【Product Name】Hipure Pathogen RNA/DNA Kit

【Product specifications】50 Preps/Kit

【Intended Use】

This kit is used for extracting total pathongen nucleic acid from low cell content biological samples such as body fluid, serum, plasma, immersion solution, tissue homogenate, culture, etc. The purified DNA/RNA is ready for downstream clinical in vitro detection such as Real Time PCR, biochip analysis, NGS and other related detection.

【Principle】

This product is based on silica gel purification. The sample is lysed and digested with lysate and protease, DNA/RNA is released into the lysate. Transfer to an adsorption plate and filter column. DNA/RNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, DNA / RNA is finally eluted by elution buffer AVE.

【Main Composition】

Cat.No	IVD4179	Contents
HiPure Viral Mini Column	50	Silica Spin column
2ml Collection Tubes	100	PP Tubes
2ml Bead Tubes	50	Glass beads
Proteinase K	50 mg	Protease K
Protease Dissolve Buffer	5 ml	Glycerol/Tris/CaCl2
DNase I (Powder)	10 mg	DNase I
DNase Buffer	6 ml	Tris/CaCl2
Buffer CLB	100 ml	Guanidine Salt/Surfactant
Buffer SDS	5 ml	Tris/EDTA/SDS
Reagent DX	1.5 ml	Anti-former
Buffer ACL	30 ml	Guanidine Salt/Surfactant
Buffer VHB*	22 ml	Guanidine Salt
Buffer RW2*	20 ml	Tris/NaCl
Buffer AVE	15 ml	DEPC treated water

【Storage conditions and validity】

Proteinase K and DNase I (Powder) should be stored at 2–8°C upon arrival. However, short-term storage (DNase I up to 1 week, Proteinase K 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 18 months under these conditions.

【Preparation before Use】

- Add 2.5mL Protease Dissolve Buffer to the bottle of Proteinase K, and store at -20-8°C.
- Add 0.6 ml DNase Buffer to the bottle of DNase I (Powder), and store at -20°C.
- Add 28 ml absolute ethanol to the bottle of Buffer VHB.
- Add 80 ml absolute ehtanol to the bottle of Buffer RW2.

【 Protocol 1: Rich microbial DNA/RNA from cell-rich samples】

1. Sample pre-treatment:

Anticoagulant blood: Transfer ~1.0ml whole blood to a 2ml centrifuge tube, centrifuge at 2000 x g for 10 minutes. Transfer plasma into a new centrifuge tube for the preparation of total viral nucleic acid. Add 1ml Buffer CLB into the sample, mix by vortex for 15-20 seconds and centrifuge at 13,000 x g for 10 minutes to collect microbial DNA, remove the supernatant.

Body fluid samples such as plasma/ascites: Take 1~1.5ml body fluid samples such as plasma/ ascites/ effusion into a 2ml centrifuge tube, centrifuge at 13,000 x g for 10 minutes to collect microorganisms and cells. Transfer all the supernatant to a new centrifuge tube for the preparation of total viral nucleic acid. The precipitation is used for extraction of microbial DNA.

Tissue: Take 50-100mg tissue sample, fully homogenized with 1ml normal saline or Buffer PBS, centrifuge at 13,000 x g for 10 minutes to collect microorganisms and cells. Transfer all the supernatant to a new centrifuge tube for the preparation of total viral nucleic acid. The precipitation is used for extraction of microbial DNA.

Sputum: Take appropriate amount of sputum, fully vortex with normal saline or Buffer PBS, centrifuge at 2,000 x g for 5 minutes. Transfer ~0.5ml supernatant for extraction of total virus nucleic acid. The balance is used for rich and extraction of microbial DNA. Add appropriate amount of DTT or trypsin to dissolve the precipitation. After full dissolved, centrifuge at 13,000 x g for 10 minutes to collect microorganisms and cells.

2. Add 1.0 buffer CLB into the precipitation, dispersed by vortex, place at room temperature for 5 minutes to lyse eukaryotic cells. Centrifuge at 13,000 x g for 10 minutes to collect microorganisms, remove the supernatant.

