Patho Gene-spin™ DNA/RNA Extraction Kit

QUICK GUIDE

50 prep : 17154
200 prep : 17154.2

1. KIT DESCRIPTION

Patho Gene-spin™ DNA/RNA Extraction Kit is designed for the rapid, highly sensitive, and reproducible isolation of viral and bacterial DNA/RNA from a broad range of biological and pathogen-infected samples. Optimized for both diagnostic research and veterinary in vitro diagnostic (IVD) testing, the kit delivers high-purity nucleic acids suitable for stringent downstream molecular assays.

Compatible sample matrices include fresh or frozen plasma, serum, whole blood (treated with non-heparin anticoagulants), swabs, cell-free body fluids, and pathogen-infected cell cultures or tissue homogenates. Utilizing advanced silica-gel membrane technology combined with an optimized chaotropic lysis system, the kit rapidly inactivates endogenous nucleases (DNase/RNase) to preserve nucleic acid integrity. The streamlined workflow yields purified pathogen DNA/RNA within 30 minutes without organic extraction or ethanol precipitation, removing proteins and PCR inhibitors to ensure high analytical sensitivity in PCR, RT-PCR, and qPCR/qRT-PCR.

2. CHARACTERISTICS

- **Broad Sample Compatibility :** Optimized for diverse clinical, veterinary, and environmental sample matrices (blood, fluids, swabs, tissue).
- **High Sensitivity & Purity :** Efficiently isolates trace pathogen nucleic acids while completely removing enzymatic inhibitors.
- **Enhanced Analytical Sensitivity :** Compatible with micro-elution volumes (down to 30 µl) to concentrate low-copy pathogen target nucleic acids.
- **Rapid Workflow :** Completes nucleic acid isolation within 30 minutes without organic solvents or alcohol precipitation.
- **Effective Nuclease Inactivation :** Chaotropic salt lysis buffer ensures immediate and complete inactivation of cellular DNases and RNases.
- **Standardized Quality :** Engineered for high reproducibility in routine molecular diagnostic research and veterinary IVD workflows.

3. INTENDED USE

This product is intended for professional use by personnel trained in molecular biology techniques. It is designed for extracting pathogen genomic DNA and viral RNA from diverse clinical, environmental, and veterinary specimens to support pathogen identification, surveillance, and diagnostic research.

4. KIT CONTENTS AND STORAGE

Contents	17154 (50 prep)	17154.2 (200 prep)
Lysis Buffer	35 ml	80 ml
Binding Buffer	35 ml	80 ml
Washing Buffer A	30 ml	120 ml
Washing Buffer B	10 ml (add 40ml EtOH)	25 ml (add 100ml EtOH)
Elution Buffer	20 ml	20 ml
Spin Columns (with collection tubes)	50 columns	200 columns

- Storage & Stability: Store all components at room temperature (15–25°C). Stable for up to 24 months.
- Documentation: Quick Guide / Instruction Manual included.

Note 1 : Add specified volume of ethanol (96–100%) to Washing Buffer B before initial use (40 ml for Cat. 17154 / 100 ml for Cat. 17154.2).

Note 2 : Lysis Buffer contains a high concentration of chaotropic salts. Handle with care.

5. NOTICE & SAFETY PRECAUTIONS

1) For professional research and veterinary diagnostic use.
2) Wear appropriate protective equipment (gloves, lab coat, eye protection) when handling chemicals and biological samples.
3) Prevent sample cross-contamination during sample preparation and pipetting.
4) Periodically decontaminate work surfaces and pipettes using 10% household bleach or a validated RNase decontamination solution (e.g., RNase WIPER, Cat. No. 21131).
5) Autoclave or treat all biological waste in accordance with local biosafety and hazard disposal regulations.

