iNtRON Biotechnology

Patho Gene-spin™ HS
DNA/RNA Extraction Kit

Ver. INT-IFU-17156-R00

Cat. No. 17156

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INTRODUCTION

1. The Patho Gene-spin™ HS DNA/RNA Extraction Kit is designed for rapid and sensitive isolation of DNA and 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.

2. One of the main advantages of the Patho Gene-spin™ HS DNA/RNA extraction kit is that it can effectively lysis a variety of samples using **Proteinase K** in the lysis phase, especially the **carrier RNA**, which allows for high sensitivity nucleic acid extraction, making it suitable for downstream applications

3. The kit quickly and effectively purifies DNA/RNA using silica-gel membrane technology. The usage of this product is so simple that users can purify DNA/RNA from various target sources within 30 minutes.

PRODUCT COMPONENTS

Contents	Unit	Q'ty
Proteinase K[A] (Lyophilized)	6 mg/vial	1
Carrier RNA[A] (Lyophilized)	300 µg/vial	1
Lysis Buffer[B]	35 ml	1
Binding Buffer	35 ml	1
Washing Buffer A[B]	30 ml	1
Washing Buffer B[C]	10 ml* (Add 40 ml EtOH)	1
Elution Buffer[D]	20 ml	1
Spin Columns[E]	50 columns	1
Instruction Manual	1 sheet	-

A. The lyophilized Proteinase K & carrier RNA can be shipping at room temperature (15–25°C) until the kit's expiration date. It should be dissolved in distilled water (D.W.) and stored at -20°C after dissolution.
B. Lysis Buffer & Washing Buffer A is composed high concentration of chaotropic salt.
C. Washing Buffer is supplied as concentrate. (Add ethanol according to the bottle label before use.)
D. DNase/RNase Free Water
E. Inserted into a 2.0 ml collection tube

STORAGE CONDITIONS

The Patho Gene-spin™ HS DNA/RNA Extraction Kit should be stored dry, at room temperature (15–25°C). Under these conditions, the Kit can be stored for up to 24 months without showing any reduction in performance.

The Freeze-dried proteinase K and carrier RNA should be avoided for long-term storage at room temperature. Store at -20°C after opening the product. Stabilize until the kit expires (24 months).
The proteinase K & carrier RNA reconstituted by resuspension in D.W. should be avoided for prolonged use at room temperature.
Store at 20°C and avoid repeating freezing and thawing.

ADDITIONAL REQUIRED EQUIPMENT and REAGENT

The Patho Gene-spin™ HS DNA/RNA Extraction Kit provides all reagents for high–sensitivity extracting DNA/RNA, including Proteinase K & carrier RNA. Prepared the following instruments for a quick and easy extraction.

- Pipettes and pipette tips
- Vortex mixer
- Microcentrifuge with rotor for 2.0 ml tubes
- Microcentrifuge tubes (not provided)
- Heating block
- Other general lab equipment
- Ethanol (96-100%)

SAFETY INFORMATION

The reagents provided contain high-concentrations of chaotropic salt. Guanidine thiocyanate and guanidine hydrochloride, which are components of the lysis buffer and washing buffer A, are harmful and irritating.
Protective equipment should always be worn when handling chemicals, and the inspection should be handled by a professionally trained person.


DO NOT add bleach or acidic solutions directly to the sample preparation waste.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

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

CONSIDERATION BEFORE USE

1. Lyophilized Proteinase K (6 mg)

Note : Dissolve the Proteinase K in 0.3 ml of pure D.W.

2. Lyophilized Carrier RNA (300 μg)

Note : Dissolve the Carrier RNA in 0.3 ml of pure D.W.

3. Centrifugation : All centrifugation steps are carried out at RT (15 - 25°C).

NOTICE

1. For research purpose only.

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. Surface of workspace and pipette should be regularly sterilized by 10% bleach solution.

