MagPure Blood RNA Kit

Introduction

This product is suitable for extracting RNA from anticoagulant blood, lymphocytes, buffy coat, bone marrow, cultured cells and other samples. This product is based on the purification method of high binding magnetic particles. The sample is lysed and digested by lysis buffer and protease, and RNA/DNA is released into the lysis buffer. Add binding solution and magnetic particles to adsorb RNA/DNA, while proteins are not adsorbed and removed. The particles adsorbed with DNA/RNA are washed with washing buffer to remove proteins and other impurities, then washed with ethanol to remove salt, and finally digested with DNase to remove DNA. RNA is recovered by adding binding solution, and finally the RNA is eluted with low salt buffer. The eluted RNA can be directly used for experiments such as RT-PCR,NGS and virus detection.

Kit Contents

Cat.No.	R661101	R661102	R661103
Purification times	48 Preps	96 Preps	480 Preps
10 x RBC Lysis Buffer	50 ml	2 x 50 ml	4 x 100 ml
Proteinase K	12 mg	24 mg	120 ml
Protease Dissolve Buffer	1.8 ml	1.8 ml	10 ml
DNase I	600 µl	2 x 600 µl	10 x 600 µl
DNase Buffer	20 ml	30 ml	150 ml
MagPure Particles N	1.2 ml	2.5 ml	11 ml
Buffer RTL	30 ml	60 ml	300 ml
Buffer ALB2	40 ml	60 ml	300 ml
Buffer MW1*	22 ml	44 ml	220 ml
Buffer MW2*	20 ml	50 ml	2 x 100 ml
RNase Free Water	10 ml	20 ml	60 ml

Storage and Stability

DNase I should be shipped with ice pack or dry ice and stored at -20°C upon arrival. MagPure Particles N and Proteinase K 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 store at room temperature and are stable for up to 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
- Dissolve the Proteinase K with 0.6ml (48 Preps), 1.2ml (96 Preps) or 6ml (480 Preps) protease Dissolve Buffer to the Proteinase K and store at -20~8℃.
- Dilute 10 x RBC Buffer with 450ml (48 Preps), 2 x 450ml (96 Preps) or 4 x 900ml (480 Preps) nuclease free water and store at room temperature.

Protocol 1: Manual or Liquid station protocol

1. **Add 1-1.5ml anticoagulant blood or 0.5-1ml bone marrow into a 15ml centrifuge tube. Add 3 times volume of 1x RBC Lysis Buffer into the tube, invert and mix 5-10 times. Place on ice for 10 minutes, during which invert and mix twice.**

   This kit provides with 10 x RBC Lysis Buffer, it must dilute to 1 x with nuclease free water before use. As the patient's white blood cells may increase, the blood sample amount needs to be adjusted to ensure that the white blood cell count does not exceed 1 x 10^7. For example, add 4.5ml 1 x RBC Lysis Buffer to 1.5ml blood sample. During the lysis process, the blood will change from a mist to a transparent solution. A clear solution indicates that red blood cells have been lysed. When processing patients' blood, it may be necessary to extend lysis to 20 minutes.

2. **Centrifuge at 2,000 x g for 10 minutes at 4℃, discard the supernatant carefully.**

3. Add 2 times volume of 1 x RBC Lysis Buffer to the tube (e.g. 1.5ml blood sample add 3ml 1 x RBC Lysis buffer), resuspend the cells by vortex. Centrifuge at 2,000 x g for 10 minutes at 4℃. Pipet and discard the supernatant carefully, leaving ~50µl of residual liquid and lymphocytes, resuspend the lymphocytes by vortex.

   When processing with bone marrow samples, control the sample to 500µl. If whole blood and bone marrow are mixed together and can not recognize bone marrow volume, it can be judged by the amount of cell precipitation after centrifugation. Most bone marrow samples get large amount of white blood cells precipitation after centrifugation, it is 3~5 times amount comparing to whole blood samples. It is necessary to control the amount of white blood cells in bone marrow samples, as excessive white blood cells may cause the lysate become too viscous and affect the extraction results. If the amount of white blood cell precipitation is too high, leave more residual liquid (such as 200-300µl) when discard the supernatant. After resuspension by vortex, keep 50µl cell suspension for RNA extraction, store the excess suspension at -70℃ for further use. If the amount of cell precipitation is normal and equivalent to conventional blood sample, only leave 50µl residual liquid. Resuspension by vortex and follow step 4 operation.

4. Add 500µl Buffer RTL, immediately vortex for 10 seconds to mix. Use a 1ml syringe to aspirate 5 times or use a pipette to aspirate repeatedly to homogenize the sample .

5. Transfer 500µl homogenate to a new centrifuge tube, add 350µl isopropanol and 20µl MagPure Particles N to the sample, vortex for 10 seconds to mix. Stay at room temperature for 6 minutes, during which invert and mix several times. Place the tube to the magnetic rack for 2 minutes, until the MagPure Particles N have formed a tight pellet, then remove the supernatant.

6. Add 500µl Buffer MW1 and vortex for 15 seconds to resuspend the particles. Place the tube to the magnetic rack for 1 minute, then remove the supernatant. Spin shortly to collect liquid on tube and remove all liquid carefully. Dry on air for 3 minutes.

7. Add 200µl DNase mixture (180µl DNase Buffer+10µl DNase I+10µl Proteinase K) to the sample, shake at room temperature for 15~20 minutes gently to digest and remove DNA.

8. Add 500µl Buffer ALB2 to the sample, invert 10-15 times to mix. Stay at room temperature for 5 minutes, during which invert and mix for 3~5 times. Place the tube to the magnetic rack for 1 minute, then remove the supernatant.

9. **Add 500µl Buffer MW2 and vortex for 15 seconds.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant.
10. **Repeat step 9 once**.
11. Spin shortly to collect liquid on tube, place the tube to the magnetic rack. Remove all liquid carefully. Dry at room temperature for 10 minutes.
12. **Add 50µl RNase Free Water to the sample** and mix the particles by vortex. Stay at room temperature for 3 minutes.
13. Place the tube to the magnetic rack for 3 minutes. Transfer the supernatant containing the purified RNA to a new 1.5ml centrifuge tube. Store RNA at -80°C or -20°C.

Protocol 2: Auto Pure by KingFisher Flex

1. Sample lyses (follow Step 1-4 in Protocol 1)
2. Add the Reagents/sample to the well of deep well plate according to the table below.

Name of the Plate	Pre-loaded reagents
Sample plate	500 µl sample homogenate 350 µl Isopropanol 20 µl MagPure Particles N
Wash Plate 1	500µl Buffer MW1, Put in 96 magnetic Tip
DNas Plate	180µl DNase Buffer + 10µl DNase I + 10µl Proteinase K After pause:add 500µl Buffer ALB2
Wash Plate 2	500µl Buffer MW1
Wash Plate 3	500µl Buffer MW2
Elution plate	70µl RNase Free Water

3. Place a 96 tip comb for deep well magnets on Wash Plate 1.
4. Start the R6611 with the KingFisher Flex 96 and load the plates.
5. **Add 500µl Buffer ALB2 to the DNase Plate plate during the pause step.**
6. Place the DNase plate back into the instrument and press Start.
7. After the program is completed, remove the plates, transfer purified RNA to new 1.5ml centrifuge and store at -80°C or -20°C.