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	R6672-02B
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	1.5 ml	10 ml
Reagent DX	1.5 ml	3 ml
MagBind Particles	1.0 ml	4.0 ml
Buffer MLB	15 ml	60 ml
Buffer MW1*	13 ml	44 ml
Buffer MW2*	6 ml	25 ml
Buffer AVE	5 ml	30 ml

Storage and Stability

MagBind Particles 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 100ml (96 Preps) 100% ethanol to the bottle of MW2.

Protocol

Part A: Sample Pre-treatment

1. Add 50μl Buffer SDS (20%) and 2μl Reagent DX to a 2ml Bead Tube.
2. Add 20μl Protease K and 0.5ml plasma, serum, body fluid, homogenate suspension, culture solution, cell suspension, soaking solution or concentrate pathogen solution to the 2ml Bead Tube.
   - When processing samples rich in cells (such as whole blood, fluid accumulation or tissue homogenate solution), centrifuge at 2,000 x g for 10 minutes to remove excess body cells, then transfer the supernatants for next process.
   - When processing Sputum samples, fully liquefied with DTT before operation.
3. 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.
4. Incubate at 55°C for 15 minutes.
5. Briefly centrifuge, process according to the manual operation in Part II or the process of 32-channel nucleic acid extractor in Part III.

Part B: Manual operation

1. Transfer 200~300μl of the sample into a new centrifuge tube. Add 30μl MagBind Particles and 500μl Buffer MLB to the sample. Mix by upside down for 10-15 times. Incubate at room temperature for 10 minutes, during which mix by upside down several times. Place on the

magnet plate for 5 minutes, discard the solution.

2. **Add 500μl Buffer MW1 and vortex for 10 seconds.** Place on the magnet plate for 1 minutes, discard the solution.

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

4. **Add 500μl Buffer MW2 and vortex for 10 seconds.** Place on the magnet plate for 1 minutes, discard the solution.

5. Briefly centrifuge and remove the solution, air dry for 3 minutes.

6. **Add 50~100μl Buffer AVE, vortex to disperse the magnetic beads.** Place for 5-10 minutes and vortex for several times to dissolve the nucleic acid.

7. Place on the magnetic plate for 3 minutes. Transfer the DNA/RNA solution into a new 1.5 ml centrifuge tube.

Part C: Process of 32-channel nucleic acid extractor

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

2. Transfer 200~300μl homogenate from part A to Row 1/7.

Row of hole	Pre-loaded reagents	Addition before use
Row 1/7	500 μl Buffer MLB	250μl~300μl of homogenate from Part Ⅰ step 3/ step 7.
Row 2/8	500μl Buffer MW1	
Row 3/9	500μl Buffer MW2, 30μl MagBind particles	
Row 4/10	500μl Buffer MW2	
Row 5/11		
Row 6/12	50~100μl Buffer NFW	

3. Turn on the machine, insert the magnetic tip, place the 96-well plate in machine.

4. Start the program. It takes about 30 minutes. Take out the 96-well plate and magnetic tip.

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

Part D: Process of 96-channel nucleic acid extractor

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

2. Transfer 200~300μl homogenate from part A to Sample Plate.

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

Name of Plate	Pre-loaded reagents	Addition before use
Sample Plate	500 μl Buffer MLBN	200~300μl homogenate mixture
Washing Plate1	500μl Buffer MW1，place 96 tip comb	
Washing Plate2	500μl Buffer MW2, 30μl MagBind Particles	
Washing Plate3	500μl Buffer MW2	
Elution plate	50~100μl Buffer NFW	

4. It takes about 20 minutes on machine running.

5. Take out the 96-well plate and tip comb when finish.

6. Transfer DNA/RNA to a new 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	3	500	20s	8	0	0	60s	0	0	/	/
2	Bind	1	700	300s	8	0	0	90s	50	50	/	/
3	W1	2	500	90s	8	0	0	90s	30	30	/	/
4	W2	3	500	60s	8	0	0	90s	0	0	/	/
5	W3	4	500	60s	8	0	0	60s	0	0	/	/
6	Dry	4	500	0	8	5	0	0	0	0	/	/
7	Elute	6	100	360s	9	0	0	60s	0	50	6	55
8	Drop	4	500	30s	9	0	0	0	0	0	/	/