MagPure Total RNA Kit

Introduction

This product supplies a simple and rapid extraction of total RNA from tissue and culture cells samples. The kit is based on superparamagnetic particles purification technology, no phenol-chloroform extraction or alcohol precipitation. The whole extraction process is only 60 minutes. Purified RNA is ready for downstream applications such as RT-PCR, virus RNA testing and so on. MagPure LQ Kits buffers can be used for both manual extraction process and automatic nucleic acid extraction machines.

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.	R662201	R662202	R662203
Purification times	48 Preps	96 Preps	5 x 96 Preps
MagPure RNA Particles	1.7 ml	4.0 ml	18 ml
Proteinase K	24 mg	50 mg	240 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
RTL Lysis Buffer	40 ml	80 ml	400 ml
Buffer MCB*	18 ml	30 ml	150 ml
Buffer MW1*	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

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

- Add 56ml (48 Preps), 84ml (96 Preps) or 2 x 280ml (5 x 96 Preps) 100% ethanol to the bottle of Buffer MW1
- Add 80ml (48 Preps), 200ml (96 Preps) or 2 x 400ml (5 x 96 Preps) 100% ethanol to the bottle of Buffer RW2
- Add 42ml (48 Preps), 70ml (96 Preps) or 350ml (5 x 96 Preps) isopropanol to the bottle of Buffer MCB.
- Add 1.2ml (48 Preps), 2.5ml (96 Preps) or 12ml (5 x 96 Preps) Protease Dissolve Buffer to the bottle of Proteinase K and store at -20℃.
- (Optional) Add 20µl 2-mercaptoethanol (or 2M DTT) per 1mL RTL Lysis Buffer. This mixture can be stored for 2 weeks at room temperature

Protocol for sample prepare

1. Homogenization and lysis of samples.

1A. Cell: Harvest cells no more than 1 x 10^7 cells. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube and add the appropriate volume of 500µl Buffer RTL. For direct lysis of cells grown in a monolayer, add 500µl Buffer RLT to the cell-culture dish. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-Free Sringe.

1B. Animal Tissue : Do not use more than 20 mg Animal Tissue. Disruption and homogenization of sample, then add 600µl Buffer RTL. After lysate and centrifuge at 14,000 x g for 3 minute at room temperature.

1C: Plant Tissue: Disruption Plant sample by liquid nitrogen, Transfer up to 50mg power to 1.5ml Tube, then add 600µl Buffer RTL and mix well by vortexing.Centrifuge at 14,000 x g for 3 minute at

room temperature.

1D: Yeast Cell: Collect 5 x 10^6 yeast cells, then add 300mg 0.4-0.6g Glass Beads and 600µl RTL Lysis Buffer. Vortex at maxi speed for 10min. Centrifuge at 10,000 x g for 3 minute at room temperature.

1E: Bacterial Cell: Collect 1 x 10^8 bacterial cells, then add 300mg 0.1-0.26g Glass Beads and 600µl RTL Lysis Buffer. Vortex at maxi speed for 10min. Centrifuge at 10,000 x g for 3 minute at room temperature.

Manual Purification

1. Transfer 500µl of the lysate to a new clean 1.5ml Tube.
2. **Add 500µl Buffer MCB, 30µl MagPure RNA Particles and 20µl Proteinase K 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 MW1 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 MCB 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 MW1 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℃ 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	500 µl Buffer MCB 500 µl of the supernatant 20 µl Proteinase K
Wash Plate 1	600µl Buffer MW1, Put in 96 magnetic Tip 30µl MagPure RNA Particles
DNas Plate	300µl DNase Buffer and 10µl DNase I After pause:add 450µl Buffer MCB
Wash Plate 2	600µl Buffer MW1
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 MCB 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.