

## 1. DESCRIPTION

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens that has lymphoid tissue as its primary target with a special predilection for the bursa of Fabricius. It was first recognized as a specific disease entity by Cosgrove in 1962 and was referred to as javian nephrosis because of the extreme kidney damage found in birds that succumbed to infection. Since the first outbreaks occurred in the area of Gumboro, Delaware, Gumboro disease was a synonym for this disease and is still frequently used. The incubation period is very short, and clinical signs of the disease are seen within 2-3 days after exposure. One of the earliest signs of infection in a flock is the tendency for some birds to pick at their own vents. Soiled vent feathers, whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, severe prostration, and finally, death. Affected birds became dehydrated, and in terminal stages of the disease, had a subnormal temperature.

VetPCR™ IBDV Detection Kit is the direct detection of Infectious bursal 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 4 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

## 2. STORAGE

The components of VetPCR™ IBDV Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

## 3. CONTENTS

	Kit	Kit	
	48	96	
VetPCR™ IBDV RT-PCR Pre-mixture			
VetPCR™ IBDV PCR Pre-mixture	48	96	tubes
BrigRT-PCR™ solution	48	96	vial
Biotech™ Transcriptase solution	1	1	vial
DNase/RNase-free water	1	1	vial
IBDV RT-PCR Positive control	1	1	vial
IBDV RT-PCR Positive control Pre-mixture	1	1	vial
IBDV PCR Positive control Pre-mixture	4	8	tubes
Brig™ Molecular Weight marker	4	8	tubes
Mineral Oil	1	1	vial
RNA extraction kit (see step 6.1)	1	2	vial(s)
	50	100	tests

## 4. SPECIMEN

0.5 ml feces or cloacal swab.

## 5. ADDITIONAL REQUIRED MATERIALS

- Pipettes, Sterile pipette tip, Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cyler, Electrophoresis kit, UV transilluminator

## 6. PROCEDURE

*Please read through the entire procedure before starting.*

### 6.1 RNA PREPARATION

Various manufacturers offer RNA isolation kits. Please carry out the RNA isolation according to the manufacturers instructions. The following standard RNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Total RNA Purification Kit (50 test)	230041(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Total RNA Purification Kit(100 test)	230041(100)	Bioingentech Biotechnology Inc.

### 6.2 AMPLIFICATION

1.- Prepare appropriate RT-PCR Premix tubes and one RT- PCR Premix tube for Positive control. Label.

2.- Add 5µl of DNase/RNase-free water into the RT-PCR Premix tube to total volume as 8,5µl.

3.- Add 1,5µl of template RNA into the RT-PCR Premix tube to total volume as 10µl.

4.- Add 5µl of DNase/RNase-free water and 1,5µl of RT-PCR Positive control into a RT-PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11µl). This step is necessary, even when using a thermal cyler that employs a top heating method.

6.- Perform RT-PCR reaction (RT-PCR 1) of samples as the below process using a PCR thermal cyler.

7.- Add 0,3µl of BrigRT-PCR™ solution and 0,5µl of Biotech™ Transcriptase solution.

8.- Perform RT-PCR reaction (RT-PCR 2) of samples as the below process, using a PCR thermal cyler.

RT-PCR cycle			Temp.	Time
RT-PCR 1	1Cycle	Initial Denaturation	80°C	10 min.
	1Cycle	Stop	4°C	5 min.
Add 0,3µl of BrigRT-PCR™ and 0,5µl of Biotech™ Transcriptase				
RT-PCR 2	1Cycle	Denaturation	80°C	10 min.
	1Cycle	Annealing	25°C	10 min.
	1Cycle	Extension	37°C	50 min.

9.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

10.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

11.- Add 2µl of template (cDNA) into the PCR Premix tube to total volume as 13µl.

12.- Add 6µl of DNase/RNase-free water and 2µl of Positive control (Positive control tube from RT-PCR) into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

13.- Add mineral oil (11µl). This step is necessary, even when using a thermal cyler that employs a top heating method.

14.- Perform PCR reaction of samples as the below process, using a PCR thermal cyler.

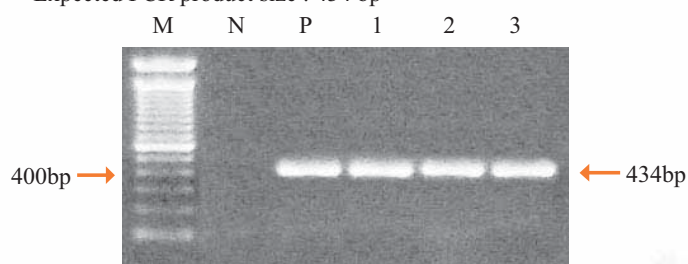
PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
30 Cycles	Denaturation	94°C	30 sec.
	Annealing	55°C	30 sec.
	Extension	72°C	30 sec.
1 Cycle	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

- 1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
- 2.- Load 7 $\mu$ l of PCR product, 7 $\mu$ l of Positive control and 2 $\mu$ l of Brig<sup>TM</sup> 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.

### 6.4 INTERPRETATION

- Expected PCR product size : 434 bp



**Fig 1.** Electrophoresis of PCR product by VetPCR<sup>TM</sup> IBDV Detection Kit  
 Lane M : Brig<sup>TM</sup> Molecular Weight Marker (Bioingentech Ltd.)  
 Lane N : Negative control  
 Lane P : Positive control  
 Lane 1–3 : IBDV Positive sample

### 7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. Bioingentech Biotechnology Inc. does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

### 8. TROUBLE SHOOTING

- 1.- In the case of difficult to interpret results due to non-specific bands; reduce amount of template by 1/10 dilution, heated at 65°C for 5 min. and reacts again.
- 2.- Preparation of PCR reaction at room temperature may cause the non-specific band.
- 3.- All procedure should be carried out on ice.

### 9. ORDERING INFORMATION

Product	Catalog No.
VetPCR <sup>TM</sup> IBDV Detection Kit 48	VET0006AR(48)
VetPCR <sup>TM</sup> IBDV Detection Kit 96	VET0006AR(96)
Brig <sup>TM</sup> Molecular Weight Marker	24012



**Bioingentech Ltd.**

Salas 350, piso 2, Concepción, Chile  
 Telephone (56)-(41)-2790435  
 Fax (56)-(41)-2790435  
 info@bioingentech.com  
 www.bioingentech.com

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