iNtRON Biotechnology
(#701-704, JungAng Industry, Sangdaewon-dong) 137, Sigimdaero-ro, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea
(주)인트론바이오테크놀로지
경기도 성남시 중원구사기막골로 137 (상대원동, 중앙인더스피아) 5층 701-704호

Patho Gene-spin™ DNA/RNA Extraction Kit

IVD reagents for extraction nucleic acids
MFDS Certificate No. 17-169

REF 17154 Σ 50 REF 17154.2 Σ 200 15℃ 25℃ CE IVD

INTRODUCTION

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 (15-25 ℃).
- 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

INTENDED TO USE

This product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.

The product is developed, designed and tested for both Research purposes (RUO) and in vitro diagnosis (IVD). Tissue, cell culture, blood and serum in the presence of pathogen nucleic acid extraction and detection of its research.

MATERIALS REQUIRED BUT NOT PROVIDED

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

KIT CONTENTS

Label	Contain	
	17154 (50T)	17154.2 (200)
Lysis Buffer[1]	35 ml	80 ml
Binding Buffer	35 ml	80 ml
Washing Buffer A[1]	30 ml	120 ml
Washing Buffer B[2](Concentrates)	10 ml* (Add 40 ml EtOH)	25 ml** (Add 100 ml EtOH)
Elution Buffer[3]	20 ml	20 ml
Spin Columns[4]	50 columns	200 columns
Instruction Manual	1 sheet	1 sheet

1. Lysis Buffer is composed high concentration of chaotropic salt. Carefully handle it.
2. Washing Buffer B is supplied as concentrate.
   * 17154 (50T) : Add 40 ml of ethanol (96~100 %) according to the bottle label before use.
   **17154.2 (200T) : Add 100 ml of ethanol (96~100 %) according to the bottle label before use.
3. DNase/RNase Free Water
4. Inserted into a collection tubes. Do not reuse. (2.0 ml tubes)

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.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products are undergone 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.

NOTICE

1. This product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.
2. The product is developed, designed and tested for both Research purposes (RUO) and in vitro diagnosis (IVD).
3. Always wear protective gear during handling chemical materials and the test should be handled by professionally trained person.
4. Be careful and prevent the contamination and direct contact from the test samples .
5. Surface of workspace and pipette should be regularly sterilized by 10% bleach solution.
6. All the waste should be sterilized before discarding.
7. 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.

PROTOCOLS

※ Before You Begin

1. **Transfer 150 µl plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infection tissue or cell in the 1.5 ml microcentrifuge tube.**
   [Note] A sample can be used as forms of swab-storage media, cell-free fluid, cell culture media, plasma, serum, urine, or other body fluid.
   [Note] If sample volume is less than 150 µl, sample should be adjusted to 150 µl with DEPC treated water.
2. **Add 300 µl of Lysis Buffer.[Note] It is critical for proper lysis to make the mixture homogenized.**
3. **Mix by vortexing for 15 sec.**
4. **Incubate at room temperature (15-25 ℃) for 10 min.**
5. **Add 300 µl Binding buffer, and completely mix well by gently vortexing.**
   [Note] 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.**

Customer & Technical Service
Do not hesitate to ask us any question
shop.intronbio.com
Tel : +82-505-550-5600
Fax : +82-505-550-5660
Mail : intronbio@intronbio.com

Belief & Relief, LiliF Diagnostics Mdx Kit
QUICK QUIDE
English (영문 표기)

LiliF Diagnostics

TROUBLE SHOOTING 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 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.

TECHNICAL INFORMATIONS

※ 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.

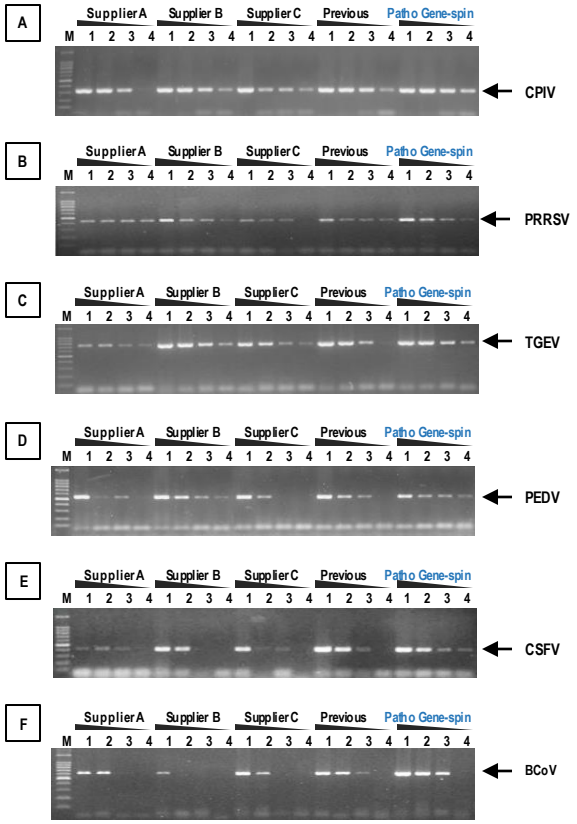

Fig. 1. RT-PCR Amplification for several virus detection with Maxime RT-PCR PreMix Kit of iNtRON

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.

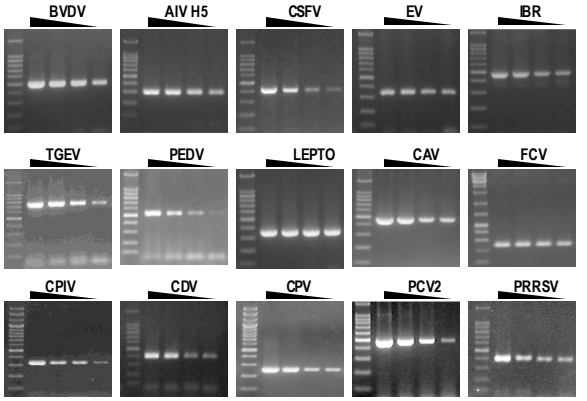

Fig. 2. Amplification for pathogen genomic extraction with Maxime PCR PreMix Kit (*i*-StarTaq) or Maxime RT-PCR PreMix of iNtRON

RELATED PRODUCTS

Product Name	Cat. No.
Maxime PCR PreMix (*i*-Taq)	25025 / 25026
Maxime PCR PreMIx (*i*-StarTaq)	25165 / 25167
Maxime RT-PCR PreMix	25131
SiZer™-100 DNA Marker Solution	24073

EXPLANATION OF SYMBOLS

Manufactured by | Attention | Manufacturing date | Expire date | Keep away from sunlight | Sufficient for tests | Consult Instructions For Use | Storage temperature limitation | Batch number | In vitro diagnostic | Product number

LOT | IVD | REF

• Document No. : QG-17154-R00
• Date : 2020. 09. 01

LiliF Diagnostics