MycoScope™

Mycoplasma PCR Detection Kit

Cat. #	Description	Contents	Quantity
MY01100	MycoScope™ MycoPlasma PCR Detection Kit (100 reactions)	dNTP/Buffer Mixture (1X)	3 x 1.4 ml
		Primer Mix (5X)	950 µl
		Positive Control Reaction Tube	120 µl
		Molecular Biology Grade Water	1.8 ml
MY01050	MycoScope™ MycoPlasma PCR Detection Kit (50 reactions)	dNTP/Buffer Mixture (1X)	2 x 1.25 ml
		Primer Mix (5X)	550 µl
		Positive Control Reaction Tube	60 µl
		Molecular Biology Grade Water	1.8 ml

Shipping	Shipped on Dry Ice.
Storage	For best results, all reagents included in the kit should be kept frozen at -20°C. Repeated freeze-thaw cycles should be avoided.

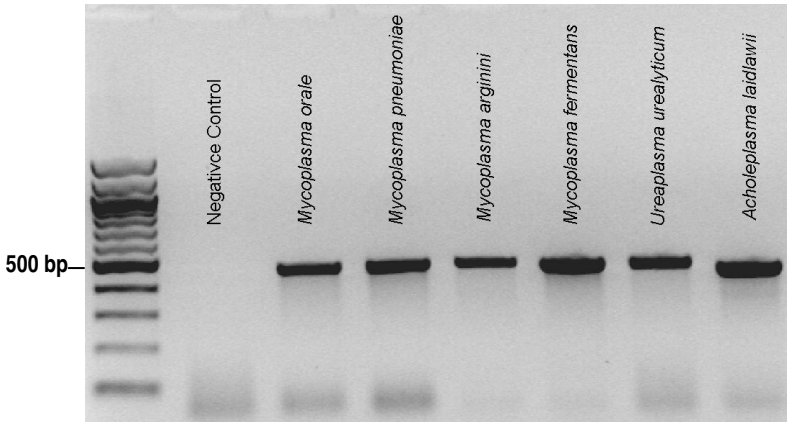
Introduction: *Mycoplasma* infections are known to elicit numerous deleterious effects upon cells. Unlike other bacterial or fungalcontaminants, *mycoplasma* infections do not manifest themselves in terms of pH changes or turbidity in the cell culture medium.Although agar cultures as well as DNA fluorochrome staining methods can be used for *Mycoplasma* detection, polymerase chainreaction (PCR) is the established method of choice for high-sensitivity detection.

The MycoScope™ *Mycoplasma* PCR Detection Kit utilizes PCR to detect *mycoplasma* infections in cell cultures in less than threehours. With the highly sensitive PCR assay, the MycoScope kit is capable of detecting less than 5 *mycoplasma* genomes permicroliter of sample. The primer set supplied with the kit is specific to the highly conserved 16S rRNA coding region in themycoplasma genome, allowing for detection of all *mycoplasma* species commonly encountered in cell culture.

The MycoScope Kit has been tested for use with a variety of commercially available DNA polymerases. The kit contains a positive control DNA, and a successfully performed reaction is indicated by a distinct 500 bp band on an agarose gel. The same band also indicates the possible presence of *mycoplasma* species in the cell culture.

Species Specificity- The MycoScope Kit detects the following mycoplasma species		
A. laidlawii	*M. agalactiae*	*M. arginini*
M. arthritidis	*M. bovis*	*M. cloacale*
M. falconis	*M. faucium*	*M. fermentans*
M. hominis	*M. hyorhinis*	*M. hyosynoviae*
M. opalescens	*M. orale*	*M. pneumoniae*
M. primatum	*M. pulmonis*	*M. salivarium*
M. Spermatophilum	*M. timone*	*U. urealyticum*

Sample gel image of a reaction using the recommended cycling conditions



METHODS AND PROCEDURES

A. Preparation of sample materials

Samples should be derived from cultures that are 90-100% confluent. Cell culture supernatants can be tested directly without further preparation. Stable templates for PCR analysis can be prepared using the following protocol:

1. Transfer 100 µl of supernatant from the test culture to a sterile microcentrifuge tube. The lid should be tightly sealed to prevent opening during heating.
2. Boil or incubate the sample supernatant at 95 °C for 5 minutes.
3. Briefly centrifuge (5 seconds) the sample supernatant to pellet cellular debris before adding to the PCR mixture. The templates are stable at 2–8 °C for at least 1 week.

Note 1: For a sample from an older culture, a DNA extraction is required prior to testing. We recommend using standardised spin columns, magnet beads or automated methods to obtain clean high quality total DNA

Note 2: Molecular Biology Grade Water may be used as a negative control

B. PCR Reaction

1. For the 100-reaction MysoScope kit (Cat. No. MY01100), add 300 µl of the 5X Primer Mix to each tube of dNTP/Buffer Mixture. For the 50-reaction MycoScope kit (Cat. No. MY011050), add 250 µl of the 5X Primer Mix to each tube of dNTP/Buffer Mixture.
2. Add sample materials from Part A and Taq polymerase to the premixed reaction mixture from step 1:

Reaction Mixture:

Samples from culture	10 µl
Premixed reaction mixture	40 µl
Taq Polymerase (not provided*)	0.5 µl

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

3. To set up a positive control reaction, use 5 ul of Positive Control Reaction and 5ul of Molecular Biology Grade Water as a template.
4. Mix well by pipetting up and down 4 times and start cycling on a certified thermal cycler.

Cycling Conditions:

Temperature	Time	Cycles
94°C	3 minutes	1X
94°C 58°C 72°C	35 seconds 45 seconds 45 seconds	35X
72°C	3 minutes	
4°C	Forever	1X

5. Use 1.2% standard agarose gel with 5 mm comb and load 8 µl of PCR products with loading buffer into each lane for electrophoresis evaluation.

C. Technical Tips

1. Use clean disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
2. Keep your reagents and PCR mixture tubes on a cold block during reaction setup.
3. Use positive displacement pipettes.
4. Add DNA last and cap each tube before proceeding to the next tube.
5. The amplification and detection areas should be physically separated, i.e. do not use the same bench area to setup the PCR reactions and run your gels.

D. Troubleshooting

1. No signal in positive control lane: Incompatible Taq polymerase: Use Taq brands that work with a $MgCl_2$ buffer; do not use Taq brand that works with a $MgSO_4$ buffer. Annealing temperature is 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 is 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, following the manufacturer's instructions, you may need more than 3 minutes used in this protocol.
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.