Patho Gene-spin™ DNA/RNA Extraction Kit

For rapid and sensitive isolation of DNA or RNA from a variety of pathogen such as virus, bacteria and etc

RUO REF 17154 Σ 50 RT

DESCRIPTION

Patho Gene-spin™ DNA/RNA Extraction Kit is designed for rapid and sensitive isolation of DNA or RNA from a variety of pathogen such as virus, bacterium and etc. Samples can be either fresh or frozen plasma/blood (treated with anticoagulants other than heparin), serum, other cell-free body fluids and pathogen-infected cell/tissue. Patho Gene-spin™ DNA/RNA Extraction Kit is specifically designed to isolate high-quality nucleic acids from a variety of pathogen and specimen using low elution volumes that allow sensitive downstream analysis. The purified RNA/DNA is free of proteins and nucleases, and is suitable for use in downstream applications that allow pathogen detection. Patho Gene-spin™ DNA/RNA Extraction Kit uses the chaotropic salt in lysis buffer inactivates immediately DNase/RNase to ensure isolation of intact DNA/RNA. Patho Gene-spin™ 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 Patho Gene-spin™ DNA/RNA Extraction Kit is very simple; users may purify DNA/RNA from a variety of target sources within 30 min.

CHARACTERISTICS

- **Room Temperature Storage** : Can store 2 years at room temperature.
- **Maximized DNA/RNA Recovery**
  - ✓ Possible to extract high yield and purity of DNA/RNA from a variety of pathogen.
  - ✓ Chaotropic salt in lysis buffer inactivates immediately DNase/RNase to ensure isolation of intact DNA / RNA.
  - ✓ Rapid and efficient purification of high-quality nucleic acid using spin column-based centrifugation with no sample cross-contamination.
  - ✓ Ability to elute viral nucleic acids in low elution volumes of 30–60 µl to allow sensitive downstream analysis.
- **Application** : Pathogen detection, PCR, RT-PCR, Quantitative PCR (qPCR, qRT-PCR)
- **Rapid and Effective** : Purification of DNA/RNA without organic extraction or ethanol precipitation.

KIT CONTENTS

Label	Description	Contain
Lysis Buffer[1]		35 ml
Binding Buffer		35 ml
Washing Buffer A		30 ml
Washing Buffer B[2]	Add 40 ml of EtOH before use	10 ml
Elution Buffer		20 ml
Spin Columns	Inserted into a collection tubes. (2.0 ml tubes)	50 columns
Instruction Manual		1 sheet

1. Lysis Buffer is composed high concentration of chaotropic salt. Carefully handle it.
2. Washing Buffer B is supplied as concentrates. Add 40 ml of ethanol (96~100 %) according to the bottle label before use.

STORAGE AND STABILITY

All buffers of Patho Gene-spin™ DNA/RNA Extraction Kit should be stored at room temperature (15–25 °C) for up to 24 months without showing any reduction in performance and quality.

APPLICATIONS

Tissue, culture, blood and serum in the presence of pathogen nucleic acid extraction and detection of its research

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.

MATERIALS REQUIRED BUT NOT PROVIDED

- 100% Ethanol
- Disposable gloves
- 1.5 ml Tube
- PBS Buffer
- Microcentrifuge
- Vortex mixer
- General lab equipments

