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For research use only
Cat. No. IP21422 | **48 Tests**

Mycoplasma haemocanis Detection Kit

Test for the detection of *Haemobartonella canis* (*Mycoplasma haemocanis*) by one-step PCR

User Manual

REV.2.2

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Mycoplasma haemocanis 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.
Mycoplasma haemocanis Detection Kit	IP21422
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
100bp Ladder Molecular Weight DNA Marker	24012

1. DESCRIPTION

Haemobartonellosis in dogs is caused by *Mycoplasma haemocanis*, formerly known as *Haemobartonella canis*. *Mycoplasma haemocanis* is not a typical bacteria, but belongs to a group of microorganisms called mycoplasma, which are the smallest free-living type of 'germs.' *Mycoplasma haemocanis* is termed "hemotropic mycoplasmas" or "hemoplasmas" because they are blood(hemo)-associated(tropic). Fleas and ticks become infected with hemotropic mycoplasmas by feeding on an infected animal. When the flea or tick then feeds on another animal, the mycoplasma are passed on. Because they live in the blood cells, they could be spread via a blood transfusion from an infected animal to a noninfected one. In the dog, the disease is generally not apparent unless the dog has previously had its *spleen* removed (splenectomy), has a suppressed immune system (e.g., from taking cancer chemotherapy), or is infected with other organisms such as *Ehrlichia*.

In acute disease, the dog will usually show depression, loss of appetite, weight loss, and fever. In severe cases, death can occur. A chronic form of the disease has been reported rarely and may cause some weakness, an increase in appetite, and *pica*.

Mycoplasma haemocanis Detection Kit is direct detection of *Haemobartonella canis* 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 ***Mycoplasma haemocanis Detection Kit*** should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

VeTeK™ HBN-C PCR Pre-mixture ····· 48 tubes
DNase/RNase-free water (white cap) ····· 1 vial
HBN-C 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 *Haemobartonella canis*

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 : **460 bp**

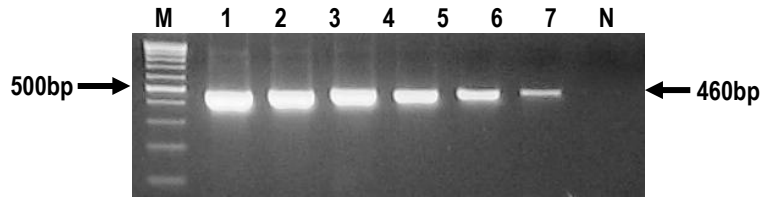

Fig 1. Electrophoresis of PCR product by **Mycoplasma haemocanis Detection Kit**
Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
Lane 1~7 : *Haemobartonella canis* positive sample
Lane N : Negative control

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.