MagPure Blood RNA Kit II

Introduction

This product is suitable for extracting RNA from anticoagulant blood, lymphocytes, buffy coat, bone marrow, cultured cells and other samples. It can specially extract high quality RNA from frozen blood 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: Bottle

Cat.No.	R661301	R661302	R661303
Purification times	48 Preps	96 Preps	480 Preps
DNase I	600 µl	2 x 600 µl	10 x 600 µl
DNase Buffer	20 ml	30 ml	150 ml
MagPure Particles N	2.5 ml	5.0 ml	28 ml
Buffer ALB2	40 ml	60 ml	350 ml
MagZol 3BD	65 ml	140 ml	3 x 200 ml
Buffer BCP2	10 ml	15 ml	80 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, MagZol 3BD and Buffer BCP2 should be stored at 2–8°C upon arrival. However, short-term storage 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 up to 18 months under these conditions.

Materials and Equipment to be Supplied by User

- 100% ethanol
- Dilute Buffer MW2 with 80ml (48 Preps), 200ml (96 Preps) or 2 x 400ml (480 Preps) 100% ethanol and store at room temperature

Protocol 1: Single tube operation

1. Add 1.3ml MagZol 3BD into a 5-15ml centrifuge tube.
2. Add 1ml of anticoagulant blood, buffy coat, serum, plasma or other liquid samples, shake vigorously for 10-15 seconds immediately to fully disperse the sample. Vortex at high speed for 15 seconds to form a homogeneous solution, place at room temperature for 3 minutes.
    - Fresh whole blood: Collect whole blood into anticoagulant vacuum collection tubes that containing EDTA, and then transfer to centrifuge tubes that containing Magzol 3BD (Step 1) as soon as possible. Sufficient mixing is crucial for RNA extraction yield.
    - Low temperature preservation 1: Transfer 1ml blood to a 5-15ml centrifuge tube and store at -70℃. When extracting RNA, add 1.2ml MagZol 3BD to frozen blood without thawing and invert or shake until the sample is completely thawed. Do not thaw blood samples without reagents, as this will cause RNA degradation.
    - Low temperature preservation 2: Transfer 1ml blood to a 5-15ml centrifuge tube, then add 1.2ml MagZol 3BD. Invert vigorously to mix for 10-15 seconds, place for 5 minutes at room temperature. Incubate at 60℃ with high-speed oscillation (1,200-1,500rpm) for 10 minutes. The lysate can be stored at -70℃ for at least 2 years, at -20℃ for at least 6 months, at 2-8℃ for at least 15 days, and at room temperature for 7 days.

- **DNA rich samples:** When processing bone marrow, buffy coat or animal blood which is rich in DNA, it is recommended to control the sample amount between 500 ~ 1,000μl. Transfer 300-500μl sample to a 10-15ml centrifuge tube, add sterile water or RNase Free Water to make up to 1,000μl. Invert and mix 3-5 times. Then add 1.2ml MagZol 3BD to the tube, shake vigorously immediately for 15 seconds and vortex to mix for 15 seconds to form a non-viscous and homogeneous homogenate. If the sample is still viscous and in-homogeneous after vortexing, pipetting repeatedly for 5-10 times to mix throughly. If the sample is too viscous, add an appropriate amount of MagZol 3BD and RNase Free Water in proportion to dilute the sample, and then shake vigorously to mix until the sample forms a homogeneous homogenate.

3. **Incubate in Water bath at 60°C for 10 minutes, during which invert and mix once.**
4. **Optional: Add 160μl Buffer BCP2 to the mixture, shake quickly to mix for 15 seconds.**
   After processing with Buffer BCP2, the supernatant will become more clear.
5. Centrifuge at 4,500~5,000 x g for 15 minutes at room temperature.
6. **Transfer 1ml supernatant to a 2ml centrifuge tube, add 700μl isopropanol and 30μl MagPure Particles N to the sample, invert 10-15 times to mix**. Place at room temperature for 6 minutes, during which invert several times to mix. Place the tube to the magnetic rack for 3 minutes, until the MagPure Particles N have formed a tight pellet, then remove the supernatant.
7. **Add 800μl Buffer MW2 and vortex for 10 seconds.** 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.
8. **Add 250μl DNase mixture (240μl DNase Buffer+10μl DNase I) to the sample**, shake gently at room temperature for 15-20 minutes to digest and remove DNA.
9. **Add 500μl Buffer ALB2 to the sample, invert and mix 10-15 times**. Place at room temperature for 6 minutes, during which invert and mix for 3~5 times. Place the tube to the magnetic rack for 1 minute, then remove the supernatant.
10. **Add 800μl Buffer MW2 and vortex for 15 seconds.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant.
11. Spin shortly to collect liquid on tube, place the tube to the magnetic rack. Remove all liquid carefully. Dry at room temperature for 5 minutes.

12. Add 50-100µl RNase Free Water to the sample and vortex to disperse the magnetic beads, stay at room temperature for 5 minutes.
13. Place the tube to the magnetic rack for 3 minutes. Transfer the supernatant containing the purified RNA to new 1.5ml centrifuge tubes. Store RNA at -80°C or -20°C.

Protocol 2: Auto Pure by KingFisher Flex

1. Sample lyses (follow Step 1-5 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 1	500 µl sample homogenate 350 µl Isopropanol 15 µl MagPure Particles N
Sample plate 2	500 µl sample homogenate 350 µl Isopropanol 15 µl MagPure Particles N
Wash Plate 1	700µl Buffer MW2, Put in 96 magnetic Tip
DNas Plate	240µl DNase Buffer 10µl DNase I After pause:add 500µl Buffer ALB2
Wash Plate 2	700µl 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 R6613 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.