18 ml
Proteinase K	24 mg	50 mg	220 mg
Protease Dissolve Buffer	1.8 ml	5 ml	15 ml
DNase I	600 µl	2 x 600 µl	10 x 600 µl
DNase Buffer	30 ml	40 ml	200 ml
Buffer MLB	40 ml	80 ml	350 ml
Buffer MCB*	15 ml	30 ml	150 ml
Buffer MW1*	22 ml	44 ml	220 ml
Buffer MW2*	20 ml	50 ml	2 x 100 ml
RNase Free Water	10 ml	15 ml	120 ml

Storage and Stability

MagPure RNA Particles and Proteinase K should be stored at 2–8°C upon arrival. DNase I should be stored at -20°C. However, short-term storage (DNase I up to 1 weeks, MagPure RNA Particles and Proteinase K 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

- 100% ethanol
- Dilute Buffer MW1 with 28ml (48 Preps), 56ml (96 Preps) or 280ml (480 Preps) 100% ethanol and store at room temperature
- Dilute Buffer MW2 with 80ml (48 Preps), 200ml (96 Preps) or 2 x 400ml (480 Preps) 100% ethanol and store at room temperature
- Dilute Buffer MCB with 35ml (48 Preps), 70ml (96 Preps) or 350ml (480 Preps) isopropanol and store at room temperature
- Dissolve the Proteinase K with 1.2ml (48 Preps), 2.5ml (96 Preps) or 12ml (480 Preps) protease Dissolve Buffer to the Proteinase K and store at -20~8℃.

Manual or Liquid station protocol

1. Pipet 20µl Proteinase K and 30µl MagPure RNA Particles into the bottom of a 96 well Plate (2.2ml).

2. Add 200~300µl sample to the 96 well plate and mix for 5 seconds.
   - Use 200~300µl whole blood, plasma, serum or body fluids.
   - 200µl buffy coat and 150µl bone marrow
   - or up to 5 x 10^6 lymphocytes or Culture Cells in 200 µl PBS.

3. Add 600µl Buffer MLB to the sample and immediately pipette mix 10 times. Mix by shaking at 900~1000rpm for 10 min.
   To ensure efficient lysis, it is essential that the sample and Buffer MLB are mixed thoroughly to yield a homogeneous solution.

4. 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.

5. **Add 600µl Buffer MW1 and shaking for 2 minute to re-suspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant.

6. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the MagPure RNA Particles for an additional 5 minutes.

7. **Add 300µl DNase Mixture (290µl DNase Buffer + 10µl DNase I) to the sample.** Mix by shaking at 600-900rpm for 10~15 minutes.
   DNase I and DNase Buffer can be premixed.

8. **Add 450µl Buffer MCB to the sample, shaking at 900-1200rpm for 6 minutes.** Place the tube to the magnetic rack for 1 minutes, then remove the supernatant.

9. **Add 600µl Buffer MW2 and shaking 900~1200rpm for 1 minute to re-suspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.

10. Repeat step 9 once.

11. Leave the plate on the magnetic separation device. Wait 1 minute and remove residual liquid with a pipettor.

12. Dry the Mag-Pure Particles for an additional 10 minutes.

13. **Add 60µl RNase Free Water to sample and mix by shaking for 5 minutes.** Place the tube to the magnetic rack for 3 minutes.

14. Transfer the supernatant containing the purified RNA to a new Plate and store RNA at -80°C.

KingFisher 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	1. Pipet 20µl Proteinase K and 30µl MagPure RNA Particles 2. Pipet 150~300µl of the sample into the well of plate: ● 200~300 µl whole blood, plasma, serum, body fluids ● 200µl buffy coat or 150µl bone marrow ● up to 5 x 10^6 lymphocytes or Culture Cells in 200µl PBS. 3. Add 600µl Buffer MLB	
Wash Plate 1	600µl Buffer MW1, Put in 96 magnetic Tip	

DNase	290µl DNase Buffer and 10µl DNase I
Wash Plate 2	600µl Buffer MW2
Wash Plate 3	600µl Buffer MW2
Elution plate	60µl RNase Free Water

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.
3. Start the R6611_Flex protocol with the KingFisher Flex 96 and load the plates.
4. **Add 450µl Buffer MCB to the DNase plate during the dispense step.**
5. Place the DNase plate back into the instrument and press Start. After the pause, the protocol will continue to the end.
6. After the run is completed, remove the plates and store the purified total RNA.

Troubleshooting Guide

1. Low RNA yields

- **Incomplete resuspension of MagPure RNA Particles:** Resuspend the MagPure RNA Particles by vortexing before use.
- **Loss of MagPure RNA Particles during procedure:** Be careful not to remove the MagPure RNA Particles during the procedure.
- **MagPure RNA Particles ot resuspended during binding:** Vortex vigorously for 2 minutes after addition of Buffer MCB.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min.

2. Low A260/A280 value

- Water 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..