REF PCR-V053-48R
PCR-V053-96R

VetPCR™ NDV Detection Kit

SYMBOLOGY

Manufacturer

−20 ° C 30 ° C Limits Temperature

Caution

Expiration Date

REF Catalog Number

Consult Instructions for Use

1. DESCRIPTION

Newcastle disease is caused by an RNA virus, paramyxovirus-1 (PMV-1), also known as Newcastle disease virus (NDV). NDV is a contagious and fatal viral disease affecting most species of birds. Clinical signs are extremely variable depending on the strain of virus, species and age of bird, concurrent disease, and preexisting immunity. NDV is so virulent that many birds die without showing any clinical signs. A death rate of almost 100 percent can occur in unvaccinated poultry flocks. NDV can infect and cause death even in vaccinated poultry.

VetPCR™ NDV Detection Kit is the direct detection of Newcastle disease virus on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 3 hours for detection.

Therefore, it is a very fast, accurate and reliable technique.

2. STORAGE

The VetPCR™ NDV Detection Kit is shipped at room temperature (15–25°C) because contains a chemical stabilizer.

The VetPCR™ NDV Detection Kit should be stored immediately upon receipt at –20°C in a constant-temperature freezer. For routine use should be stored al 4°C. When stored under these conditions and handled co-rrectly, these products can be kept at least until the expiration date without showing any reduction in performance.

3. KIT CONTENTS

KIT	48	96	
VetPCR™ NDV RT-PCR Premixture	1	1	vial
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Brig™ RT-PCR solution	1	1	vial
Biotech™ Transcriptase solution	1	1	vial
DNase/Rnase free water	1	1	vial
NDV PCR Positive control	1	1	vial
PCR Negative control	1	1	vial
PCR Internal Control (white cap)	1	1	vial
Mineral Oil solution	1	2	vial
Brig™ Molecular Weight marker	1	1	vial

4.MATERIALS

Materials required but not provided:

- Microcentrifuge and PCR tubes, disposable gloves powderless, pipettes, sterile pipette tip
- Vortex mixer, Centrifuge for microcentrifuge tubes
- Thermal cycler
- Tube racks
- Electrophoresis kit, UV transilluminator
- Biohazard waste container.

5.PROCEDURE

Please read through the entire procedure before starting.

5.1 PREPARATION OF NDV RT-PCR MIXTURE

1) Prepare the reaction mixture for sample by combining the reagents as shown in the table 1. The final reaction volume should be 10.5µl.

Notes:

- The mineral oil is necessary, even when using a termal cycler that employs a top heating method.

Table 1. Reaction components for RT-PCR

Kit components	samples
VetPCR™ NDV RT-PCR Premixture	4.5µl
DNase/RNase free water	4µl
RNA isolated from the sample	2µl
Mineral Oil Solution	11µl

2)Place the tubes in a thermal cycler and perform RT-PCR according to the program outlined in Table 2.

Table 2. RT-PCR cycling parameters

PCR cycle			Temp	Time
RT-PCR 1	1 cycle	Initial denaturation	65 ºC	5 min.
	1 cycle	Stop	4 ºC	5 min.
Add 1.0 µl of Brig™ RT-PCR solution				
Add 1.0 µl of Biotech™ Transcriptase solution				
RT-PCR 2	1 cycle	Annealing	25 ºC	10 min.
	1 cycle	Extension	42ºC	60 min.
	1 cycle	Denaturation	65 ºC	5 min.

3) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 3. The final reaction volume should be 13.5µl.

Notes:

- Run a positive control, a negative control, and an internal control each 12 samples.
- The mineral oil is necessary, even when using a termal cycler that employs a top heating method.

Table 3. Reaction components for PCR

KIT	Sample	Positive control	Negative control	Internal control
VetPCR™ NDV PCR Premixture	5.5 µl	5.5 µl	5.5 µl	
PCR Internal Control (white cap)				5.5 µl
DNase/Rnase free water	6 µl	6 µl	6 µl	6 µl
cDNA from the sample	2 µl			2 µl
NDV PCR Positive control		2 µl		
PCR Negative control			2 µl	
Mineral Oil solution	11 µl	11 µl	11 µl	11 µl

4) Place the tubes in a thermal cycler and perform amplification according to the program outlined in Table 4.

Table 4. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	95ºC	3 min.
30 cycles	Denaturation	95ºC	30 sec.
	Annealing	56ºC	30 sec.
	Extension	72ºC	30 sec.
1 cycle	Final extension	72ºC	3 min.
1 cycle	Hold	10ºC	5 min.

5.2 DETECTION OF AMPLIFIED PRODUCTS

1) Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
2) Load 7µl of PCR product, 7µl of positive control, 7µl of negative control, 7µl of internal control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.
3) Run electrophoresis by 100V (required about 30~40 minutes).
4) Identify the result on ultra-violet (UV) transilluminator.

5.3 INTERPRETATION OF THE TEST RESULTS

- Expected PCR product size : 162bp

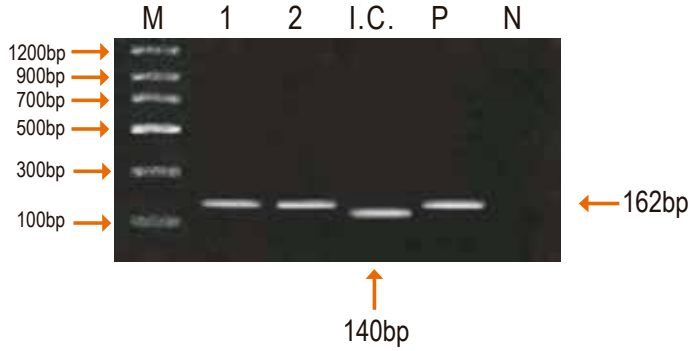

Fig. 1 Results:

Lane M: Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane 1~2: NDV Positive samples
Lane I.C.: Internal control
Lane P: Positive control
Lane N: Negative control

6. TROUBLESHOOTING

1.No band in positive control

• Check Internal control band: If internal control band is seen, PCR has been performed properly.
• Check PCR machine: check the temperature and make sure to check that the machine is working properly.

2.No internal control band

• Check template cDNA concentration: Competition can occur by high template concentration. Proceed with a lower concentration of cDNA.
• Check template RNA quality: Even tough RNA is isolated from the sample, the RT-PCR reaction can be inhibited depending on RNA purity in some cases. In this case, extracted RNA should be diluted 10 times with distilled water and used to run the RT-PCR reaction again. If still no band is seen, please inquire with our technical support staff.

3.Amplicon bands in the negative control

• Check contamination of distilled water: Distilled water can be contaminated. Perform PCR again with fresh sterile water.
• Check contamination of laboratory instruments and other environments: We recommend that you use filter tips and a pipette after sterilization to reduce contamination. Proceed with all procedures on a clean bench and keep the location where you procedures are performed sterile.

4.Poor resolution on agarose gel

• We recommend using a 1.5~2% agarose gel and run electrophoresis for 40 minutes at 100 V.

7. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS), available online in our website, where you can find, view, and print the MSDS for each Bioingentech kit and kit component.