ISO 9001/14001 Certified company

Viral Gene-spin™ Viral DNA/RNA Extraction Kit

Cat. No. 17151 50 Columns

DESCRIPTION

Viral Gene-spin™ Viral DNA/RNA Extraction Kit is designed for rapid isolation of DNA or RNA from a variety of sample sources including fresh or frozen plasma, serum, other cell-free body fluids and virus-infected cell/tissue. The purified DNA/RNA is free of contaminants and impurities, and ideal for PCR and RT-PCR.

Viral Gene-spin™ Viral DNA/RNA Extraction Kit uses advanced silica-gel membrane technology for rapid and effective purification of DNA or RNA without organic extraction or ethanol precipitation. Furthermore, the buffering conditions are finely adjusted to provide optimum binding of the DNA/RNA to the column. Procedural directions of Viral Gene-spin™ Kit is very simple; users may purify DNA/RNA from a variety of target sources within 15min.

STORAGE

Store all components at RT.

KIT CONTENTS

Label	Description	Contents
Lysis Buffer[1]		30 ml
Binding Buffer		40 ml
Washing Buffer A		30 ml
Washing Buffer B[2] **(concentrate)**	Add 40ml of EtOH before use	10 ml
Elution Buffer	Elution Buffer	20 ml
Spin Columns (Orange color column)	Inserted into a collection tubes. (2.0ml tubes)	50 columns

[1] Lysis Buffer is composed high concentration of guanidium salt. The salt of Lysis Buffer is easy to precipitate, when the buffer is stored in low temperature (below 20℃). If the Lysis Buffer become solid, incubate in 80 ℃ for 10min.

[2] Washing Buffer B is supplied as concentrates. Add 40 ml of ethanol (96~100%) according to the bottle label before use.

PRECAUTIONS AND SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotrophic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

PROTOCOL

1. Transfer 150 (300)µl plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infection tissue or cell in the 1.5ml microcentrifuge tube.
   Note : If sample volume is less than 150µl, sample should be adjusted to 150µl with DEPC treated water.
2. Add 250 (500) µl of Lysis buffer.
   Note: If the sample volume is larger than 150µl, increase the amount of Lysis buffer (e.g., a 300µl sample will require 500µl of Lysis buffer) and if the Lysis buffer become solid, incubate in 80°C for 10min.
3. Mix by vortexing for 15sec.
4. Incubate at room temperature (15-25°C) for 10 min.
   Note : If the target virus is composed envelope structure (e.g., HBV), following the below step for recovery enhancing.
   1) Add 20µl of Proteinase K Solution(20mg/ml, not provided)
   2) incubate at 55°C for 10min.
5. Add 350 (700) µl of Binding buffer, and completely mix well by gently vortexing.
   Note : If the sample volume is more than 150µl, increase the amount of Binding buffer (e.g., a 300µl sample will require 700µl of Binding buffer). This step is conducive efficient passage of cell lysates through a column and to increase binding onto column resins and important for effective deproteinization.
6. Place a spin column in a provided 2ml collection tube.
7. Load lysates on the column and centrifuge at 13,000rpm for 1min.
   Note : The maximum volume of the column reservoirs 800µl. For sample volumes of more then 800µl, simply load and spin again. If the solution has not completely passed through the membrane, centrifuge again at higher speed until all of the solution passed through.
8. Discard solution in collection tube and place the column back in the same 2ml collection tube.
9. Add 500µl of Washing buffer A to column and centrifuge for 1min at 13,000rpm.
10. Discard solution in collection tube and place the spin column back in the same 2ml collection tube.
11. Add 500µl of Washing buffer B to the column and centrifuge for 1min at 13,000rpm.
12. Discard solution in collection tube and and place the spin column back in the same 2ml collection tube. Centrifuge for 1min at 13,000rpm.
    Note : It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
13. Place the column in a RNase-free 1.5ml microcentrifuge tube (not provided), and add 30-60µl of Elution buffer directly onto the membrane.
14. Incubate at RT for 1min, and then centrifuge for 1min at 13,000rpm.
15. Use 2-5µl of eluted solution for PCR or RT-PCR.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

• *Extraction efficiency comparison with phenol based method and Viral Gene-spin™ Viral DNA/RNA Extraction Kit*

Efficiency of similar products of various manufacturers are compared with Viral Gene-spin™ DNA/RNA Extraction Kit by testing different types of samples and determining by RT-PCR method.
Panel A, B exhibit equally superior extraction efficiency of Viral Gene-spin™ DNA/RNA Extraction Kit from different types of sample, while the performance of the similar kit is highly depend on the sample types.

