

# VetPCR™ EAV Detection Kit

## 1. DESCRIPTION

Equine arteritis virus (EAV) is a positive stranded RNA virus in the family *Arteriviridae*, order *Nidovirales*, and infects horses worldwide. Sporadic respiratory disease and sudden death in foals, abortion in mares, and mild or subclinical infections in adult horses have been described resulting from this infection. Adult stallions may become chronically infected; they can become a reservoir and spread the virus via their semen.

Clinical signs of EAV infection include depression, anorexia, jaundice, rash, nasal discharge, cough, swelling of the lower limbs, scrotum or mammary gland, periobital swelling and conjunctivitis (pink eye). Mares and geldings eliminate infection within 21 to 30 days. However, 30-60 % of infected stallions become carriers and shed the virus in their semen. Only one serotype of EAV is reported, but there are several strains that differ in their virulence and neutralization phenotype.

VetPCR™ EAV Detection Kit is the direct detection of Equine arteritis 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™ EAV 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™ EAV RT-PCR Pre-mixture .....	48	96	tubes
VetPCR™ EAV 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
EAV RT-PCR Positive control .....	1	1	vial
EAV RT-PCR Positive control Pre-mixture .....	4	8	tubes
EAV 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

Nasopharyngeal swab, or 1 ml whole blood in EDTA (purple top) tube, or 1 ml semen, 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.

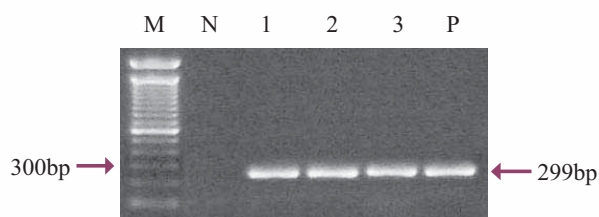
PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
30 Cycles	Denaturation	94°C	30 sec.
	Annealing	55°C	30 sec.
	Extension	72°C	30 sec.
1 Cycle	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

- 1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
- 2.- Load 7 $\mu$ l of PCR product, 7 $\mu$ l of Positive control and 2 $\mu$ l of Brig<sup>TM</sup> 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 : 299 bp



**Fig 1.** Electrophoresis of PCR product by VetPCR<sup>TM</sup> EAV Detection Kit  
 Lane M : Brig<sup>TM</sup> Molecular Weight Marker (Bioingentech Ltd.)  
 Lane P : Positive control  
 Lane 1~3 : EAV 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 <sup>TM</sup> EAV Detection Kit 48	VET0001ER(48)
VetPCR <sup>TM</sup> EAV Detection Kit 96	VET0001ER(96)
Brig <sup>TM</sup> Molecular Weight Marker	24012



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