



# **MycoGuard™ Mycoplasma PCR Detection Kit**

**For quick detection of mycoplasma contamination**

**Cat. No. MPD-T-050 (50 PCR reactions)**

## **User Manual**

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## USER MANUAL

### MycoGuard™ Mycoplasma PCR Detection Kit

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#### I. Description

The MycoGuard mycoplasma PCR detection kit is a quick, simple and sensitive PCR-based mycoplasma detection kit. The kit contains an optimized master mix, ultrapure water and a positive control. It specifically detects mycoplasma in cell cultures or cell culture derived products.

The primers in the MycoGuard master mix are highly specific to the conserved rDNA region in the mycoplasma genomes and can detect all well-known mycoplasma genera, including the commonly encountered ones in cell cultures, such as *M. arginini*, *M. arthritidis*, *M. bovis*, *M. fermentans*, *M. genitalium*, *M. hominis*, *M. hyorhinitis*, *M. neurolyticum*, *M. orale*, *M. pirum*, *M. pneumoniae*, *M. pulmonis*, *M. salivarium*, and *U. urealyticum*. Mycoplasma positive samples can be easily recognized by a distinct PCR product ranging in size from 150 to 400 bp.

#### Key advantages

- |                  |  |
|------------------|--|
| <b>Simple</b>    | <ul style="list-style-type: none"> <li>• PCR reaction is ready to go</li> <li>• No need to pre-treat your cell culture medium</li> </ul> |
| <b>Fast</b>      | <ul style="list-style-type: none"> <li>• Get the result in as short as 2 hours</li> </ul>  |
| <b>Sensitive</b> | <ul style="list-style-type: none"> <li>• Require as little as 0.03 µl of cell culture medium</li> </ul>                                  |

#### II. Contents and Storage

| Content                 | Quantity | Storage and shipping conditions   |
|-------------------------|----------|---|
| 4x MycoGuard master mix | 250 µl   | Stored at –20°C. Avoid freeze/thaw cycles. Divide it into smaller aliquots if necessary. Shipped on Ice pack. |
| Ultrapure water         | 1.0 ml   | Stored at room temperature. Shipped on Ice pack.  |
| Positive control        | 20 µl    | Stored at –20°C.<br>Shipped on Ice pack.  |

#### Additional materials/equipments required but not supplied

- Phosphate buffered saline (PBS), cell culture grade
- 1.5 ml centrifuge tubes with cap
- 0.2 ml thin wall PCR reaction tubes
- Thermo cycler
- 100 bp DNA ladder
- Agarose gel
- Horizontal electrophoresis apparatus and power supply

### III. Sample preparation

#### IMPORTANT NOTES:

Prior to PCR detection, the cells should have been grown continuously for over 2 weeks in the absence of any antibiotics. The cells should be over 80% confluent and the culture medium should be at least 2 days old at the time of sampling.

#### Option 1 Fast and simple procedure

1. Remove 2-5  $\mu\text{l}$  of culture medium, and dilute 20-fold with molecular biology grade water. Then use 2-6  $\mu\text{l}$  of diluted culture medium (equivalent to 0.1-0.3  $\mu\text{l}$  of original culture medium) for PCR.
2. Alternatively, transfer 50  $\mu\text{l}$  of culture medium to a 1.5 ml tube. Incubate the tightly capped tube for 5-10 minutes at 95°C. Dilute the boiled samples 20-fold with molecular biology grade water. Centrifuge the tube for 1 minute at 20,000 $\times$ g to pellet denatured proteins. Then use 2-6  $\mu\text{l}$  of diluted culture medium (equivalent to 0.1-0.3  $\mu\text{l}$  of original culture medium) for PCR.

#### Note:

- For suspension cells it is not necessary to remove cells from the culture medium.
- The volume of culture medium should not exceed 1.0  $\mu\text{l}$  per one 20- $\mu\text{l}$  PCR reaction. Serum proteins and other components in the cell culture medium can inhibit the PCR reaction.
- The boiled samples can be stored at 4°C for 1-2 months.

#### Option 2 Enrich mycoplasma DNA from culture medium

To help detect low abundant mycoplasma in the culture, it is necessary to enrich mycoplasma using the following procedure before PCR analysis.

1. Transfer 1 ml of culture medium to a 1.5 ml tube. Centrifuge the tubes for 5 minutes at 20,000 $\times$ g at room temperature. Carefully aspirate the supernatant.
2. Suspend the pellet with 1 ml of PBS. Centrifuge the tubes for 5 minutes at 20,000 $\times$ g at room temperature. Carefully aspirate the supernatant.
3. Wash the pellet 2 more times with PBS. Carefully aspirate the supernatant.
4. Suspend the pellet with 100  $\mu\text{l}$  of molecular biology grade water.
5. Incubate the tube for 5-10 minute at 95°C with the cap tightly closed. Centrifuge the tube for 1 minute at 20,000 $\times$ g to pellet denatured proteins.
6. Use 0.1-10  $\mu\text{l}$  of the boiled sample for PCR.

