

1. DESCRIPTION

Epizootic Haematopoietic Necrosis (EHN) is caused by a double-stranded DNA, non-enveloped Iridovirus known as Epizootic Haematopoietic Necrosis Virus (EHNV). This virus shares at least one antigen with iridoviruses infecting sheat fish (*Silurus glanis*) and the cat-fish (*Ictalurus melas*) in Europe and with amphibian iridoviruses from North America (frog virus 3) and Australia (Bohle iridovirus). Recently, the OIE included the two agents, European catfish virus and European sheatfish virus, as causative agents of EHN. There are no specific clinical signs associated with EHN. Mortalities are characterised by necrosis of liver (with or without white spots), spleen, haematopoietic tissue of the kidney and other tissues. Disruption of blood function leads to osmotic imbalance, haemorrhagic lesions, build up of body fluids in the body cavity. The body cavity fluids (ascites) plus enlarged spleen and kidney may cause abdominal distension (dropsy). Clinical disease appears to be associated with poor water quality, as well as water temperature. In rainbow trout, disease occurs at temperatures from 11 to 17°C (in nature). No disease is found in redfin perch at temperatures below 12°C under natural conditions. Both juvenile and adult redfin perch can be affected, but juveniles appear more susceptible.

VetPCR™ EHNV Detection Kit is the direct detection of *Epizootic Haematopoietic Necrosis 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™ EHNV 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™ EHNV PCR Pre-mixture.....	48	96	tubes
DNase/RNase-free water	1	1	vial
EHNV PCR Positive control	1	1	vial
EHNV 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
1 Cycle	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
	Annealing	57.5°C	30 sec.
30 Cycles	Extension	72°C	30 sec.
	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 : 465 bp

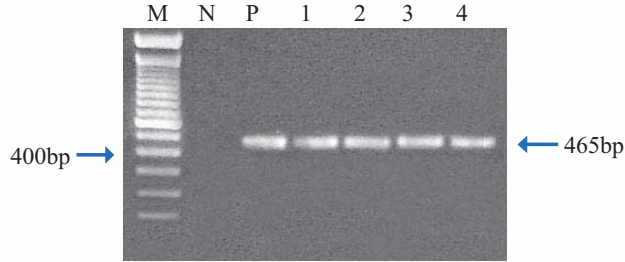


Fig1. Electrophoresis of PCR product by VetPCR™ EHNV Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane N : Negative control
 Lane P : Positive control
 Lane 1~4 : EHNV Positive sample

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™ EHNV Detection Kit 48	VET0002Q(48)
VetPCR™ EHNV Detection Kit 96	VET0002Q(96)
Brig™ Molecular Weight Marker	24012



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