BioinGentech Ltd.
Genetic Engineering

VetPCR™ APV Detection Kit

1. DESCRIPTION

Avian polyomavirus (APV) causes budgerigar fledgling disease. The virus has a double-stranded DNA genome. It has a worldwide distribution and is one of the most significant pathogens of fledglings of caged birds such as macaws, conures, eclectus parrots, ring-necked parrots, lovebirds, and budgerigars. Polyomavirus is highly infectious, although many infections may be asymptomatic. Disease in adult birds is rare and may require a simultaneous infection with Psittacine Beak and Feather Disease Virus. Birds infected with APV can develop abdominal distention and a feather abnormality known as "French molt". The disease causes a lack of down feathers on the back and abdomen, filoplumes on the head and neck, and subcutaneous hemorrhage, sometimes culminating in mortality. APV and PBFD (psittacine beak and feather disease) virus cause similar feather abnormalities and it is difficult to differentiate the viral etiology based on clinical presentations. However, it is important to differentiate them since treatments and countermeasures differ for the two diseases.

VetPCR™ APV Detection Kit is the direct detection of Avian polyomavirus 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 components of VetPCR™ APV Detection Kit should be stored at -20ºC. Under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

	Kit 48	Kit 96	
VetPCR™ APV PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
APV PCR Positive control	1	1	vial
APV PCR Positive control Pre-mixture	4	8	tubes
Brig™ Molecular Weight marker	1	1	vial
Mineral Oil	1	1	vial
DNA purification kit (see step 6.1)	50	100	tests

4. SPECIMEN

0.5 ml whole blood in EDTA (purple top) tube, or cloacal swab, or swab of the fresh outer surface of liver, spleen or kidney, or tissue.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- Pipettes
- Sterile pipette tip
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Electrophoresis kit
- UV transilluminator

6. PROCEDURE

Please read through the entire procedure before starting.

6.1 DNA PREPARATION

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

Product	Catalog No.	Manufacturer
Bioingentech™ Genomic DNA Purification Kit (50 test)	230040(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Genomic DNA Purification Kit (100 test)	230040(100)	Bioingentech Biotechnology Inc.

6.2 AMPLIFICATION

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

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

3.- Add 2µℓ of template DNA into the PCR Premix tube to total volume as 13µℓ.

4.- Add 6µℓ of DNase/RNase-free water and 2µℓ of PCR Positive control into a 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 cycler that employs a top heating method.

6.- Perform PCR reaction of samples as the below process, using a PCR thermal cycler.

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94ºc	2 min.
30 Cycles	Denaturation	94ºc	30 sec.
	Annealing	56ºc	30 sec.
	Extension	72ºc	30 sec.
1 Cycle	Final extension	72ºc	5 min.

6.3 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 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.

6.4 INTERPRETATION

- Expected PCR product size : 500 bp

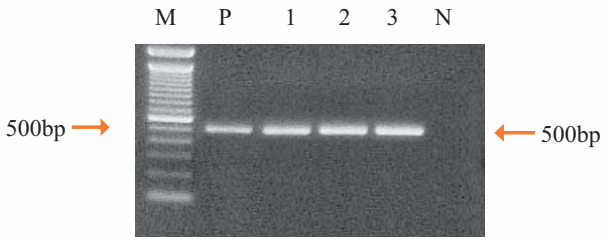

Fig1. Electrophoresis of PCR product by VetPCR™ APV Detection Kit
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane P : Positive control
Lane 1~3 : APV Positive sample
Lane N : Negative control

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 65º 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™ APV Detection Kit 48	VET0002A(48)
VetPCR™ APV Detection Kit 96	VET0002A(96)
Brig™ Molecular Weight Marker	24012

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