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Viral Nucleic Acid Extraction Kit Instructions

Composition

Viral Nucleic Acid Extraction Kit Cat. No.	5 Preps 4002005	50 Preps 4002050	250 Preps 4002250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
Proteinase K	120 µl	1.2 ml	1.2 ml×5
Carrier RNA	40 µl	400 µl	400 µl×5
Buffer VL	1.5 ml	15 ml	75 ml
Buffer WBR (concentrate)	1.5 ml	6.5 ml×2	32 ml×2
Buffer TE	0.5 ml	5 ml	25 ml
Instructions	1	1	1

Storage

1. Store Proteinase K and Carrier RNA at -20°C.
2. Other reagents and items, if stored at room temperature (0~30℃), can be used within 2 years without significant changes in performance; if stored at 2~8℃, the validity can be extended to more than 2 years.

Technical Support

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Introduction

This product is suitable for the extraction of viral RNA or viral DNA from plasma, cell-free body fluids (including plasma, serum, urine, CSF and cell culture supernatant), viral stock solutions and virus-infected tissues. Viral RNA or viral DNA can be detected in samples of body fluids with a viral copy number of up to 50 copies/ml (DNA viruses) using this kit. The detection sensitivity can be increased 10-50 times compared with the traditional boiling method for viral DNA extraction and 5-10 times compared with the traditional Trizol method for viral RNA extraction. After the nucleic acid in the lysed virus binds to the Spin Column, Buffer WBR washes to remove PCR inhibitors remaining on the Spin Column, and then elutes with Buffer TE, which can be used for PCR or RT-PCR reactions.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tube (DNase-free & RNase-free 1.5 ml tubes are recommended).
3. Pipettes and tips (to avoid cross contamination between samples, use DNase-free & RNase-free pipette tips with filter cartridges).
4. Disposable gloves and protective gear and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml microcentrifuge tubes).
6. Water bath and Vortexer.
7. PBS solution and normal saline may be required.

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 25°C.
2. Set the water bath temperature to 56°C and incubate the Buffer TE to 56°C.
3. Add absolute ethanol to the Buffer WBR according to instructions on the reagent bottle label and tick the box on the label to mark "Ethanol Added".
4. Calculate the volume of Buffer VL to be used according to the number of nucleic acid samples to be prepared (200 µl Buffer VL/tube, note that it is recommended to increase the volume of Buffer VL by 300-500 µl due to the possibility of error during the addition process), add 25 µl of Carrier RNA per 1 ml of Buffer VL volume, vortex for a few seconds to mix.

Protocol

Treatment for different samples sources:

A. Plasma, serum, cell-free body fluid, viral stock fluid, urine, cerebrospinal fluid, herpes fluid, CSF and cell culture supernatants:
Use 200 µl of sample directly to extraction; if the sample volume is less than 200 µl, add PBS solution to 200 µl.

* Extraction of viral nucleic acids using freshly isolated or freeze-thawed samples no more than one time, whenever possible.

B. Pharyngeal swab elution, genital tract swab elution, mouthwash fluid:
Transfer 300 µl sample into a 1.5 ml centrifuge tube, centrifuge at 12000 rpm for 5 min, then transfer 200 µl supernatant for extraction of viral nucleic acids.

C. Tissue lysate infected with the virus:
Take 10 mg of virus infected tissue, add liquid nitrogen to immerse the tissue for grinding, after grinding, add 300 µl PBS solution for suspension, then transfer 200 µl tissue suspension for extraction of viral nucleic acids.

D. Stool
Add 1 ml of normal saline to a 1.5 ml centrifuge tube, take about 200 mg stool with a sterilized toothpick (if the stool is in liquid form, aspirate 200 µl directly), add to the 1.5 ml centrifuge tube and vortex until the stool are completely dispersed. Centrifuge at 12,000 rpm for 1 min and transfer 200 µl the top supernatant for extraction of viral nucleic acids.

1. **Add 20 µl Proteinase K to a 1.5 ml centrifuge tube, then add 200 µl body fluid sample.**

* If the body fluid sample is less than 200 µl, add normal saline to bring the final volume to 200 µl.
* Do not add Proteinase K directly to Buffer VL.

2. **Add 200 µl Buffer VL (Carrier RNA added), vortex for about 15 sec to mix well.**
3. **Incubate the 1.5 ml tubes in a 56°C water bath for 10 min.**
4. **Add 320 µl absolute ethanol and gently invert 4-6 times to mix well.**

* To avoid cross contamination between samples, spin down the solution on the cap settles to the bottom of the tube before open the lid.

5. **Transfer the solution from step 4 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid and centrifuge it at 12000 rpm for 30 sec.**

* Be careful not to get the solution on the edge of the Spin Column mouth, otherwise the Spin Column will not be cleaned in subsequent washing steps.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 700 µl Buffer WBR to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the collection tube mouth, you can slap the 2 ml Collection Tube upside down once on a paper towel.
* Ensure that absolute ethanol has been added to the Buffer WBR.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge for 2 min at full speed.
* Do not omit this step, as the subsequent PCR may be affected by the presence of ethanol in the purified nucleic acid.

8. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 50 µl pre-warmed Buffer TE at 56°C to the Spin Column center, close the lid and incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof cap, change the centrifugation condition to centrifugation at 8000 rpm for 1 min to avoid damage to the centrifuge if the cap falls off.

9. **Discard the Spin Column and the eluted viral nucleic acid can be used**

immediately for various molecular biology experiments or stored below -70°C for later use.