3. Add 300μl buffer AVE to the sample and vortex to resuspend the pelledt, add 100μl DNase Buffer and 10μL DNase to the suspension, mix well and place for 20 minutes to digest cell DNA and RNA.

4. Transfer all the digestion solution to the 2ml Bead Tube, and dd 50μL Buffer SDS and 2μL Reagent DX. vortex at maximum speed for 10 minutes or place on a bead beater machine (such as FastPrep-24) grind for 3 minutes to lysate microorganisms.

5. Add 300μL homogenate mixture (step 4) and 200μL supernatant (supernatant keep at step 1) into a 2ml centrifuge tube, then add 500μl Buffer ACL and 40μl proteinase K into the sample, mix by upside down for several times, incubate at 55℃ for 15 minutes.

6. Add 500μl absolute ethanol to the sample, mix by upside down for several times.

7. Insert a HiPure Viral Mini Column into a 2ml collection Tube. Transfer 750μl mixture to the column. Centrifuge at 10,000 x g for 30~60 seconds.

8. Discard the filtrate and insert the column back into the collection tube. Transfer 750μl mixture to the column. Centrifuge at 12,000 × g for 30-60 seconds. Repeat the step until all the mixture (step 6) is transferred.

9. Discard the filtrate and insert the column back into the collection tube. Add 500μl buffer VHB (diluted by absolute ethanol) to the column. Centrifuge at 10,000 x g for 30~60 seconds.

10. Discard the filtrate and insert the column back into the collection tube. Add 500μl RW2 (diluted by absolute ethanol) to the column. Centrifuge at 10,000 x g for 30~60 seconds.

11. Discard the filtrate and insert the column back into the collection tube. Add 500μl RW2 (diluted by absolute ethanol) to the column. Centrifuge at 10,000 x g for 30~60 seconds.

12. Discard the filtrate and insert the column back into the collection tube. Centrifuge at 13,000 x g for 3 minutes to dry the column.

13. Insert the column into a new 1.5 ml centrifuge tube. Add 30~50μl Buffer AVE to the center of the membrane. Centrifuge at 13,000 x g for 1 minute.

14. Discard the column and store DNA/RNA at -20℃.

【 Protocol 2: Total nucleic acid extraction】

1. Take 0.5ml sample to a 1.5ml centrifuge tube, Add 100μl DNase Buffer and 10μL DNase I, mix it upside down, place for 30 minutes to digest cell DNA and RNA.

2. Transfer all the digestion solution to 2ml homogenization tube. Add 50μL Buffer SDS and 2μL Reagent DX, vortex at maximum speed for 10 minutes or place on a bead beater machine (such as FastPrep-24) grind for 3 minutes to lysate microorganisms.

3. Briefly centrifuge, transfer 500μl digestion solution into a new centrifuge tube. Add 40μl protease K and 500μl Buffer ACL, mix by upside down, incubate at 55℃ for 15 minutes.

4. Add 500μl absolute ethanol to the sample, mix by upside down for several times.

5. Insert a HiPure Viral Mini Column into a 2ml collection Tube. Transfer one half mixtures to the column. Centrifuge at 10,000 x g for 30~60 seconds.

6. Discard the filtrate and insert the column back into the collection tube. Transfer the rest mixture to the column. Centrifuge at 10,000 × g for 30-60 seconds.

7. Discard the filtrate and insert the column back into the collection tube. Add 500μl buffer VHB (diluted by absolute ethanol) to the column. Centrifuge at 10,000 x g for 30~60 seconds.

8. Discard the filtrate and insert the column back into the collection tube. Add 500μl RW2 (diluted by absolute ethanol) to the column. Centrifuge at 10,000 x g for 30~60 seconds.

9. Discard the filtrate and insert the column back into the collection tube. Add 500μl RW2 (diluted by absolute ethanol) to the column. Centrifuge at 10,000 x g for 30~60 seconds.

10. Discard the filtrate and insert the column back into the collection tube. Centrifuge at 13,000 x g for 3 minutes to dry the column.

11. Insert the column into a new 1.5 ml centrifuge tube. Add 30~50μl Buffer AVE to the center of the membrane. Centrifuge at 13,000 x g for 1 minute.

12. Discard the column and store DNA/RNA at -20℃.

【Basic Information】

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