

1. DESCRIPTION

Canine ehrlichiosis is a disease of dogs, wolves and other canids. Occurring worldwide, canine ehrlichiosis is also known by other names such as ‘tracker dog disease’, ‘tropical canine pancytopenia’, ‘canine hemorrhagic fever’ and ‘canine typhus.’ The disease is caused by *Ehrlichiae*, tick-transmitted, Gram-negative, obligately intracellular bacteria that infect the leukocytes of specific mammalian hosts. There are several ehrlichiae that can infect dogs but *Ehrlichia canis* is the most common and severe one.

Disease caused by *E. canis* typically occurs in three phases. The initial acute phase is characterized by fever, malaise, lymphadenomegaly, splenomegaly, thrombocytopenia, leukopenia, and nonregenerative anemia. Symptoms subside in 2 to 4 weeks but are followed by a sub-clinical phase that persists for 2–3 months to years, during which infected dogs are carriers. Some dogs subsequently enter a chronic phase, a period when severe clinical ehrlichiosis occurs. *E. canis* causes ocular disease and meningitis during this phase. *Ehrlichia* is transmitted by the Brown Dog tick, *Rhipicephalus sanguineus*. The immature form of the tick feeds on an animal infected with *Ehrlichia*. When the tick later feeds on another animal, the *Ehrlichia* is passed on.

VetPCR™ EHR spp. Detection Kit is the direct detection of *Ehrlichia spp.* 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™ EHR.spp 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™ EHR.spp PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
EHR.spp PCR Positive control	1	1	vial
EHR.spp 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	54°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 : 451 bp

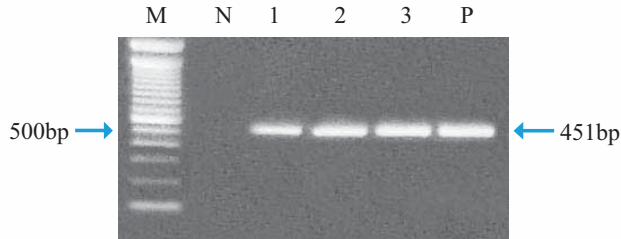


Fig1. Electrophoresis of PCR product by VetPCR™ EHR.spp Detection Kit
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane N : Negative control
Lane 1~3 : EHR.spp Positive sample
Lane P : Positive control

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™ EHR.spp Detection Kit 48	VET0017C(48)
VetPCR™ EHR.spp Detection Kit 96	VET0017C(96)
Brig™ Molecular Weight Marker	24012



Bioingentech Ltd.
Salas 350, piso 2, Concepción, Chile
Telephone (56)-(41)-2790435
Fax (56)-(41)-2790435
info@bioingentech.com
www.bioingentech.com

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