Viral DNA/RNA Extraction Kit

Item No. V669947

Storage conditions: room temperature.

Products

individual parts making up a compound	50T
Buffer GL	15ml
Buffer GW1 (concentrate)	13ml
Buffer GW2 (concentrate)	15ml
Buffer RE	10ml
Proteinase K	12.5mg
Proteinase K Storage Buffer	1.25ml
Spin Columns RS with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	50

Products

This kit is suitable for the extraction of viral RNA and DNA from fresh or frozen plasma, serum and cell-free body fluids. It is easy to operate as it does not require the use of organic solvents such as phenol and chloroform for extraction. The kit uses a unique buffer system to enable efficient and specific binding of viral nucleic acids in lysate to silica gel centrifugal adsorption columns. Inhibitors of PCR and enzyme reactions as well as residual impurities can be efficiently removed in a two-step effective rinsing step, and finally high purity viral nucleic acids can be obtained by using a low-salt buffer or water for elution. The purified viral nucleic acid is free of protein, nuclease and other impurities, and can be used directly in PCR, RT-PCR, Real-Time PCR, blotting experiments and so on.

Self-contained reagent: anhydrous ethanol.

Pre-experiment and Important Notes

1. Add 1.25ml 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. Do not add Proteinase K directly into Buffer GL.
2. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller DNA fragments and a decrease in the amount of extracted DNA.
3. Avoid repeated freezing and thawing of serum or plasma, which can lead to protein denaturation or precipitation, reducing the viral titer and thus affecting the yield of extracted viral nucleic acids.
4. Anhydrous ethanol should be added to Buffer GW1 and Buffer GW2 according to the label instructions of the reagent bottle before first use.
5. Check Buffer GL for crystallization or precipitation before use. If crystallization or precipitation occurs, redissolve Buffer GL in a water bath at 56℃.

Procedure

1. Take a 1.5 ml centrifuge tube (self-provided) and add 20 μl Proteinase K.
2. Add 200 μl serum or plasma to the centrifuge tube. Add 200 μl Buffer GL and vortex and shake for 15 seconds.
Note: 1) Sample volume less than 200 μl can be made up by adding 0.9% NaCl (self-provided). 2) In order to ensure effective lysis of the sample, the sample needs to be mixed well with Buffer GL after adding Buffer GL.
3. Incubate at 56° C for 15 minutes, centrifuge briefly, and collect the solution from the wall of the tube to the bottom of the tube.
4. 250 μl of anhydrous ethanol was added, vortexed and shaken for 15 seconds, left at room temperature for 5 minutes, centrifuged briefly, and the solution on the wall of the tube was collected at the bottom of the tube.
Note: If the ambient temperature exceeds 25° C, anhydrous ethanol should be used after pre-cooling on ice.
5. Add the solution obtained in step 4 to the adsorbent column (RNase-Free Columns RS) that has been loaded into the collection tube, and if the solution cannot be added at one time, it can be transferred in several times. centrifuge the column at 12,000 rpm (~13,400 × g) for 1 min, pour off the waste liquid in the collection tube, and put the column back into the collection tube.
6. Add 500 μl of Buffer GW1 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
7. Add 500 μl of Buffer GW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
Note: Step 7 can be repeated if further DNA purity is required.
8. Add 500 μl of anhydrous ethanol to the adsorbent column and centrifuge at 12,000 rpm for 1 min. Pour off the waste liquid in the collection tube and put the adsorbent column back into the collection tube.

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

Note: The purpose of this step is the removal of residual ethanol from the adsorbent column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

10. Place the adsorption column in a new collection tube (RNase-Free Centrifuge Tube), add 20-150 μl of Buffer RE or sterilized water overhanging the middle of the adsorption column membrane, leave it at room temperature for 2-5 minutes, and then centrifuge it at 12,000 rpm for 1 minute to collect the nucleic acid solution.

Note: 1) If the downstream experiment is sensitive to pH or EDTA, you can use sterilized water for elution. The pH of the eluent has a great influence on the elution efficiency, if water is used as the eluent it should be ensured that its pH is 7.0-8.5 (the pH of water can be adjusted to this range with NaOH), and the elution efficiency is not high when the pH is lower than 7.0.

(2) For long-term storage, please store the DNA solution at -20℃ and the RNA solution at -70℃.

3) If the final concentration of DNA/RNA is to be increased, the DNA/RNA eluate obtained in step 10 can be re-spiked onto the adsorbent membrane and step 10 repeated.