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Mycoplasma PCR Detection Kit

Cat. #: C0044 Size: 40 T

Introduction

Mycoplasma PCR Detection Kit allows for quick and reliable screening of cell cultures for contamination with mycoplasmas. Mycoplasma DNA in the cell culture supernatant is amplified via PCR and visualized using gel electrophoresis. In addition to the short detection process (less than 2 hours), the easy handling and high sensitivity makes this Mycoplasma PCR Detection Kit a convenient tool for routine examination of cell cultures and media. The kit contains an optimized PCR master mix and a positive control. The primers in the PCR master mix are highly specific to the conserved rDNA region in the mycoplasma genomes and can detect all well-known mycoplasma genera, including the commonly encountered ones in cell cultures, such as *M. orale, M. hyorhinis, M. laidlawii, M. salivarium, M. arginini, M. fermentans, M. hominis, and M. pneumoniae*. Mycoplasma positive samples can be easily recognized by a distinctive PCR product ranging in size from 400 to 600 bp.

Package Information

Components	M0060
2× PCR Master Mix	2×300 µl
Positive Control	60 µl

Storage

Store at -20°C and avoid repeated freeze-thaw cycles.

Protocols

1. The cells should have been in culture for at least 24 hours prior to screening for the presence of mycoplasmas.

2. Withdraw 0.5 ml of cell culture medium and centrifuge it for 5 mins at 2000 g to pellet cells/ debris. The supernatant from this centrifugation step will serve as the Test sample for PCR.

3. Set-up the various reactions according to the table below:

	Test Sample	Positive Control	Negative Control
2X PCR Master Mix	14.5 µl	14.5 µl	14.5 µl
Test sample	2 µl	-	-
Positive Control	-	2 µl	-
Nuclease-free H_2O	8.5 µl	8.5 µl	10.5 µl
Final volume per reaction	25 µl	25 µl	25 µl

4. Perform 40 cycles of PCR as follows:

Step	Temperature	Duration	Cycle(s)
Enzyme activation	94 °C	90 secs	-
Denaturation	94 °C	30 secs	20 cycles (every two cycle, decrease annealing temperature by 1 °C)
Annealing	70-61 °C	30 secs	
Extension	72 °C	45 secs	
Denaturation	94 °C	30 secs	20 cycles
Annealing	60 °C	30 secs	
Extension	72 °C	45 secs	
Final extension	72 °C	4 mins	-

5. Resolve the amplification products by agarose gel electrophoresis and visualize by LiGreen™ DNA Gel Stain (Cat. # M0049) or ethidium bromide staining.

6. The presence of PCR products approximately 500 bp in length indicates that the cell culture tested is contaminated with mycoplasmas. Note that the length of the PCR product will vary between 400-600 bp depending on the different Mycoplasma species/strains.

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