**Introduction:** Mycoplasma infections are known to elicit numerous deleterious effects upon cells. Unlike other bacterial or fungal contaminants, 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 chain reaction (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 three hours. With the highly sensitive PCR assay, the MycoScope kit is capable of detecting less than 5 mycoplasma genomes per microliter of sample. The primer set supplied with the kit is specific to the highly conserved 16S rRNA coding region in the mycoplasma 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. cloacae**
- **M. falconis**
- **M. fauclium**
- **M. fermentans**
- **M. hominis**
- **M. hyorhinis**
- **M. hyosynoviae**
- **M. opalescens**
- **M. oralis**
- **M. pneumoniae**
- **M. primatum**
- **M. pulaonis**
- **M. salivarum**
- **M. Spermatophilum**
- **M. timone**
- **U. urealyticum**

Sample gel image of a reaction using the recommended cycling conditions

![Sample gel image of a reaction using the recommended cycling conditions](image)

**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 | 100 µl |
|  |  | Molecular Biology Grade Water | 1.5 ml |

**MY01050** | MycoScope™ Mycoplasma PCR Detection Kit (50 reactions) | dNTP/Buffer Mixture (1X) | 2 x 1.25 ml |
|  |  | Primer Mix (5X) | 530 µl |
|  |  | Positive Control Reaction Tube | 50 µl |
|  |  | Molecular Biology Grade Water | 1.5 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.
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: For a sample from an older culture, a DNA extraction is required prior to testing.

B. PCR Reaction
1. For the 100-reaction MycoScope 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 0.5 µl

3. Mix well by pipetting up and down 4 times and start cycling on a certified thermal cycler.

Cycling Conditions:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>94°C</td>
<td>3 minutes</td>
<td>1X</td>
</tr>
<tr>
<td>94°C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>58°C</td>
<td>30 seconds</td>
<td>35X</td>
</tr>
<tr>
<td>72°C</td>
<td>30 seconds</td>
<td></td>
</tr>
<tr>
<td>72°C</td>
<td>2 minutes</td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>Forever</td>
<td></td>
</tr>
</tbody>
</table>

4. 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: 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.
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.

LIMITED LICENSE: The purchase price paid for the MycoScope™ Mycoplasma PCR Detection Kit (MycoScope) grants end users a non-transferable, non-exclusive license to use the kits and/or their components for internal research use only as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis, a division of Gene Therapy Systems, Inc. (GTS) -- separate licenses are available for non-research use or applications. MycoScope and/or its components are not to be used for human diagnostic or included/used in any drug intended for human use. Care and attention should be exercised in handling the kit components by following appropriate research laboratory practices. Purchasers may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license. The laws of the State of California shall govern the interpretation and enforcement of the terms of this License.