MagPure Pathogen DNA/RNA Kit B

Introduction

This kit is suitable for extracting total pathogen nucleic acid from a variety of clinical samples (including serum and plasma). The kit is based on super paramagnetic particles purification technology. Purified DNA/RNA is ready for downstream applications such as Real Time PCR, biochip analysis, NGS and other related experiments.

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, DNA/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 DNA/RNA was eluted by Elution Buffer.

Kit Contents

Cat.No.	R667200B	R667202B
Purification times	24 Preps	96 Preps
2ml Bead Tubes	24	96
Proteinase K	12 mg	50 mg
Protease Dissolve Buffer	1.8 ml	3 ml
Buffer SDS (20%)	1.8 ml	6 ml
Particles MPN9	0.6 ml	2.5 ml
Buffer MLB	15 ml	60 ml
Buffer MW1*	13 ml	44 ml
Buffer MW2*	6 ml	50 ml
Buffer AVE	5 ml	30 ml

Storage and Stability

Particles MPN9 and 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 stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

Preparation before Use

- Add 0.6ml (24 Preps) or 2.5ml (96 Preps) Protease Dissolve Buffer to the bottle of Proteinase K, and store at -20-8°C.
- Add 17ml (24 Preps) or 56ml (96 Preps) 100% ethanol to the bottle of MW1.
- Add 24ml (24 Preps) or 200ml (96 Preps) 100% ethanol to the bottle of MW2.

Protocol

Part I: Sample Pre-treatment

1. **Add 50µl Buffer SDS (20%) to a 2ml Bead Tubes, transfer ~0.5ml sample (plasma, serum, body fluid, homogenate suspension, culture solution, cell suspension, soaking solution or concentrate pathogen solution) to the tube and screw the lid.**

- When processing samples rich in cells (such as whole blood, culture cells, fluid accumulation or tissue homogenate solution), centrifuge at 500 x g for 5 minutes to remove excess body cells, then transfer the supernatants for next process.
- When processing Sputum or lavage fluid samples, fully liquefied the sample with fresh prepared DTT reagent before operation.

2. Vortex at maximum speed for 10 minutes or place on a bead grinding machine for fast grinding with 60~120 seconds.

- PowerLyzer grinder: recommend 2000rpm for 30s, pause for 30s and then repeat once.
- FastPrep 24 grinder: recommend 5m/s for 30s, pause for 30s, and then repeat once.
- Tissue Lysis II grinder: recommend 25Hz for 5min, reposition and then repeat once.

3. Centrifuge the bead tubes at 13,000 x g for 5 minutes, process according to the manual operation in Part II or automated extraction machine in Part III or Part IIII.

Part II: Manual operation

1. **Transfer 250µl of the sample (supernatant at Step 3 in Part I) into a new centrifuge tube. Add**

20µl Proteinase K, 20µl Particles MPN9 and 500µl binding Buffer MLB to the sample. Invert for 10-15 times to mix. Place at room temperature for 10 minutes, during which invert several times. Place the tube to the magnetic rack for ~5 minutes, until the Particles MPN9 have formed a tight pellet. Remove the supernatant carefully, spin shortly to collect the liquid on the tube, then remove the residual liquid.

2. **Add 500µl Buffer MW1 and vortex for 10 seconds.** Place the tube on the magnet plate for 1 minutes, then remove the supernatant.

3. **Add 500µl Buffer MW2 and vortex for 10 seconds.** Place on the magnet plate for 1 minutes, then remove the supernatant.

4. Repeat step 3 once.

5. Spin shortly to collect liquid on tube, place the tube to the magnetic rack. Remove all liquid carefully. Dry at room temperature for 3~5 minutes.

6. **Add 50~100µl Buffer AVE, incubate at 55** °C with oscillating for 5~10 minutes to dissolve the DNA/RNA. (Magen Thermostatic oscillating metal bath machine cat# MagMix B)

7. Place the tube to the magnetic rack for 3 minutes. Transfer the supernatant containing the purified DNA/RNA to new 1.5ml centrifuge tubes. Store DNA/RNA at -20~8°C.

Part III: Process of 32-channel nucleic acid extractor

1. Add the Reagent/sample to the deep well plate according to the following table.

2. Transfer 250µl sample (supernatant at Step 3 in Part I) to Row 1/7.

Row of hole	Pre-loaded reagents	Addition before use
Row 1/7	500 µl Buffer MLB	250µl sample, 20µl Proteinase K.
Row 2/8	500µl Buffer MW1	
Row 3/9	500µl Buffer MW2, 20µl Particles MPN9	
Row 4/10	500µl Buffer MW2	
Row 5/11		
Row 6/12	50~100µl Buffer AVE	

3. Turn on the machine, start the program, place the magnetic tip and the plates in to the machine.

4. After the program finish at about 30 minutes, take out the plates and magnetic tip.

5. Transfer DNA/RNA to a new 1.5 ml centrifuge tube. Store at -20~8°C.

Part IIII: Process of 96-channel nucleic acid extractor

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

2. Transfer 250μl sample (supernatant at Step 3 in Part I) to Sample Plate.

Name of Plate	Pre-loaded reagents	Addition before use
Sample Plate	500 μl Buffer MLB	250μl sample, 20μl Proteinase K.
Washing Plate1	500μl Buffer MW1	
Washing Plate2	500μl Buffer MW2, 20μl Particles MPN9	
Washing Plate3	500μl Buffer MW2	
Elution plate	50~100μl Buffer AVE	

3. Turn on the machine, start the program, place the tip comb and plates in to the machine.
4. Start the program. After the program finish at about 30 minutes, take out the plates and tip comb.

5. Store the Elution plate containing purify DNA/RNA at -20~8°C.

Recommend program for MagMix 32 extractor (Magen

Step	Name	Well	Volume	Mix		Wait		Magnet			HEAT	
				Time	Speed	Time	Pos	Up& Down	Up	Bottom	Well	Tem.
1	Collect	3	500	0.5min	8	0	0	90s	0	0	/	/
2	Bind	1	800	5min	8	0	0	90s	30s	30s	/	/
3	W1	2	500	1min	8	0	0	60s	10s	10s	/	/
4	W2	3	500	0.5min	8	0	0	60s	0	0	/	/
5	W3	4	500	0.5min	8	0	0	60s	0	0	/	/
6	Dry	4	500	0	0	2	dry	0	0	0	/	/
7	Elute	6	100	5min	9	0	0	90s	0	40s	6	55
8	Drop	2	500	0.5min	9	0	0	0	0	0	/	/