

1. DESCRIPTION

West Nile virus (WNV) belongs to the genus *Flavivirus* of the family *Flaviviridae* and is an arthropod-borne virus. It possesses a single-stranded plus-sense RNA genome of approximately 11,000 nucleotides. This virus can cause encephalitis or meningitis, infection of the brain and the spinal cord. WNV circulates in natural transmission cycles involving primarily *Culex* species mosquitoes and birds; humans and other mammals, such as primates and horses, are thought to be incidental hosts. Infection with West Nile Virus does not always lead to signs of illness in people or animals. Horses appear to be a species that is susceptible to infection with the virus. In horses that do become clinically ill, the virus infects the central nervous system and may cause symptoms of encephalitis. Clinical signs of encephalitis in horses may include a general loss of appetite and depression, in addition to any combination of the following signs: fever, weakness of hind limbs, paralysis of hind limbs, impaired vision, ataxia, head pressing, aimless wandering, convulsions, inability to swallow, walking in circles, hyperexcitability and coma.

VetPCR™ WNV Detection Kit is the direct detection of West Nile 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™ WNV 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™ WNV RT-PCR Pre-mixture	48	96	tubes
VetPCR™ WNV 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
WNV RT-PCR Positive control	1	1	vial
WNV RT-PCR Positive control Pre-mixture	4	8	tubes
WNV 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 CSF or tissue. Less preferred samples: 1 ml whole blood in EDTA (purple top) tube, serum or plasma.

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 : 273 bp

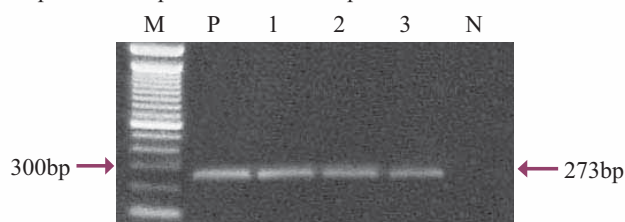


Fig 1. Electrophoresis of PCR product by VetPCRTM WNV Detection Kit
 Lane M : BrigTM Molecular Weight Marker (Bioingentech Ltd.)
 Lane P : Positive control
 Lane 1~3 : WNV Positive sample
 Lane N : Negative control

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 TM WNV Detection Kit 48	VET0009ER(48)
VetPCR TM WNV Detection Kit 96	VET0009ER(96)
Brig TM Molecular Weight Marker	24012



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