【Product Name】 MagPure Universal RNA Kit

【Product specifications】 20Preps, 200 Preps/Kit

【Intended Use】

This product is suitable for rapid RNA extraction from tissue , cells, and other clinical samples. RNA can be used directly for RT-PCR, quantitative RT-PCR and so on.

【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 I. The magnetic particles are efficiently washed, and RNA is eluted in RNase-free water

【Main Composition】

Cat.No.	IVD3020-20	IVD3020	Main Composition
Purification times	20	200	-
MagPure RNA Particles	1 ml	7 ml	Magnetic Particles
DNase I	600 µl	4 x 600 µl	DNase I
DNase Buffer	15 ml	80 ml	Tris/MgCl2
RTL Lysis Buffer	15 ml	150 ml	Guanidine Salt
Buffer MCB*	9 ml	75 ml	Guanidine Salt
Buffer MW1*	13 ml	110 ml	Guanidine Salt
Buffer MW2*	6 ml	50 ml	Tris/NaCl
RNase Free Water	5 ml	60 ml	DEPC-Treated Water

【Storage conditions and Validity】

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.

【Preparation before Use】

- Add 17ml (20 Preps) or 140ml (250 Preps) 100% ethanol to the bottle of MW1.
- Add 24ml (20 Preps) or 200ml (250 Preps) 100% ethanol to the bottle of MW2.
- Add 21 ml (20 Preps) or 175 ml (250 Preps) isopropanol to the bottle of MCB.
- Optional) Add 20µl 2-mercaptoethanol (or 2M DTT)per 1mL RTL Lysis Buffer. This mixture can be stored for 2 weeks at room temperature.

Part 1: Sample preparation protocol

a. **Cells Grown in Suspension (3~5 x 10^6 cells)**
Transfer cells culture fluid to a new centrifuge tube, centrifuge at 500 x g for 10 min. Remove culture fluid completely, and loose the cell pellet throughly by vortex or flicking the tube. Add 500~600µl of RTL Lysis Buffer to the tube and vortex vigorously to mix the cells throughly.

b. **Cells Grown in Monolayer (3~5 x 10^6 cells)**
Lyse cells directly in a culture dish by adding 600µl of RTL Lysis Buffer and pipetting the cell lysate several times through a blue pipette tip. Use more RTL Lysis Buffer if in the lysate is too viscous to aspirate with a pipette.

c. **Animal Tissue (10~20 mg Tissue)**
Homogenize no more than 20mg tissue samples in 600µl RTL Lysis Buffer using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube and add RTL Lysis Buffer. After lysate, centrifuge at 14,000 x g for 3 minute at room temperature.

d. **Plant Tissue (Do not use more than 100 mg Tissue)**
Disruption Plant sample by liquid nitrogen and transfer up to 100mg power to 1.5ml Tube.Add 600µl RTL Lysis Buffer to the sample and mix well by vortex vigorously. Centrifuge at 14,000 x g for 3 minute at room temperature.

e. **Trizol/MagZol Regeant (without chloroform extraction):**
Following Trizol/MagZol Reagent protocol, add 600µl MagZol Reagent to lyse the sample. After lysate, centrifuge at 12,000 x g for 10 minutes at 2-8°C.

f. **Trizol/MagZol Regeant (witht chloroform extraction):**
Following Trizol/MagZol Reagent protocol, add 1ml Trizol/MagZol Reagent to lyse the sample. After lysate, add 200µl chloroform to the lysate and centrifuge at 12,000 x g for 15 minutes at 2-8°C.

Part 2: Manual Purification Protocol

1. Add 30µl MagPure RNA Particles and 500µl Buffer MCB to a new clean 1.5ml tube.
2. **Transfer 500µl of the lysate or the supernatants from Sample Preparation (Part 1) step into the tube.** Mix up and down 15~30 times.Stay at room temperature for 10 minutes, and mix up and down for several times. Place the tube to the magnetic rack for 2 minutes, until the MagPure RNA Particles have formed a tight pellet, then remove the supernatant.
3. **Add 500µl Buffer MW1 and vortex for 10 seconds to resuspend the particles.** Place the tube to the magnetic rack for 1 minute, then remove the supernatant.

4. Spin shortly to collect liquid on tube and remove all liquid carefully. Dry on air for 5 minutes
5. **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 10~15 min to lysis DNA.
6. **Add 450µl Buffer MCB to the sample and vortex for 10 sec.** 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.
7. **Add 500µl Buffer MW1 and vortex for 10 sec to resuspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.
8. **Add 500µl Buffer MW2 and vortex for 10 sec to resuspend the particles.** Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.
9. Repeat step 8 once.
10. Spin shortly to collect liquid on tube and remove all liquid carefully. Dry on air for 10~15 minutes .
11. **Add 50~100µl RNase Free Water to sample and,** mix the particles by vortex. Stay at room temperature for 3 minutes.
12. 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 2: Auto purification by 32/48 channel Extractor

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

Raw in the Plate	Pre-loaded reagents	Addition before use
Raw 1/7	500µl Buffer MCB	400~450µl cell lysate or lysate supernatant from Part 1.
Raw 2/8	500µl Buffer MW1 30µl MagPure RNA Particle	
Raw 3/9	290µl DNase Buffer and 10µl DNase I	
Raw 4/10	500µl Buffer MW1	
Raw 5/11	500µl Buffer MW2	
Raw 6/12	50~100µl RNase Free Water	

2. Turn on the machine, put the plate and 8 strip tip into the machine, start the IVD3020-TL-06 protocol.
3. The program pause at about 25 minutes. Take out the plate and **add 450µl Buffer MCB to each well of Raw 3/9.**
4. Place the plate back into the instrument and continue the program.
5. After the run is completed, take out the plates and tips.
6. Transfer the purified total RNA into new 1.5ml centrifuge tubes and store at -20 °C.

Part 2: Auto purification by 96 channel Extractor

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

Name of the Plate	Pre-loaded reagents	Addition before use
Sample plate	500µl Buffer MCB	400~450µl cell lysate or lysate supernatant from Part 1.
Wash Plate 1	500µl Buffer MW1, Put in 96 magnetic Tip 30µl MagPure RNA Particle	
DNase Plate	290µl DNase Buffer and 10µl DNase I	
Wash Plate 2	500µl Buffer MW1	
Wash Plate 3	500µl 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 IVD3020-F-96 protocol and load the plates.
4. The program pause at about 25 minutes. Take out the DNase plate and **add 450µl Buffer MCB** to the plate.
5. Place the DNase plate back into the instrument and continue the program.
6. After the run is completed, remove the plates and store the purified total RNA at -20°C.