MagPure Universal RNA Kit II

Introduction

This product supplies an fast extraction of total RNA from universal samples such as culture cells, tissue, blood, blood vessels, bones, plant, fungal, bacteria samples. Purified RNA is ready for downstream applications such as RT-PCR, virus RNA testing and so on. This kit can be used for both manual extraction and automatic nucleic acid extraction systems.

Principle

The product is based on superparamagnetic particles purification technology. Samples are lysed and digested by Magzol Reagent, and DNA/RNA is released into the lysis buffer. Genomic DNA and protein impurities are removed after adding Buffer BCP (or chloroform). RNA is absorb on magnetic particles with binding buffer. Protein and other impurities are removed by wash buffer, and salts are remove by ethanol condition. Purified is eluted in low salt buffers (RNase Free Water). .

Kit Contents

Cat.No.	R662300	R662301	R662302	R662303
Purification times	20 Preps	48 Preps	96 Preps	480 Preps
MagPure Particles N	1.0 ml	1.7 ml	3.5 ml	17 ml
MagZol Reagent	25 ml	60 ml	120 ml	2 x 250 ml
Buffer BCP	5 ml	10 ml	15 ml	60 ml
Buffer MW1*	11 ml	22 ml	44 ml	2 x 110 ml
Buffer MW2*	10 ml	20 ml	50 ml	3 x 50 ml
RNase Free Water	10 ml	15 ml	30 ml	150 ml

Storage and Stability

MagPure Particles N, MagZol Reagent and Buffer BCP 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 at least 18 months under these conditions.

Preparation before use

- Add 17ml (20 Preps), 28ml (48 Preps), 56ml (96 Preps) or 2 x 170ml (480 Preps) 100% ethanol to the bottle of Buffer MW1
- Add 40ml (20 Preps), 80ml (48 Preps), 200ml (96 Preps) or 3 x 200ml (480 Preps) 100% ethanol to the bottle of Buffer MW2

Part 1: Protocol for sample prepare

1. Homogenize and lyse according to the sample type.

- Animal tissue: Take 10-50mg tissue blocks into centrifuge tubes, add 1ml MagZol Reagent, and homogenize thoroughly with a grinding pestle or homogenizer.
- Plant tissue: Grind the plant sample into powder using liquid nitrogen, add 30-100mg of the sample into a centrifuge tube, immediately add 1ml MagZol Reagent, vortex thoroughly to disperse the sample.
- Adhesive cells: Remove the culture medium throughly and add 1ml MagZol Reagent (for 10cm^2 culture area), Pipette 3-5 times to fully lyse the cells.
- Suspension cells: Collect <5 x 10^6 cells by centrifuge at 500 x g and remove the culture medium. Vortex or tap with fingers to loose cell clusters. Add 1ml MagZol Reagent and pipette 3-5 times to fully lyse the cells.
- Whole blood/bone marrow: Take ~1ml whole blood or 0.5ml bone marrow, separate lymphocytes using lymphocyte separation medium or red blood cell lysis buffer with 50-100μl of residual liquid and precipitate left. Vortex to disperse the precipitate. Add 1ml MagZol Reagent and pipette 3-5 times to fully lyse the cells.

  Note: For bone marrow possessing a large amount of lymphocyte precipitation, when separating lymphocytes and discarding the supernatant, the amount of lymphocytes used can be visually observed and controlled. When there are too many lymphocytes, resuspend them and absorb the excess cells until the remaining amount of cell suspension is appropriate (50-100μl) for RNA extraction. It is necessary to control the white blood cell precipitation of bone marrow samples. Excessive white blood cells may cause the lysis buffer to be too viscous or decrease the extraction quality.
- Bacteria: Collect 1 x 10^8 bacteria by centrifugation, add 100μl TE/lysozyme (not provided, Magen Cat# C12136), after 10 minutes add 1ml MagZol Reagent and vortex for 1 minute.
- Trace amount fungi: Transfer <10mg fungal sample to a 2ml fungal/bacterial homogenate tube (not provided, Magen Cat# CB6-60), add 1ml MagZol reagent, and vortex at high speed for 5-10 minutes to lyse the fungi.
- Bone: Grind the bone/teeth sample into powder by liquid nitrogen with a bone meal grinder/bead mill. Transfer 100~200mg sample into a centrifuge tube, immediately add 1ml MagZol Reagent, vortex thoroughly to disperse the sample.
- Blood vessels: Take 10-100mg blood vessels into centrifuge tubes, add 1ml MagZol Reagent, and homogenize thoroughly with a grinding pestle or homogenizer.

