

1. DESCRIPTION

Canine leishmaniasis is a parasitic worldwide zoonotic disease due to an unicellular blood protozoa of the genus *Leishmania*, transmitted by a tiny insect (female phlebotomidae). Leishmaniasis also affects the man and a few other mammals, but the *Leishmania* species involved can be very different.

The phlebotome absorbs *Leishmania* when pumping the blood of a parasited dog, and it can retransmit them to another dog one or two weeks after the infecting meal. Man is an exceptional host; when he happens to be contaminated, he may develop a lesion at the inoculation point (cutaneous leishmaniasis), or an internal disease, called “Kala-Azar”, inducing the enlargement of the spleen and the liver.

Dogs present a suspicious injury on the nose, becomes apathetic and loses weight. Most often, the loss of temporal muscles gives him the air of an old dog. Some other signs are less constant: claws lengthening, dandruff in the hair, enlargement of lymphatic nodes, nose bleed, ocular inflammation and renal troubles.

VetPCR™ LEISH spp. Detection Kit is the direct detection of *Leishmania* spp. 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™ LEISH.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™ LEISH.spp PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
LEISH.spp PCR Positive control	1	1	vial
LEISH.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, or skin simples, or tissue.

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
30 Cycles	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
	Annealing	53°C	30 sec.
1 Cycle	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 : 195 bp

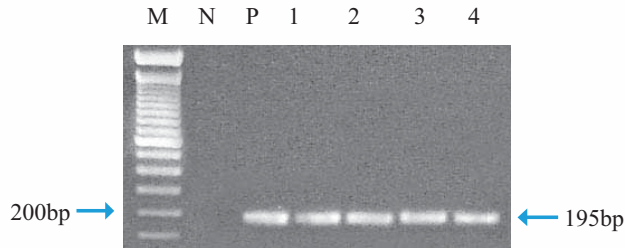


Fig1. Electrophoresis of PCR product by VetPCR™ LEISH.spp Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane N : Negative control
 Lane P : Positive control
 Lane 1~4 : LEISH.spp Positive sample

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™ LEISH.spp Detection Kit 48	VET0019C(48)
VetPCR™ LEISH.spp Detection Kit 96	VET0019C(96)
Brig™ Molecular Weight Marker	24012



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