PROTOCOL

1. **Transfer 150 (300) µl plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infection tissue or cell in the 1.5 ml microcentrifuge tube.**
   Note : If sample volume is less than 150 µl, sample should be adjusted to 150 µl with DEPC treated water. If sample volume is more than 150 µl, you need to use 15 ml centrifuge tube.
2. **Add 300 (600) µl of Lysis Buffer.**
   Note : If the sample volume is larger than 150 µl, increase the amount of Lysis buffer (e.g., a 300 µl sample will require 600 µl of Lysis buffer)
3. **Mix by vortexing for 15 sec.**
4. **Incubate at room temperature (15-25 °C) for 10 min.**
5. **Add 300 (600) µl Binding buffer, and completely mix well by gently vortexing.**
   Note : If the sample volume is more than 150 µl increase the amount of Binding buffer (e.g., a 300 µl sample will require 600 µl 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 2 ml collection tube.**
7. **Load lysates on the column and centrifuge at 13,000 rpm for 1 min.**
   Note : The maximum volume of the column reservoirs 800 µl. For sample volumes of more then 800 µl 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 2 ml collection tube.**
9. **Add 500 µl of Washing Buffer A to column and centrifuge for 1 min at 13,000 rpm.**
10. **Discard solution in collection tube and place the spin column back in the same 2 ml collection tube.**
11. **Add 500 µl of Washing Buffer B to the column and centrifuge for 1 min at 13,000 rpm.**
    Note : Washing Buffer is supplied as a concentrate. Before using for the first time, add ethanol (96~100 %) as indicated on the bottle.
12. **Discard solution in collection tube and place the spin column back in the same 2 ml collection tube. Centrifuge for 1 min at 13,000 rpm.**
    Note : It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
13. **Place the column in a RNase-free 1.5 ml microcentrifuge tube (not provided), and add 30 - 60 µl of Elution Buffer directly onto the spin column membrane.**
    Note : It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
    Note : Avoid touching membrane with the pipet tip.
14. **Incubate at RT for 1 min, and then centrifuge for 1 min at 13,000 rpm.**
15. **Use 2-5 µl of eluted solution as template for PCR or RT-PCR.**

EXPERIMENTAL INFORMATION

Comparative test of extraction efficiency with Patho Gene-spin™ DNA/RNA Extraction Kit and competitor's

Total DNA/RNA from vaccine samples mixed with whole blood were extraction with Patho Gene-spin™ DNA/RNA Extraction Kit or competitor's. Samples were used 1/10 diluted with whole blood. After extraction, each of 5 µl of extracted pathogens were used as template of RT-PCR analysis.

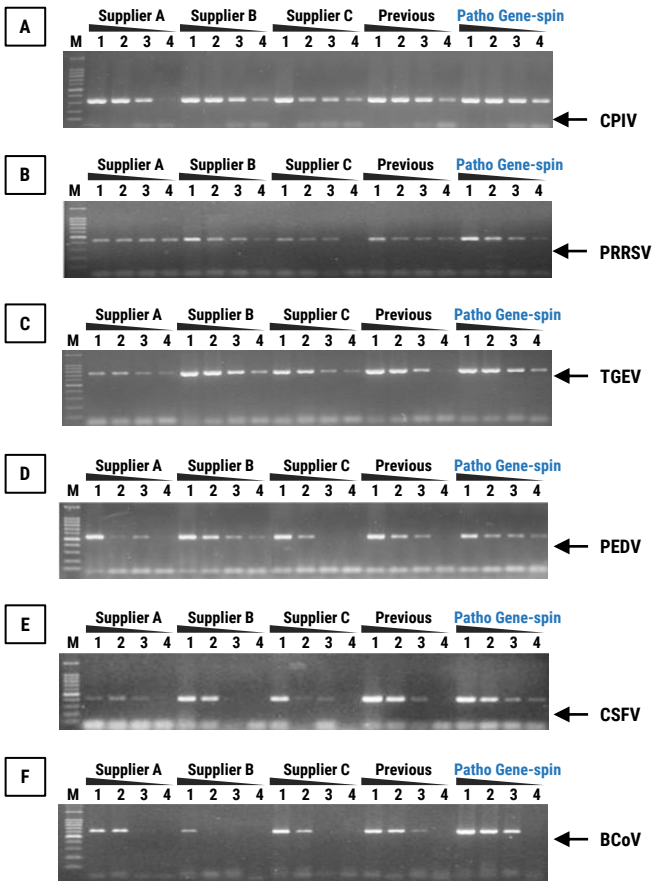

Fig. 1. RT-PCR Amplification for several virus detection with Maxime RT-PCR PreMix Kit of iNtRON
Panel A, Canine parainfluenza virus**; Panel B,** Porcine reproductive & respiratory syndrome virus; **Panel C,** Transmissible gastroenteritis virus; **Panel D,** Porcine epidemic diarrhea virus; **Panel E,** Classical swine fever virus; **Panel F,** Bovine Corona virus
lane M, DNA marker; **lane 1,** 10^0 diluted Sample; **lane 2,** 10^{-1} diluted Sample; **lane 3,** 10^{-2} diluted Sample; **lane 4**, 10^{-3} diluted Sample

Application of various pathogen DNA/RNA extracted with Patho Gene-spin™ DNA/RNA Extraction Kit

Total DNA/RNA samples were used 1/10 diluted with PBS Buffer, than pathogen from the samples were extraction with Patho Gene-spin™ DNA/RNA Extraction Kit. After extraction, each of 5 µl of extracted DNA/RNA were used as template of PCR/RT-PCR analysis.