2. Add 100µl Buffer BCP (for every 1ml MagZol Reagent) to the lysis buffer . Shake vigorously by hand for 15 seconds and place at room temperature for 3 minutes.

   Note: Buffer BCP is a safe substitute of Chloroform. This step requires a high speed and fiercely shaking by hand, while slow inverting and mixing can lead to insufficient extraction. DO NOT use vortex to replace shaking, as vortex mixing can lead to more DNA contamination.

3. Centrifuge at 12,000 x g for 15 minutes at 4°C, get the sample in supernatant . Follow the protocol in Part 2/3.

Part 2: Manual Purification Protocol

1. Add 500µl isopropanol and 30µl MagPure Particles N into a 1.5ml centrifuge tube, add 500µl supernatant (Step 3 in Part 1), invert 15-20 times to mix. Place at room temperature for 10 minutes, during which invert several times to mix. Place the tube to the magnetic rack for 2 minutes, until the MagPure Particles N have formed a tight pellet, then remove the supernatant.
2. Add 500µl Buffer MW1 and vortex for 15 seconds. Place the tube to the magnetic rack for 1 minute, until the MagPure Particles N have formed a tight pellet, then remove the supernatant.
3. Add 500µl Buffer MW2 and vortex for 15 seconds. Place the tube to the magnetic rack for 1 minute, until the MagPure Particles N have formed a tight pellet, then remove the supernatant.
4. Repeat Step 3 once.
5. Centrifuge shortly to collect the liquid on the tube wall, and remove all the reagent. Dry at room temperature for 10 minutes.
6. Add 30-100µl RNase Free Water to the sample, and vortex to disperse the magnetic beads. Place at room temperature for 5 minutes.
7. 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 -20°C or -80°C.

Part 3: Auto purification by 32/48 channel Extractor

1. Add the Reagents/sample to the well of the 96 well deep plate according to the table below.

Well of the Plate	Pre-loaded reagents
Row 1/7	500µl isopropanol + 30µl MagPure Particles N
Row 2/8	500µl Buffer MW1
Row 3/9	empty
Row 4/10	500µl Buffer MW2
Row 5/11	500µl Buffer MW2
Row 6/12	70µl RNase Free Water

2. Add 400-500μl supernatant to the well of Row 1/7 (steps 3 of Part 1).
3. Insert the 8 strip Tip into the corresponding position of the instrument. Place the plate into the corresponding position of the instrument. (The well of A1 is placed towards the left inner corner)
4. Start the program #R6623.
5. Finish the operation after ~30 minutes. Remove the 96-well plate and magnetic Tip.
6. Transfer RNA to a 1.5ml centrifuge tube and store the product at -20°C or -80°C

Recommend program for MagMix 32/48 extractor

No	Name	Well	Volume	Mix		Wait		Magnet			Magnet	Heat	
			μl	Time	Speed	Time	Position	Up/ Down	Surface	Bottom	Auto	Plate	Temp.
1	Magnet	4	500	20s	8	0	0	60s	0	0	Auto	/	/
2	Bind1	1	900	400s	7	0	0	90s	30	30	Auto	/	/
3	Wash1	2	500	90s	8	0	0	90s	10	10	Auto	/	/
4	Wash2	4	500	60s	8	0	0	60s	0	0	Auto	/	/
5	Wash3	5	500	60s	8	0	0	60s	0	0	Auto	/	/
6	Dry	5	500	0	8	4min/Dry		0	0	0	Auto	/	/
7	Elute	6	100	240s	8	0	0	60s	0	50	Auto	/	/
8	Remove	5	500	30s	9	0	0	0	0	0	Auto	/	/