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Viral RNA Extraction Kit Instructions

Composition

Viral RNA Extraction Kit Cat. No.	5 Preps 4001005	50 Preps 4001050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Carrier RNA	20 µl	180 µl
Buffer L	2 ml	18 ml
Buffer WA（concentrate）	1.9 ml	12 ml
Buffer WBR（concentrate）	1.5 ml	10 ml
Buffer TE	0.6 ml	2 ml×2
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Storage

1. Carrier RNA should be stored at -20℃.
2. If other reagents and items are stored at room temperature (0~30℃), can keep their performance unchanged for 2 years, and if stored at 2~8℃, the validity period can be extended to more than 2 years.

Technical Support

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Introduction

This kit is for extracting viral RNA from 100 µl fresh or frozen cell-free body fluid samples, including plasma, serum, urine, CSF, and cell culture supernatant. Purified viral nucleic acids extracted with this kit can detect RNA viruses at concentrations up to 500 copies/ml in body fluids. After the viral nucleic acid in the body fluid is bound to the Spin Column, the residual PCR inhibitors remaining on the Spin Column are washed by two wash buffers, and finally eluted with Buffer TE and can be used for RT-PCR reaction.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml tubes (RNase-free 1.5 ml tubes must be selected).
3. Pipettes and tips (RNase-free pipette tips with filters must be selected).
4. Disposable gloves, masks and other protective equipment and paper towels.
5. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer and water bath.

Preparation Before Use

1. Check if white precipitates appeared in Buffer L before use. If there is precipitate, dissolve it by incubating at 70℃ (note: Buffer L is highly susceptible to precipitation when the room temperature is below 20℃).
2. If the centrifuge has refrigeration function, set the temperature to 25℃.
3. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the bottle label and tick the box on the label to mark "Ethanol Added".
4. According to the number of samples to be prepared, calculate the volume of Buffer L (300 µl Buffer L/tube, note that there may be errors in the dosing process, it is recommended to increase the volume of 300~500 µl Buffer L during the calculation), add Carrier RNA at the ratio of 10 µl Carrier RNA per 1 ml Buffer L volume, and vortex for a few seconds to mix.
   !! Note: Buffer L with Carrier RNA added must be used up within 12 h.

Protocol

Cautions:

A. **Try to use freshly isolated or freeze-thaw samples no more than once for extraction of viral RNA. Repeated freeze-thaw samples will result in a decrease in the sensitivity of the detection, which can be manifested as a high CT value or a false negative.**

B. **If it is necessary to use more than once freeze-thaw plasma or serum for viral RNA extraction, the sample should be centrifuged at 6800 rpm for 3 min, then transfer 100 µl supernatant for viral RNA extraction.**

1. **Add 300 µl Buffer L with Carrier RNA to a 1.5 ml centrifuge tube, add 100 µl sample, vortex for a few seconds to mix well, and incubate at room temperature for 10 min.**

* In order to avoid false positives caused by contamination of Buffer L by positive serum, Buffer L should be added before the serum to be tested.
* To avoid cross-contamination between samples before open the lid, spin down the solution to the bottom of the tube.

2. **Add 320 µl absolute ethanol to each tube, gently invert the centrifuge tube 3~5 times and mix well.**

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

3. **Transfer the mixture from step 2 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Be careful not to touch the edge of the Spin Column to avoid subsequent washing steps that fail to wash.

4. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA 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 adhered to the mouth of the centrifuge tube, you can clap the 2 ml Collection Tube upside down on a paper towel once.
* Ensure that absolute ethanol has been added to Buffer WA.

5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WBR to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

6. **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 at full speed for 2 min.
* Do not omit this step, otherwise the subsequent RT-PCR effect may be affected due to the ethanol mixed in the purified nucleic acid.

7. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 50 µl Buffer TE to the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 30 sec.**

* DNase-free & RNase-free 1.5 ml tubes must be used to collect viral RNA.
* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm centrifugation for 1 min to avoid the tube lid coming off and damaging the centrifuge.

8. **Discard the Spin Column, the eluted RNA can be immediately used in various molecular biology experiments or stored at -70℃ for later use.**

* 25 µl (50 µl RT-PCR one-step reaction) of eluted viral RNA was used as a template for RT-PCR will increase the sensitivity of virus detection.