Mycoplasma PCR Detection Kit (MYCO)

Catalog #8208, 100 tests

Product Description

Mycoplasma, the smallest and self-replicating prokaryotes, are common contaminants of primary cells and cell lines. Mycoplasma contamination can affect cell growth, morphology and metabolism. Mycoplasma are able to pass through 0.2 µm filters due to their small size. Furthermore, unlike bacteria and fungi, mycoplasma contamination is resistant to commonly used antibiotics, and can not be identified visually. Due to the difficulties in detecting mycoplasma, cells should be regularly screened to control mycoplasma contamination. Conventional screening methods include microbiological culture, fluorescent DNA staining, and biochemical detection methods, and are typically time consuming and lack sensitivity. As a result, polymerase chain reaction (PCR) based methods have been developed as a quick and convenient detection of low level mycoplasma infection, while providing high sensitivity and specificity.

Alignment studies of mycoplasma 16S rRNA sequences revealed the existence of highly conserved sequences. Primers targeting these sequences allow for the specific amplification of the mycoplasma DNA fragment. ScienCell™ Mycoplasma PCR Detection Kit is a 16S rRNA-based PCR assay consisting of 2×PCR master mix (which includes PCR buffer, DNA polymerase and dNTPs), mycoplasma primer set, and a positive control, which is noninfectious genomic DNA (gDNA) of *M. fermentans*. The genus-specific primer set 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 contamination, as well as members of the genera *Ureaplasma*, *Spiroplasma*, and *Acholeplasma,* which account for the remaining 2% of contamination. Eukaryotic and bacterial DNA is not amplified by this kit. The size of the expected band is 280 bp.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8208a	2	2× PCR master mix, hot start	1.25 ml	-20˚C
8208b	1	Mycoplasma primer set	100 µl	-20˚C
8208c	1	*M. fermentans* positive control (0.05 ng/µl)	20 µl	-20˚C

Additional Materials Required (Not Included in Kit)

PCR grade H2O (ScienCell, cat #GQ100)
Thermal cycler
Electrophoresis system
gDNA extraction kit (optional)

Quality Control

The ScienCell™ Mycoplasma PCR Detection Kit is applied to gDNA of *M. fermentans* with serially diluted concentrations (1 ng/µl to 1 pg/µl). The reaction mixtures are prepared according to Table 1 (*M. fermentans* positive control, see page 3). The diagnostic band at 280 bp can be observed, as shown in Figure 1. Results indicate that this kit can detect as little as 1 pg of gDNA of *M. fermentans*.

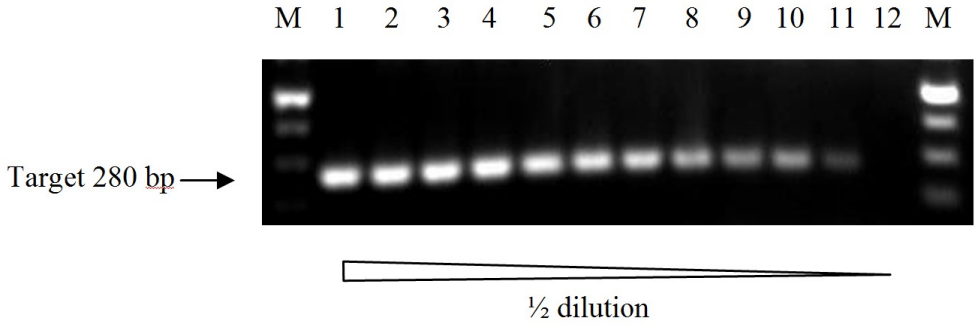

Figure 1. The PCR results obtained with 1 µl of different concentrations of *M. fermentans* positive sample control. Lane M: DNA ladder; lanes 1-11: PCR products with gDNA of *M. fermentans* with concentrations of 1 ng/µl (lane 1), 0.5 ng/µl (lane 2), 0.25 ng/µl (lane 3), 0.125 ng/µl (lane 4), 62.5 pg/µl (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 DNA-free H_2O.

Product Use

MYCO is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, store the product at -20°C in a manual defrost freezer.

Procedures

A. Preparation of testing sample

ScienCell™ Mycoplasma PCR Detection Kit is sensitive enough that cell culture supernatant can be used to detect mycoplasma contamination. For those cultures with possible PCR inhibitors or for detection of early contamination, gDNA is recommended to avoid false negative results. For both protocols, cells should be cultured in antibiotic-free medium for several days to near confluence to maximize test sensitivity.

1. Preparation 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) at 1,000 x g to pellet the cellular debris before adding the supernatant to the PCR mix.

2. Alternatively, preparation of gDNA from 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, cat #80204). Check the quality and quantity of the extracted gDNA according to the manufacturer's manual.

B. Preparation of the PCR mixture and perform PCR reactions

For each PCR reaction, prepare a PCR reaction mixture of 50 µl in a PCR tube according to Table 1. Scale up/down when necessary. Mix well and spin briefly. If the thermal cycler is not equipped with a heated lid, overlay each reaction mixture with 50 µl of PCR grade mineral oil to prevent evaporation. Perform PCR reactions with the program shown in Table 2. Interpretation of control sample patterns are explained in Table 3.

Table 1. Preparation of PCR mixture.

Reagent	**Amount per reaction**		
	Test sample	***M. fermentans* positive control**	**"No template" negative control**
PCR grade water	X µl	23 µl	24 µl
2× PCR buffer	25 µl	25 µl	25 µl
Mycoplasma primer set	1 µl	1 µl	1 µl
Test Sample	5 µl of supernatant OR 1-50 ng of gDNA	-	-
M. fermentans positive control	-	1 µl of 0.05 ng/ µl	-
Total reaction mixture	50 µl	50 µl	50 µl

Table 2. Thermal cycle program of PCR reaction.

Cycle number	Temperature	Time
1	95˚C	10 min
40	95˚C	20 sec
	55˚C	20 sec
	72˚C	20 sec
1	72˚C	2 min

Table 3. Interpretation of control sample patterns.

PCR sample	Band pattern	Result interpretation
M. fermentans positive control	No band	PCR failed
	280 bp band	Expected
"No template" negative control	No band	Expected
	280 bp band	external contamination present

C. 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. See example results in Figure 1.