MagPure Pathogen DNA/RNA Enrich Kit

Introduction

This kit is suitable for extracting total pathogen nucleic acid from a variety of clinical samples such as blood, serum, plasma, swab soaking solution, fluid accumulation and homogenate solution. This kit is designed to remove host cells background nucleic acid and enrich pathogen nucleic acid (including viral/bacterial/fungal DNA/RNA) from the sample. Purified DNA/RNA is ready for downstream applications such as PCR, virus detection, 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 (0.4g)	24	96
DNase I (Powder)	10 mg	15 mg
Protease Dissolve Buffer	3 ml	10 ml
DNase Buffer	5 ml	20 ml
Lysis Buffer LBX1	40 ml	180 ml
Buffer TL	5 ml	30 ml
Proteinase K	24 mg	120 mg
Particles MPN9	1.2 ml	5 ml
Buffer MLB	30 ml	120 ml
Buffer MW1*	13 ml	110 ml
Buffer MW2*	10 ml	50 ml
Buffer AVE	10 ml	20 ml

Storage and Stability

Proteinase K, DNase I powder and Particles MPN9 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 1.2ml (24 Preps) or 6ml (96 Preps) Protease Dissolve Buffer to the bottle of Proteinase K, and store at -20-8°C.
- Add 1.0ml (24 Preps) or 1.5ml (96 Preps) Protease Dissolve Buffer to the tube of DNase I and store at -20°C.
- Add 17ml (24 Preps) or 140ml (96 Preps) 100% ethanol to the bottle of MW1.
- Add 40ml (24 Preps) or 200ml (96 Preps) 100% ethanol to the bottle of MW2.

Part I: Sample pretreatment process

Plan A: Total nucleic acid extraction from pathogens (rapid)

1. Transfer 1.0~1.5 ml body fluid samples such as whole blood, blood water, fluid accumulation, serum, plasma, homogenate, swab soaking solution, etc. into a 2 ml centrifuge tube, and centrifuge at 500 x g for 10 minutes to remove eukaryotic cells.
2. Add 200µl Buffer AL and 40µl Proteinase K to the 2ml Bead Tube, transfer 0.4ml sample (supernatant from step 1) to the tube and screw the lid tightly.
3. Vortex at maximum speed for 10 minutes or place on a bead grinding machine for fast grinding with 30~90 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

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

Plan B: Total nucleic acid extraction from pathogens with enrichment

1. Transfer 1.0~1.5 ml body fluid samples such as whole blood, homogenate, cells suspension, swab soaking solution, etc. into a 2 ml centrifuge tube, and centrifuge at 10,000 x g for 10minutes. Transfer 0.5~0.75ml supernatant (including virus and mycoplasma) into a new centrifuge tube, process later according to the manual operation in Part II or automated extraction machine in Part III or Part IIII. Keep no more than 0.7ml residual liquid in the tube with cells sediment, and follow step 2 process.

2. Vortex the tube to resuspend the cells. Add 1.0ml Lysis Buffer LBX1 and 5ul Proteinase K, invert at room temperature for 10 minutes to lyse the eukaryotic cells. Centrifuge at 10,000 x g for 10 minutes to collect microbial cells, and remove all supernatant carefully.

   Note: if the cells sediment is too much,add another 0.5ml Lysis Buffer LBX1 to the sample and resuspend. Then centrifuge at 10,000 x g for 10 minutes to collect the cells and remove all supernatant carefully.

3. Add 150µl DNase I Buffer and 10µl DNase I to the sample, invert to mix. Incubate at 37 °C for 30 minutes with oscillating (600~900rpm) to remove eukaryotic cells DNA. (Magen Thermostatic oscillating metal bath machine cat# MagMix B)

4. Add 200µl Buffer TL and 20µl Proteiase K, vortex to mix throughly. Then transfer all liquid to the 2ml Bead Tubes, screw the lid tightly.
   Vortex at maximum speed for 10 minutes or place on a bead grinding machine for fast grinding with 30~90 seconds.

