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Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

For research use only
Cat. No. IP21143 | **48 Tests**

Ehrlichia spp. Detection Kit

Test for the detection of *Ehrlichia spp.* by one-step PCR

User Manual

REV.2.2

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Ehrlichia spp. Detection Kit

■ **NOTE :**

7. NOTICE

- This product was designed to detect more than 100 copies of target gene(or gene segment). When the copy number of target present in the test reaction is less than 100, a false-negative(a negative test result when the attribute for which the subject is being tested actually exists in that subject) may occur. Use this product For Research Use Only.
- Do not use any reagent after the expiration date.
- Do not use together with reagents of other products.
- Follow the instructions.
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. iNtRON 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 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.
Ehrlichia spp. Detection Kit	IP21143
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
100bp Ladder Molecular Weight DNA Marker	24012

1. DESCRIPTION

The *Ehrlichia* are a group of small, gram-negative, pleiomorphic, obligate intracellular cocci that infect different blood cells in various animal species and in humans. There has recently been a reclassification of the family *Anaplasmata -ceae* to which the *Ehrlichia* belong.

According to this new classification there are two leukotrophic diseases in dogs that are caused by bacteria in the genus *Ehrlichia*, namely, Canine Monocytic Ehrlichiosis (caused mainly by *Ehrlichia canis*) and Canine Granulocytic Ehrlichiosis (caused by *Ehrlichia ewingii*). It should be noted that cross-reactivity and co-infection is common among the ehrlichia. The pathogenesis of infection with *E. canis* is occurs through salivary secretions of the tick at the attachment site during ingestion of a blood meal or through blood transfusions. Transmission by *Rhipicephalus sanguineus* is transstadial: the tick acquires the bacteria by feeding on an infected dog in either the larvae or nymph form and the tick transmits the disease to another dog as either the nymph or adult form.

The life cycle of *Ehrlichia* is not yet completely understood but it is thought that it occurs in three intracellular forms. The initial bodies are small spherical structures (1-2 mm) which are believed to develop into larger multiple membrane-bound units known as morulae. The morulae are inclusions within the cytoplasm of the leukocyte.

Ehrlichia spp. Detection Kit is 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 2~3 hours for detection. Therefore, it is a very fast accurate, reliable technique.

2. STORAGE

The components of ***Ehrlichia spp. Detection Kit*** should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

VeTeK™ EHR PCR Pre-mixture ····· 48 tubes
DNase/RNase-free water (white cap) ····· 1 vial
EHR positive control (Yellow cap) ····· 2 vial

Component in 20㎕ reaction
i-StarTaq™ DNA Polymerase dNTPs PCR Reaction buffer
Chemical stabilizer Gel loading buffer
8-MOP (dissolved in DMSO) Primers for *Ehrlichia*

4. SPECIMEN

Performs the test with whole blood. The specimen should be stored at -20℃ prior to use.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- DNA extraction kit (see 6.1 DNA preparation method)
- 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 manufacturer's instructions. The following standard extraction kit is recommended.

Product	Catalog No.	Manufacturer
Viral gene-spin™ Viral DNA/RNA Extraction Kit	17151	iNtRON Biotechnology, Inc.

6.2 Amplification

① Prepare appropriate PCR premix tubes and label. And one PCR premix tube for positive control.
② Add 2㎕ of template DNA into the PCR premix tube.
③ Add 18㎕ of DNase/RNase-free water into the PCR premix tube to total volume as 20㎕.
④ Add 2㎕ of positive control and 18㎕ of RNase-free water into a PCR premix tube for monitoring of amplification and easy interpretation.
⑤ Dissolve the blue pellet by pipetting.
Note :The pellet is easily dissolved, by letting the mixture stand at R.T. for 1-2minutes after adding water.
⑥ (*Optional)* Add mineral oil. This step is unnecessary when using a thermal cycler that employs a top heating method (general methods).
⑦ Perform PCR reaction of samples as the below process using PCR machine.

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94℃	5 min.
40 Cycles	Denaturation	94℃	30 sec.
	Annealing	52℃	30 sec.
	Extension	72℃	40 sec.
1 Cycle	Final extension	72℃	5 min.

6.3 Detection of Amplified Products

① Prepare 1.5% agarose gel containing RedSafe™ Nucleic Acid Staining Solution. (Cat. No. 21141)
② Load 7㎕ of PCR product and positive control 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 : **336 bp**

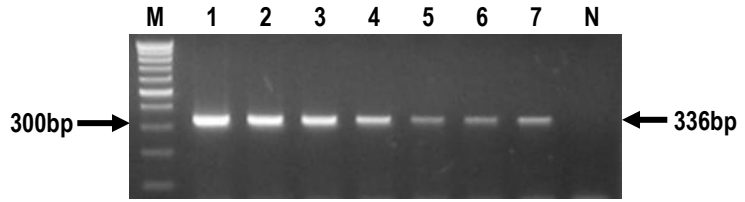

Fig 1. Electrophoresis of PCR product by **Ehrlichia spp. Detection Kit**
Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
Lane 1~7 : *Ehrlichia* positive sample
Lane N : Negative control

6.5 Elimination of carry-over contamination

- Each PCR/RT-PCR Pre-mixture contains 8-methoxypsoralen (8-MOP) for preventing of carry-over contamination.
- All PCR products should be discarded after UV irradiation (10 min/365nm) for preventing from carry-over contamination.