VetPCR™ MULTIPLEX KIT PCR Mycoplasma Detection Kit

SYMBOLOGY

Manufacturer
Caution
REF Catalog Number
Expiration Date
Consult Instructions for Use

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 human 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™ MULTIPLEX KIT PCR Mycoplasma Detection Kit is the direct detection of Mycoplasma gallisepticum and Mycoplasma synoviae 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 VetPCR™ MULTIPLEX KIT PCR Mycoplasma Detection Kit is shipped at room tempera-ture (15–25°C) because contains a chemical stabilizer. The VetPCR™ MULTIPLEX KIT PCR Mycoplasma Detection Kit should be stored immediately upon receipt at 4°C in a constant temperature freezer. For routine use should be stored al 4°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date without showing any reduction in performance.

3. KIT CONTENTS

KIT	48	96	
VetPCR™ MULTIPLEX KIT PCR Mycoplasma Premixture	1	1	vial
PCR Internal Control (white cap)	1	1	vial
DNase/RNase free water	1	1	vial
MULTIPLEX KIT PCR Mycoplasma PCR Positive control	1	1	vial
PCR Negative control	1	1	vial
Mineral Oil Solution	1	1	vial
Brig ™ Molecular Weight marker	1	1	vial

4.MATERIALS

Materials required but not provided:

- Microcentrifuge and PCR tubes
- Disposable gloves, powderless
- Pipettes
- Sterile pipette tip
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Tube racks
- UV transilluminator
- Biohazard waste container

5.PROCEDURE

Please read through the entire procedure before starting.

5.1 PREPARATION OF MULTIPLEX KIT PCR Mycoplasma PCR MIXTURE

1) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 1. The final reaction volume should be 13.5 µL

Notes:

- **Run a positive control, a negative control, and an internal control each 12 samples.**
- The mineral oil is necessary, even when using a thermal cycler that employs a top heating method.

Table 1. Reaction components for PCR

Kit components	Sample	Positive control	Negative control	Internal control
VetPCR™ MULTIPLEX KIT PCR Mycoplasma Premixture	5.5µL	5.5µL	5.5µL	
PCR Internal control (white cap)				5.5µL
DNase/RNase free water	6µl	6µl	6µl	6µl
DNA isolated from the sample	2µl			2µl
MULTIPLEX KIT PCR Mycoplasma Positive control		2µl		
PCR Negative control				
Mineral Oil Solution	11µl	11µl	11µl	11µl

2) Place the tubes in a thermal cycler and perform amplification according to the program outlined in Table 2.

Table 2. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	94ºC	2 min.
30 cycles	Denaturation	94ºC	30 sec.
	Annealing	58ºC	30 sec.
	Extension	72ºC	30 sec.
1 cycle	Final extension	72ºC	5 min.

5.3. 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, 7µl of negative control, 7µl of internal 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.

5.4. INTERPRETATION OF THE TEST RESULTS

- Expected PCR product size : M. Galli 163pb and My. syno 215

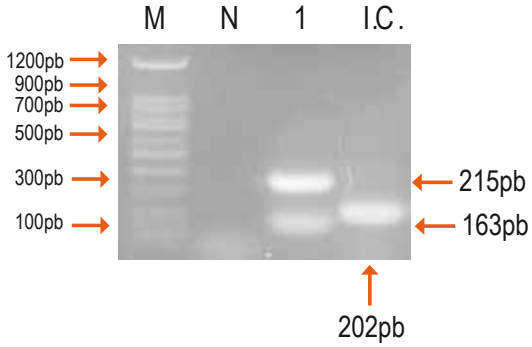

Fig. 1 Result:
Lane M: Brig™ Molecular Weight Marker (Bioingentech Ltd.)
Lane 1: MULTIPLEX KIT PCR M. Galli 163 and My. syno 215 Mycoplasma Positive samples
Lane I.C.: Internal control (202 pb)
Lane N: Negative control

6. TROUBLESHOOTING

1) No band in positive sample

• Check Internal control band: If internal control band is seen, PCR has been performed properly. It is not a problem of the product.
• Check template DNA quality: the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with DNA rehydration solution and used to perform PCR again.
• Check PCR machine: check the temperature and make sure to check that the machine is working properly.

2) No internal control band

• Check template DNA concentration: Competition can occur by high template concentration. Proceed with a lower concentration of DNA.
• Check template DNA quality: Even tough DNA is isolated from the sample, the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with distilled water and used to run the PCR reaction again. If still no band is seen, please inquire with our technical support staff.

3) Amplicon bands in the negative control

• Check contamination of distilled water: Distilled water can be contaminated. Perform PCR again with fresh sterile water.
• Check contamination of laboratory instruments and other environments: We recommend that you use filter tips and a pipette after sterilization to reduce contamination. Proceed with all procedures on a clean bench and keep the location where you procedures are performed sterile.

4) Poor resolution on agarose gel

• We recommend using a 1.5~2% agarose gel and run electrophoresis for 40 minutes at 100 V.

7. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS), available online in our website www.kitpcr.com, where you can find, view, and print the MSDS for each Bioingentech kit and kit component.

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