

1. DESCRIPTION

Babesia spp. are protozoal organisms that parasitize erythrocytes, causing anemia in the host. Many different species exist with varying host specificity. *B. canis* and *B. gibsoni* are two organisms commonly known to infect dogs. Both organisms have Ixodid tick vectors and are found throughout Asia, Africa, Europe, the Middle East, and North America, with *B. canis* being more prevalent. Cases of canine babesiosis may present with a wide variation of severity of clinical signs, ranging from a hyperacute, shock-associated, hemolytic crisis to an inapparent, sub-clinical infection. Dogs typically present with the acute form of babesiosis, which is characterized by general findings such as pyrexia, weakness, mucous membrane pallor, depression, lymphadenopathy, splenomegaly, and general malaise. Laboratory studies may document anemia, thrombocytopenia, hypoalbuminemia, and bilirubinuria. Initially, the anemia is normocytic, normochromic, and nonregenerative, but later develops into a macrocytic, hypochromic, regenerative anemia with reticulocytosis. The anemia is hypochromic because the reticulocytes have not yet formed their adult concentrations of hemoglobin.

2. STORAGE

The components of VetPCR™ BAB.C 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.C PCR Pre-mixture.....	48	96	tubes
DNase/RNase-free water	1	1	vial
BAB.C PCR Positive control.....	1	1	vial
BAB.C 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.

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 : 294 bp

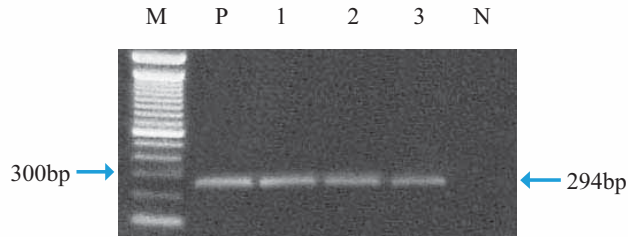


Fig1. Electrophoresis of PCR product by VetPCR™ BAB.C Detection Kit

Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)

Lane P : Positive control

Lane 1~3 : BAB.C Positive sample

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.C Detection Kit 48	VET0003C(48)
VetPCR™ BAB.C Detection Kit 96	VET0003C(96)
Brig™ Molecular Weight Marker	24012



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