MagPure Plant RNA Kit

Introduction

This product supplies a simple and rapid extraction of total RNA from Plant and Fungal samples. The kit is based on super paramagnetic particles purification technology, no phenol-chloroform extraction or alcohol precipitation. The whole extraction process takes only 60 minutes. Purified RNA is ready for downstream applications such as RT-PCR, virus RNA testing and so on. MagPure RNA Kits buffers can be used for both manual extraction process and automatic nucleic acid extraction machines. This Kits is suitable for extracting RNA from $\leqslant 5x10^6$ cultured cells, 20mg tissue and <50mg plant samples.

Principle

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. After adding magnetic particles and binding solution, RNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing solution to remove proteins and impurities, washed with ethanol to remove salts, and finally RNA was eluted by Elution Buffer.

Kit Contents

Cat.No.	R664101	R664102	R664103
Purification times	48 Preps	96 Preps	480 Preps
MagPure RNA Particles	1.7 ml	3.5 ml	18 ml
DNase I	600 µl	2 x 600 µl	10 x 600 µl
DNase Buffer	30 ml	40 ml	200 ml
Buffer PRC1	40 ml	70 ml	350 ml
Buffer RL	40 ml	70 ml	350 ml
Buffer MCB*	18 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 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

- 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 42ml (48 Preps), 70ml (96 Preps) or 350ml (480 Preps) isopropanol and store at room temperature
- (Optional) 2-mercaptoethanol can be added to an aliquot of Buffer PRC1/RL before use. Add 20µl 2-mercaptoethanol per 1mL Buffer PRC1/RL. This mixture can be stored for 2 weeks at room temperature. Alternatively, add 20µl of 1M dithiothreitol (DTT) per 1 ml Buffer PRC1/RL. The stock solution of 1 M DTT in water should be prepared fresh or frozen in single-use aliquot Buffer. Buffer PRC1/RL containing DTT can be stored at room temperature for up to 1 month.

Protocol

1. **Determine the amount of plant material. Do not use more than 50 mg.**
It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity. A maximum amount of 50 mg plant material can generally be processed. For most plant materials, the RNA binding capacity of the Particles and the lysing, we recommend starting with no more than 30 mg plant material. Depending on RNA yield and purity, it may be possible to use up to 50 mg plant material in subsequent preparations.

2. **Immediately place the tissue in liquid nitrogen and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an 2ml microcentrifuge tube.** Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.
RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. **Add 600µl Buffer PRC1 or Buffer RL to a maximum of 50mg tissue powder. Vortex vigorously** and then centrifuge for 5 min at ⩾14, 000 x g.
   This Kit provides a choice of lysis buffers. Buffer RL is the lysis buffer of choice but Buffer RL can cause solidification of some samples, depending on the amount and type of secondary metabolites in the tissue. In these cases, Buffer PRC1 should be used.

4. **Add 500µl Buffer MCB and 30µl MagPure RNA Particles to 2.2 ml 96-well Plate.**

5. **Transfer 500µl of the supernatants from step 3 into the well of plate. Pipette mix 10 times and then shaking to mix at 700~900rpm for 10 minutes.**
   The liquid should appear homogeneous after mixing.

6. **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.

7. **Add 600µl Buffer MW1 and shaking at 900~1200rpm for 2 minute to resuspend the particles.** Place the plate to the magnetic plate for 1 minute, then remove the supernatant.

8. Leave the plate on the Magnet plate. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Pure Particles for an additional 3 minutes.

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

10. **Add 450µl Buffer MCB to the sample, shaking for 5 minutes.** Place the plate to the magnet plate for 1 minutes, then remove the supernatant.

11. **Add 600µl Buffer MW1 and shaking for 1 minute to resuspend the particles.** Place the plate on the magnet plate for 1 minutes, then remove the supernatant.

12. **Add 600µl Buffer MW2 and shaking for 1 minute to resuspend the particles.** Place the plate on the magnet plate for 1 minutes, then remove the supernatant.

13. Repeat step 12 once.

14. Leave the plate on the magnet plate. Wait 1 minute and remove residual liquid with a pipettor. Dry the Mag-Pure Particles for an additional 10 minutes.

15. **Add 50~100µl RNase Free Water to sample and mix by shaking for 5 minutes.** Place the plate to the magnet plate for 3 minutes.

16. 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	450 µl Buffer MCB 30µl MagPure RNA Particle	450µl of lysate supernatant from step 3.
Wash Plate 1	600µl Buffer MW1, Put in 96 magnetic Tip	
DNase	290µl DNase Buffer and 10µl DNase I After Pause: 450µl Buffer MCB	
Wash Plate 2	600µl Buffer MW1	
Wash Plate 3	900µl Buffer MW2	
Elution plate	50~100µl RNase Free Water	

2. Place a 96 tip comb for deep well magnets on Wash Plate 1.
3. Start the R6641_Flex protocol with the KingFisher Flex 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. 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 Particles:** Resuspend the MagPure Particles by vortexing before use.
- **Loss of MagPure Particles during procedure:** Be careful not to remove the MagPure Particles during the procedure.
- **MagPure Particles not resuspend during binding:** Vortex vigorously for 2 minutes after addition of Buffer MCB.

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..