Mycoplasma PCR Detection Kit
Cat. No. 8208, 100 tests

Introduction

Mycoplasma, the smallest and self-replicating prokaryotes, are common contaminant of primary and continuous cell line cultures. It has been shown that micoplasma contamination affects cell growth, morphology and metabolism. Because of their small size and flexibility, mycoplasma can pass through commonly used filters of 0.2 µm. Furthermore, unlike bacterial and fungi, micoplasma contamination is resistant to commonly used antibiotics, and not easy to be spotted visually. These drawbacks emphasize the need for regular screening in control of mycoplasma contamination. Conventional screening methods include microbiological culture, fluorescent DNA staining and biochemical detection methods. However, mycoplasma culture is restricted to specialized laboratories and takes 2-4 weeks. Results of DNA fluorochrome staining are difficult to interpret due to the presence of broken nuclei. Various biochemical detections are time consuming and often lack of sensitivity. Recently, polymerase chain reaction (PCR) based methods have been developed as a quick and convenient detection of low level mycoplasma infection, which offers great advantage over the conventional methods because of its extreme sensitivity and specificity.

Computer alignment studies of mycoplasmal 16S rRNA sequences have revealed the existence of highly conserved sequences. Primers designed according to these sequences allow the specific amplification of mycoplasma DNA fragment and thus highly sensitive detection of mycoplasma. ScienCell™ Mycoplasma PCR Detection Kit is a 16S rRNA-based PCR assay including 2× reaction buffer, primer set, as well as internal amplification control (IAC) and positive sample control. The genus-specific primer set we select recognizes the five typical contaminating mycoplasma species (i.e. *M. hyorhinis*, *M. arginini*, *M. orale*, *M. fermentans*, and *A. laidlawi*), which account for 98% of contaminations, as well as members of the genera *Ureaplasma*, *Spiroplasma*, and *Acholeplasma*, which account for the remaining 2% of contaminations. Eukaryotic and bacterial DNA is not amplified by this kit. The IAC is designed to be co-amplified simultaneously with the target mycoplasma sequence by the same set of primers. The resulted control band, which is distinguished from the target band by a difference in molecular mass, indicates the occurrence of DNA amplification. Therefore, false negative results due to the presence of PCR inhibitors in some cell cultures can be identified. Our kit also provides a positive sample control, which is noninfectious genomic DNA (gDNA) of *M. fermentans*. On the other hand, a no template (i.e. DNA-free DI H_2O) negative sample control can help to determine whether there is a false positive result due to carry-over contamination. Each experiment should include both positive and negative sample controls, as well as the IAC. The size of the expected bands and the interpretation of possible band patterns are given in the Tables 1 and 2.

Kit Component

Cat. No.	# of vials	Reagent	Quantity	Storage
8208a	2	2× PCR buffer	1.25 ml	-20˚C
8208b	1	Mycoplasma primer	100 µl	-20˚C
8208c	1	Internal amplification control (IAC)	100 µl	-20˚C
8208d	1	*M. fermentans* positive sample control (0.05 ng/µl)	20 µl	-20˚C

Materials to be Supplied by the User

Thermal cycler
Electrophoresis system
PCR grade H_2O, mineral oil, tips and tubes
Microcentrifuge
gDNA extraction kit (optional)
Spectrophotometer (optional)

Quality Control

The ScienCell™ Mycoplasma PCR Detection Kit is applied to gDNA of *M. fermentans* with serially diluted concentrations (1 ng/µl to 0.98 pg/µl). The reaction mixtures are prepared according to Table 3 (*M. fermentans* positive sample control w/IAC). The IAC band at 750 bp and the diagnostic band at 280 bp can be observed, as shown in Figure 1. Results indicate that this kit can detect as little as 0.98 pg of gDNA of *M. fermentans*. It is also noticed that as the concentration of the gDNA of *M. fermentans* increases, the band of IAC is fading, probably due to its competition with the gDNA.

Procedures

A. Preparation of testing sample

ScienCell™ Mycoplasma PCR Detection Kit is sensitive enough that cell culture supernatant can be used for detection of mycoplasma contamination. However, for those cultures with possible PCR inhibitors, indicated by the absence of IAC band, or for detection of early contamination, gDNA is recommended as testing sample to avoid false negative results. For both protocols, cells can be cultured in antibiotics-free medium for several days to near confluence to maximize test sensitivity. Cares must be taken to avoid carry-on contaminations, including using PCR grade tubes, tips and water, wearing gloves and performing pre-PCR steps in an area separated from post-PCR area.

1. Preparation of boiling extract of cell culture supernatant:

1.1 Transfer 100 µl of supernatant from the cell culture to a PCR tube.

1.2 Heat the supernatant for 5 min at 95˚C.

1.3 Spin the tube briefly (10-30 seconds) in a microcentrifuge to pellet the cellular debris before adding the supernatant to the PCR mix.

