

1. DESCRIPTION

Viral haemorrhagic septicaemia (VHS) is caused by ssRNA enveloped rhabdovirus, known as viral haemorrhagic septicaemia virus (VHSV). VHSV is synonymous with Egtved virus. VHSV is found in continental Europe, the Atlantic Ocean and Baltic Sea. Although VHSV-like infections are emerging in wild marine fish in North America, VHS continues to be considered a European-based disease, until the phylogenetic identities of the VHSV-like viruses which do not cause pathology in rainbow trout can be clearly established.

The virus infects blood cells (leucocytes), the endothelial cells of the blood capillaries, haematopoietic cells of the spleen, heart, nephron cells of the kidney, parenchyma of the brain and the pillar cells of the gills. Spread of the virus causes haemorrhage and impairment of osmoregulation. This is particularly severe in juvenile fish, especially during periods when water temperatures ranging between 4 – 14 °C.

VetPCR™ VHSV Detection Kit is the direct detection of *Viral haemorrhagic septicaemia 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™ VHSV 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™ VHSV RT-PCR Pre-mixture	48	96	tubes
VetPCR™ VHSV 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
VHSV RT-PCR Positive control.....	1	1	vial
VHSV RT-PCR Positive control Pre-mixture.....	4	8	tubes
VHSV 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

Tissue, ovas, sperm, feces.

5. ADDITIONAL REQUIRED MATERIALS

- Pipettes, Sterile pipette tip, Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cyler, 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 cyler that employs a top heating method.

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

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 cyler.

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 cyler that employs a top heating method.

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

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
30 Cycles	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 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 : 376 bp

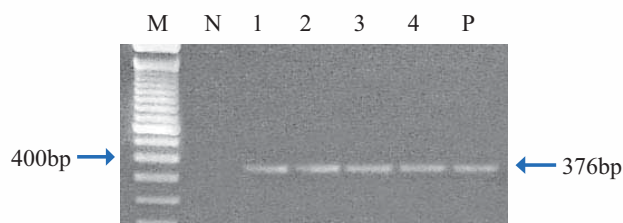


Fig 1. Electrophoresis of PCR product by VetPCR™ VHSV Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane N : Negative control
 Lane 1–4 : VHSV Positive sample
 Lane P : Positive control

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™ VHSV Detection Kit 48	VET0005QR(48)
VetPCR™ VHSV Detection Kit 96	VET0005QR(96)
Brig™ Molecular Weight Marker	24012



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