Product Name】 MagPure Blood RNA Precast Kit (Auto Pure 32)

【Product Specification】 16 Preps/Kit, 96 Preps/Kit

【Intended Use】

This product is suitable for extracting RNA from anticoagulant blood, lymphocytes, buffy coat, bone marrow, cultured cells and other clinical samples. The kit is based on the purification method of high binding magnetic particles. The purified RNA can be directly used for experiments such as RT-PCR,NGS and virus detection.

【Principle】

The sample is lysed and digested by lysis buffer and protease, and RNA/DNA is released into the lysis buffer. Add binding solution and magnetic particles to adsorb RNA/DNA, while proteins are not adsorbed and removed. The particles adsorbed with DNA/RNA are washed with washing buffer to remove proteins and other impurities, then washed with ethanol to remove salt, and finally digested with DNase to remove DNA. RNA is recovered by adding binding solution, and finally the RNA is eluted with low salt buffer.

【Main Composition】

Cat.No	Pre-filled Reagent	R6611-TL-01	R6611-TL-06
10 x RBC Buffer		10 ml	2 x 50 ml
Proteinase K		12 mg	24 mg
Protease Dissolve Buffer		1.8 ml	1.8 ml
DNase I		600 µl	2 x 600 µl
DNase Buffer		15 ml	30 ml
RTL Lysis Buffer		15 ml	60 ml
Buffer ALB2		15 ml	60 ml
AS Tip		2 PCS	12 PCS
2.0ml V-bottom plate	Row 1/7: 500µl Buffer MCB	1 Plates	6 Plates
	Row 2/8: 500µl Buffer MW1		
	Row 3/9: empty		
	Row 4/10: 500µl Buffer MW2 20µl Magpure Particles N		
	Row 5/11: 500µl Buffer MW2		
	Row 6/12: 70µl RNase Free Water		

【Storage conditions and validity】

DNase I should be shipped with ice pack and stored at -20°C after arrival. Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 8 weeks) at room temperature (15–25°C) does not affect their performance.The remaining kit components can be store at room temperature and are stable for up to 18 months under these conditions.

【Applicable Instrument】

Nucleic Acid Extraction Machine such as Auto Pure 32 (Allsheng) or similar.

【Preparation before Use】

- Dissolve the Proteinase K with 0.6ml (R6611-TL-01), 1.2ml (R6611-TL-06) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃.
- Dilute 10 x RBC Buffer with 90ml (R6611-TL-01), 2 x 450ml (R6611-TL-06) nuclease free water and store at room temperature..

【Part 1: Sample Preparation】

1. **Add 1-1.5ml anticoagulant blood or 0.5-1ml bone marrow into a 15ml centrifuge tube. Add 3 times volume of 1x RBC Lysis Buffer into the tube, invert 5-10 times to mix. Place on ice for 10 minutes, during which invert twice to mix.**
   - This kit provides with 10 x RBC Lysis Buffer, it must dilute to 1 x with nuclease free water before use. As the patient's white blood cells may increase, the blood sample amount needs to be adjusted to ensure that the white blood cell count does not exceed 1 x 10^7. For example, add 4.5ml 1 x RBC Lysis Buffer to 1.5ml blood sample. During the lysis process, the blood will change from a mist to a transparent solution. A clear solution indicates that red blood cells have been lysed. When processing patients' blood, it may be necessary to extend lysis to 20 minutes.
2. Centrifuge at 2,000 x g for 10 minutes at 4℃, remove the supernatant carefully.
3. **Add double volume of 1 x RBC Lysis Buffer to the tube (e.g. 1.5ml blood sample add 3ml 1 x RBC Lysis buffer), resuspend the cells by vortex. Centrifuge at 2,000 x g for 10 minutes at 4℃. Remove the supernatant carefully by pipette, leaving ~50µl of residual liquid and lymphocytes, resuspend the lymphocytes by vortex.**
   - When processing with bone marrow samples, use the sample less than 500µl.

- If whole blood and bone marrow are mixed together and can not recognize bone marrow volume, it can be judged by the amount of cell precipitation after centrifugation.
- Most bone marrow samples get large amount of white blood cells precipitation after centrifugation, it is 3~5 times amount comparing to whole blood samples. It is necessary to control the amount of white blood cells in bone marrow samples, as excessive white blood cells may cause the lysate become too viscous and affect the extraction results.
- If the amount of white blood cell precipitation is too high, leave more residual liquid (such as 200-300μl) when discard the supernatant. After resuspension by vortex, keep 50μl cell suspension for RNA extraction, store the excess suspension at -70℃ for further use.
- If the amount of cell precipitation is normal and equivalent to conventional blood sample, only leave 50μl residual liquid. Resuspension by vortex and follow step 4 operation.

4. Add 500μl Buffer RTL, immediately vortex for 10 seconds to mix. Use a 1ml syringe to aspirate 5 times or use a pipette to aspirate repeatedly to homogenize the sample .

Recommend program for Auto Pure 32 machine

No	Step Name	Well	Volume (ul)	Mix time (sec)	Mix speed (1-10)	Drying time (sec)	Tem (37~120℃)	Magnet Segments (1-5)	Every magnet (sec)	Liquid level magnetic (sec)	Cycle (1-10)	Magnet speed (1-10)
1	Mix	1	900	120	7	0	/	0	0	0	0	0
2	Wash	2	500	20	8	0	/	0	0	0	0	0
3	Collect	4	500	20	8	0	/	1	60	0	1	1
4	Sample	1	900	360	7	0	/	1	100	10	1	1
5	Wash 1	2	500	90	8	0	/	1	100	10	1	1
6	Dry	2	500	0	0	180	/	0	0	0	0	0
7	DNase	3	200	600	8	0	/	0	0	0	0	0
8	Pause	3	300	0	0	0	/	0	0	0	0	0
9	Rebind	3	700	300	8	0	/	1	100	15	1	1
10	Wash 2	4	500	60	8	0	/	1	60	0	1	1
11	Wash 3	5	500	60	8	0	/	1	60	0	1	1
12	Dry	5	500	0	0	180	/	0	0	0	0	0
13	Elute	6	70	240	8	0	/	1	60	0	1	1
14	Drop	5	500	30	9	0	/	0	0	0	0	0

【Part 2: Auto Pure 32 nucleic acid extractor operation】

1. Take out the components of the kit. Invert the Plates several times to re-suspend the magnetic beads. Remove the sealing bag and sealing film.
2. Transfer 500μl homogenate (from Step 4 in Part 1) into each well of Raw 1/7.
3. Add 200μl DNase Mixture (180μl DNase Buffer +10μl DNase I +10μl Proteinase K) into each well of Raw 3/9.
4. Turn on the machine, load the AS-tip and reagent plates (A1 on left inner corner) on the machine.
5. Edit the R6611-TL-06 program and save. Start the program.
6. The program pause after about 20 minutes. Take out the plate and add 500μl Buffer ALB2 to teach well of Raw 3/9.
7. Place the plate back into the instrument and continue the program.

Note: if the instrument doesn't have pause setting, edit in two programs.

8. The program finish after about 30 minutes, take out the plates and tips.
9. Transfer the purified RNA into new 1.5ml centrifuge tubes and store at -20~8 ℃.