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Viral Nucleic Acid Extraction Kit

Catalog Number: EXR0233

Size: 50T/250T

Storage: Store at 2-8℃, valid for one year.

product formation

Viral nucleic acid purification kit Cat .No .	5 samples 4002005	50 preparation 4002050	250 preparation 4002250
Nucleic acid purification column 2 ml centrifuge tube Protease K storage solution Carrier RNA Buffer VL Buffer WBR (Concentrate) Buffer TE specification	5 5 120 µl 40 µl 1.5 ml 1.5 ml 0.5 ml 1	50 50 1.2 ml 400 µl 15 ml 6.5 ml×2 5 ml 1	250 250 1.2 ml×5 400 µl×5 75 ml 32 ml×2 25 ml 1

product storage

1. Protease K storage solution and Carrier RNA should be stored at -20℃.
2. Other reagents and articles if stored at room temperature (0~30℃), can maintain no significant change in performance within two years; If the product is stored at 2~8 ° C, the validity period of the product can be extended to more than two years.

Product introduction

This product is suitable for the extraction of various viral RNA or viral DNA from plasma, cell-free body fluids (including plasma, serum, urine, CSF and cell culture supernatant), viral stock solution and infected tissue. Viral nucleic acid can be detected with this kit from body fluid samples (DNA viruses) with a maximum viral copy number of 50 copies/ml. Compared with the traditional boiling method, the detection sensitivity can be increased by 10-50 times. Compared with the traditional Trizol method, the detection sensitivity can be increased by 5-10 times. After the nucleic acid of the dissolved virus is bound to the purification column, the Buffer WBR washes to remove the remaining PCR inhibitors on the purification column, and then elution with Buffer TE, which can be used for PCR or RT-PCR reactions.

Reagents and items to be prepared by the user

1. Anhydrous ethanol
2. 1.5ml centrifuge tube (DNase-free & RNase-free 1.5ml centrifuge tube is recommended)
3. Pipettes and pipettes (To avoid cross-contamination between samples, please use DNase-free & RNase-free pipette pipettes with filter elements)
4. Disposable gloves, protective equipment and paper towels
5. Table type small centrifuge (can be equipped with 1.5 ml centrifuge tube and 2 ml centrifuge tube rotor)
6. Water bath with vortex oscillator
7. PBS solution and saline solution may be required

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the temperature of the water bath to 56 ° C and incubate the Buffer TE at 56 ° C.
3. Add anhydrous ethanol to Buffer WBR according to the instructions on the label of the reagent bottle, and tick the box on the label to mark "ethanol has been added".
4. Calculate the required Buffer VL volume (200 µl Buffer VL/ tube) according to the number of nucleic acid samples to be prepared. Note that due to possible errors during liquid loading, it is recommended to increase the volume of 300~500 µl Buffer VL during calculation. Carrier RNA was added at a ratio of 25 µl Carrier RNA per 1 mlBuffer VL volume, and was mixed with vortex oscillation for several seconds.

Operation procedure

Sample processing before use

A. Plasma, serum, acellular body fluid, viral stock fluid, urine specimen, cerebrospinal fluid, herpes fluid, CSF and cell culture supernatant: 200 µl samples were directly absorbed for the isolation and purification of viral nucleic acid; If the sample volume is less than 200 µl, the PBS solution is added to 200 µl.

* The isolation and purification of viral nucleic acids should be carried out as far as possible using freshly isolated or freeze-thawed samples not more than once.

B. Throat swab lotion, reproductive tract swab lotion and gargle: 300μl sample was absorbed into 1.5ml centrifuge tube, centrifuged at 12000 rpm for 5 min, and 200μl supernatant was absorbed for the separation and purification of viral nucleic acid.

C. Tissue lysate infected with virus: 10 mg of tissue infected with virus was ground in liquid nitrogen, then 300 μl PBS solution was added to suspension after grinding, and 200 μl tissue suspension was absorbed for the separation and purification of viral nucleic acid.

D. Feces: Add 1 ml of normal saline into 1.5ml centrifuge tube, pick up about 200 mg with sterilized toothpick (if feces are liquid, directly absorb 200 μl), add to 1.5ml centrifuge tube, swirl and oscillate until feces are completely dispersed. Centrifuge at 12000 rpm for 1 minute and take 200 μl of the top supernatant for the separation and purification of viral nucleic acid.

1. Add 20 μl of protease K storage solution into 1.5 ml centrifuge tube, and add 200 μl of body fluid sample.

* If the fluid sample is less than 200 μl, add saline to bring the final volume of the fluid sample to 200 μl.

* Do not add protease K directly to Buffer VL.

2. Add 200 μl Buffer VL containing Carrier RNA, swirl and mix for about 15 seconds.

3. Place the centrifuge tube in a 56℃ water bath for 10 minutes.

4. Add 320 μl of anhydrous ethanol and gently turn 4~6 times to mix well.

* To avoid cross-contamination between samples when opening the cap, centrifuge at low speed for a few seconds before opening the cap, so that the solution on the cap settles to the bottom of the tube.

5. Absorb the solution in step 4 and add it to the nucleic acid purification column (the nucleic acid purification column is placed in 2 ml centrifuge tube), cover the tube cover, and centrifuge at 12000 rpm for 30 seconds.

* Be careful not to dip the solution onto the edge of the tube mouth of the purification column, so as not to wash the purification column in subsequent washing steps

6. Discard the filtrate in the 2 ml centrifuge tube, put the nucleic acid purification column back into the 2 ml centrifuge tube, add 700 μl Buffer WBR into the nucleic acid purification column, cover the tube, and centrifuge at 12000 rpm for 30 seconds.

* The filtrate does not need to be completely discarded. If you want to avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the centrifuge tube, you can reverse tap the 2 ml centrifuge tube on a paper towel once.

* Confirm that anhydrous ethanol has been added to Buffer WBR.

7. Discard the filtrate in the 2 ml centrifuge tube, place the nucleic acid purification column back into the 2 ml centrifuge tube, and centrifuge at 14,000 rpm for 1 minute.

* If the centrifuge does not reach 14000 rpm, centrifuge at the highest speed for 2 minutes.

* Do not omit this step, otherwise the effect of subsequent PCR may be affected due to ethanol mixed in the purified nucleic acid.

8. Discard 2 ml centrifuge tube, place the nucleic acid purification column in a clean 1.5ml centrifuge tube, add 50μl Buffer TE preheated at 56℃ to the center of the membrane of the purification column, cover the tube, stand at room temperature for 1 minute, and centrifuge at 12000rpm for 30 seconds.

* If the centrifuge does not have a leak-proof cover, change the centrifuge condition to 8000 rpm for 1 minute to prevent the cover from falling off and damaging the centrifuge.

9. Discard purification column, elution of viral nucleic acid can be immediately used in various molecular biology experiments; Or store the viral nucleic acid below -70 ° C for backup.