

## 1. DESCRIPTION

The members of the order Chlamydiales are obligate intracellular bacteria with a 2-stage developmental cycle of replication. After infection, the bacteria reside within a cytoplasmic inclusion where they replicate by binary fission, then break out of the host cell to be disseminated. *Chlamydomphila psittaci* has a worldwide distribution. Birds are natural hosts but transmission of *C. psittaci* to humans occurs by inhalation of contaminated dust or through contact with the excretions of infected animals. Infections occur worldwide and have been identified in at least 150 avian species. Clinical signs vary greatly in severity and depend on the species and age of the bird. Avian chlamydiosis can produce upper respiratory signs, conjunctivitis, gastrointestinal dysfunction and hepatitis. Current commonly used methods of *C. psittaci* diagnosis are culture, ELISA, immunofluorescent stains and serological tests. Over the last 15 years, advances in the field of molecular biology have allowed for the development of extremely sensitive and specific nucleic acid detection methods. The efficiency of molecular diagnostic techniques generally exceeds that of other methodologies.

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

## 2. STORAGE

The components of VetPCR™ CHLA.PSI 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.PSI PCR Pre-mixture.....	48	96	tubes
DNase/RNase-free water .....	1	1	vial
CHLA.PSI PCR Positive control.....	1	1	vial
CHLA.PSI 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

Choanal slit, cloacal or conjunctival swabs, biological fluids, blood samples.

## 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.
30 Cycles	Extension	72°C	30 sec.
	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

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

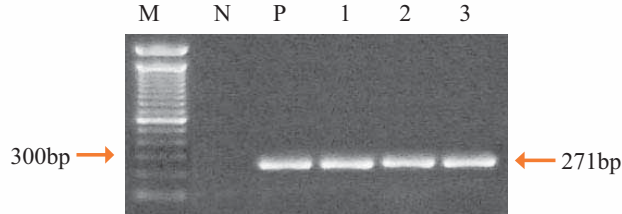
2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

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 : 271bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ CHLA.PSI Detection Kit  
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
 Lane N : Negative control  
 Lane P : Positive control  
 Lane 1~3 : CHLA.PSI 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.PSI Detection Kit 48	VET0005A(48)
VetPCR™ CHLA.PSI Detection Kit 96	VET0005A(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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