

1. DESCRIPTION

Bovine babesiosis is a major tick-borne disease of cattle caused by protozoan parasites (*Babesia spp.*). In tropical areas the agents are *Babesia bovis* and *Babesia bigemina*.

Cattle with advanced babesiosis have a low exercise tolerance and sometimes collapse and die while being driven to a yard for treatment. Older animals are more acutely affected (clinical babesiosis is rare in cattle younger than six months). Abortions may occur when pregnant animals are severely affected. Similar clinical signs develop during infection with both organisms. However, the course of the disease differs markedly. Infections are characterized by; fever, anorexia, depression, increased respiratory rate particularly following exertion, muscle tremor, reluctance to move, and haemoglobinuria, occasionally signs of cerebral derangement such as circling, head pressing, mania and convulsions. While these signs are seen very early in the course of *B. bovis* infections, they only develop in *B. bigemina* infections in the latter stages when parasitaemia is well advanced. Anaemia and jaundice develop steadily with *B. bovis*, but much more precipitously with *B. bigemina*. *B. bigemina*-affected animals may exhibit irritability and aggression but not the central nervous system signs.

VetPCR™ BAB.BIGE Detection Kit is the direct detection of *Babesia bigemina* 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 components of VetPCR™ BAB.BIGE 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.BIGE PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
BAB.BIGE PCR Positive control	1	1	vial
BAB.BIGE 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

0.5 ml whole blood in EDTA (purple top) tube, or egg (albumen), thymus or spleen.

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	55.5°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 : 453 bp

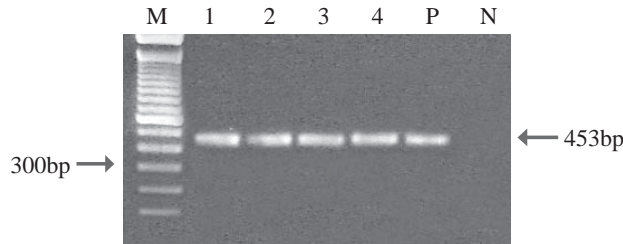


Fig1. Electrophoresis of PCR product by VetPCR™ BAB.BIGE Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane 1~4 : BAB.BIGE Positive sample
 Lane P : Positive control
 Lane N : Negative control

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™ BAB.BIGE Detection Kit 48	VET0002B(48)
VetPCR™ BAB.BIGE Detection Kit 96	VET0002B(96)
Brig™ Molecular Weight Marker	24012



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