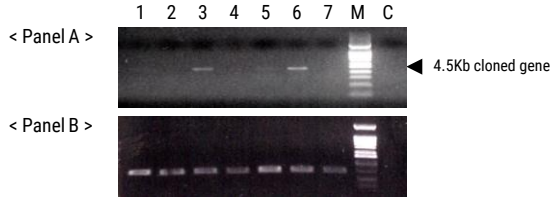

Fig. 1. Agarose gel electrophoresis of RT-PCR products from isolated total RNA.
Panel A, tested with kits applying Phenol-based-method; Panel B, tested with Viral Gene-spin™ DNA/RNA Extraction Kit.
Lane M, 100bp Ladder DNA Marker; **lane C,** Negative control; **lane 1-7,** various virus sample

• *Analysis of specific amplification of HCV RNA purified with Viral Gene-spin™ Viral DNA/RNA Extraction Kit.*

Sensitivity of the Viral Gene-spin™ Kit is tested by decimal dilution of total 5×10^5 $TCID_{50}$ HCV/ml samples. Viral RNA is obtained from 300ml samples by using the Viral Gene-spin™ DNA/RNA Extraction Kit. From 50ml eluted solution, 5ml were taken for RT-PCR with ONE-STEP RT-PCR PreMix Kit (Cat.No. 25101). About 5 $TCID_{50}$ HCV/ml can be detected.

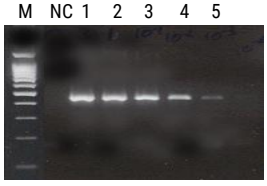

Fig. 2. RT-PCR amplification with ONE-STEP RT-PCR PreMix Kit (Cat.No. 25101)
RT-PCR results of HCV(Hog Cholera virus) specific RNA were obtained by using Viral Gene-spin™ DNA/RNA Extraction Kit. RT-PCR was performed with ONE-STEP RT-PCR PreMix Kit (Cat.No. 25101).
Lane M, Maker DNA; **lane NC,** Negative control; **lane 1,** 5×10^4 $TCID_{50}$/ml; **lane 2,** 5×10^3 $TCID_{50}$/ml; **lane 3,** 5×10^2 $TCID_{50}$/ml; **lane 4,** 5×10^1 $TCID_{50}$/ml; **lane 5,** 5 $TCID_{50}$/ml

• *Analysis of specific amplification of NDV (Newcastle disease Virus) RNA purified with Viral Gene-spin™ Viral DNA/RNA Extraction Kit.*

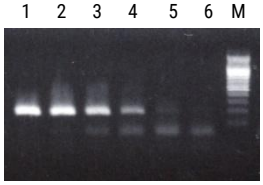

Fig. 3. Analysis of sensitivity of the ONE-STEP RT-PCR PreMix Kit (Cat.No. 25101) or detection of NDV(Newcastle disease Virus)
Total RNA was purified from virus using Viral Gene-Spin™ DNA/RNA Extraction Kit. Then the RT-PCR reaction was performed using ONE-STEP RT-PCR PreMix Kit (Cat.No. 25101).
Lane M, Maker DNA; **lane 1,** 10^{-2} diluted viral RNA; **lane 2,** 10^{-3} diluted viral RNA; **lane 3,** 10^{-4} diluted viral RNA; **lane 4,** 10^{-5} diluted viral RNA; **lane 5,** 10^{-6} diluted viral RNA; **lane 6,** 10^{-7} diluted viral RNA

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Little or no viral gene in the eluate	Low concentration of virus in the sample	- Concentrate the sample volume to 300 (150)ml using a microconcentrator (Centricom-100 or Microcep 100).
	Inefficient virus lysis in Lysis Buffer	- Precipitate, formed in Lysis Buffer after storage at 15℃ below, was not redissolved by heating before starting the procedure
	Lysis Buffer prepared incorrectly	- Check Lysis Buffer for precipitate. Dissolve precipitate by incubation at 80℃
	RNA degraded	- Often RNA is degraded by RNases in the starting material. It is recommended to work quickly during sample preparation. if necessary, add RNase inhibitor to the sample
	Too much starting material (In case of virus infected animal tissues)	- Do not overload the sample, overloading significantly reduces purity and yield. After tissues sample homogenization and brief centrifugation, transfer 300(150)ml supernatant to a new tube and add 500(250)ml Lysis buffer. Do not apply homogenized pellet.
	Buffer binding, washing A and washing B used in the wrong order	- Ensure that Buffer are used in the correct order in the protocol.
Viral gene does not perform well in subsequent enzymatic reactions	Ethanol carryover	- Ensure that after the Washing Buffer B wash, the column is spun at maximum speed for 1minute to dry the Viral-Gene spin™ membrane.

RELATED PRODUCTS

Product Name	Cat.No.
Maxime PCR PreMix (*i*-Taq)	25025 / 25026
Maxime PCR PreMIx (*i*-StarTaq)	25165 / 25167
Maxime PCR PreMix (*i*-pfu)	25185
Maxime RT PreMix (Oligo dT_{15} Primer)	25081
Maxime RT PreMix (Random Primer)	25082
Maxime RT-PCR PreMix	25131
i-StarMAX™ II DNA Polymerase	25173
i-Taq™ DNA Polymerase	25021 / 25022
Power cDNA Synthesis Kit	25011 / 25012
ONE-STEP RT-PCR PreMix Kit	25101 / 25102
MEGAquick-spin™ Total Fragment DNA Purification Kit	17286 / 17287/17288
easy-spin™ Total RNA Extraction Kit	17221
easy-BLUE™ Total RNA Extraction Kit	17061 / 17062
SiZer™-100 DNA Marker Solution	24073