6. MATERIALS REQUIRED BUT NOT PROVIDED

- 100% Ethanol
- Vortex mixer
- Disposable gloves
- PBS Buffer
- Microcentrifuge
- 1.5 ml microcentrifuge tubes

7. PROTOCOL

✱ Before you begin

- Ensure that ethanol (96–100%) has been added to Washing Buffer B concentrate as indicated on the bottle label prior to initial use.
- Preheat Elution Buffer (or sterile nuclease-free water) to room temperature or 56°C if required.

1) **Transfer 150 µl of sample (plasma, serum, urine, cell-free fluid, or swab media) into a 1.5 ml microcentrifuge tube.**

Note : If sample volume is less than 150 µl, adjust the total volume to 150 µl using DEPC-treated water or PBS.

2) **Add 300 µl of Lysis Buffer and vortex thoroughly for 15 seconds.**

Note : Complete lysis and homogenization are critical for optimal nucleic acid yield.

3) **Incubate at room temperature (15–25°C) for 10 minutes.**
4) **Add 300 µl of Binding Buffer and mix thoroughly by gentle vortexing.**

Note : Complete lysis and homogenization are critical for optimal nucleic acid yield.

5) **Pipette the lysate (~750 µl) onto a Spin Column inserted in a 2 ml collection tube.**

Note : Maximum column reservoir capacity is 800 µl. For sample volumes over 800 µl, load and centrifuge sequentially.

6) **Centrifuge at 13,000 rpm for 1 minute. Discard the flow-through and reinsert the column.**

Note : If liquid has not completely passed through the membrane, centrifuge again at a higher speed.

7) **Add 500 µl of Washing Buffer A and centrifuge at 13,000 rpm for 1 minute. Discard the flow-through and reinsert the column.**
8) **Add 500 µl of Washing Buffer B (ethanol added) and centrifuge at 13,000 rpm for 1 minute. Discard the flow-through and reinsert the column.**
9) **Centrifuge at 13,000 rpm for an additional 1 minute to completely dry the membrane.**

Note : Complete removal of residual ethanol is essential, as carryover ethanol inhibits downstream PCR/RT-PCR reactions.

10) **Transfer the spin column to a clean, RNase-free 1.5 ml tube, and add 30–60 µl of Elution Buffer directly onto the center of the membrane. Incubate for 1 minute at room temperature.**
11) **Centrifuge at 13,000 rpm for 1 minute to elute purified DNA/RNA.**

8. TROUBLE SHOOTING GUIDE

Problem	Possible cause	Recommendation
Low nucleic acid yield	Low pathogen concentration in sample	• Concentrate sample to 150 µl using a microconcentrator prior to lysis.
	Incomplete medium removal (Cell cultures)	• Ensure complete removal of cell culture media before harvesting cells.
	Incorrect protocol or reagent preparation	• Verify that ethanol (96–100%) was added to Washing Buffer B concentrate before use.
	RNA degradation	• Use fresh samples. Work quickly and add RNase inhibitors if necessary.
	Overloaded starting material	• Do not overload the column. Homogenize tissue samples thoroughly and use 150 µl of clarified supernatant.
	Washing buffers used in wrong order	• Strictly follow the sequential application of Washing Buffer A followed by Washing Buffer B.
Smeared bands / Inhibited downstream PCR	Ethanol carryover	• Ensure the spin column is centrifuged at maximum speed (13,000 rpm for 1 min) during the drying step (Step 9) to completely eliminate residual ethanol.

9. TECHNICAL INFORMATIONS

※ Experimental Data 1 : Comparative Extraction Efficiency

Comparative analysis of extraction efficiency between Patho Gene-spin™ DNA/RNA Extraction Kit and competitor kits. Pathogen DNA/RNA samples were prepared by 1/10 serial dilutions with whole blood or PBS buffer. After extraction using each kit, 5 µl of eluted DNA/RNA was used as a template for RT-PCR analysis.

