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.	R667200C	R667202C
Purification times	24 Preps	96 Preps
2ml Beads Tubes	24	96
Reagent DX	1.0 ml	3ml
Buffer SDS	1.5 ml	10 ml
Buffer CLB2	10 ml	40 ml
Proteinase K	12 mg	50 mg
Nuclease	-	25 KU
Nuclease Buffer	1.5 ml	5 ml
Protease Dissolve Buffer	1.8 ml	6 ml
MagBind Particle	1.0 ml	3.5 ml
Buffer MLB	15 ml	60 ml
Buffer MW1	13 ml	44 ml
Buffer MW2	10 ml	50 ml
Buffer AVE	10 ml	20 ml

Storage and Stability

MagBind Particles Nuclease 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 1.2ml (96 Preps) Protease Dissolve Buffer to the bottle of Nuclease and store at -20°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.

Part I: Sample pretreatment process

- A: Total DNA/RNA extraction from pathogens (rapid)

1. Transfer 0.5 to 1.5 ml of body fluid samples such as whole blood, blood water, effusion, serum, plasma, homogenate, swab immersion solution, etc. into a 2 ml centrifuge tube, and centrifuge for 10 minutes at 2000 x g to remove exfoliated cells.

2. Transfer 0.5ml of supernatant into a 1.5ml centrifuge tube. Add 25μl Nuclease Buffer and 10μl Nuclease, mix them upside down. Incubate at room temperature for 15 minutes to digest extracellular nucleic acids.

3. Add 2μl Reagent DX and 50μ l Buffer SDS to 2ml Beads Tubes. Then add 0.5ml of Nuclease treated sample (Step 2), and finally add 20μ l of Protein K. Tighten the cover and transfer to a vortex meter for 10 minutes or transfer to a ball mill for ball grinding.

4. Incubate at 65°C for 20 minutes, centrifuge 13000 x g for 3 minutes, and proceed as described in Part 2/3.

- B: Enrichment and extraction of pathogenic total nucleic acid

1. Take 1.0~1.5ml of body fluids such as plasma, effusion, whole blood, homogenate, cell suspension, swab immersion solution, etc. into a 2ml centrifuge tube, and centrifuge 5000 x g for 5 minutes to remove exfoliated cells. Transfer 250μl of supernatant to a new centrifuge tube for virus and mycoplasma extraction (Step 4).

2. **Retain the remaining residual liquid and sediment and vortex resuspension precipitation. Add 0.25 times the volume of Buffer CLB2 (200~300μ l), invert and mix for 10-15 times.** Place at room temperature for 10 minutes, invert and mix several times. Centrifuge 13000 x g for 10

minutes to collect microbial cells, and carefully suck and discard the supernatant.

3. **Add 250μl Buffer AVE to the precipitation, vortex resuspension precipitation, add 25μl Nuclease Buffer and 10μl Nuclease.** Mix well and incubate at room temperature for 20 minutes.

4. **In 2ml Beads Tubes, first add 50μl Buffer SDS and 2μl Reagent DX.**

5. **Transfer all the digestive fluid from Step 4 and 250μl of virus supernatant (Step 1) into a homogenate tube, and finally add 20μl of Protein K. Tighten the lid and transfer it to a vortex meter for 10 minutes or to a ball mill for cracking microorganisms.**

6. Incubate at 5.65 degrees Celsius for 20 minutes, centrifuge 13000 x g for 3 minutes, and proceed as described in Part 2/3.

Part II: 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 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 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.

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	/	/