REF: PCR-V158-48D
PCR-V158-96D

VetPCR™ Equine piroplasmosis (EP) Detection Kit

SYMBOLOGY

Manufacturer
Caution
REF Catalog Number
−20 °C 30 °C Limits Temperature
Expiration Date
Consult Instructions for Use

1. DESCRIPTION

Equine piroplasmosis (EP) is a tick-borne disease of horses caused by the intraerytrocytic protozoan parasites Babesiacaballi and Theileria equi of the Order Piroplasmida. Theileria equi was previously designated as Babesia equi but compelling evolutionary, morphologic, biochemical, and genetic evidence supports its reclassification as a Theileria. Infected animals may remain carriers of these blood parasites for long periods and act as sources of infection for other ticks. The introduction of carrier animals into areas where competent tick vectors are prevalent can lead to an epizootic spread of the disease. The Equine piroplasmosis multiplex polymerase chain reaction (PCR) kit detects the genes for the Babesia caballi and Theileria equi on the basis of a genetic database, so it can diagnose very fast and accurately.

2. STORAGE

The VetPCR™ Equine piroplasmosis (EP) Detection Kit is shipped at room temperature (15–25°C) because contains a chemical stabilizer. The VetPCR™ Equine piroplasmosis (EP) 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™ Equine piroplasmosis (EP) Premixture	1	1	vial
PCR Internal Control (white cap)	1	1	vial
DNase/RNase free water	1	1	vial
Equine piroplasmosis (EP) PCR Positive control	1	1	vial
PCR Negative control	1	1	vial
Mineral Oil Solution	1	1	vial
Brig™ Molecular Weight marker	1	1	vial
DNA 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 DNA PREPARATION

This kit includes all reagents necessary for purification of DNA from different samples. Carry out the DNA 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 DNA 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 Equine piroplasmosis (EP) PCR MIXTURE

1) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 1. 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 thermal cycler that employs a top heating method.

Table 1. Reaction components for PCR

Kit components	Sample	Positive control	Negative control	Internal control
VetPCR™ Equine piroplasmosis (EP) Premixture	5.5µL	5.5µL	5.5µL	
PCR Internal control (white cap)				5.5µL
DNase/RNase free water	6µl	6µl	6µl	6µl
DNA isolated from the sample	2µl			2µl
Equine piroplasmosis (EP) PCR Positive control		2µl		
PCR Negative control			2µl	
Mineral Oil Solution	11µl	11µl	11µl	11µl

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

Table 2. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	94ºC	2 min.
30 cycles	Denaturation	94ºC	30 sec.
	Annealing	58ºC	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: 300bp

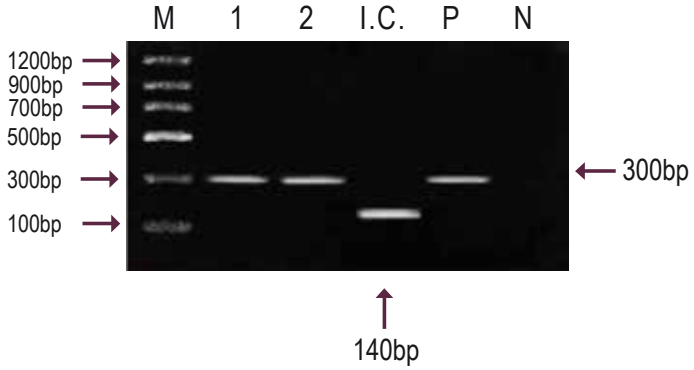

Fig. 1 Result:
Lane M: Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane 1~2: Equine piroplasmosis (EP) Positive samples
Lane I.C.: Internal control
Lane P: Positive control
Lane N: Negative control

6. TROUBLESHOOTING

1) No band in positive sample

• Check Internal control band: If internal control band is seen, PCR has been performed properly. It is not a problem of the product.
• Check template DNA quality: the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with DNA rehydration solution and used to perform PCR again.
• Check PCR machine: check the temperature and make sure to check that the machine is working properly.

2) No internal control band

• Check template DNA concentration: Competition can occur by high template concentration. Proceed with a lower concentration of DNA.
• Check template DNA quality: Even tough DNA is isolated from the sample, the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with distilled water and used to run the 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 DNA 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 DNA Purification Kit (50 test)	PU-A001(50)	Bioingentech
Bioingentech™ Genomic DNA Purification Kit (100 test)	PU-A002(100)	Bioingentech