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MycoCheck™ Mycoplasma PCR Detection Kit

Catalog Number: A058-1, A058-2

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
MycoCheck™ Mycoplasma PCR Detection Kit (Cat. No. A058-1)			The product is stable for at least one year when stored as directed.
2X PCR Master Mix (Component A)	320 µL	-20 °C Avoid repeated freeze-thaw cycles	
Positive Control (Component B)	24 µL		
MycoCheck™ Mycoplasma PCR Detection Kit (Cat. No. A058-2)			
2X PCR Master Mix (Component A)	640 µL	-20 °C Avoid repeated freeze-thaw cycles	
Positive Control (Component B)	45 µL		

Product Description

Mycoplasma infections are relatively common in laboratory cell cultures; it has been estimated that between 5% and 35% of all cell cultures are infected. Mycoplasmas have been shown to alter the growth rate of cells in culture, induce chromosomal aberrations, influence amino acid and nucleic acid metabolism and cause membrane aberrations. Several methods have been developed to detect mycoplasma including direct culture in special growth media, enzyme-linked immunoassay, immunofluorescence staining, PCR, biochemical detection and fluorescent nucleic acid stains.

The MycoCheck™ 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.

Protocol Overview

For screening, a small aliquot of the cell culture medium is spun to remove any cells/debris and then applied as a template (Test sample) in a PCR. In parallel, a No-Template-Control (NTC) reaction is set-up as a negative control to rule out the possibility of any other source of contamination (there should be no amplification in this reaction) while the set-up of a positive control reaction helps assess whether the PCR itself ran unhindered. Targeted amplification of *Mycoplasma* DNA from the test sample confirms the presence of mycoplasmas in the cell culture while lack of amplification indicates absence of contamination.

Protocol

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	12.5 µl	12.5 µl	12.5 µl
Test sample	2 µl	-	-
Positive Control	-	2 µl	-
Nuclease-free H_2O	10.5 µl	10.5 µl	12.5 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 ^{o}C	90 secs	-
Denaturation	94 ^{o}C	30 secs	20 cycles (every two cycle, decrease annealing temperature by 1 ^{o}C)
Annealing	70-61 ^{o}C	30 secs	
Extension	72 ^{o}C	45 secs	
Denaturation	94 ^{o}C	30 secs	20 cycles
Annealing	60 ^{o}C	30 secs	
Extension	72 ^{o}C	45 secs	
Final extension	72 ^{o}C	4 mins	-

5. Resolve the amplification products by agarose gel electrophoresis and visualize by GreenView DNA Gel Stain (Cat. No. N100) 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.

***Mycoplasma* species**	**PCR product**
M. orale	462 bp
M. hyorhinis	464 bp
A. laidlawii	438 bp
M. salivarium	466 bp
M. arginini	465 bp
M. fermentans	461 bp
M. hominis	463 bp
M.pneumoniae	470 bp