

ISO 9001/14000 certified

For research use only

Cat. No. INT-IP31456 | **48 Tests**

Neospora caninum Detection Kit

Test for the detection of *Neospora caninum* by one-step PCR

User Manual



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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.
Neospora caninumDetection Kit	IP31456
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer™ 100 DNA Marker	24073

1. DESCRIPTION

Neospora is an important pathogen in cattle and dogs. It was not discovered until 1984 in Norway, where it was found in dogs. It is highly transmissible and some herds can have up to a 90% prevalence. Neospora causes abortions in cattle and up to 33% of pregnancies can result in aborted fetuses on one dairy farm. Diagnosis is hard because the parasite is not found in adults. The best way to detect the parasite is by its pathological effects on fetuses. Cows are usually the intermediate host. No horizontal cow-to-cow transmission have been shown, although salival interactions have been suggested. Vertical transmission can occur when an infected cow gives birth to an infected calf the calf survives the infection and grows into an adult.

The life cycle is similar to Toxoplasma. An infected dog will pass the oocysts through its feces and infect food or water. A cow or other animal will then up take the parasite. The parasite will undergo asexual reproduction in the animal's muscle until it is eaten by a dog. There, sexual reproduction will occur and oocysts will be created and passed through the feces.

Neospora caninum Detection Kit is direct detection of *Neospora caninum* 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 **Neospora caninum Detection Kit** should be stored at -20 °C, under this condition, the kit is stable until expiration date stated on the label

3. CONTENTS

Neospora PCR Pre-mixture	48 tubes
DNase/RNase-free water (white cap)	1 vial
Blackleg positive control (yellow cap)	1 vial

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

4. SPECIMEN

Performs the test with whole blood, feces, amniotic fluid, CSF or tissue. The specimen should be stored at -20 °C 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 2µl of template DNA into the PCR premix tube.
- ③ Add 18µl of DNase/RNase-free water into the PCR premix tube to total volume as 20µl.
- ④ Add 2µl of positive control and 18µl 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 °C	5 min.
	Denaturation	94 °C	30 sec.
40 Cycles	Annealing	58 °C	30 sec.
	Extension	72 °C	40 sec.
1 Cycle	Final extension	72 °C	5 min.

6.3 Detection of Amplified Products

- ① Prepare 1.5% agarose gel containing RedSafe™ Nucleic Acid Staining Solution (20,000x).
- ② Load 7µl 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 : **250bp**

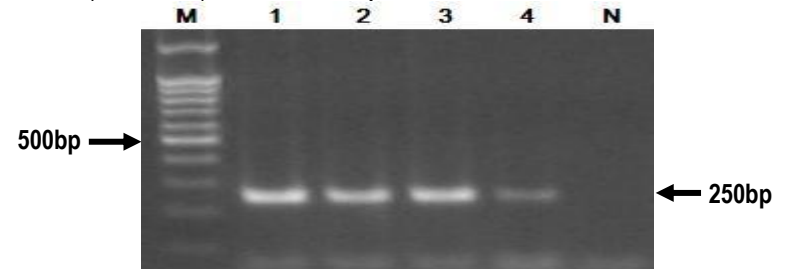


Fig 1. Electrophoresis of PCR product by **Neospora caninum Detection Kit**

Lane M : 100bp Molecular ladder (iNtRON Biotechnology)
 Lane 1~4 : Neospora positive sample (1/10 serial dilution)
 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.