【Product Name】MagPure Pathogen RNA Kit

【Product specifications】50Preps/Kit, 200 Preps/Kit

【Intended Use】

This product is suitable for extracting total pathogen RNA from biological samples with no/low cell content such as body fluid, serum, plasma, soaking solution, tissue homogenate supernatant, culture medium supernatant, etc. The purified RNA can be used for clinical in vitro detection.

【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 Buffer NFW.

【Main Composition】

Cat.No	IVD6672C50	IVD6672C	Contents
2ml Bead Tube	50	4 x 50	Glass beads
MagPure Particle	1.6 ml	7.0 ml	Magnetic beads
Proteinase K	24 mg	100 mg	Protease K/Poly A
Protease Dissolve Buffer	1.8 ml	6 ml	Glycerol
DTT （Powder)	0.5 g	2 g	DTT
Buffer SDS	3 ml	15 ml	SDS
Buffer MLBN	60 ml	220 ml	Tween-20/Guanidine Salt
DNase I	0.6 ml	4 x 0.6 ml	DNase I
DNase Buffer	15 ml	60 ml	MgCl2, CaCl2, Tris,
Buffer MW1*	22 ml	53 ml	Guanidine Salt
Buffer MW2*	20 ml	50 ml	Tris/NaCl
Buffer NFW	10 ml	30 ml	DEPC treated water

【Storage conditions and validity】

MagPure Particles and Proteinase K, DNase I should be stored at 2–8°C upon arrival. However, short-term storage (up to 2 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 18 months under these conditions.

【Preparation before Use】

- Add 28ml (50 Preps) or 67ml (200 Preps) absolute ethanol to the bottle of Buffer MW1.
- Add 80ml (50 Preps) or 200ml (200 Preps) absolute ethanol to the bottle of Buffer MW2.
- Add 1.2ml (50 Preps) or 2.5mL (200 Preps) Protease Dissolve Buffer and store at -20-8°C.

【 Protocol 】

A: Manual operation

1. **Add ~0.5ml samples, 50μl Buffer SDS and 20μl Proteinase K into 2ml Bead tube.** Close the lid and vortex at maximum speed for 10 minutes or place on a bead beater machine (such as FastPrep-24) grind for 60~90 seconds.
   - It is recommended to use MagMix A for vortex, which can process 24 samples at the same time.
   - For samples rich in somatic cells (whole blood, blood water, fluid accumulation, sputum liquefaction, tissue homogenate, saliva, etc.): Centrifuge at 1,000~1,500 x g for 10 minutes to remove excess somatic cells, then transfer the supernatant for next process.
   - For dry swab/solid tissue samples, transfer the samples directly to the bead tube, then add 500μl buffer PBS or normal saline.
   - Sputum samples shall be fully liquefied with 4~5 volume of 0.1% DTT before operation. Transfer 1-2ml sputum to 15ml centrifuge tube and add 4~5 volume of 0.1% DTT. Vortex to mix well and incubate at room temperature for 15-30min. During the incubation, it is necessary to continuously swirl and oscillate until the clumps disappear and the sputum is completely homogenized. Centrifuge at 2,500~4,000 x g for 10min to collect cells. Remove excess supernatant and remaining 500ul of supernatant and sediment. Vortex to resuspend the cell.
2. **Incubate at 55°C for 10 minutes, Briefly centrifuge to collect the droplets on the tube.**
   - For tissue samples, centrifuge at 13,000 x g for 5 minutes to remove cell debris.
3. **Transfer 250~300μl of the sample into a new centrifuge tube, add 30μl MagPure Particles and 500μl Buffer MLBN.** Mix by upside down for 10-15 times. Incubate at room temperature for 6 minutes, during which mix by upside down several times. Place on the magnetic rack for 2 minutes, discard the supernatant.
4. **Add 500μl Buffer MW1 and vortex for 15 seconds.** Place on the magnetic rack for ~1 minutes, discard the supernatant. Spin shortly to collect liquid on tube and remove all liquid carefully. Dry on air for 3 minutes.
5. **Add 250μl DNase Mixture (240μl DNase Buffer + 10μl DNase I) to the sample,** shake slightly to resuspend the particles and incubate at room temperature for 10 min.

