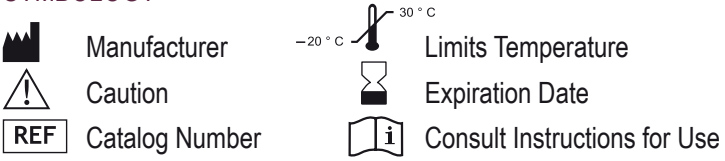
VetPCR™ EAV Detection Kit

SYMBOLOGY



1. DESCRIPTION

Equine arteritis virus (EqAV) is a positive stranded RNA virus that 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.

VetPCR™ EAV Detection Kit is the direct detection of Equine viral arteritis 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, reliable technique.

2. STORAGE

The VetPCR™ EAV Detection Kit is shipped at room temperature (15–25°C) because contains a chemical stabilizer. The VetPCR™ EAV Detection Kit should be stored immediately upon receipt at –20°C in a constant-temperature freezer. For routine use should be stored al 4°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date without showing any reduction in performance.

3. KIT CONTENTS

KIT	48	96	
VetPCR™ EAV RT-PCR Premixture	1	1	vial
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Brig™ RT-PCR solution	1	1	vial
Biotech™ Transcriptase solution	1	1	vial
DNase/Rnase free water	1	1	vial
EAV PCR Positive control	1	1	vial
PCR Negative control	1	1	vial
PCR Internal Control (white cap)	1	1	vial
Mineral Oil solution	1	2	vial
Brig™ Molecular Weight marker	1	1	vial
RNA purification Kit	50	100	test

4.MATERIALS

Materials required but not provided:

• Microcentrifuge and PCR tubes, Disposable gloves powderless, Pipettes, Sterile pipette tip, Vortex mixer, Centrifuge for microcentrifuge tubes, Thermal cycler,Tube racks, Electrophoresis kit, UV transilluminator, Biohazard waste container.

5.PROCEDURE

Please read through the entire procedure before starting.

5.1 RNA PREPARATION

This kit includes all reagents necessary for purification of RNA from different samples. Carry out the RNA isolation according to the instructions available inside the kit, the instructions also can be downloaded of our website www.bioingentech.com. If you need an additional RNA Purification kit, use only the following kits that are standardized for the process: See Item

9. ADDITIONAL PRODUCTS.

Note:Completely thaw the components of the kit prior to use; homogenize the solutions for several seconds prior to pipetting.

5.2 PREPARATION OF EAV RT-PCR MIXTURE

1) Prepare the reaction mixture for sample by combining the reagents as shown in the table 1. The final reaction volume should be 10.5µl.

Notes:

• The mineral oil is necessary, even when using a termal cycler that employs a top heating method.

Table 1. Reaction components for RT-PCR

Kit components	samples
VetPCR™ EAV RT-PCR Premixture	4.5µL
DNase/RNase free water	4µl
RNA isolated from the sample	2µl
Mineral Oil Solution	11µl

2)Place the tubes in a thermal cycler and perform RT-PCR according to the program outlined in Table 2.

Table 2. RT-PCR cycling parameters

PCR cycle			Temp	Time
RT-PCR 1	1 cycle	Initial denaturation	65 ºC	10 min.
	1 cycle	Stop	4 ºC	5 min.
Add 1.0 µl of Brig™ RT-PCR solution				
Add 1.0 µl of Biotech™ Transcriptase solution				
RT-PCR 2	1 cycle	Annealing	25 ºC	10 min.
	1 cycle	Extension	37ºC	60 min.
	1 cycle	Denaturation	70 ºC	10 min.

3) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 3. The final reaction volume should be 13.5µl.

Notes:

• Run a positive control, a negative control, and an internal control each 12 samples.

• The mineral oil is necessary, even when using a termal cycler that employs a top heating method.

Table 3. Reaction components for PCR

KIT	Sample	Positive control	Negative control	Internal control
VetPCR™ EAV PCR Premixture	5.5 µl	5.5 µl	5.5 µl	
PCR Internal Control (white cap)				5.5 µl
DNase/Rnase free water	6 µl	6 µl	6 µl	6 µl
cDNA from the sample	2 µl			2 µl
EAV PCR Positive control		2 µl		
PCR Negative control			2 µl	
Mineral Oil solution	11 µl	11 µl	11 µl	11 µl

4) Place the tubes in a thermal cycler and perform amplification according to the program outlined in Table 4.

Table 4. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	94ºC	2 min.
30 cycles	Denaturation	94ºC	30 sec.
	Annealing	55ºC	30 sec.
	Extension	72ºC	30 sec.
1 cycle	Final extension	72ºC	5 min.

5.3 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, 7µl of negative control, 7µl of internal 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.

5.4 INTERPRETATION OF THE TEST RESULTS

- Expected PCR product size : 297bp

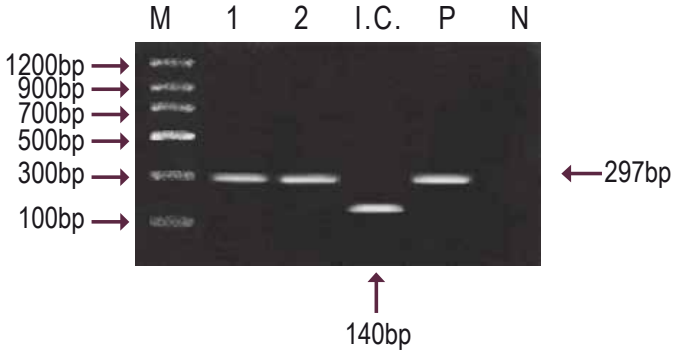

Fig. 1 Results:
Lane M: Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane 1~2: EAV Positive samples
Lane I.C.: Internal control
Lane P: Positive control
Lane N: Negative control

1.No band in positive control
• Check Internal control band: If internal control band is seen, PCR has been performed properly.
• Check PCR machine: check the temperature and make sure to check that the machine is working properly.
2.No internal control band
• Check template cDNA concentration: Competition can occur by high template concentration. Proceed with a lower concentration of cDNA.
• Check template RNA quality: Even tough RNA is isolated from the sample, the RT-PCR reaction can be inhibited depending on RNA purity in some cases. In this case, extracted RNA should be diluted 10 times with distilled water and used to run the RT-PCR reaction again. If still no band is seen, please inquire with our technical support staff.
3.Amplicon bands in the negative control
• Check contamination of distilled water: Distilled water can be contaminated. Perform PCR again with fresh sterile water.
• Check contamination of laboratory instruments and other environments: We recommend that you use filter tips and a pipette after sterilization to reduce contamination. Proceed with all procedures on a clean bench and keep the location where you procedures are performed sterile.
4.Poor resolution on agarose gel
• We recommend using a 1.5~2% agarose gel and run electrophoresis for 40 minutes at 100 V.

7. TECHNICAL ASSISTANCE

At Bioingentech we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Bioingentech products. If you have any questions or experience any difficulties regarding Bioingentech Genomic RNA Detection Kits or Bioingentech products in general, please do not hesitate to contact us.

8. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS), available online in our website, where you can find, view, and print the MSDS for each Bioingentech kit and kit component.

9. ADDITIONAL PRODUCTS

Product	Product Code	Manufacturer
Bioingentech™ Genomic RNA Purification Kit (50 test)	230041(50)	Bioingentech
Bioingentech™ Genomic RNA Purification Kit (100 test)	230041(100)	Bioingentech