RNApure Virus Kit

Viral RNA Extraction Kit

Project No. R666005 (50 preps)
Storage conditions: room temperature (15-30° C)

Product content

individual parts making up a compound	R666005 50 preps
Buffer GL	15 ml
Buffer RW1	40 ml
Buffer RW2 (concentrate)	11 ml
Proteinase K	12.5 mg
Proteinase K Storage Buffer	1.25 ml
RNase-Free Water	10 ml
Spin Columns RS with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Product Introduction

This kit is designed for the isolation of viral RNA from cell-free body fluids such as serum, plasma, urine, cerebrospinal fluid, and cell culture supernatants using an adsorbent column that specifically binds viral RNA and a unique buffer system that allows the isolation of viral RNA. viral RNA specifically binds to a silica-based membrane, through which contaminants are passed. Proteins and other impurities are completely removed by two high-performance washes, and the high-purity viral RNA is eluted with RNase-free water or RNase-Free Water provided in the kit.The viral RNA extracted from this kit can be used directly for RT-PCR, Real-time RT-PCR, and blotting analysis.

Self-contained reagents: anhydrous ethanol, 0.9% NaCl.

Pre-experiment Preparation and Important Notes

1. Add 1.25 ml Proteinase K Storage Buffer to Proteinase K to dissolve it and store it at -20℃. Do not leave the prepared Proteinase K at room temperature for a long time, and avoid repeated freezing and thawing to avoid affecting its activity.

2. To prevent RNase contamination, attention should be paid to the following aspects:
1) Use RNase-free plastics and tips to avoid cross-contamination.
(2) Glassware should be dry baked at a high temperature of 180 ℃ for 4 hours before use, plasticware can be immersed in 0.5 M NaOH for 10 minutes, rinsed thoroughly with water and autoclaved.
3) RNase-free water should be used to prepare the solution.
(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.
3. Avoid repeated freezing and thawing of serum or plasma, which may result in denaturation of proteins or precipitation, reducing the viral titer and thus the yield of the extracted viral nucleic acid.
4. Anhydrous ethanol should be added to Buffer RW2 according to the instructions on the label of the reagent bottle before first use.
5. Buffer GL may be heated at 56° C to dissolve if a precipitate is produced and then left at room temperature.
6. All centrifugation steps are carried out at room temperature unless otherwise indicated and all steps are performed quickly.

procedure

1. Add 200 μl of serum or plasma to a 1.5 ml centrifuge tube (supplied) at room temperature. Note: If 200 μl is not enough, add 0.9% NaCl (supplied by the customer) to make up the difference.
2. Add 20 μl of Proteinase K to the solution in the previous step and mix well.
3. Add 200 μl Buffer GL and vortex for 15 seconds. Note: Do not add Proteinase K directly to Buffer GL.
4. Incubate at 56 ° C for 15 min, centrifuge briefly and collect the solution from the wall to the bottom of the tube.
5. Add 250 μl of anhydrous ethanol, vortex and shake for 15 s. Incubate for 5 min at room temperature, centrifuge briefly, and collect the solution from the wall to the bottom of the tube.
6. Add all of the solution from step 5 to the Spin Columns RS in the collection tube. If you cannot add all of the solution to the column at once, transfer it in two passes, centrifuge the column at 12,000 rpm (~13,400 x g) for 1 minute, pour off the waste liquid in the tube, and place the column back into the collection tube.
7. Add 500 μl of Buffer RW1 to the column, centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and return the column to the collection tube.
8. Add 500 μl of Buffer RW2 to the adsorbent column (check that anhydrous ethanol is added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and put the adsorbent column back into the collection tube.
9. Add 500 μl of anhydrous ethanol to the column, centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube, and put the column back into the collection tube.

10. Centrifuge at 12,000 rpm for 3 minutes and pour off the waste liquid from the collection tube. Leave the column at room temperature for several minutes to dry thoroughly.

Attention:

1) The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

2) Recommended procedure: Place the column into a new 1.5 ml centrifuge tube (supplied), open the cap and incubate at 56° C for 3 minutes to dry the membrane of the column thoroughly.

11. Place the adsorbent column in a new RNase-free centrifuge tube, add 20-50 μl RNase-Free Water to the center of the adsorbent column overhanging the column, let it stand at room temperature for 5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the RNA solution, and store the RNA at -70° C to prevent degradation.

Attention:

1) The volume of RNase-Free Water should not be less than 20 μl, too small volume affects the recovery.

2) If you want to increase the RNA yield, repeat step 11 with 20-50 μl of fresh RNase-Free Water.

3) If the RNA concentration is to be increased, the resulting solution can be reintroduced into the adsorption column and step 11 repeated.