VetPCR-RFLP™ K2 Detection Kit

1. DESCRIPTION

Fowl adenoviruses (FAdVs) belong to the group I avian adenovirus that includes conventional avian adenoviruses sharing a common group antigen. FAdVs have been classified by their serological relationships. According to the American classification, 12 fowl serotypes (FAdV1 to FAdV12) have been recognized to date. These12 FAdV serotypes have been grouped in five species (Fowl adenovirus A to E) by the International Committee on taxonomy of viruses. Such viruses are regularly isolated from chickens but thepathogenic role of most of the FAdVs is still questionable. The widespread occurrence of antibodies and the isolation of FAdVs from clinically healthy chicks argue against a primary role of FAdVs in a specific poultry disease. However, some FAdVs were shown to be involved in the aetiology of respiratory disease, inclusion body hepatitis and infectious hydropericardium, Angara disease or hepatitis/hydropericardium syndrome.

VetPCR™ K2 Detection Kit is the direct detection of Fowl adenoviruses serotypes B5, E6, E7, E8, E9, D2, D11 and D12 on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify and digest only specific gene using the PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) methods, and take only 6 hours for detection. Therefore, it is a very fast, accurate, reliable technique.

2. STORAGE

The components of Detection Kit should be stored at -20ºC, under this condition, the kit is stable until expiration date stated on the label.

3. KIT CONTENTS

KIT		
K2-FAdV.D2-11-12 Premix	1	Tubes
K2-FAdV.B-5/E-6-7-8-9	1	Tubes
E1	1	Tubes
E2	1	Tubes
E3	1	Tubes
E4	1	Tubes
E5	1	Tubes
E6	1	Tubes
DNase/RNase-free water (caps10)	4	Tubes
Brig™ Molecular Weight marker	1	Tubes
Mineral Oil	1	Tubes
Buffer 1 (caps 2)	1	Tubes
Buffer 2 (caps 2)	1	Tubes
Buffer 3 (caps 2)	1	Tubes
Buffer 4 (caps 2)	1	Tubes
Buffer 5 (caps 2)	1	Tubes
Buffer 6 (caps 2)	1	Tubes

4.MATERIALS

- Alantoid liquid, Tissue, Cloacal swab or feces.

5. ADDITIONAL REQUIRED MATERIALS

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

6. PROCEDURE

Please read through the entire procedure before starting.

Table 1. Reaction components for PCR

Kit components	Sample K2.FADV.B5E6789	Sample K2.FADV.D21112	Internal control
K2.FADV.B5E6789	5.5µl		
K2.FADV.D21112		5.5µl	
PCR Internal control			5.5µl
DNase/RNase free water	6µl	6µl	6µl
DNA isolated from the sample	2µl	2µl	2µl
PCR Negative control			
Mineral Oil Solution	11µl	11µl	11µl

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

Table 2. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	94ºC	2 min.
35 cycles	Denaturation	94ºC	30 sec.
	Annealing	60ºC	30 sec.
	Extension	72ºC	1,20 min.
1 cycle	Final extension	72ºC	10 min.

6.2 DETECTION OF AMPLIFIED PRODUCTS

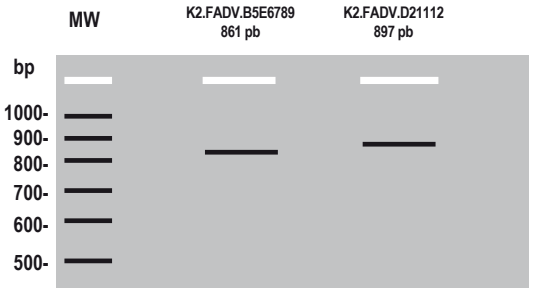
1.- Use 1.2% agarose gel containing Ethidium bromide (Et-Br).

2.- Load 5 of PCR product and 2 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.