2. Preparation of genomic gDNA of cells:

2.1 Harvest cells, count the number of cells and pellet appropriate number of cells ($1\text{-}3\times10^6$ cells).

2.2 Extract the gDNA from the cells using a gDNA extraction kit (e.g. Qiagen Allprep DNA/RNA Mini Kit). Check the quality and quantity of the extracted gDNA using a spectrophotometer.

B. Preparation of the PCR mixture

For each PCR reaction, prepare a PCR reaction mixture of 50 µl in a PCR tube according to Table 3. Scale up based on the number of PCR reactions to be preformed when necessary. Mix well and spin briefly. If the thermal cycler is not equipped with a heated cover, overlay each reaction mixture with 50 µl of PCR grade mineral oil to prevent evaporation.

C. PCR Reaction

Perform PCR reaction with the program shown in Table 4.

D. Electrophoresis analysis of PCR product

1. Use 1.5% agarose gel for electrophoresis.
2. Mix 10 µl of PCR product with 2 µl of 6× Loading buffer, load 10 µl per lane. Add 10 µl of DNA ladder to the reference lane.
3. Run the gel for 25 min at 120 V.
4. Stop electrophoresis and stain the bands with ethidium bromide. Visualize the gel under UV light.

Table 1. Size of expected bands.

IAC band	750 bp
Mycoplasma diagnostic band	280 bp

Table 2. Interpretation of possible band patterns.

PCR sample	**Band Pattern**	**Result Interpretation**
Test sample w/IAC	No band	PCR failed. False negative.
	750 bp band only	Test sample has no mycoplasma infection.
	280 bp band only	Test sample has heavy mycoplasma infection. Try to dilute the test sample and the 750 bp band should show.
	Both bands	Test sample has some mycoplasma infection.
M. fermentans positive sample control w/IAC	No band	PCR failed.
	750 bp band only	Positive sample control has gone bad.
	280 bp band only	IAC has gone bad.
	Both bands	Expected.
Negative sample control (DNA-free DI H_2O) w/ IAC	No band	PCR failed.
	750 bp band only	Expected.
	280 bp band only	Heavy carry-on contamination or some carry-on contamination with non-working IAC. False positive.
	Both bands	Carry-on contamination. False positive.

Table 3. Preparation of PCR mixture.

Reagent	**Amount per reaction**		
	Test sample w/IAC	***M. fermentans* positive sample control w/IAC**	**Negative sample control (DNA-free DI H_2O) w/ IAC**
PCR grade water	X µl	22 µl	23 µl
2× PCR buffer	25 µl	25 µl	25 µl
Mycoplasma primer	1 µl	1 µl	1 µl
Internal amplification control (IAC)	1 µl	1 µl	1 µl
Test Sample	5 µl of supernatant OR 1-50 ng of gDNA	-	-
M. fermentans positive sample control	-	1 µl of 0.05 ng/ µl	-
Total reaction mixture	50 µl	50 µl	50 µl

Table 4. Thermal cycle program of PCR reaction.

Cycle number	Temperature	Time
1	94˚C	30 sec
40	94˚C	30 sec
	55˚C	30 sec
	72˚C	1 min
1	72˚C	5 min

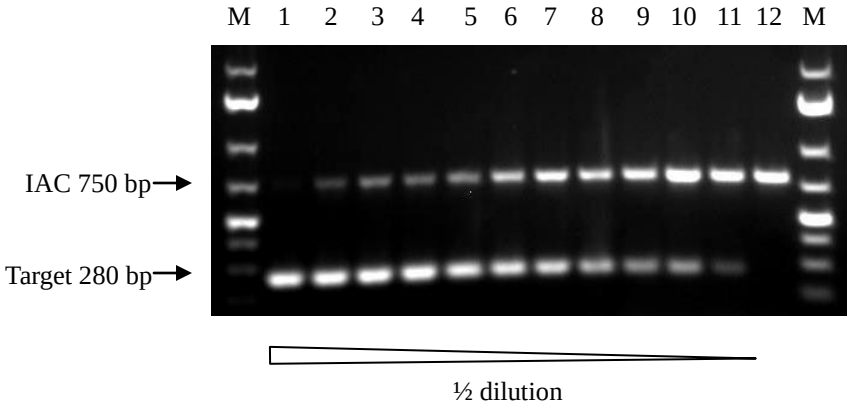

Figure 1. The PCR results obtained with reaction mixture containing 1 µl of IAC and 1 µl of different concentrations of *M. fermentans* positive sample control. Lane M: DNA ladder; lanes 1-11: PCR products of IAC and gDNA of *M. fermentans* with concentrations of 1 ng/µl (lane 1), 0.5 ng/µl (lane 2), 0.25 ng/µl (lane 3), 0.125 ng/µl (lane 4), 62.5 pg/µl (lane 5), 31.25 pg/µl (lane 6), 15.63 pg/µl (lane 7), 7.81 pg/µl (lane 8), 3.91 pg/µl (lane 9), 1.95 pg/µl (lane 10), 0.98 pg/µl (lane 11); Lane 12: PCR product of negative control with IAC and DNA-free H_2O.