

1. DESCRIPTION

Feline chlamydiosis (also known as feline pneumonitis) is a relatively mild, chronic upper respiratory disease caused by the bacterium *Chlamydomyphila 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). The inflammation leads to discharge, swelling, and pain; the cats 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.

VetPCR™ CHLA.FELIS Detection Kit is the direct detection of *Chlamydomyphila felis* 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 3 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

2. STORAGE

The components of VetPCR™ CHLA.FELIS Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

	Kit 48	Kit 96	
VetPCR™ CHLA.FELIS PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
CHLA.FELIS PCR Positive control	1	1	vial
CHLA.FELIS PCR Positive control Pre-mixture.....	4	8	tubes
Brig™ Molecular Weight marker	1	1	vial
Mineral Oil	1	1	vial
DNA purification kit (see step 6.1)	50	100	tests

4. SPECIMEN

0.5 ml whole blood in EDTA (purple top), feces, ocular swab, nasal swab, placenta, liver or spleen.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- 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 manufacturers instructions. The following standard DNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Genomic DNA Purification Kit (50 test)	230040(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Genomic DNA Purification Kit (100 test)	230040(100)	Bioingentech Biotechnology Inc.

6.2 AMPLIFICATION

1.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

2.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

3.- Add 2µl of template DNA into the PCR Premix tube to total volume as 13µl.

4.- Add 6µl of DNase/RNase-free water and 2µl of PCR Positive control into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11µl). This step is necessary, even when using a thermal cycler that employs a top heating method.

6.- Perform PCR reaction of samples as the below process, using a PCR thermal cycler.

PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
	Annealing	55°C	30 sec.
	Extension	72°C	30 sec.
30 Cycles			
1 Cycle	Final extension	72°C	5 min.

6.3 DETECTION OF AMPLIFIED PRODUCTS

1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).

2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker 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 : 490 bp

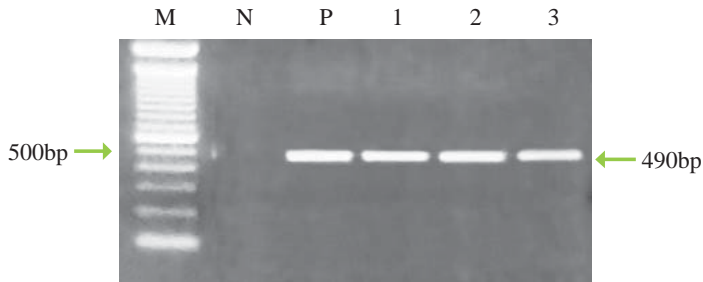


Fig1. Electrophoresis of PCR product by VetPCR™ CHLA.FELIS Detection Kit

Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)

Lane N : Negative control

Lane P : Positive control

Lane 1~3 : CHLA.FELIS Positive sample

7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. Bioingentech 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, heated at 65° C for 5 min. 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.
VetPCR™ CHLA.FELIS Detection Kit 48	VET0002F(48)
VetPCR™ CHLA.FELIS Detection Kit 96	VET0002F(96)
Brig™ Molecular Weight Marker	24012



Bioingentech Ltd.

Salas 350, piso 2, Concepción, Chile

Telephone (56)-(41)-2790435

Fax (56)-(41)-2790435

info@bioingentech.com

www.bioingentech.com

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