6.3 INTERPRETATION



Enzyme cutting site

K2.FADV.B5E6789	E3 416 pb

Diferentiation Enzyme cutting:

FADV.B5	Only E3
FADV.E6	Only E1 and E5
FADV.E7	Only E4
FADV.E8	Only E5
FADV.E9	Only E6

K2.FADV.D21112	E2 500 pb

Diferentiation Enzyme cutting:

FADV.D2	Only E1 and E5
FADV.D11	Only E1
FADV.D12	Only E1 and E6

6.4 DIGESTION:

Example Digestion of PCR Product K2.FADV.B5E6789:
Digestión E1

1. Add 2 uL of E1 (cap1)
2. Add 2 uL of Buffer 1 (caps 2)
3. Add 10 uL of DNase/RNase-free water (caps10)
4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.B5E6789:
Digestión E2

1. Add 2 uL of E2 (cap1)
2. Add 2 uL of Buffer 2 (caps 2)
3. Add 10 uL of DNase/RNase-free water (caps10)
4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.B5E6789:
Digestión E3

1. Add 2 uL of E3 (cap1)
2. Add 2 uL of Buffer 3 (caps 2)
3. Add 10 uL of DNase/RNase-free water (caps10)
4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.B5E6789:
Digestión E4

1. Add 2 uL of E4 (cap1)

2. Add 2 uL of Buffer 4 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.D21112:
Digestión E1

1. Add 2 uL of E1 (cap1)

2. Add 2 uL of Buffer 1 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.B5E6789:
Digestión E5

1. Add 2 uL of E5 (cap1)

2. Add 2 uL of Buffer 5 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.D21112:
Digestión E2

1. Add 2 uL of E2 (cap1)

2. Add 2 uL of Buffer 2 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.B5E6789:
Digestión E6

1. Add 2 uL of E6 (cap1)

2. Add 2 uL of Buffer 6 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.D21112:
Digestión E3

1. Add 2 uL of E3 (cap1)

2. Add 2 uL of Buffer 3 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.D21112:
Digestión E4

1. Add 2 uL of E4 (cap1)

2. Add 2 uL of Buffer 4 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.D21112:
Digestión E5

1. Add 2 uL of E5 (cap1)

2. Add 2 uL of Buffer 5 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

Example Digestion of PCR Product K2.FADV.D21112:
Digestión E6

1. Add 2 uL of E6 (cap1)

2. Add 2 uL of Buffer 6 (caps 2)

3. Add 10 uL of DNase/RNase-free water (caps10)

4. Add 6 uL product PCR.

20 uL total

Time	
60 min	37°C
10 min	70°C

6.5 DETECTION OF DIGESTED PRODUCTS

1.- Use 2% agarose gel containing Ethidium bromide (Et-Br).

2.- Load 8 of digested products and 2 of BrigTM 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.- For the interpretation of result, see the instructive associate "INTERPRETATION OF TEST RESULT"

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	Product Code
VetPCR-RFLP™ K2 Detection Kit	020A2012
Bioingentech™ DNA Extraction Kit	230040
Brig™ Molecular Weight Marker	24012

Product use limitations warranty disclaimer

Bioingentech manufactures product for a number of Intended uses.Please refer to the product label for the Intended use statements for specific product. Bioingentech products contain chemicals which may be harmful if misused. Due care should be exercised whith all Bioingentech products to prevent direct human contact. Each Bioingentech product is shipped whith documentation Stating specifications and other technical information. Bioingentech products are warranted to meet or exceed the stated specifications. Bioingentech ´s sole the obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Bioingentech make no other warranty of any whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, INCLUDING,WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTE WITH RESPECT TO BIOINGENTECH PRODUCTS. In no event shall Bioingentech be Liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Bioingentech products to perform in accordance with the stated specifications.

Product use limitations warranty disclaimer

Products may be covered by pending or issued patents or may have certain limitations. Please visit our web site for more information.
All prices and specifications are subject to change without prior notice.
Product claims are subject to change. Please contact Bioingentech technical services or access the Bioingentech online catalog for the most up-to-date information on Bioingentech products.