

## 1. DESCRIPTION

Avian influenza is an infection caused by avian (bird) influenza (flu) viruses. These influenza viruses occur naturally among birds. Wild birds worldwide carry the viruses in their intestines, but usually do not get sick from them. However, avian influenza is very contagious among birds and can make some domesticated birds, including chickens, ducks, and turkeys, very sick and kill them. Infected birds shed influenza virus in their saliva, nasal secretions, and feces. Susceptible birds become infected when they have contact with contaminated secretions or excretions or with surfaces that are contaminated with secretions or excretions from infected birds. Domesticated birds may become infected with avian influenza virus through direct contact with infected waterfowl or other infected poultry, or through contact with surfaces (such as dirt or cages) or materials (such as water or feed) that have been contaminated with the virus.

Avian	Influenza	Virus	H9
• Potentially	nine	different	subtypes
• Documented	only	in low	pathogenic form
• At least three	H9	infections	in humans have been confirmed

VetPCR™ AIV-H9 Detection Kit is the direct detection of avian influenza virus serotype H9 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™ AIV-H9 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™ AIV-H9 RT-PCR Pre-mixture .....	48	96	tubes
VetPCR™ AIV-H9 PCR Pre-mixture .....	48	96	vial
BrigRT-PCR™ solution .....	1	1	vial
Biotech™ Transcriptase solution .....	1	1	vial
DNase/RNase-free water .....	1	1	vial
AIV-H9 RT-PCR Positive control .....	1	1	vial
AIV-H9 RT-PCR Positive control Pre-mixture .....	4	8	tubes
AIV-H9 PCR Positive control Pre-mixture .....	4	8	tubes
Brig™ Molecular Weight marker .....	1	1	vial
Mineral Oil .....	1	2	vial(s)
RNA extraction kit (see step 6.1) .....	50	100	tests

## 4. SPECIMEN

Tracheal swab.

## 5. ADDITIONAL REQUIRED MATERIALS

- 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 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 cycler that employs a top heating method.

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

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 cycler.

		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 cycler that employs a top heating method.

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

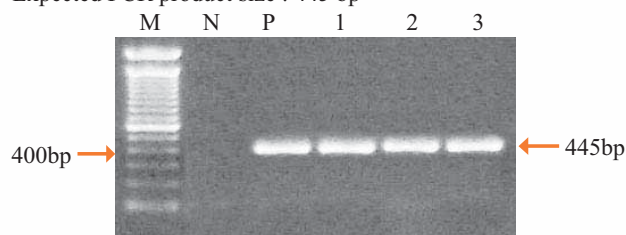
PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
30 Cycles	Denaturation	94°C	30 sec.
	Annealing	55.5°C	30 sec.
	Extension	72°C	30 sec.
1 Cycle	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

- 1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
- 2.- Load 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 : 445 bp



**Fig 1.** Electrophoresis of PCR product by VetPCR™ AIV-H9 Detection Kit  
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
 Lane N : Negative control  
 Lane P : Positive control  
 Lane 1–3 : AIV-H9 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™ AIV-H9 Detection Kit 48	VET0004AR(48)
VetPCR™ AIV-H9 Detection Kit 96	VET0004AR(96)
Brig™ Molecular Weight Marker	24012



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