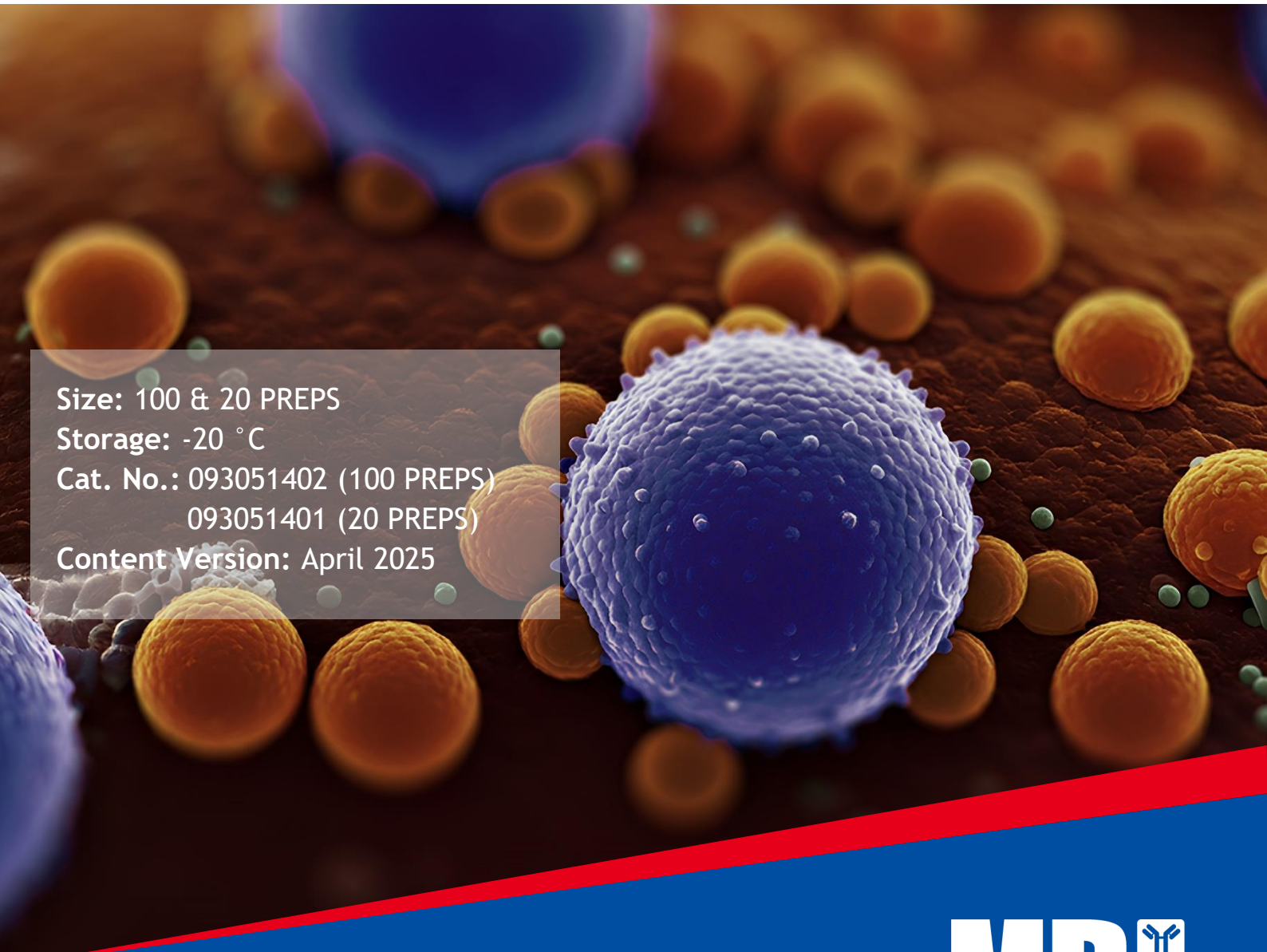
Instruction Manual
Research Use Only

Myco-Visible Mycoplasma PCR Detection Kit

For simple and standardized detection of mycoplasma contamination in cell culture



