

1. DESCRIPTION

Equine infectious anemia (EIA), also known as Swamp Fever, is a viral disease known to occur in all members of *Equidae*, including horses, mules and donkeys. Equine infectious anemia virus (EIAV) is a member of the genus *Lentivirus* of the family *Retroviridae*. EIAV is unique among the lentiviruses in that the initial acute febrile response and associated viremia are followed by recurrent cycles of the disease and, finally, a prolonged asymptomatic period. Acute cases experience fever and hemorrhaging 7-30 days post infection. Very few horses with this initial fever are detected by owners. Chronic infection is marked by episodes of fever, weight loss, depression, progressive weakness, anemia and edema. These signs occur every two weeks in recurring cycles. Other symptoms that may occur during the course of the disease include loss of appetite, frequent urination, diarrhea, weakness, paralysis of the hindquarters, paleness of the mucous membranes, yellowish discoloration of the conjunctiva, small pinpoint hemorrhages beneath the tongue, rapid breathing and accelerated pulse. Pregnant mares may abort. The EIAV most frequently is transmitted between horses in close proximity by large biting insects, such as horseflies and deerflies.

VetPCR™ EIAV Detection Kit is the direct detection of Equine infectious anemia 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™ EIAV 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™ EIAV RT-PCR Pre-mixture	48	96	tubes
VetPCR™ EIAV 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
EIAV RT-PCR Positive control	1	1	vial
EIAV RT-PCR Positive control Pre-mixture	4	8	tubes
EIAV 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 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	57°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 : 408 bp

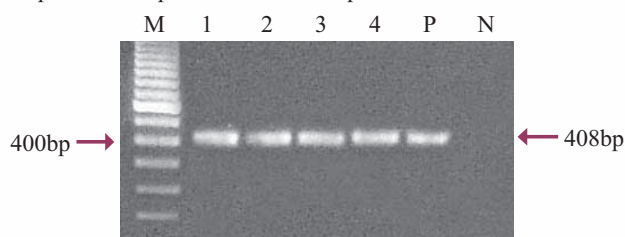


Fig 1. Electrophoresis of PCR product by VetPCRTM EIAV Detection Kit
 Lane M : BrigTM Molecular Weight Marker (Bioingentech Ltd.)
 Lane 1~4 : EIAV Positive sample
 Lane P : Positive control
 Lane N : Negative control

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 EIAV Detection Kit 48	VET0003ER(48)
VetPCR TM EIAV Detection Kit 96	VET0003ER(96)
Brig TM Molecular Weight Marker	24012



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