

## 1. DESCRIPTION

*Flavobacterium columnare* is a thin Gram-negative rod bacterium of the genus *Flavobacterium*. Fish with columnaris usually have brown to yellowish-brown lesions (sores) on their gills, skin and/or fins. The bacteria attach to the gill surface, grow in spreading patches, and eventually cover individual gill filaments. This results in cell death. Portions of the gills are eroded by protein and cartilage-degrading enzymes produced by the bacteria. Skin lesions produced by columnaris initially are very shallow. More advanced lesions may be round or oval in shape, yellowish-brown in color, with an open ulcer in the center. A characteristic lesion produced by columnaris is a pale white band encircling the body, often referred to as saddleback condition. As the infection progresses, a yellowish-brown ulcer often is found in the center of the “saddle”.

VetPCR™ FLAV.COLUM Detection Kit is the direct detection of *Flavobacterium columnare* 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™ FLAV.COLUM 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™ FLAV.COLUM PCR Pre-mixture .....	48	96	tubes
DNase/RNase-free water .....	1	1	vial
FLAV.COLUM PCR Positive control .....	1	1	vial
FLAV.COLUM 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

Tissue, ovas, sperm, feces.

## 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
30 Cycles	1 Cycle	Initial Denaturation	94°C 2 min.
		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).

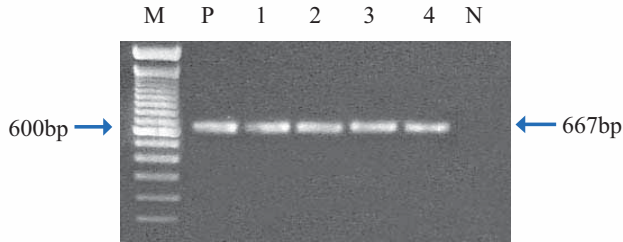
2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

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 : 667 bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ FLAV.COLUM Detection Kit  
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
Lane P : Positive control  
Lane 1~4 : FLAV.COLUM 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 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™ FLAV.COLUM Detection Kit 48	VET0003Q(48)
VetPCR™ FLAV.COLUM Detection Kit 96	VET0003Q(96)
Brig™ Molecular Weight Marker	24012



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