Size: 100 & 20 PREPS
Storage: -20 °C
Cat. No.: 093051402 (100 PREPS)
093051401 (20 PREPS)
Content Version: April 2025

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1. Introduction to Myco-Visible Mycoplasma PCR Detection Kit

Mycoplasmas are commonly found in research laboratories and are resistant to many antibiotics, especially those that target cell wall formation, such as penicillin. The lack of a cell wall also allows some mycoplasmas to pass through 0.2 µm filters. Several studies have examined the prevalence of mycoplasma contamination in cell cultures. Some have estimated that it could be as high as 80%, while studies in the USA suggest a lower contamination rate of 10-15%. An average figure between 25% and 50% worldwide seems quite plausible. This presents a significant problem for the industry, potentially leading to serious economic losses. Most cases of cell culture contamination are caused by a group of about six to eight species with human, porcine, or bovine hosts. The most common species are *Mycoplasma orale* and *Mycoplasma hyorhinis*.

PCR-based detection methods have become the standard protocol for mycoplasma detection, replacing direct mycoplasma culture method, according to European (EP 2.6.7), American (USP <63>) and Japanese (JP 17) pharmacopoeias. The **Myco-Visible Mycoplasma PCR Detection Kit** has been shown to be a highly sensitive, specific and simple method for the detection of mycoplasma contamination in cell cultures. The kit achieves this by targeting and amplifying unique regions of the mycoplasma genome at loci that are well conserved in all Mollicutes, including *Acholeplasma laidlawii, Mycoplasma arginini, Mycoplasma fermentans, Mycoplasma hominis, Mycoplasma hyorhinis Mycoplasma orale, Mycoplasma salivarium* and *Mycoplasma pneumoniae*. The detection procedure can be completed within 3 hours and the detection limit is as low as 10 Colony-forming Units/mL (CFU/mL) or 10 fg of Mycoplasma genomic DNA per reaction. An exogenous internal control is provided to distinguish negative reactions resulting from the absence of mycoplasma contamination from PCR inhibition. The primer sets included in the kit are used to amplify the internal control and target DNA, which can be differentiated by size. Furthermore, a positive control is included in the kit to validate template DNA performance and confirm the expected PCR product size in positive samples. Each kit provides sufficient reagents for either 100 or 20 reactions, based on a 25 µL reaction volume.

2. Kit Components and User Supplied Materials

2.1 Myco-Visible Mycoplasma PCR Detection Kit Component

	100 TESTS (Cat. No.: 093051402)		20 TESTS (Cat. No.: 093051401)	
Components	Package	Cat. No.	Package	Cat. No.
Sample Buffer	3.5 mL X 3	093051406	2.5 mL	093050903
2X PCR Master Mix	1.4 mL	093051407	270 µL	093051403
Primer Mix	275 µL	093051408	55 µL	093051404
Nuclease-free water	1.1 mL	093051409	220 µL	093051405
Positive Control	110 µL	093051410	50 µL	093050906
Quick-Start Protocol	1 ea	-	1 ea	-
Instruction Manual	Available at www.mpbio.com			
MSDS & CoA	Available at www.mpbio.com			

2.2 User Supplied Materials

- PCR tubes
- Sterile microcentrifuge tubes
- Heat block
- Microcentrifuge
- Micropipettes and sterile pipette tips
- Thermal cycler for PCR
- Agarose gel and gel electrophoresis apparatus

3. Storage and Kit Stability

All Myco-Visible Mycoplasma PCR Detection Kit components are guaranteed until the expiry date when stored appropriately. Upon receipt, store the Kit at -20 °C.

4. Important Consideration Before Use

- Thaw the reagents at 4 °C or room temperature. Keep kit vials on ice when in use.
- It is recommended to prepare aliquots of the **2X PCR Master Mix**, **Primer Mix** and **Positive Control** upon first use to prevent frequent freeze-thaw and contamination.
- Clean the work area with 70 % alcohol or 10 % household bleach before the assay setup.

- Preparation of reaction mix should be performed in a dedicated clean area that is separate from PCR amplification and gel electrophoresis.

  IMPORTANT: Do not bring open reaction tubes back into the reaction preparation area.

