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PCR Mycoplasma Test Kit

Catalog Number 409010

For research use only

INTRODUCTION

The PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma contaminating biological materials including cultured cells. Mycoplasma detection by the direct culture method and nucleic acid testing (NAT). Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate. With the NAT method, polymerase chain reaction (PCR) is used to amplify mycoplasma specific DNA, and the results are obtained within a few hours. Using the PCR Mycoplasma Test Kit means that there is no need to prepare PCR primers, label probes with radioisotopes, or to determine polymerase, dNTP's or buffer concentrations. Instead, a ready-to-use, optimized PCR master mix (Reaction Mix) is supplied. Using the Reaction Mix allows the direct loading of PCR products onto agarose gel. The primer set allows detection of various mycoplasma species (e.g., M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. bovis, M. pneumoniae, M. pirum and M. capricolum), as well as Acholeplasma and Spiroplasma species, with high sensitivity and specificity. The kit contains positive control (DNA template) and internal control(DNA template) to exclude the possibility for PCR inhibition by the test sample(false negative).

KIT COMPONENTS

Enough reagents for 20 tests:

1.	Reaction Mix	200µL
2.	Buffer Solution	1.0mL
3.	Positive Template Control	20µL
4.	Internal Control Primers Mix	100µL
5.	Internal Control DNA Template	20µL

REAGENTS REQUIRED

1. Mineral Oil
2. Agarose gel
3. Reagents for gel electrophoresis
4. Distilled Sterilized water

EQUIPMENT REQUIRED

1. Authorized thermal cycler for PCR
2. Microcentrifuge tubes
3. Agarose gel electrophoresis apparatus
4. Microcentrifuge
5. Micropipettes and pipette tips (autoclaved)

STORAGE

-20°C.
Note: Avoid repeated changes in the Reaction Mix temperature. When in use, always keep the Reaction Mix on ice.

REFERENCE

Rottem, S., Barile, F.M. (1993), TIBTECH, 11:143-150

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PRINCIPLE

rRNA gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the lengths and sequences of the spacer region in the rRNA operon (for example, the region between 16S and 23S gene) differ from species to species. The detection procedure utilizing the PCR process with this primer set consists of:

1. Amplification of a conserved and mycoplasma-specific 16S rRNA gene region using two primers.
2. Detection of the amplified fragment by agarose gel electrophoresis.

This system does not allow the amplification of DNA originating from other sources, such as mammalian cells or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection. Amplified products are then detected by agarose gel electrophoresis.

SAMPLE PREPARATION

Transfer 1.0 mL cell culture supernatant into a 1.5 mL centrifuge tube. To pellet cellular debris, centrifuge the sample at 250 x g briefly. Transfer the supernatant into a fresh sterile tube and centrifuge at 15,000 - 20,000 x g for 10 minutes to sediment mycoplasma. Carefully decant the supernatant and keep the pellet (the pellet will not always be visible). Re-suspend the pellet with 50 µL of the Buffer Solution and mix thoroughly with a micropipette. Heat to 95 °C for 3 minutes. The test sample can be stored at this stage at -20 °C for later use.

PCR AMPLIFICATION

1. Prepare the reaction mixture in a PCR tube by combining the reagents shown below. Keep all preparations and components on ice.

Test Samples Preparation

Distilled H_2O	29µL
Reaction Mixture	10µL
Test Sample	5µL
Internal Control DNA Template	1µL
Internal Control Primers Mix	5µL
Total	**50µL**

Positive Control Preparation

Distilled H_2O	33µL
Reaction Mixture	10µL
Internal Control DNA	1µL
Internal Control Primers Mix	5µL
Positive Control DNA	1µL
Total	**50µL**

An optional negative control can be run in addition to the positive control using 5µL Buffer Solution.

2. Overlay mineral oil (approximately 40µL) to avoid the evaporation of the reaction mixture.

3. Place all tubes in DNA thermal cycler. Set the parameters for the following conditions and perform PCR.

94 °C	30 secs.	
94 °C	30 secs.	
60 °C	120 secs.	35 cycles
72 °C	60 secs.	
94 °C	30 secs.	
60°C	120 Secs.	
72 °C	5 min.	

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ANALYSIS BY GEL ELECTROPHORESIS

1. Apply 20µL of the PCR product to the gel electrophoresis using a 2% agarose gel.
2. Perform agarose gel electrophoresis with PCR amplified samples to verify the amplified product and its size.

- The size of DNA fragments amplified using the mycoplasma specific primers in this kit is 270bp.
- Control Templates: By the use of 1µL of Positive Template Control, PCR efficiency can be checked. The size of the PCR product obtained using the positive template is 270bp.
- The use internal control is to check for PCR inhibition by the test sample (false negative). The sized of the PCR product obtained using the internal control template is 357bp.

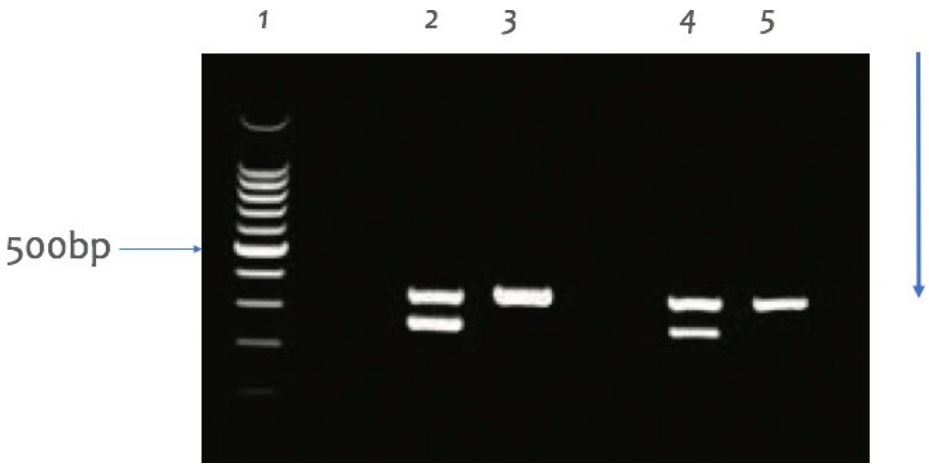
INTERPRETATION OF THE RESULTS

- Mycoplasma positive sample shows a 270bp band as well as 357bp band.
- Mycoplasma negative sample shows a 357bp band only.
- PCR inhibition yields no band
- Negative control: one band of 357bp.
- Primer self-annealing may yield a band of <100bp in size. This does not affect the sensitivity and precision of the test.

Band at 270bp	Internal control band at 357bp	Interpretation
Positive	Irrelevant*	Mycoplasma positive sample
Negative	Negative	PCR inhibitor (test no valid)
Negative	Positive	Mycoplasma negative sample

Note: If the mycoplasma concentration in the sample is high the Internal Control band might be absent due to competition.

A TYPICAL AGAROSE GEL



1. DNA size marker (100bp)
2. Internal and positive controls
3. Negative control
4. Test sample: positive
5. Test sample: negative

- The PCR Mycoplasma Test Kit is adequate to diagnose cell cultures infected with mycoplasmas. Infections usually result in mycoplasma titers of 10^5 - 10^8 CFU/mL (Mc Garrily 1982).

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