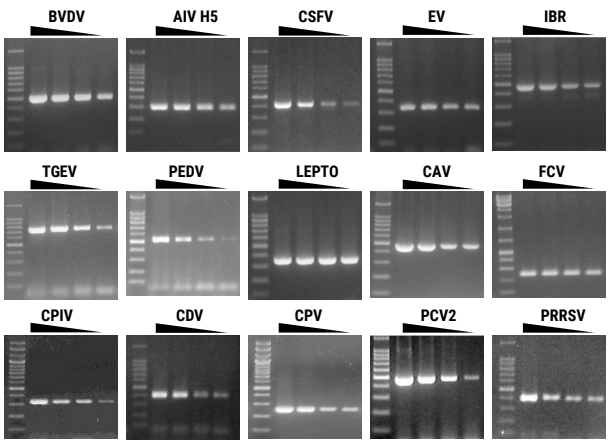

Fig. 2. Amplification for pathogen genomic extraction with Maxime PCR PreMix Kit (*i*-StarTaq) or Maxime RT-PCR PreMix of iNtRON

NOTICE

1. For research purpose only. Not intended for the diagnosis, prevention, or treatment of a disease.
2. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
3. Be careful and prevent the contamination and direct contact from the test samples .
4. Centrifuge and pipette should be regularly sterilized by 10% bleach solution.
5. All the waste should be sterilized before discarding.
6. The contamination should be considered very seriously. The work station should be kept with extreme cleanness not to have false-positive. Use RNase WiPER (iNtRON. Cat. 21131) to clean the desk or 1/20 diluted household bleach can be used alternatively.

SYMBOLS

LOT	Lot (batch) number	RUO	For research use only
REF	Product (Catalogue) number		Storage temperature limitation
Σ XX	Sufficient for XX tests		

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Little or no nucleic acid in the eluates	Low concentration of pathogen in the sample	• Concentrate the sample volume to 150 (300)µl using a microconcentrator (Centricom-100 or Microcep 100).
	Incomplete removal of medium (Cell samples)	• When processing cultured cells, ensure complete removal of the cell-culture medium after harvesting cells.
	Step were not followed correctly or wrong reagent used	• Check the protocol; Washing buffer B is Supplied as a concentrate. Ensure that ethanol Is added to Washing buffer B before use.
	RNA degraded	• Often RNA is degraded by RNase 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	• Do not overload the sample, overloading significantly reduces purity and yield. After tissues sample homogenization and brief centrifugation, transfer 150 (300) µl supernatant to a new tube and add 300 (600) µl Lysis buffer. Do not apply homogenized pellet.
	washing A and washing B used in the wrong order	• Ensure that Buffer are used in the correct order in the protocol.
Primer dimer or product bands are smeared	Ethanol carryover	• Ensure that after the Washing Buffer B wash, the column is spun at maximum speed for 1 minute to dry the Patho-Gene spin™ membrane.

RELATED PRODUCT

Product Name	Cat. No.
Maxime PCR PreMix (*i*-Taq)	25025 / 25026
Maxime PCR PreMIx (*i*-StarTaq)	25165 / 25167
Maxime PCR PreMix Kit (*i*-StarTaq™ GH)	26050 / 26051
HiSenScript™ RH(-) RT-PCR PreMix Kit	25135
Maxime RT-PCR PreMix	25131
RealMOD™ Probe qPCR mix	25341 / 25342
RealMOD™ Probe HiSenScript qRT-PCR mix	26010
i-StarTaq™ GH DNA Polymerase	26030 / 26031
SiZer™-100 DNA Marker Solution	24073