- Always use filter pipette tips that are sterile and nuclease-free.
- Samples, amplified PCR products, and DNA gels are biohazards that must be sterilized before discarding.

5. Safety Precautions

Kit vials contain components that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

6.1 Sample Preparation

1. Transfer 1 mL of cell culture supernatant to a 1.5 mL tube (not provided).
   Note: The cell culture should be >80% confluent. The supernatant can be stored at 4 °C for up to 3 days or long term at -20 °C before use.
2. Centrifuge at maximum speed (20,000 *g*) for 2 min. Carefully aspirate the supernatant and leave approximately 15 µL behind to ensure the pellet is not disturbed.
   Note: The pellet may be invisible. The presence of the pellet will not affect the result.
3. Add **100 µL Sample Buffer** to the tube. Mix well by pipetting up and down for **3 times**.
4. Heat the sample at **95 °C** for **5 min** using a heat block.
5. Centrifuge for **2 min @ 14,000 *g*** and transfer the supernatant to a **fresh** 1.5 mL tube (not provided).
6. This supernatant is ready for immediate use in **Section 6.2** or can be stored at -20°C for up to 2 weeks.

6.2 Reaction Mix Preparation

1. Prepare the appropriate number of PCR tubes (not provided). The number of tubes should include your **samples**, **a positive control (PC)** and a **no template control (NTC)**.
2. Determine the volume of **Nuclease-free water** (X µL) to be added to each tube (refer to the table below).
3. Add the following to each PCR tube:
   - **12.5 µL 2X PCR Master Mix**
   - **2.5 µL Primer Mix**
   - up to **5 µL** DNA sample (or **1 µL** Control)
   - **X µL Nuclease-free water**

Note: This step should be performed in a clean and separate location to prevent carry-over contamination.

Reaction Mix	Volume
2X PCR Master Mix	12.5 µL
Primer Mix	2.5 µL
Sample / Control*	up to 5 µL / 1 µL
Nuclease-free water	X (to a total of 25 µL)

* For the positive control (PC) and no template control (NTC), use 1 µL Positive Control and Nuclease-free water, respectively (both supplied by the kit).

6.3 Mycoplasma detection

1. Use the thermal cycling conditions presented in the table below to run PCR reactions in a thermal cycler.

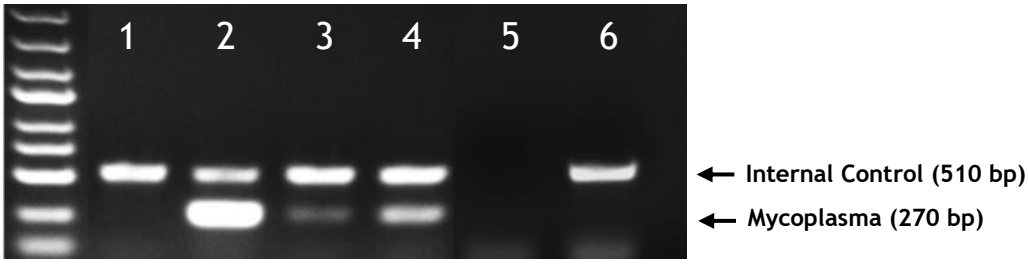
PCR Condition		Temp.	Time
Initial denaturation		95 °C	2 min
35 Cycles	Denaturation Annealing Extension	95 °C 56 °C 72 °C	30 sec 30 sec 30 sec
Final extension		72 °C	5 min
Storage		4 °C	∞

2. Prepare a **1.5%** agarose gel. Load **5-10 µL** of completed PCR products from each tube for electrophoresis analysis. Stop electrophoresis after the DNA has run approximately **2-2.5 cm** (e.g., after running for 25-30 minutes at **100 V**).
3. Results interpretation:

Mycoplasma band at 270 bp	Internal control band at 510 bp	Interpretation
Positive	Irrelevant *	Mycoplasma positive
Negative	Negative	PCR inhibition (invalid result)
Negative	Positive	Mycoplasma negative

