

**【Product Name】** MagPure Universal RNA Kit B

**【Product specifications】** 20Preps, 200 Preps/Kit

**【Intended Use】**

This product is suitable for rapid extraction of RNA from low RNA yield samples such as tissue (<10mg), cells, bone marrow, fresh blood, and other clinical samples. RNA can be used directly for RT-PCR, Real time PCR, NGS, Viral RNA detection 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.	IVD3020B-20	IVD3020B-96	IVD3020B
Purification times	20	96	200
MagPure Particles N	0.6 ml	2.5 ml	5 ml
DNase I	600 µl	2 x 600 µl	4 x 600 µl
DNase Buffer C	15 ml	60 ml	120 ml
Buffer RLC	15 ml	60 ml	120 ml
Buffer MCB*	15 ml	60 ml	150 ml
Buffer GW1 *	13 ml	44 ml	110 ml
Buffer MW2*	10 ml	50 ml	100 ml
RNase Free Water	10 ml	20 ml	60 ml

**【Storage conditions and Validity】**

DNase I should be shipped with ice pack and stored at -20°C after arrival. MagPure Particles N should be stored at 2–8°C for long time storage. The remaining kit components can be stored at room temperature (15–25°C) for up to 18 months under these conditions.

**【Preparation before Use】**

- Add 35 ml (20 Preps), 140 ml (96 Preps) or 350 ml (200 Preps) isopropanol to the bottle of MCB.
- Add 17ml (20 Preps), 56 ml (96 Preps) or 140ml (200 Preps) 100% ethanol to the bottle of GW1.
- Add 40ml (20 Preps), 200 ml (96 Preps) or 400ml (200 Preps) 100% ethanol to the bottle of MW2.

**【Part 1: Sample Preparation】**

**a. Cells Grown in Suspension (no more than  $3 \times 10^6$  cells )**

Transfer appropriate cells culture to a centrifuge tube, centrifuge at 500 x g for 10 minutes to collect cells. Remove the supernatant completely. Loosen the cell pellets by vortex or flicking the tube, add 500µl lysis Buffer RLC and pipette several times to mix. When the lysate mixture is viscous, pipette 3~5 times by 1ml syringe to make a non-viscous mixture ( break the genomics).

**b. Cells Grown in Monolayer/adherent cells( no more than  $3 \times 10^6$ cells )**

Pipette to remove the culture, then add 500µl lysis Buffer RLC to the culture dish with cells. Pipette the buffer several times to spreading cells from dish. Then transfer the lysate mixture to a 1.5ml centrifuge tube. When the lyse mixture is viscous, pipette 3-5 times to make a non-viscous mixture (break the genomics).

**c. Animal Tissue ( Do not use more than 10 mg Tissue)**

Transfer tissue sample into a 1.5ml centrifuge tube and add 500µl Buffer RLC to lysis, Centrifuge at 14,000 x g for 3 minutes at room temperature.

**d. Plant Tissue ( Do not use more than 30 mg Tissue)**

Grind the Plant sample into powder by liquid nitrogen, and then transfer the powder to a 1.5ml centrifuge tube. Add 500µl lysis Buffer RLC to the sample and vortex for 10 seconds immediately to mix. Centrifuge at 13,000 x g for 3 minute at room temperature.

Note: for difficult extraction plant sample that contains polysaccharide and polyphenol, recommend to use Buffer RLF (purchase separately) to replace Buffer RLC. Buffer RLF will reduce the oxidation of polysaccharide and polyphenols. For sample rich in starch that is easy in gelatinization (such as cereal and potato), recommend to use Buffer PAL/ phenol chloroform (purchase separately) to replace Buffer RLC.

**e. Whole Blood (0.5~1.0 ml fresh blood or bone marrow and fresh blood mixture)**

Separate the leukocytes cell from 0.5~1.5ml Whole blood by red blood cells lysis buffer (RBC Buffer purchase separately). Discard the supernatant and leave ~30µl liquid, resuspend the leukocytes pellets

completely by vortex. Add 500µl lysis Buffer RLC, pipette several times to mix the lysate. When the lysate mixture is too viscous, pipette 3~5 times by 1ml syringe to make a non-viscous mixture ( break the genomics).

**Part 2: Manual Purification Protocol**

1. Add 20µl MagPureParticles N and 500µl Buffer MCB to a new clean 1.5ml centrifuge tube.
2. Transfer 500µl of the lysate or the supernatants from Sample Preparation (Part 1) step into the tube. Mix by inverting 15~20 times. Stay at room temperature for 10 minutes, and invert for several times. Place the tube to the magnetic rack for 2 minutes, until the MagPure Particles N have formed a tight pellet, then remove the supernatant.
3. Add 500µl Buffer GW1 and vortex for 10 seconds to resuspend the particles. Place the tube to the magnetic rack for 1 minute, then remove the supernatant.
4. Add 500µl DNase Mixture (490µl DNase Buffer C + 10µl DNase I) to the sample. Oscillating and incubate at room temperature for 15 min to lysis DNA. (Cat# MagMix B). Place the tube to the magnetic rack for 1 minutes, then remove the supernatant.
5. Add 500µl Buffer GW1 and vortex for 10 sec to resuspend the particles. Place the tube on the magnetic rack for 1 minutes, then remove the supernatant.
6. 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.
7. Repeat Step 6 once.
8. Spin shortly to collect liquid on tube and remove all liquid carefully. Dry on air for 10~15 minutes .
9. Add 30~100µl RNase Free Water to sample and, mix the particles by vortex. Stay at room temperature for 3 minutes.
10. 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 GW1 20µl MagPure Particles N
Raw 3/9	490µl DNase Buffer C and 10µl DNase I
Raw 4/10	500µl Buffer GW1
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 IVD3020B-TL-06 protocol.
3. After the run is completed at about 35 minutes, take out the plates and tips.
4. 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 GW1, Put in 96 magnetic Tip 20µl MagPure Particles N	
DNase Plate	490µl DNase Buffer C and 10µl DNase I	
Wash Plate 2	500µl Buffer GW1	
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 IVD3020B-F-96 protocol and load the plates.
4. After the run is completed at about 35 minutes, take out the plates and tips
5. Store the purified total RNA at -20°C.