Clongen laboratories, llc
Committed to Scientific Excellence
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Mycoplasma Detection Polymerase Chain Reaction Kit (16S rRNA gene)

Product Name: MycoFind™ Version 1.0

Catalog # MF001: 100 Reactions (50 µl each)

Technical Bulletin

Introduction:

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It has been documented in the literature that 30-80% of all cell lines are contaminated *with Mycoplasma species*. Unlike other bacterial or fungal contaminants, *Mycoplasma* infections do not manifest themselves in terms of pH changes or turbidity in the cell culture medium. *Mycoplasma* infections may elicit numerous deleterious effects and their presence has a major impact on cell biology. Although broth and agar cultures as well as the Hoechst DNA fluorochrome staining method are commonly used for *Mycoplasma* detection, polymerase chain reaction (PCR) is becoming increasingly available and has become a routine part of any major cell culture operation's regular maintenance. It is essential that cells get tested regularly for *Mycoplasma* contamination and if detected, treat the cells as soon as possible to eliminate the infection.

MycoFind™ Kit:

The kit provides all the necessary ingredients to perform a PCR reaction with the exception of Taq Polymerase. The primer mix and Buffer/dNTP solutions are shipped in separate tubes to ensure a longer kit shelf life. For best results, all reagents included in the kit should be kept frozen at –20° C and the user should avoid repeated freeze-thaw cycles.

Kit components:

1. Buffer/dNTP 1x mixture
2. Multiplex primer mix (5X)
3. One tube of Positive Control
4. One tube of Molecular Biology Grade Water

Reagent Preparation:

For preparation of the mastermix, add 300 µl of the primer mix to a tube of Buffer/dNTP mix (each tube contains 1400 µl of Buffer/dNTP mixture). Mix by vortexing for 5 seconds. Add the template DNA and Taq Polymerase to the reaction mixture, and start cycling on a certified thermal cycle.

Reaction Mixture:

Template DNA	10 µl (You can use 5 µl of positive control)
Premixed reaction mixture	40 µl
Taq Polymerase (not provided)*	0.5 µl

Mix well by pipetting up and down 4 times and start cycling.

Cycling Conditions:

Temperature	Time	Cycles
94° C	3 minutes	1 X
94° C 58° C 72° C	30 Seconds 30 Seconds 35 Seconds	35X
72° C	2 minutes	
4° C	Forever	1X

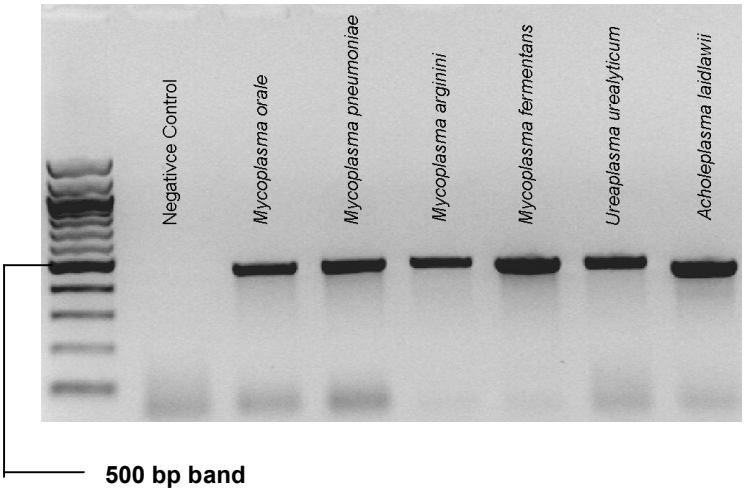
*We recommend New England Biolabs Standard Taq Polymerase for use with this kit.

These conditions have been validated on a Bio-Rad Gene Cycler, and a Stratagene Gradient 96 Robocyler.

Technical tips:

1. It is recommended to use a DNA extraction methodology that is proven to yield high quality DNA and that is manufactured under GMP conditions.
2. Use clean disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
3. Keep your reagents and PCR mixture tubes on a cold block during reaction setup.
4. Use positive displacement pipettes.
5. Avoid splashes and if splashing occurs, change gloves.
6. Add DNA last and cap each tube before proceeding to the next tube.
7. The amplification and detection areas should be physically distant (do not use the same bench area to setup the PCR reactions and run your gels. PCR product contamination can cause serious problems.

The gel image below is a sample of what a typical reaction using the recommended cycling conditions should look like:



Troubleshooting:

1. No signal in positive control lane: annealing temperature too high. Use recommended annealing temperature and make sure that your cycler is calibrated and the temperature on the display is the actual block temperature.
2. Too many bands: annealing temperature too low, increase annealing temperature gradually. This could also be due to PCR mis-priming prior to cycling. Make sure your PCR reaction tubes are kept cool to avoid priming before cycling. The initial cycles are critical. Alternatively, use a hotstart Taq Polymerase.
3. Negative control shows a PCR product: this is due to contamination of either the mastermix, the water template used in the negative control tube or the pipette tip used to mix the negative control reaction mixture.

Feel free to contact Tech Support at Clongen Labs if you have any questions. We do offer a Mycoplasma testing and elimination service.