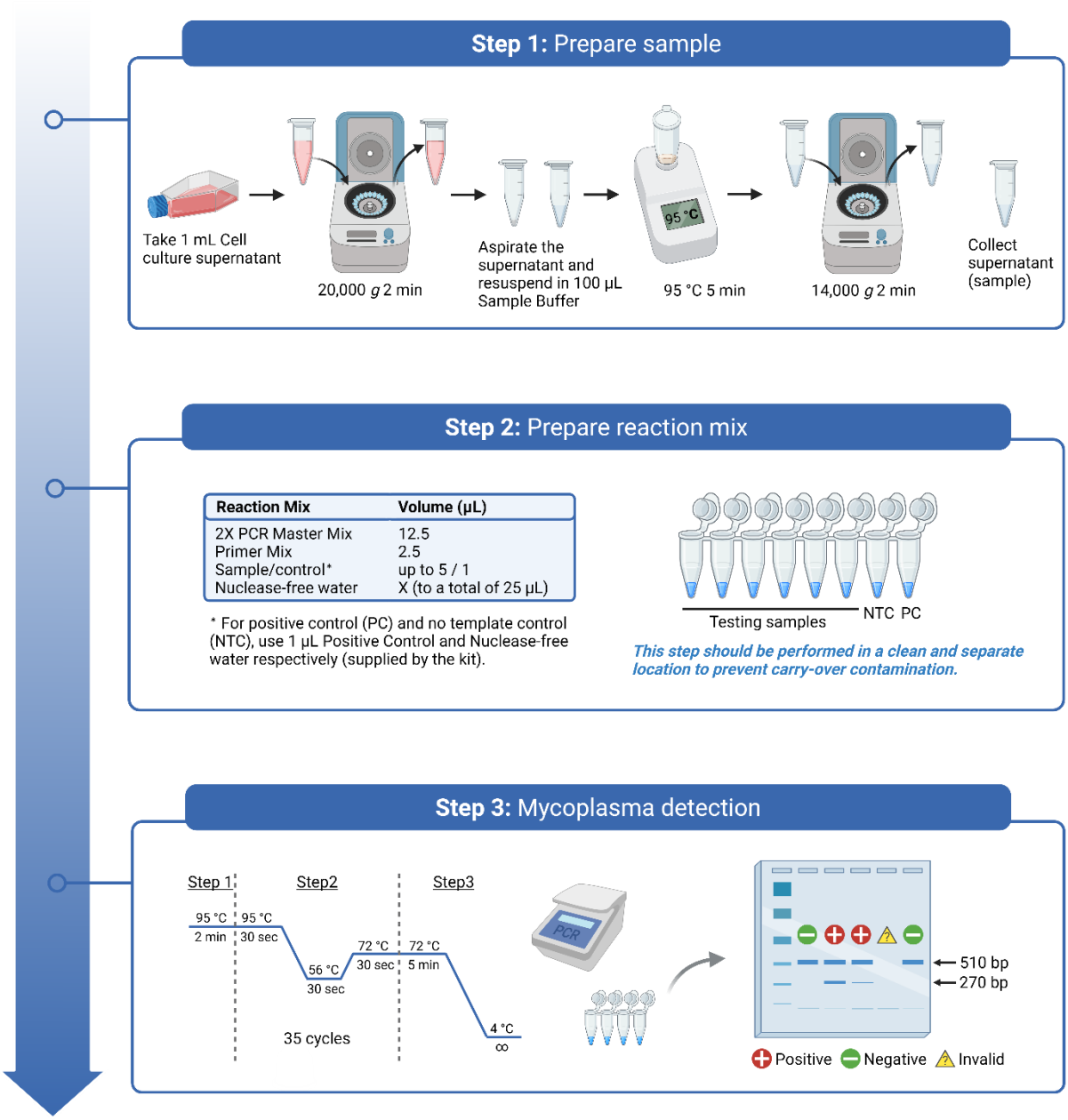
* The internal control band might be absent if the mycoplasma concentration in the sample is high.

Note: Primer dimer may yield a band of <100 bp in size. This does not affect the sensitivity and precision of the test.



