ISO 9001/14000 certified

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. IP21037 | **48 Tests**

Babesia canis & B. gibsoni Detection Kit

Test for the detection of *Babesia(B. canis & B. gibsoni)* by one-step PCR

User Manual

REV.2.2

iNtRON Biotechnology

Table of Contents

Babesia canis & B. gibsoni 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.
Babesia canis & B. gibsoni Detection Kit	IP21037
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
100bp Ladder Molecular Weight DNA Marker	24012

1. DESCRIPTION

Babesia spp. are protozoal organisms that parasitize erythrocytes, causing anemia in the host. Many different species exist with varying host specificity.

B. canis and *B. gibsoni* are two organisms commonly known to infect dogs. Both organisms have Ixodid tick vectors and are found throughout Asia, Africa, Europe, the Middle East, and North America, with *B. canis* being more prevalent.

Cases of canine babesiosis may present with a wide variation of severity of clinical signs, ranging from a hyperacute, shock-associated, hemolytic crisis to an inapparent, subclinical infection.

Dogs typically present with the acute form of babesiosis, which is characterized by general findings such as pyrexia, weakness, mucous membrane pallor, depression, lymphadenopathy, splenomegaly, and general malaise. Laboratory studies may document anemia, thrombocytopenia, hypoalbuminemia, and bilirubinuria. Initially, the anemia is normocytic, normochromic, and nonregenerative, but later develops into a macrocytic, hypochromic, regenerative anemia with reticulocytosis. The anemia is hypochromic because the reticulocytes have not yet formed their adult concentrations of hemoglobin.

Babesia canis & B. gibsoni Detection Kit is direct detection of *Babesia(B. canis, B. gibsoni)* on the basis of a genetic database, so it can detect very fast and accurately. It can amplify the specific gene using the PCR (Polymerase Chain Reaction) method, it takes 2~3 hours that it can be detected, therefore it must be a very fast and susceptible technique.

2. STORAGE

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

3. CONTENTS

Babesia canis & B. gibsoni PCR Pre-mixture ····· 48 tubes
DNase/RNase-free water (white cap) ····· 1 vial
BAB-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 *Babesia*

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 : **235 bp**

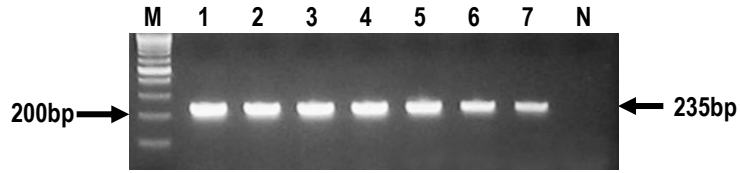

Fig 1. Electrophoresis of PCR product by **Babesia canis & B. gibsoni Detection Kit**
Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
Lane 1~7 : *Babesia* positive sample
Lane N : Negative control

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.