MagPure Stool RNA Kit

Introduction

This product is specially designed for stool RNA extraction. The kit is suitable for extracting high-purity microbial or host cell RNA from ≤0.15g stool samples. The purified RNA can be directly used in RT-PCR and Northern hybridization.

Principle

The Kit combines the speed and efficiency of silica-based technology with the convenient handling of magnetic particles for purification of total RNA. Samples are lysed and RNA is purified from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet and DNA is removed by treatment with RNase-free DNase. The magnetic particles are efficiently washed, and RNA is eluted in RNase-free water.

Kit Contents

Cat.No.	R662601	R662602	R662603
Purification times	48 Preps	96 Preps	5 x 96 Preps
MagPure RNA Particles	1.7 ml	4.0 ml	18 ml
DNase I	600 µl	2 x 600 µl	10 x 600 µl
DNase Buffer	30 ml	40 ml	200 ml
Buffer SPL	30 ml	60 ml	270 ml
Buffer PHC	30 ml	60 ml	270 ml
Buffer MCB*	9 ml	15 ml	75 ml
Buffer ALB3*	10 ml	20 ml	100 ml
Buffer GW1*	44 ml	66 ml	2 x 220 ml
Buffer RW2*	20 ml	50 ml	2 x 100 ml
RNase Free Water	10 ml	30 ml	120 ml
2ml Beads Tubes	48	96	5 x 96

Storage and Stability

MagPure RNA Particles 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 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

- Add 56ml (48 Preps), 84ml (96 Preps) or 2 x 280ml (5 x 96 Preps) 100% ethanol to the bottle of Buffer GW1
- Add 80ml (48 Preps), 200ml (96 Preps) or 2 x 400ml (5 x 96 Preps) 100% ethanol to the bottle of Buffer RW2
- Add 21ml (48 Preps), 35ml (96 Preps) or 175ml (5 x 96 Preps) isopropanol to the bottle of Buffer MCB.
- Add 20ml (48 Preps), 40ml (96 Preps) or 200ml (5 x 96 Preps) 100% ethanol to the bottle of Buffer ALB3.

Protocol for sample prepare

1. **Transfer 100~150mg Stool or 200~300mg other environmental samples to 2ml Bead Tubes.**
2. **Add 500μL Buffer SPL and 500μL Buffer PHC to the sample.** Lyse sample by vortex at maximum speed for 10 minutes or by Fastpreps 24 (6 .5 m/s twice for 45s) .For best results, a mixer mill, such as GenoGrinder 2010, Fastprep-24® , or Omni Bead Ruptor should be used.
3. Incubate at 65°C for 15 minutes, during which vortex for 10 seconds every 1 minute to mix well. Centrifuge for 5 seconds to remove drops of liquid from the lid.
4. **Add 250~500μL chloroform and vortex to mix thoroughly.** Incubate for 3 minutes .
5. Centrifuge at maximum speed (⩾13,000 x g) for 10 min at 4°C.

Manual Purification

1. Transfer 400µl of the lysate to a new clean 1.5ml Tube.
2. **Add 400µl Buffer MCB and 20µl MagPure RNA Particles to the sample.** Mix up and down 20~30 times.Stay at room temperature for 10 minutes, and mix up and down for several times. Place the tube to the magnetic rack for1 minutes, until the MagPure RNA Particles have formed a tight pellet, then remove the supernatant.
3. **Add 600µl Buffer GW1 and vortex for 20 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 2 minutes.
4. **Add 300µl DNase Mixture (290µl DNase Buffer + 10µl DNase I) to the sample,** shake slightly to resuspend the particles and incubate at room temperature for 15 min.
5. **Add 450µl Buffer ALB3 to the sample** and vortex for 20 seconds. Stay at room temperature for 5 minutes and mix up and down for 2~3 times. Place the tube to the magnetic rack for 1 minutes, then remove the supernatant.
6. **Add 600µl Buffer GW1 and vortex for 10 seconds to resuspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.
7. **Add 600µl RW2 and vortex for 10 seconds to resuspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.
8. Repeat step 7.
9. Spin shortly to collect liquid on tube, place the tube to the magnetic rack. Remove all liquid carefully. Dry at room temperature or 37°C for 10 minutes.
10. **Add 30~100µl RNase Free Water to sample,** mix the particles by vortex. Stay at room temperature for 3 minutes.
11. 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.

Auto Pure by KingFisher Flex

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
Sample plate	400 µl Buffer MCB 400 µl of the supernatant
Wash Plate 1	600µl Buffer GW1, Put in 96 magnetic Tip 20µl MagPure RNA Particles
DNas Plate	290µl DNase Buffer and 10µl DNase I After pause:add 450µl Buffer ALB3
Wash Plate 2	600µl Buffer GW1
Wash Plate 3	900µl Buffer RW2
Elution plate	100µl RNase Free Water

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.
3. Start the R6622 with the KingFisher Flex 96 and load the plates.
4. **Add 450µl Buffer ALB3 to the Sample plate during the dispense step.**
5. Place the sample plate back into the instrument and press Start.
6. After the run is completed, remove the plates and store the purified total RNA at −80°C.