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PCR Mycoplasma Test Kit

Catalog Number: 409010

INTRODUCTION

The PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma contaminating biological materials such as cultured cells. Mycoplasma detection by the direct culture procedure is time-consuming, and some mycoplasma species are difficult to cultivate. With PCR testing, results are obtained within a few hours since the presence of contaminant mycoplasma can be easily detected simply by verifying the bands of amplified DNA fragments in electrophoresis. There is no need to prepare probes labeled with radioisotopes or calculate enzyme, dNTP's or buffer concentrations. Instead, a ready-to-use, optimized PCR mix is supplied. The primer set allows detection of various mycoplasma species (*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.

KIT COMPONENTS

Enough reagents for 20 tests:

1.	Reaction Mix	200 µL
2.	Buffer Solution	1mL
3.	Positive Template Control	20 µL

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. Micropipets 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

For research use only.

PRINCIPLE

rRNA gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the lengths and sequences of the spacer region in the rRNA operon (the region between 16S and 23S gene, for example) 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 cultured 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 0.5–1.0 mL cell culture supernatant into a 2 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 at15,000 - 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 micropipet. 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:

Test Sample Preparation		Positive Control Preparation*	
Distilled H_2O	35 µL	Distilled H_2O	35 µL
Reaction Mixture	10 µL	Reaction Mixture	10 µL
Sample	5 µL	Positive Control	1 µL
		Buffer Solution	4 µL

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

2. Overlay mineral oil (approximately 40 µL) 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.	} 35 cycles
60 °C	120 secs.	
72 °C	60 secs.	
94 °C	30 secs.	
60 °C	120 secs.	
72 °C	5 min.	

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 the PCR amplified samples to verify the amplified product and its size.
    - The size of DNA fragments amplified using the specific primers in this kit is 270bp.
    - Control Template: By the use of 1µL of Positive Template Control as a test sample, PCR efficiency can be checked. The size of the PCR product obtained using the positive template with primer pairs is 270 bp.

METHOD COMPARISON

PCR-Based Method	Microbiological Culture
Rapid Results within 5 hours	Long and time consuming Results up to 3 weeks
Simple, one-step reaction Less than 10 minutes to prepare the sample Themixture contains everything (including Tag Poly)	Cumbersome
Can be used routinely in every lab	Requires specialized personnel
Minimal sample handling reducing risk of contamination	
Sensitive * (100-1000 CFU/mL)	Sensitive
M. Hyorhinis can be detected	hard to detect M. Hyorhinis

- 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)

SENSITIVITY

Type of Mycoplasma	Minimum Conc. Detected
M. FERMENTANS	240 CFU/mL
M. CAPRICOLOM	110 CFU/mL
PENETRANS	200 CFU/mL
HYORHINIS	210 CRU/mL

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