- MagMix A grinder (Magen): recommend 4500rpm for 45s, pause for 20s and then repeat twice. MagMix A contains 2ml tube clamp, it can process for 10~20 samples efficiently. Fast homogenization is important for sample lysis.
- 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

5. Centrifuge the bead tubes at 13,000 x g for 2 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 20μl Proteiase K, 250μl sample mixture (supernatant from Step 5 in Plan B) and 250μl sample mixture (supernatant from Step 1 in Plan B) into a new 2.0ml centrifuge tube**. Invert 3~5 timex to mix. Incubate at room temperature for 5~10 minutes.

   Note: For Plan A sample (no enrichment): **take 500μl sample mixture (supernatant from Step 4 in Plan A).**

2. **Add 1.0ml binding Buffer MLB and 40μl Particles MPN9 to the sample, invert to mix at room temperature for 6 minutes.** Place the tube to the magnetic rack for 2 minutes, until the Particles MPN9 have formed a tight pellet, then remove the supernatant.

3. **Add 500μl Buffer MW1 and vortex for 10 seconds.** Place the tube to the magnetic rack for 1 minutes, then remove the supernatant .

4. Repeat Step 3 once.

5. **Add 500μl Buffer MW2 and vortex for 10 seconds.** Place the tube to the magnetic rack for 1 minutes, then remove the supernatant .

6. Repeat Step 5 once.

7. Centrifuge shortly to collect liquid on tube, place the tube to the magnetic rack. Remove all liquid carefully. Dry at room temperature for 10 minutes.

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

9. 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 32/48 channel nucleic acid extractor

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

2. Transfer the sample mixture from part I to Row 1/7 and Raw 2/8.

Row of hole	Pre-loaded reagents	Addition before use
Row 1/7	500 μl Buffer MLB	250μl sample(supernatant in Step 1 in Plan B), 20μl Proteinase K
Row 2/8	500μl Buffer MLB	250μl sample(supernatant in Step 5 in Plan B)
Row 3/9	500μl Buffer MW1	
Row 4/10	500μl Buffer MW2, 30μl Particles MPN9	
Row 5/11	500μl Buffer MW2	
Row 6/12	80μl Buffer AVE	

Note: For Plan A sample (no enrichment), add 250μl sample (supernatant in Step 4 in Plan A) to each Raw 1/7 and 2/8.

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

4. Edit the program on machine and start the program. After the program finish at about 40 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.

Recommend program for MagMix 32/48 extractor (Magen)

Step	Name	Well	Volume	Mix		Wait		Magnet			HEAT	
				Time	Speed	Time	Pos	Up& Down	Up	Bottom	Well	Tem.
1	Collect	4	500	30s	8	0	0	60s	0	0	/	/
2	Bind 1	1	700	360s	8	0	0	90s	10	30	/	60
3	Bind 2	2	700	240s	8	0	0	90s	10	30	/	/
4	W1	3	500	90s	8	0	0	90s	0	0	/	/
5	W2	4	500	90s	8	0	0	60s	0	0	/	/
6	W3	5	500	90s	8	0	0	60s	0	0	/	/
7	Dry	5	500	0	8	180s	0	0	0	0	/	/
8	Elute	6	80	360s	9	0	0	90s	0	50	6	55
9	Drop	5	500	30s	9	0	0	0	0	0	/	/

Part IIII: Process of 96-channel nucleic acid extractor

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

2. Transfer the sample mixture from part I to Sample Plate 1 and Sample Plate 2.

Row of hole	Pre-loaded reagents	Addition before use
Sample Plate 1	500 μl Buffer MLB	250μl sample(supernatant in Step 1 in Plan B), 20μl Proteinase K
Sample Plate 2	500μl Buffer MLB	250μl sample(supernatant in Step 5 in Plan B)
Wash Plate 1	500μl Buffer MW1	
Wash Plate 2	500μl Buffer MW2, 30μl Particles MPN9	
Wash Plate 3	500μl Buffer MW2	
Elution Plate	80μl Buffer AVE	

Note: For Plan A sample (no enrichment), add 250μl sample (supernatant in Step 4 in Plan A) to each Sample Plate 1 and Sample Plate 2.

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

4. Edit the program on machine and start the program. After the program finish at about 40 minutes, take out the 96 well plate and tip comb.

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