Lane 1: NTC
Lane 2: PC
Lane 3: Weak Contamination
Lane 4: Strong Contamination
Lane 5: PCR Inhibition
Lane 6: Negative contamination

7. Flow Chart


Step 1: Prepare sample
Take 1 mL Cell culture supernatant
20,000 g 2 min
Aspirate the supernatant and resuspend in 100 µL Sample Buffer
95 °C
95 °C 5 min
14,000 g 2 min
Collect supernatant (sample)
Step 2: Prepare reaction mix
Reaction Mix
Volume (µL)
2X PCR Master Mix
12.5
Primer Mix
2.5
Sample/control*
up to 5 / 1
Nuclease-free water
X (to a total of 25 µL)
* For positive control (PC) and no template control (NTC), use 1 µL Positive Control and Nuclease-free water respectively (supplied by the kit).
Testing samples
NTC PC
This step should be performed in a clean and separate location to prevent carry-over contamination.
Step 3: Mycoplasma detection
Step 1
Step2
Step3
95 °C
2 min
95 °C
30 sec
56 °C
30 sec
72 °C
30 sec
72 °C
5 min
4 °C
∞
35 cycles
PCR
510 bp
270 bp
Positive
Negative
Invalid

8. Data

8.1 Analytical sensitivity

The Myco-Visible Mycoplasma PCR Detection Kit is designed for the detection of *Mycoplasma* and *Acholeplasma* species that commonly contaminate cell cultures. The kit offers a detection limit as low as 10 Colony-forming Units/mL. To evaluate the analytical sensitivity, inactivated mycoplasma preparations titrated to 100 and 10 CFU/mL from 8 *Mycoplasma spp*. in the matrix of a cell suspension of $1x10^6$ CHO cells/mL were tested in the study. The eight Mycoplasma species tested in this kit include major cell culture contaminants and human-infecting strains, as listed in the Pharmacopoeias (**EP 2.6.7 / USP <63> / JP G3**). The kit's detection limit of 10 CFU/mL complies with the sensitivity requirements outlined for nucleic acid amplification technology (NAT)-based methods in the European, United States, and Japanese Pharmacopoeias.

