

## 1. DESCRIPTION

*Mycoplasmas* are bacteria that lack a cell wall, but are enclosed by a lipid bilayer membrane. They colonize the mucous membranes of the respiratory and genital tracts as well as red blood cells, and are found in many animals and humans. Several species are pathogenic and a variety of infections such as anaemia, arthritis, infertility and respiratory disease have been attributed to infection with mycoplasmas. *Mycoplasmas* are approximately 0.3/0.8 µm in size and readily pass through 450 and 300 nm membranes. They are fastidious in nature and some require cholesterol, sterol or urea for growth. Most *Mycoplasma spp.* normally appear in the upper respiratory tract. They have also been isolated at necropsy from lungs of dogs and cats with various types of pneumonia but are not generally present in the lungs of healthy animals, especially cats. Also, these are opportunistic organisms normally found in the vaginal canal. In large enough numbers, they may cause infertility, early embryonic deaths, abortion, stillbirths and weak, nonviable pups.

VetPCR™ MYCO.spp. Detection Kit is the direct detection of *Mycoplasma spp.* 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™ MYCO.spp Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

## 3. CONTENTS

VetPCR™ MYCO.spp PCR Pre-mixture  
DNase/RNase-free water  
MYCO.spp PCR Positive control  
MYCO.spp PCR Positive control Pre-mixture  
Brig™ Molecular Weight marker  
Mineral Oil  
DNA purification kit (see step 6.1)

## 4. SPECIMEN

0.5 ml semen or urine, or urogenital swab, or tissue.

## 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.

### 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.

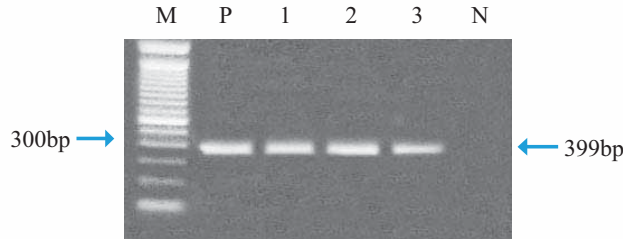
PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
	Annealing	56°C	30 sec.
30 Cycles	Extension	72°C	30 sec.
	Final extension	72°C	5 min.

### 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 : 399 bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ MYCO.spp Detection Kit  
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
Lane P : Positive control  
Lane 1~3 : MYCO.spp Positive sample  
Lane N : Negative control

## 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™ MYCO.spp Detection Kit 48	VET0022C(48)
VetPCR™ MYCO.spp Detection Kit 96	VET0022C(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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