

1. DESCRIPTION

Venezuelan equine encephalitis (VEE), eastern equine encephalitis (EEE) and western equine encephalitis (WEE) viruses are arthropod borne viruses (arboviruses). They are members of the family Togaviridae, genus Alphavirus. Members of the genus Alphavirus have a spherical, enveloped virion 60 to 65 nm in diameter and possess a single-stranded, positive-sense RNA genome over 11 kb in length. The natural transmission cycles of these three viruses involve a variety of mosquito and avian species. Under unique ecological conditions, VEE, EEE and WEE viruses are transmitted from avian hosts to dead-end hosts such as equines, non-human primates and humans. They are associated with both human and equine encephalitis throughout the Americas. Geographically, there is a difference in the distribution of these three antigenically and ecologically distinct viruses. The majority of EEE virus activity has occurred in the eastern United States within the geographic range of *Culiseta melanura*, the primary insect vector of North American EEE virus. The majority of WEE virus activity has occurred in the western United States, where *Culex tarsalis* is the primary mosquito vector of WEE virus. In contrast to both EEE and WEE, the distribution of VEE is primarily restricted to Central and South America.

VetPCR™ EEEV Detection Kit is the direct detection of Eastern equine encephalitis 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 4 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

2. STORAGE

The components of VetPCR™ EEEV 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™ EEEV RT-PCR Pre-mixture	48	96	tubes
VetPCR™ EEEV 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
EEEV RT-PCR Positive control	1	1	vial
EEEV RT-PCR Positive control Pre-mixture	4	8	tubes
EEEV 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

1 ml whole blood in EDTA (purple top) tube, or 1 ml CSF, serum, plasma or tissue.

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°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 7 μ l of PCR product, 7 μ l of Positive control and 2 μ l 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.

6.4 INTERPRETATION

- Expected PCR product size : 472 bp

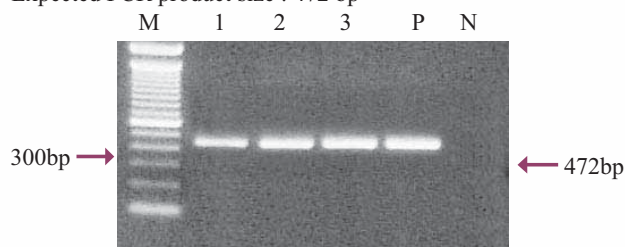


Fig 1. Electrophoresis of PCR product by VetPCRTM IPNV Detection Kit
 Lane M : BrigTM Molecular Weight Marker (Bioingentech Ltd.)
 Lane 1~3 : IPNV Positive sample
 Lane P : Positive control
 Lane N : Negative control

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 TM EEEV Detection Kit 48	VET0002ER(48)
VetPCR TM EEEV Detection Kit 96	VET0002ER(96)
Brig TM Molecular Weight Marker	24012



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