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For research use only
Cat. No. IP31246 | **48 Tests**

Heart worm Detection Kit

Test for the detection of *Dirofilaria immitis* (Heartworm) by one-step PCR

User Manual

REV.2.2

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Heart worm 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.
Heart worm Detection Kit	IP31246
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer ™ 100 DNA Marker	24073

1. DESCRIPTION

Heartworm disease is caused by a parasitic roundworm (*Dirofilaria immitis*). Usually only dogs are affected; however, cats and other mammals are susceptible to infection. In the host, usually a dog, adult worms live in the heart and the large blood vessels entering and leaving the heart. Female worms are about 10 inches long; males are somewhat smaller. The female gives birth to microscopically small worms called *microfilariae* which circulate in the host's blood.

The *microfilariae* must be ingested by a mosquito to continue their development. Inside the mosquito they develop to infective larvae. The larvae migrate to the mouthparts of the mosquito and remain there until the mosquito feeds again. They then leave the mosquito through the mouthparts and enter the new host through the skin.

The young larvae begin to grow in the tissue under the skin and muscles. In about two months they enter the right side of the heart where they grow to maturity. The route they follow from the skin tissue and muscles to the heart is unknown.

The first sign of infection is either a chronic cough which is aggravated by exercise, of tiring on exercise, or both. In advanced cases, heart failure with fainting and collapse may occur. Even light infections may cause irreversible damage to the heart, blood vessels, kidneys and liver.

Heart worm Detection Kit is direct detection of *Dirofilaria immitis* 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 ***Heart worm Detection Kit*** should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

Heart worm PCR Pre-mixture ····· 48 tubes
DNase/RNase-free water (white cap) ····· 1 vial
HW 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 *D. immitis*

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	50℃	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 : **241 bp**

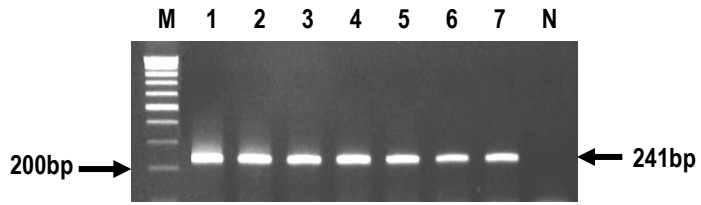

Fig 1. Electrophoresis of PCR product by **Heart worm Detection Kit**
Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
Lane 1~7 : *D.immitis* positive sample
Lane N : Negative control

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.