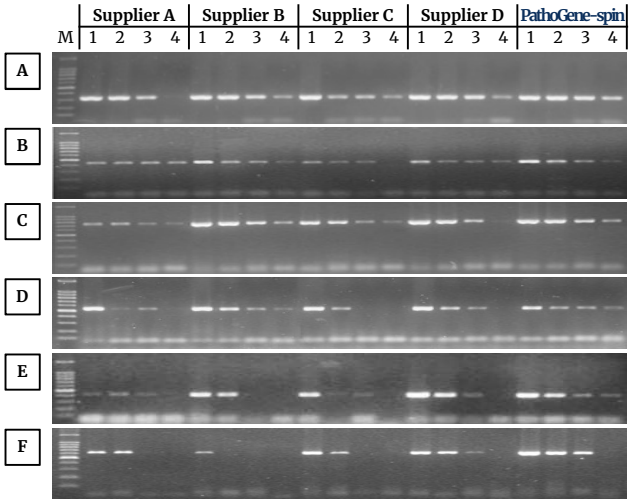

Fig. 1. Comparative Analysis of Viral RNA/DNA Extraction Efficiency Against Competitors

Comparative RT-PCR evaluation of viral nucleic acids extracted using Patho Gene-spin™ Kit, previous version, and competitor kits (Suppliers A, B, C). Target pathogens were diluted 10-fold serially (Lanes 1–4) in whole blood or buffer matrices prior to extraction.

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

※ Experimental Data 2 : Broad-Spectrum Pathogen Extraction & Downstream PCR Compatibility

To evaluate extraction versatility across diverse sample matrices and target organisms, total DNA/RNA was isolated from various pathogen-infected clinical and veterinary specimens diluted 1:10 in PBS buffer using Patho Gene-spin™ Kit. Purified nucleic acids (5 µl per reaction) were subjected to PCR or RT-PCR using Maxime PCR PreMix (i-StarTaq) or Maxime RT-PCR PreMix.The results demonstrate complete removal of PCR inhibitors and reliable, high-yield recovery across 15 distinct viral and bacterial targets (including RNA viruses, DNA viruses, and bacteria), ensuring superior sensitivity for routine diagnostic research.

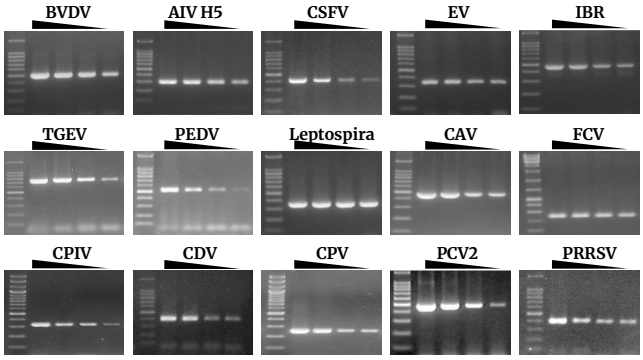

Fig. 2. Broad-Spectrum Pathogen Detection Performance Across Diverse Viral and Bacterial Targets

Validation of downstream PCR and RT-PCR compatibility using genomic DNA and viral RNA extracted with Patho Gene-spin™ Kit. Nucleic acids were extracted from 10-fold diluted specimens and amplified using Maxime PCR PreMix (i-StarTaq) or Maxime RT-PCR PreMix.

Tested Pathogen Targets (15 Species)

- **DNA Viruses & Bacteria :** IBR, CAV, CPV, PCV2, Leptospira
- **RNA Viruses :** BVDV, AIV H5, CSFV, EV, TGEV, PEDV, FCV, CPIV, CDV, PRRSV

All target pathogen nucleic acids were successfully amplified with high signal intensity and specific band clarity, demonstrating superior purity and complete removal of downstream PCR inhibitors.

10. ORDERING INFORMATION

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
RealMOD™ Probe R² 2X qPCR mix (with UDG)	25363.200 (200 rxns) 25363.500 (500 rxns) 25363.1000 (1000 rxns)
RealMOD™ Probe R² 2X qRT-PCR mix (with UDG)	25362.100 (100 rxns) 25362.500 (500 rxns) 25362.1000 (1000 rxns)