

## 1. DESCRIPTION

Canine brucellosis is a disease caused by the bacteria *Brucella canis*, a Gram-negative coccobacillus or short rod. It is found throughout the world. It is spread through contact with aborted fetuses and discharges from the uterus of infected bitches, during mating, through maternal milk and possibly through airborne transmission in some cases. The bacteria enters the body through mucous membranes and spreads from there to lymph nodes and the spleen. It also spreads to the uterus, placenta and prostate gland as well as other internal organs at times.

In female dogs, infection leads to abortion or early death of infected puppies. Infected females may have no other clinical signs. In some cases there may be decreased fertility rather than abortion. This may be due to resorption of fetuses early in their development. In male dogs, infection of the testicles can lead to infertility due to anti-sperm antibodies developed as the body attempts to fight off the bacterial infection. The testes may atrophy after the initial period of swelling. Scrotal enlargement or infection of the skin over the scrotum may be seen. In both female and male dogs there may be infection of spinal discs (diskospondylitis) which can cause back pain and rear leg weakness or even paralysis. Eye inflammation may be seen in either sex.

VetPCR™ BRU.CA Detection Kit is the direct detection of *Brucella canis* on the basis of a genetic data base, 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™ BRU.CA 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<br>48 | Kit<br>96 |       |
|---|-----------|-----------|-------|
| VetPCR™ BRU.CA PCR Pre-mixture          | 48        | 96        | tubes |
| DNase/RNase-free water                  | 1         | 1         | vial  |
| BRU.CA PCR Positive control             | 1         | 1         | vial  |
| BRU.CA 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            | 57°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).

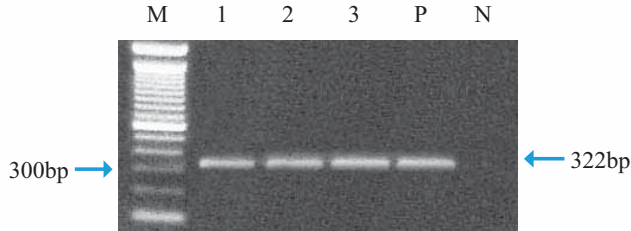
2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

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 : 322 bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ BRU.CA Detection Kit  
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
 Lane 1~3 : BRU.CA 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™ BRU.CA Detection Kit 48 | VET0006C(48) |
| VetPCR™ BRU.CA Detection Kit 96 | VET0006C(96) |
| Brig™ Molecular Weight Marker   | 24012        |



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