ELK Biotechnology

For research use only.

Viral RNAclean Kit

Component	Size(50T)	Size(200T)	Storage
Nucleic acid purification column	50	200	RT
Protease K storage solution	1.1 ml	4.4 ml	-20℃
Carrier RNA	400 μl	1.6 ml	-20℃
Buffer REL	15 ml	60 ml	RT
Buffer WBR	60 ml	240 ml	RT
Buffer TE	5 ml	20 ml	RT
manual	1	1	RT

Product storage

1. Protease K storage solution and Carrier RNA should be stored at -20℃.
2. If other reagents and materials are stored at room temperature (15~25℃), their performance can be kept unchanged for two years; If the product is stored at 2~8℃, the product life can be extended to more than two years.

Product introduction

This product is suitable for the extraction of various viral RNA from plasma, whole blood, cell-free body fluids (including plasma, serum, urine, CSF and cell culture supernatant), viral stock solution and infected tissues.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 5-10 times.After the nucleic acid of the dissolved virus is bound to the purified column, the Buffer WBR is washed to remove the residual PCR inhibitor on the purified column, and then eluted with Buffer TE for PCR or RT-PCR reaction.

Reagents and materials are required

1. Anhydrous ethanol
2. 1.5ml centrifuge tube (DNase-free & RNase-free 1.5ml centrifuge tube is recommended)
3. Pipette tips (to avoid contamination in the samples, please select the DNase-free & RNase-free Pipette tips with filter cartridges)
4. Disposable gloves, protective equipment and tissues
5. Desktop small centrifuge (rotor with 1.5ml centrifuge tube and 2ml centrifuge tube)
6. Water bath and vortex shaker
7. Normal saline may be needed

Preparation before use

1) If the centrifuge has refrigeration function, please set the temperature to 25℃.
2) Set the temperature of the water bath to 56℃ and incubate the Buffer TE at 56℃.

3) Add anhydric ethanol to the Buffer WBR according to the instructions on the label of the reagent bottle, and tick the box of the label to mark "ethanol added".

4)According to the number of nucleic acid samples to be prepared, the volume of Buffer REL to be used (200μl Buffer REL/ tube) was calculated. It is suggested to increase the volume of Buffer REL by 300~500μl due to possible errors in the infusion process. Carrier RNA was added to the ratio of 25μl Carrier RNA per 1ml Buffer REL volume, vortex and mix for a few seconds.

Sample pretreatment

A. plasma, serum, cell-free body fluids, viral stock, urine specimens, cerebrospinal fluid, herpes fluid, CSF and cell culture supernatant were directly absorbed into 200μl of specimens for the isolation and purification of viral nucleic acid. If the specimen volume is less than 200μl, PBS solution is added to 200μl.

* try to isolate and purify viral nucleic acids from fresh isolated or freeze-thaw specimens with no more than once.

B. Pharyngeal swab lotion, genital tract swab lotion, mouthwash solution

Add 300μl pharharyngeal swab lotion, genital tract swab lotion, and mouthwash solution into 1.5 ml centrifuge tube, centrifuge at 12000rpm for 5 minutes, and pipette 200 μl of supernatant for the isolation and purification of viral nucleic acids.

C. tissue lysate of infected virus:

Take 10 mg of virus-infected tissue for liquid nitrogen grinding, add 300 μl PBS solution to the ground tissue and suspend it.200 μl tissue suspension was used to separate and purify viral nucleic acid.

D. stool

Take 1ml of normal saline was added to the 1.5 ml centrifuge tube, about 200 mg was selected with sterilized toothpick (200μl were directly absorbed if the stool were liquid), and added to the 1.5 ml centrifuge tube, and the vortex oscillated until the stool were completely dispersed.Centrifugation at 12000rpm for 1 min, taken 200μl top supernatant for the isolation and purification of viral nucleic acid.

Operating Steps

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

* if the body fluid sample is less than 200μl, add normal saline to make the final volume of the body fluid sample 200μl.

* do not add protease K directly to Buffer REL.

2. 200μl Buffer REL containing Carrier RNA was added and mixed with vortex for about 15 seconds.

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

4. Add 320μl anhydrous ethanol and gently flip the mixture 4 to 6 times to mix well.

* to avoid cross-contamination between samples when the cap is opened, centrifuge at a low speed for several seconds before the cap is opened, so that the solution on the cap can be settled to the bottom of the tube.

5. The solution in step 4 was added to the nucleic acid purification column (the nucleic acid

purification column was placed in a 2ml centrifuge tube), covered with the tube cover, and centrifuged at 12000rpm for 30 seconds.
* be careful not to stick the solution to the edge of the nozzle of the purified column, in case the subsequent washing steps cannot clean the purified column.
6. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, add 700μl 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. To avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the centrifuge tube, the 2ml centrifuge tube can be flipped on the paper towel once.
* confirm that anhydrous ethanol has been added to Buffer WBR.
7. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, and centrifuge at 14000rpm for 1 minute.
* if the centrifuge speed cannot reach 14000rpm, centrifuge at the highest speed for 2 minutes.
* please do not omit this step, or the subsequent PCR effect may be affected by the presence of ethanol in the purified nucleic acid.
8. Discard the 2ml centrifuge tube, place the nucleic acid purification column in a clean 1.5ml centrifuge tube, add 50μl of preheated Buffer TE at 56℃ in the center of the membrane of the purified column, cover the tube, let it stand at room temperature for 1 minute, and centrifuge at 12000rpm for 30 seconds.
* if the centrifuge does not have a leak-proof cover, please change the centrifuge condition to 8000rpm for 1 minute, so as to prevent the tube cover from falling off and damaging the centrifuge.
9. Discard the purified column and store the viral nucleic acid at -20℃ for future use.