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Viral Genome DNA/RNA Extraction Kit

Cat. No.: EXR0225

Package: 50T/100T

Storage: At room temperature(15℃-25℃) in dry place for 1 year.

Kit Contents

Component	50T	100T
Proteinase K	1 ml	1 ml × 2
Prewashing buffer	50 ml	50 ml × 2
Binding buffer	25 ml	50 ml
Washing buffer	15 ml	15 ml × 2
RNase free ddH_2O	15 ml	15 ml × 2
RNase free adsorption column	50 Units	100 Units
RNase free collection tube	50 Units	100 Units
Instruction	1 Piece	1 Piece

Note: Proteinase K should be kept at -20℃.

Product Description

Viral Genome DNA/RNA Extraction Kit is suitable for extracting genome from serum, cell supernatant and lymph fluid. It is not suitable for extraction of viral from cells and tissues. The extracted DNA/RNA can be used for various routine operations, including enzyme-cut, PCR, library construction, RT-PCR, etc.

Protocol (for reference only)

Add fresh opened absolute ethanol in Washing buffer before use, volume is based on the label of bottle as a reference. Put cap back on bottle and shake well. All centrifuge steps are performed at 2-8℃.

1. Take 0.5mL viral supernatant, centrifuge at 12,000rpm for 5min. Discard precipitation (this step can be omitted if there is no precipitation).
2. Add 20μL Proteinase K (10mg/mL) into supernatant, and mix it thoroughly. Incubate at 65℃ for 10min. Invert tube several times during incubating.
3. Pretreatment of RNase free adsorption column: take one RNase free adsorption column out of packing bag and put into one RNase free collection tube. Add 700μL Prewashing buffer in RNase free column and incubate at RT for 2min. Centrifuge at 12,000rpm for 2min at 2-8℃. Discard waste liquid, and keep adsorption column in collection tube for next step.
4. Add 500μL Binding buffer in the mixture in step 2 and mix thoroughly. Then add 400μL absolute ethanol and mix thoroughly. At this point, flocculation precipitation may form, which does not affect extraction of DNA/RNA. Add mixture into pretreatment RNase free adsorption column and incubate

at RT for 2mins. (The maximum volume of adsorption column is 750μL, it can be divided in two times. After one time adsorption and centrifuge, add remaining mixture to adsorption column for settling and centrifuge.)

5. Centrifuge at 12,000rpm for 2min and discard waste liquid. Put adsorption column in collection tube for next step.
6. Wash the adsorption column with 700μL Washing buffer, centrifuge at 12,000 rpm for 1 min. Discard waste liquid. Put adsorption column in collection tube for next step.

   Note: Please check if absolute ethanol has been added before use.
7. Repeat step 6 with another 500μL Washing buffer.
8. Centrifuge at 12,000rpm for 2min. Incubate at room temperature or 50℃ for several minutes to remove residual Washing buffer in column, otherwise residual enethanol will affect next experiment such as enzyme-cut, PCR.
9. Place the adsorbing column into a sterile 1.5-mL microcentrifuge tube. Add 50μL-100μL RNase free ddH_2O after preheating in 65℃water bath to the center of adsorption membrane. Incubate at room temperature for 5min, centrifuge at 12,000rpm for 2 min.Obtain high quality viral genome DNA/RNA.

Notes

1. Change gloves regularly. Bacteria on the skin can lead to RNase contamination. Use RNase-free plastic products and tips to avoid cross contamination. When extracting RNA, it is best to operate in RNase free environment.
2. Repeated freezing and thawing of stored samples should be avoided, otherwise it leads to degrade DNA/RNA.
3. If there is precipitation in Binding buffer, it can be re-dissolved in a 37°C water bath.
4. If sample is not completely digested, adsorbing column may be blocked, so centrifuge time can be appropriately extended.
5. If volume of RNase free ddH_2O is less than 50μL, it may affect recovery efficiency.
6. DNA/RNA should be stored in -70℃ in case of degradation.
7. RNA detection: The size of obtained genome RNA fragments is related to preservation conditions and types of viruses. Since the virus does not contain ribosomal RNA, it can't be detected by routine electrophoresis, and it can only be detected by later experiments. D260 value 1.0 is equivalent to approximately 40μg/mL of single-stranded RNA.
8. DNA detection: The size of obtained genome DNA fragments is related to the preservation conditions and types of viruses. D260 value 1.0 is equivalent to approximately 50μg/mL double-stranded DNA or 40μg/mL single-stranded DNA. If virus content is too low, obtained genome DNA may not be detectable by electrophoresis, but other experiments(such as PCR) will have results.