#### Note:

- It is not necessary to remove cells or cell debris from the culture medium.
- The boiled samples can be stored at 4°C for 1-2 months.

#### Option 3 Isolate mycoplasma DNA from cultured cells

The following procedure is recommended for cell lines whose growth is inhibited by mycoplasma.

1. Transfer 1-10 $\times$ 10<sup>4</sup> of cells to a 1.5 ml tube. Centrifuge the tubes for 1 minute at 10,000 $\times$  g to pellet cells. Carefully aspirate the supernatant.
2. Suspend cell pellet with 1 ml of PBS. Centrifuge the tubes for 1 minute at 10,000 $\times$  g at room temperature. Carefully aspirate the supernatant.
3. Wash the pellet one more time with PBS. Discard supernatant.
4. Suspend cell pellet with 100  $\mu\text{l}$  of molecular biology grade water.
5. Incubate the tube for 5-10 minutes at 95°C with the cap tightly closed. Centrifuge the tube for 1 minute at 20,000 $\times$ g to pellet denatured proteins.

6. Use 0.1-10 µl of the boiled sample for PCR.

**Note:**

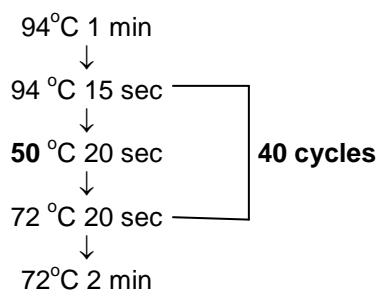
- The boiled samples can be stored at 4°C for 1-2 months.

**IV. Set up PCR reaction**

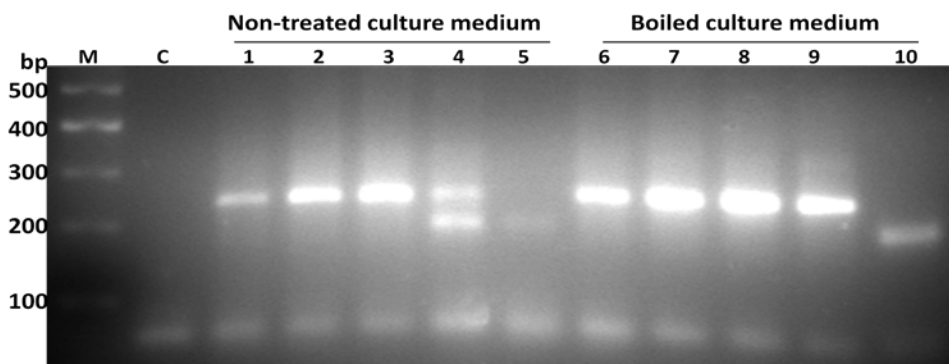
1. Thaw the MycoGuard Master Mix on ice. Tap the tube gently to mix the thawed solution. Spin the tube briefly.
2. The volume of each PCR reaction is 20 µl. Both negative and positive control reactions should be included. Set up PCR reaction tubes on ice. Use 5.0 µl 4xMaster Mix per one reaction.

| Component            | Negative control | Positive control | Test Sample |
|----------------------|------------------|------------------|-------------|
| Ultrapure water (µl) | 15.0             | 13.0             | 15-x        |
| 4xMaster Mix (µl)    | 5.0              | 5.0              | 5.0         |
| Mycoplasma (µl)      | -                | 2.0              | x           |
| Final vol (µl)       | 20               | 20               | 20          |

3. Cap the tubes after adding all of the components. Tap the tubes to mix the contents. Spin the tubes briefly to bring the contents to the bottom. Proceed immediately to the following PCR reaction:



4. Analyze the PCR products by **2% agarose gel** electrophoresis.



**Figure 1. Example of mycoplasma PCR**

M: 100 bp DNA ladder

C: Negative control

1-5: non-treated cell culture medium, 0.03, 0.1, 0.3, 1.0, and 3.0 µl, respectively

6-10: boiled cell culture medium, 0.03, 0.1, 0.3, 1.0, and 3.0 µl, respectively

**Note:** Serum proteins and other components in the cell culture medium can inhibit the PCR reaction.

## V. Limited Use License and Warranty

### Limited Use License

Following terms and conditions apply to use of all MycoGuard™ Mycoplasma PCR Detection Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

### Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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