For research use only Cat. No. IP21072 | 48 **Tests**



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Chlamydia psittaci Detection Kit

Test for the detection of Chlamydia felis or C. psittaci by one-step PCR

User Manual

REV.2.2

Chlamydia psittaci Detection Kit

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Chlamydia psittaci 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.
Chlamydia psittaci Detection Kit	IP21072
Viral Gene-spin $^{\text{TM}}$ Viral DNA/RNA Extraction kit	17151
SiZer [™] 100 DNA Marker	24073

1. DESCRIPTION

Feline chlamydiosis (also known as feline pneumonitis) is a relatively mild, chronic upper respiratory disease caused by the bacterium *Chlamydophila felis* (previously *Chlamydia psittaci var. felis*). The main symptom is conjunctivitis, an abnormal eye discharge due to inflammation of the conjunctiva, the membrane lining the inside of the eyelid. The infection can also cause nasal discharge, sneezing, and pneumonia. Left untreated, the infection tends to become chronic, lasting weeks or months.

Chlamydiosis is symptomatic only in the respiratory tract and eyes. When symptoms do appear, the most common include the following: Anorexia (loss of appetite; may occur as the disease progresses), Coughing, Difficulty breathing, Fever (may occur as the disease progresses), Pneumonia (in young kittens 2 to 4 weeks old, which could be fatal), Runny nose (rhinitis), Sneezing, Watery eyes due to conjunctivitis (either one or both eyes). Conjunctivitis is inflammation of the conjunctiva, the thin, transparent membrane that lines the inside of the eyelid. The inflammation leads to discharge, swelling, and pain; the cat's eyes may be red and weepy. This is the main symptom of chlamydiosis and usually occurs initially in one eye and eventually affects both eyes. Rarely, it becomes chronic.

Chlamydia psittaci Detection Kit is direct detection of *Chlamydophila felis* or *Chlamydia psittaci* 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 **Chlamydia psittaci Detection Kit** should be stored at $-20\,^{\circ}$ C, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

Chlamydia psittaci PCR Pre-mixture
DNase/RNase-free water (white cap) ····································
CHLA positive control (Yellow cap)

Component in 20^{μℓ} reaction

i-StarTaq[™] DNA Polymerase dNTPs

anirs

PCR Reaction buffer

Chemical stabilizer

Gel loading buffer

8-MOP (dissolved in DMSO)

Primers for Chlamydophila felis

4. SPECIMEN

Performs the test with whole blood, feces, ocular swab, nasal swab, placenta, liver or spleen. The specimen should be stored at -20 $^{\circ}$ 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

- Prepare appropriate PCR premix tubes and label. And one PCR premix tube for positive control.
- ② Add $2\mu\ell$ of template DNA into the PCR premix tube.
- 3 Add $18\mu\ell$ of DNase/RNase-free water into the PCR premix tube to total volume as $20\mu\ell$.
- 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℃	5 min.
	Denaturation	94℃	30 sec.
40 Cycles	Annealing	50℃	30 sec.
	Extension	72℃	40 sec.
1 Cycle	Final extension	72℃	5 min.

6.3 Detection of Amplified Products

- Prepare 1.5% agarose gel containing RedSafe[™] Nucleic Acid Staining Solution. (Cat. No. 21141)
- ② Load 7^{µℓ} of PCR product and positive control on agarose gel without adding a loadingdye 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 : 410 bp

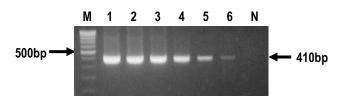


Fig 1. Electrophoresis of PCR product by *Chlamydia psittaci* Detection Kit

Lane M: 100bp Molecular ladder (iNtRON Biotechnology)

Lane 1~6: Chlamydia 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.