



MycoGuard™ Mycoplasma PCR Detection Kit 2.0

For quick detection of mycoplasma contamination

Cat. No. MP004 (50 reactions)

User Manual

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USER MANUAL

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- I. Description
- II. Contents and Storage
- III. Sample Preparation
- IV. PCR Reaction
- V. Limited Use License and Warranty

I. Description

The MycoGuard™ Mycoplasma PCR detection kit 2.0 is a quick, simple and sensitive PCR-based mycoplasma detection kit. The kit contains an optimized master mix, primer mix, ultrapure water and a positive control. It specifically detects mycoplasma in cell cultures or cell culture derived products.

The primers in the MycoGuard primer 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. hyorhinis*, *M. neurolyticum*, *M. orale*, *M. pirum*, *M. pneumoniae*, *M. pulmonis*, *M. salivarium*, and *U. urealyticum*. This kit can detect more than 50 mycoplasmas including the common mycoplasma above. Mycoplasma positive samples can be easily recognized by a distinct PCR product ranging in size from 260 to 280 bp.

Key advantages

- Simple**
 - PCR reaction is ready to go
 - No need to pre-treat your cell culture medium
- Fast**
 - Get the result in as short as 2 hours
- Specific**
 - Amplification of mycoplasma DNA only

II. Contents and Storage

Content	Quantity	Storage and shipping conditions
MycoGuard™ master mix II	450 µl	Stored at –20°C. Avoid freeze/thaw cycles. Divide it into smaller aliquots if necessary. Shipped on Ice pack.
MycoGuard™ primer mix	450 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 – 20°C. Shipped on Ice pack.
Positive control	40 µl	Stored at – 20°C. 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 µl of culture medium, and dilute 10-fold with molecular biology grade water. Then use 2-6 µl of diluted culture medium (equivalent to 0.2-0.6 µl of original culture medium) for PCR.
2. Alternatively, transfer 50 µl 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 10-fold with molecular biology grade water. Centrifuge the tube for 2 minute at 12,000 rpm to pellet denatured proteins. Then use 2-6 µl of diluted culture medium (equivalent to 0.2-0.6 µl 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 µl per one 20 µl 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 12,000 rpm at room temperature. Carefully aspirate the supernatant.
2. Suspend the pellet with 1 ml of PBS. Centrifuge the tubes for 5 minutes at 12,000 rpm 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 µl 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 2 minute at 12,000 rpm to pellet denatured proteins.
6. Use 0.1-10 µl 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 $1-10 \times 10^4$ of cells to a 1.5 ml tube. Centrifuge the tubes for 1 minute at 6,000 rpm to pellet cells. Carefully aspirate the supernatant.
2. Suspend cell pellet with 1 ml of PBS. Centrifuge the tubes for 1 minute at 6,000 rpm at room temperature. Carefully aspirate the supernatant.
3. Wash the pellet one more time with PBS. Discard supernatant.
4. Suspend cell pellet with 100 µl 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 2 minute at 12,000 rpm to pellet denatured proteins.
6. Use 0.1-10 µl of the boiled sample for PCR.

Note:

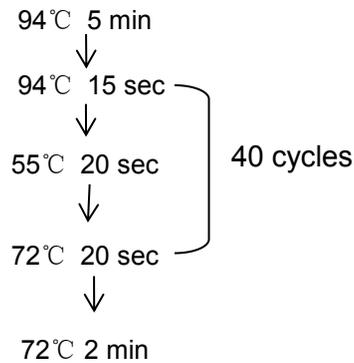
- The boiled samples can be stored at 4°C for 1-2 months.

IV. Sample preparation

1. Thaw the MycoGuard Master Mix II 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 9.0 µl MycoGuard Master Mix II per one reactions.

Component	Negative Control	Positive Control	Test Sample
MycoGuard Master Mix II	9 µl	9 µl	9 µl
MycoGuard Primer Mix	9 µl	9 µl	9 µl
Positive Control	-	2 µl	-
Mycoplasma	-	-	2 µl
Ultrapure Water	2 µl	-	-
total	20 µl		

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.

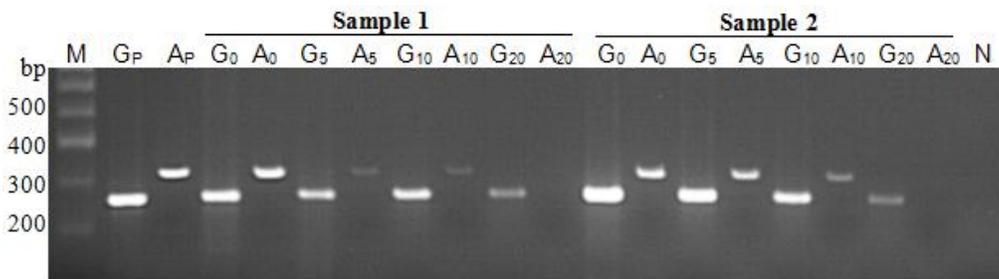


Figure1. Example of Mycoplasma PCR

G: GeneCopeia A: Competitor M: 100 bp DNA marker N: Negative Control
 Gp,Ap: Positive Control
 G0,A0: Mycoplasma sample
 G5,A5: Mycoplasma sample diluted 5 times
 G10,A10: Mycoplasma sample diluted 10 times
 G20,A20: Mycoplasma sample diluted 20 times

V. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of all MycoGuard™ Mycoplasma PCR Detection Kit 2.0 (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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