

Mycoplasma PCR Detection Kit

Rev 02/21

(Catalog # K1476-100; 100 Rxns; Store at -20°C)

I. Introduction:

BioVision's Mycoplasma PCR Detection Kit is used for quick and reliable screening of cell cultures for mycoplasma contamination. Mycoplasma infections are easily acquired, highly undesirable and notorious for an elusive onset of infection. Mycoplasmas tend to alter the infected cells at a molecular level, leading to changes in cell morphology, proliferation, metabolism, gene synthesis and processing and adhesion properties. Timely detection of mycoplasmas in cell cultures is needed to reduce wide-spread contamination and save on the costly efforts of elimination. In this kit, mycoplasma DNA in the cell culture supernatant is amplified via PCR and visualized using gel electrophoresis. **BioVision's Mycoplasma PCR Detection Kit** helps to minimize false positives while ensuring coverage of over 200 species/strains of mycoplasmas with a quick protocol for routine screening. In addition to the short detection process (less than 1 hour), the easy handling and high sensitivity makes this kit a convenient tool for routine examination of cell cultures and media. The MasterMix contains a gel loading dye, making it convenient to load for gel electrophoresis.

II. Applications:

- A convenient tool to screen for mycoplasma contamination in cell cultures

III. Key Features:

- Fast and reliable identification
- Detection in less than 1 h
- Easy handling and high sensitivity
- Minimizes false positives

IV. Sample Types:

Cell culture media

V. Shipping and Storage Conditions:

The kit is shipped in a gel pack. Upon arrival, the kit should be stored at -20°C. Avoid repeated freeze-thaw cycles of the MasterMix to retain maximum performance. The kit reagents are stable for 12 months if stored properly.

VI. Kit Contents:

Components	K1476-100 (100 Rxns)	Part Number
ExpressTaq™ 2X PCR MasterMix	1.25 ml	K1476-100-1
Mycoplasma PCR Primer Mix	100 µl	K1476-100-2
Mycoplasma Positive Control	250 µl	K1476-100-3
Nuclease-free water	1 ml	K1476-100-4

VII. User Supplied Reagents and Equipment:

- Thermal Cycler
- PCR tubes
- Nuclease-free water
- 1.5 ml microcentrifuge tubes
- Reagents and equipment for agarose gel electrophoresis
- Ethidium bromide or Safe Image™ Basic DNA Stain
- DNA ladder

VIII. PCR Detection Protocol:

1. The cells should remain in culture for at least 48-72 hours undisturbed prior to screening for the presence of mycoplasmas and should be at at least 80% confluence. Collect 2.5 µl directly from culture.
2. Mix individual components before use and assemble reaction on ice.

Component	Test Sample	Positive Control	Negative Control
ExpressTaq™ 2X PCR MasterMix	12.5 µl	12.5 µl	12.5 µl
Mycoplasma PCR Primer Mix	1 µl	1 µl	1 µl
Test sample	2.5 µl	-	-
Mycoplasma Positive Control	-	2.5 µl	-
Nuclease-free water	9 µl	9 µl	11.5 µl
Final volume per reaction	25 µl	25 µl	25 µl

3. Gently mix the reaction and briefly centrifuge. Run thermocycling conditions for standard PCR:

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4. Perform **30-40 cycles** of PCR as follows:

Step	Temperature	Duration	Cycle (s)
Enzyme activation	95 °C	3 min	1
Denaturation	95 °C	15 sec	30-40
Annealing	55 °C	15 sec	
Extension	72 °C	15 sec	
Final extension	72 °C	1 min	1
Holding	4 °C	-	-

5. After PCR, maintain the reaction at 4 °C or store at -20 °C until use.
6. Analyze the amplification products by agarose gel electrophoresis.
7. Visualize by Safe Image™ Basic DNA Stain (Cat. No. M1193) or ethidium bromide staining.
8. The presence of **PCR product of ~500 bp** in length indicates that the cell culture tested is contaminated with mycoplasma. Note that the length of the PCR product will vary between 370-550 bp depending on different mycoplasma species/strains.

IX. Recommendations for Optimal Results:

- Always keep the reaction mixture chilled on ice prior to running the PCR and start the PCR as soon as the reaction mixture is prepared.

X. Related Products:

Product Name	Cat. No.	Size
PCR-Salmonella Detection Kit	K1447	96 Rxns
PCR-Salmonella-Listeria Detection Kit	K1448	96 Rxns
PCR-Listeria monocytogenes Detection Kit	K1449	96 Rxns
PCR-Legionella spp Detection Kit	K1450	96 Rxns
PCR-Legionella spp Plus Detection Kit	K1451	96 Rxns
PCR-STEC Detection Kit	K1452	96 Rxns
PCR-Campylobacter Detection Kit	K1453	96 Rxns
Coronavirus Rapid RT-qPCR Detection Kit	K1461	100 Rxns
Mycoplasma DNA Kit	K1416	50, 250 Preps
Mycoplasma Pneumoniae IgG ELISA Kit	E4664	96 Assays
Mycoplasma Pneumoniae IgM ELISA Kit	E4665	96 Assays

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