

1. DESCRIPTION

Equine piroplasmiasis is caused by the intracellular, haemoprotozoan parasites *Babesia equi* (recently reclassified as *Theileria equi*) and *Babesia caballi*, which are transmitted by ticks of several genera. The disease is found in many tropical and subtropical areas. Clinical manifestation of the disease is variable and often includes icterus (jaundice), haemoglobinuria and fever. Both chronic and acute infection can occur. Sub-clinical infected animals are of major concern, as they can be carriers of the organism. In addition to the fact that sub-clinical babesiosis may negatively affect the animal's performance, it has been shown that strenuous exercise, such as that experienced in horse racing, can cause sub-clinical infections to become acute. Thus there is a real need for the diagnosis of both clinical and sub-clinical infections. In general, *B. caballi* causes a less severe disease, as only about 1% of the red blood cells are infected. Infections may not be apparent, but can persist 1 to 4 years before they are eventually eliminated. They may be associated with poor appetite, poor performance and weight loss. In contrast, *B. equi* infects up to 20% of red blood cells, leading to more severe clinical signs including fever, anemia, icterus, increased respiratory and heart rates and enlargement of the spleen. The parasites destroy red blood cells, causing anemia, and the released hemoglobin may cause icterus and dark urine. Colic, constipation followed by diarrhea, and swelling of the legs can occur. Foals can be infected in utero, and can be aborted or born anemic and weak. Animals with *B. equi* infections become life-long carriers.

VetPCR™ BAB.EQUI Detection Kit is the direct detection of *Babesia equi* 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 and reliable technique.

2. STORAGE

The components of VetPCR™ BAB.EQUI 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™ BAB.EQUI PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
BAB.EQUI PCR Positive control	1	1	vial
BAB.EQUI PCR Positive control Pre-mixture	4	8	tubes
Brig™ Molecular Weight marker	1	1	vial
Mineral Oil	1	1	vial
DNA purification kit (see step 6.1)	50	100	tests

4. SPECIMEN

1 ml whole blood in EDTA (purple top) tube.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- 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 DNA PREPARATION

Various manufacturers offer DNA isolation kits. Please carry out the DNA isolation according to the manufacturers instructions. The following standard DNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Genomic DNA Purification Kit (50 test)	230040(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Genomic DNA Purification Kit (100 test)	230040(100)	Bioingentech Biotechnology Inc.

6.2 AMPLIFICATION

1.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

2.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

3.- Add 2µl of template DNA into the PCR Premix tube to total volume as 13µl.

4.- Add 6µl of DNase/RNase-free water and 2µl of PCR Positive control into a 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 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.
	Denaturation	94°C	30 sec.
	Annealing	55°C	30 sec.
30 Cycles	Extension	72°C	30 sec.
	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 7µl of PCR product, 7µl of positive 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.

6.4 INTERPRETATION

- Expected PCR product size : 323 bp

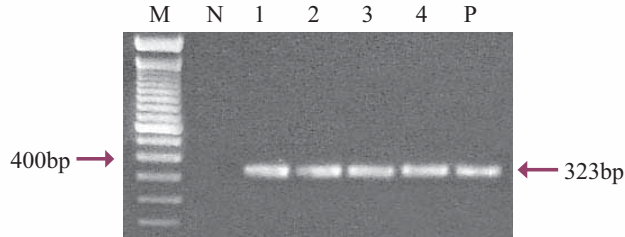


Fig1. Electrophoresis of PCR product by VetPCR™ BAB.EQUI Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane N : Negative control
 Lane 1~4 : BAB.EQUI Positive sample
 Lane P : Positive control

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™ BAB.EQUI Detection Kit 48	VET0001E(48)
VetPCR™ BAB.EQUI Detection Kit 96	VET0001E(96)
Brig™ Molecular Weight Marker	24012



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