6. **Add 500μl Buffer MLBN to the sample** and vortex for 20 seconds. 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 MW2 and vortex for 15 seconds.** Place on the magnetic rack for ~1 minutes, discard the supernatant.

8. Repeat Step7 again.

9. Briefly centrifuge, discard the supernatant, air dry for ~10 minutes.

10. **Add 50μl buffer NFW, vortex to disperse the magnetic beads.** Place for 5-10 minutes, during which vortex for several times to dissolve the nucleic acid. Place on the magnetic rack for 3 minutes.

11. **Transfer the purified RNA to a new 1.5 ml centrifuge tube. Store at -20~8°C.**

B: Process of 32/48-channel nucleic acid extractor

1. Add the buffer to the deep well plate according to the following table. Add 250-300μl of the sample (following manual operation step 1~2) to Row 1/7.

Row of hole	Pre-loaded reagents	Addition before use
Row 1/7	500 μl Buffer MLBN	200~300μl of the samples
Row 2/8	500μl Buffer MW1	
Row 3/9	240μl DNase Buffer + 10μl DNase I	
Row 4/10	500μl Buffer MW2,30μl MagPure Particles	
Row 5/11	500μl Buffer MW2	
Row 6/12	50~100μl Buffer NFW	

2. Turn on the machine, insert the magnetic tip, put the 96-well plate into the instrument.

3. **Add 500μl Buffer MLBN to the Row 3/9 during the dispense step.**

4. Place the sample plate back into the instrument and press Start.

5. It takes about 25~30 minutes. Take out the 96-well plate and magnetic tip.

6. Transfer RNA to 1.5 ml centrifuge tube. Store at -20~8°C.

Step	Name	Well	Volume	Mix		Wait		Magnet			HEAT	
				Time	Speed	Time	Pos	Up& Down	Up	Bottom	Well	Tem.
1	Collect	4	500	20s	8	0	0	60s	0	0	/	/
2	Bind	1	700	300s	8	0	0	90s	10	10	1	65
3	W1	2	500	90s	8	0	0	60s	0	0	/	/
4	dry	2	500	0	8	3min	0	0	0	0	/	/
5	DNase	3	250	600s	7	0	0	0	0	0	/	/
6	Pause	3	250	0	7	Pause		0	0	0	/	/
7	ReBind	3	800	240s	8	0	0	90s	0	0	/	/
	W3	4	500	60s	8	0	0	60s	0	0	/	/
5	W4	5	500	60s	8	0	0	60s	0	0	/	/
6	Dry	5	500	0	8	5min	0	0	0	0	/	/
7	Elute	6	100	240s	9	0	0	60s	0	50	6	55
8	Drop	5	500	30s	9	0	0	0	0	0	/	/

(Program for MagMix 32/48)

C: Process of 96-channel nucleic acid extractor

1. Add the buffer to the deep well plate according to the following table.

Name of Plate	Pre-loaded reagents	Addition before use
Sample Plate	500 μl Buffer MLBN	250~300μl homogenate mixture
Washing Plate1	500μl Buffer MW1，place 96 tip comb	
DNase Plate	240μl DNase Buffer + 10μl DNase I	
Washing Plate2	500μl Buffer MW2, 30μl MagPure Particles	
Washing Plate3	500μl Buffer MW2	
Elution plate	50~100μl Buffer NFW	

2. Add 250-300μl of the sample (following manual operation step 1~2) to sample plate.

3. Turn on the machine, start the program, place the 96-well plate in to the instrument.

4. **Add 500μl Buffer MLBN to the DNase plate during the dispense step.**

5. It takes about 20-30 minutes on machine running.

6. Take out the 96-well plate and tip comb when finish. Store RNA at -20~8°C.