5. All the waste should be sterilized before discarding.

PREPARATION

※ Before You Begin

- The samples are prepared at room temperature.
- Prepare the 56°C heating block for use in the lysis step.
- Prepare proteinase K and carrier RNA according to the instructions on page 2, 4, and verify that other reagents are ready.
- All centrifugation steps are carried out at room temperature (15 to 25°C).

PROCEDURE

1. Add 5 µL of Proteinase K solution into the microcentrifuge tube. (not provided)

2. Add 150 µL of samples into the microcentrifuge tube and vortex lightly.

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.

3. Add 300 µL of Lysis Buffer and 5 µL of carrier RNA.

Note : If you prepare a mixture of lysis buffer and carrier RNA according to the number of tests before adding the lysis buffer, you can make the experiment more convenient. For example, when conducting 10 tests, you can lightly vortex the mixture of 3.3 ml of lysis buffer and 55 µl of carrier RNA in advance according to the amount of 11 tests, and then add 305 µl to the microcentrifuge tube containing the sample.

4. Mix by vortexing for 15 sec.

5. Incubate at 56°C for 10 min.

6. Add 300 µL of 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.

7. Place the spin column. (Inserted into a 2 ml collection tube).

8. 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.

9. Discard containing solution in collection tube and place the column back in the same 2 ml collection tube.

10. Add 500 µl of Washing Buffer A to column and centrifuge for 1 min at 13,000 rpm.

11. Discard solution in collection tube and place the spin column back in the same 2 ml collection tube.

12. 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.

13. 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.

14. Place the column in a RNase-free 1.5 ml microcentrifuge tube (not provided), and add 40 - 50 µl of Elution Buffer directly onto the spin column membrane.

Note : It is recommended to use 50ul of elution volume. However, 40µl can also be used for elution depending on your requirements. By reducing the input of elution buffer, the extracted DNA and RNA concentration can be concentrated.

Note : It is important to dry the membrane since residual ethanol may interfere with downstream reactions. Avoid touching membrane with the pipet tip.

15. Incubate at RT for 1 min, and then centrifuge for 1 min at 13,000 rpm.

Note : Incubation at room temperature for about a minute before elution can increase the yield of isolated DNA and RNA.

TROUBLESHOOTING GUIDE

Problem	Possible Cause and Recommendation
Little or no nucleic acid in the eluates	If proteinase K and carrier RNA are degraded or not used properly, it may affect extraction performance. Check the conditions of use and storage of the components and use them according to the protocol. (2,4 pages) Repeated freezing/thawing may affect the stability of the reagent, so divide a certain amount according to the usage and store it in -20°C for use. If the concentration of pathogens in the sample is low, it is recommended to concentrate the sample volume to 150 µl using a microconcentrator (Centricom-100 or Microcep 100). When processing cultured cells, the cell culture medium must be removed completely after collecting the cells. You may not have followed the protocol properly or used an incorrect reagent. Check the protocol and make sure the appropriate buffer is used for each extraction step. Cleaning buffer B is provided as concentrate. Make sure ethanol is added to cleaning buffer B before use. RNA is often broken down by RNase in the starting material. It is recommended to work quickly during sample preparation. If necessary, add RNase inhibitors to the sample Using excessive samples greatly reduces purity and yield. After tissue sample homogenization and short centrifugation, transfer 150 µl supernatant to a new tube and add 300 µl dissolution buffer. Do not add homogenized pellets.
Poor amplification	If ethanol remains in the eluate, downstream application, such as the PCR reaction, may be inhibited. After cleaning buffer B, rotate the column at maximum speed for 1 minute to dry the patho gene-spin™ HS DNA/RNA Extraction kit membrane.

EXPERIMENTAL INFORMATIONS

High-sensitivity extraction performance for various pathogenic clinical samples

The Patho Gene-spin™ HS DNA/RNA extraction kit has effective in extracting nucleic acids from various samples and shows high-sensitivity extraction performance by minimizing the loss of nucleic acids extracted from low-titer samples.

