

1. DESCRIPTION

ADV, also known as pseudorabies virus (PrV, porcine herpesvirus 1), belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. Herpesvirus virions are enveloped, about 150 nm in diameter, and contain an icosahedral nucleocapsid about 100 nm in diameter, composed of 162 capsomers. The genome is linear dsDNA, 125-235 kbp in size. Replication occurs in the nucleus with sequential transcription of immediate early (α), early (β) and late (γ) genes producing α , β , and γ proteins respectively; the earlier genes and their products regulate the transcription of later genes. DNA replication and encapsidation occur in the nucleus; the envelope is acquired by budding through the inner layer of the nuclear envelope.

The virus infects the central nervous system and other organs, such as the respiratory tract, in virtually all mammals except humans and the tailless apes. Aujeszky's disease (AD) is primarily a disease of swine, which serve as a reservoir and the principal source of natural infection for a diverse range of secondary hosts, including cattle, sheep, goats, dogs, cats, and many feral species. Humans are refractory.

VetPCR™ ADV Detection Kit is the direct detection of Aujeszky's 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 components of VetPCR™ ADV 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 48	Kit 96	
VetPCR™ ADV PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
ADV PCR Positive control	1	1	vial
ADV 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

Nasal swabs, oro-pharyngeal fluid or tonsil biopsies.

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 6 μ l of DNase/RNase-free water into the PCR Premix tube to total volume as 11 μ l.

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

4.- Add 6 μ l of DNase/RNase-free water and 2 μ l of PCR Positive control into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11 μ l). 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
30 Cycles	1 Cycle	Initial Denaturation	94°C 2 min.
		Denaturation	94°C 30 sec.
		Annealing	54.5°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 μ l of PCR product, 7 μ l of positive control and 2 μ l 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 : 496 bp

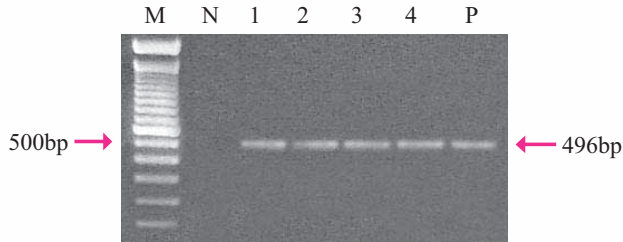


Fig1. Electrophoresis of PCR product by VetPCR™ ADV Detection Kit
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane N : Negative control
Lane 1~4 : ADV Positive sample
Lane P : Positive control

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 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™ ADV Detection Kit 48	VET0002P(48)
VetPCR™ ADV Detection Kit 96	VET0002P(96)
Brig™ Molecular Weight Marker	24012



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