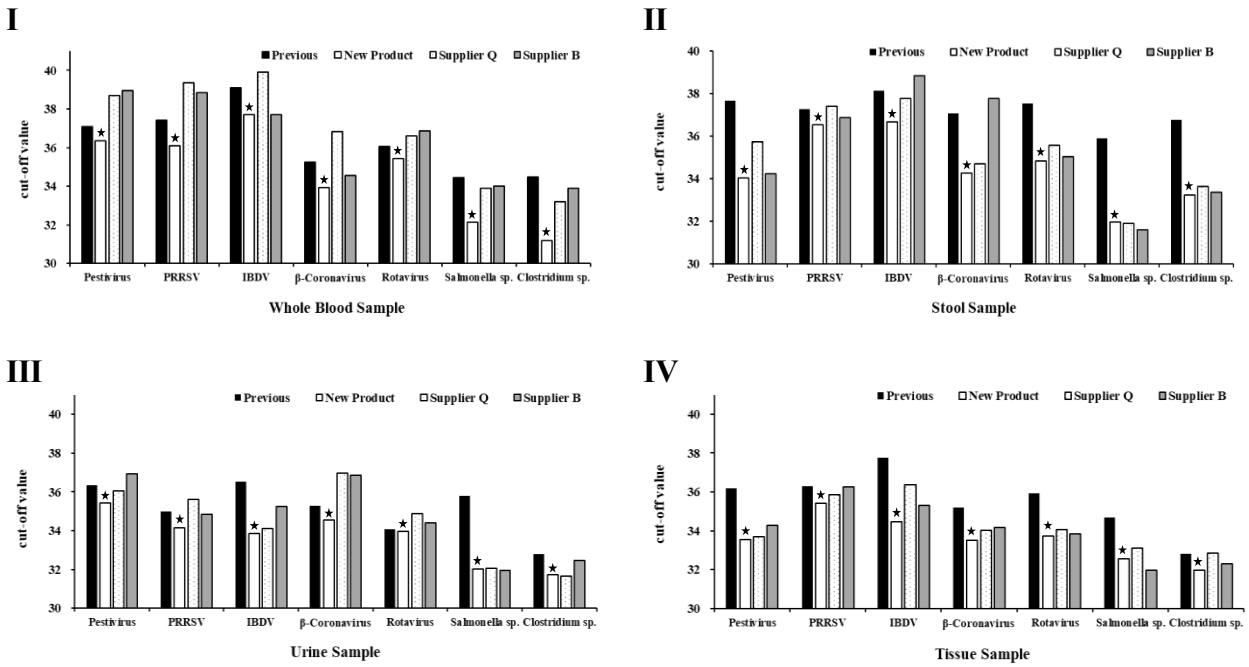

Fig. 1. Provides optimized Extraction performance for variety of pathogenic samples.
The extraction test used 3 enveloped viruses (*pestivirus, β-coronavirus*, and PRRSV) and 2 non-enveloped viruses (IBDV, *rotavirus*), Gram-positive bacteria (*Clostridium sp.*), and Gram-negative bacteria (*Salmonella sp.*) on a low-titer basis, and was performed on 4 spiked biological samples: (I) whole blood, (III) stool, (III) urine, and (IV) tissue. The Patho Gene-spin ™ HS DNA/RNA extraction Kit showed high-sensitivity extraction from a variety of samples and showed the same or superior extraction performance as other products.

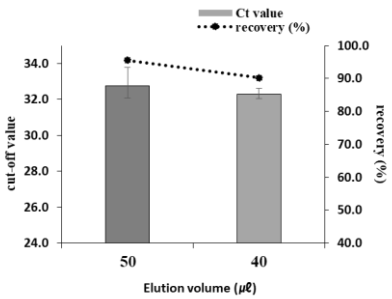

Fig. 2. Flexible Elution Volume Adjustment
The elution can be adjusted from 40 µl to 50 µl depending on the desired volume. Generally, 50 µl is recommended, but if you need to increase the nucleic acid concentration to increase the extraction sensitivity, you can use 40 µl. After extracting viral nucleic acids using pestivirus sample, the detection results was checked by qPCR, indicating an enhanced cut-off value when the elution was 40 µl compared to the results obtained using 50 µl.

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