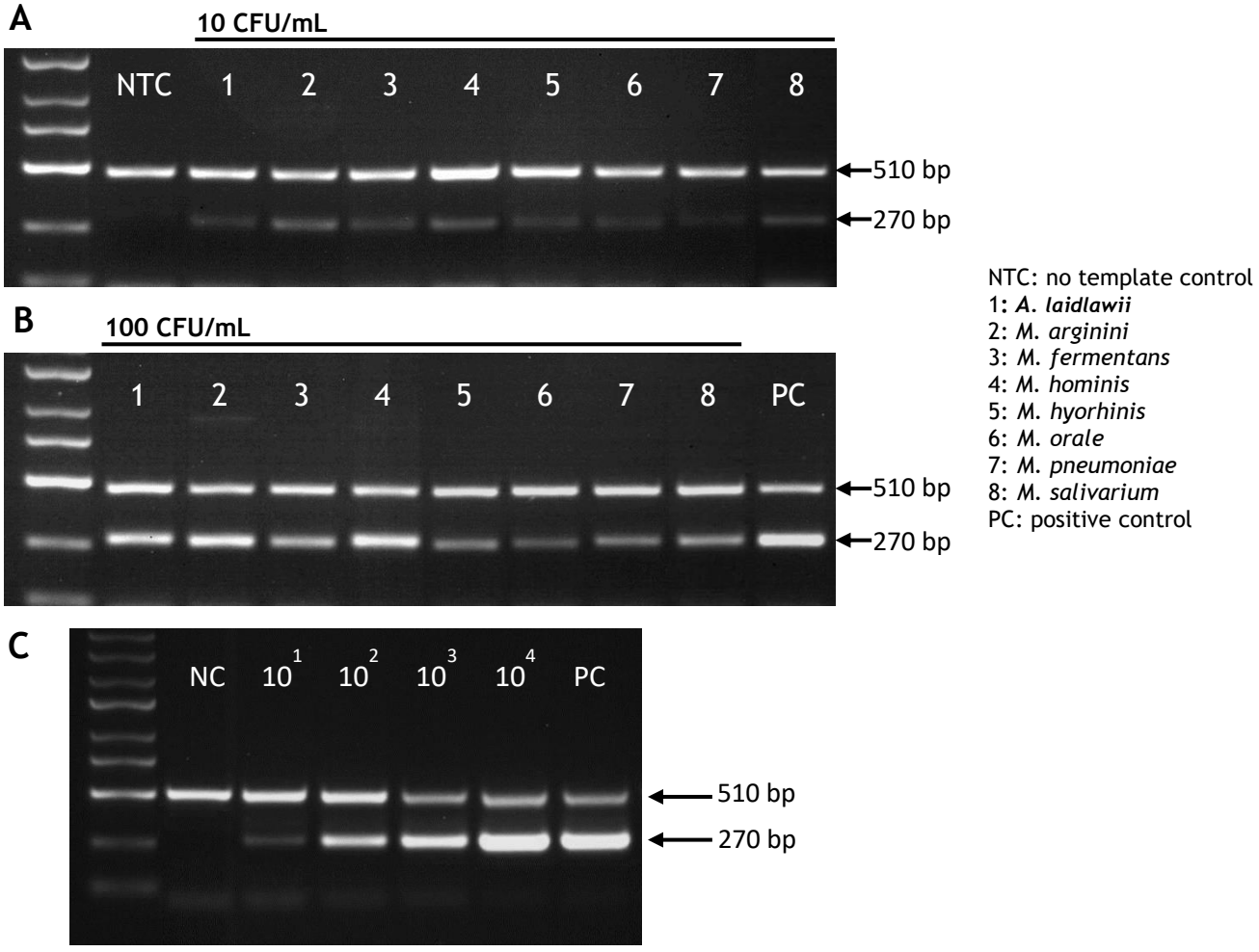


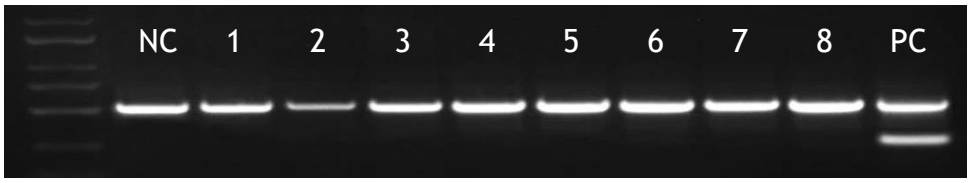
Figure 1: Analytical Sensitivity of the Myco-Visible Mycoplasma PCR Detection Kit.

(A and B), 8 strains of mycoplasmas at 100 and 10 CFU/mL were tested. All mycoplasma samples yielded positive results at both concentrations. The no template control (NTC) consisting of nuclease-free water incubated along with other samples showed no amplification. **(C)** Genomic DNA from *Mycoplasma hyorhinis* was tested at four different input levels: 10, 100, 1,000, and 10,000 copies per reaction. All samples produced

positive results, confirming the kit's ability to detect low copy numbers of mycoplasma DNA.

8.2 Specificity

The Myco-Visible Mycoplasma PCR Detection Kit specifically detects mycoplasma without cross-reactivity to other genetically related microorganisms. In this specificity test, purified gDNA at 1 x 10^5 copies/reaction from 8 off-target bacteria species were assessed. All samples returned negative results for mycoplasma detection, with only the internal control band detected. This confirms that there was no interference in the test.



NTC: No-template control
1: *Bacillus subtilis*
2: *Candida albicans*
3: *Listeria monocytogenes*
4: *Salmonella typhimurium*
5: *Staphylococcus epidermidis*
6: *Micrococcus luteus*
7: *Bacillus cereus*
8: *Staphylococcus aureus*
PC: Positive control

Figure 2: Specificity test of Myco-Visible Mycoplasma PCR Detection Kit.
Purified gDNA at 1 x 10^5 copies/reaction from 8 bacteria species that are genetically related to mycoplasma were tested. All species yielded negative results without any cross-reactivity.

9. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at **apac-techsupport@mpbio.com**

Problem	Possible Cause	Recommendation
No internal control band	Positive template concentration too high	Competition may occur when using high concentrations of positive template. The signal of target band only is still considered as a valid positive result. The internal control band may become visible if the PCR is repeated using a diluted template.
	Quality of the template	If the PCR reaction is inhibited by impurities in the sample preparation, the use of diluted sample may be helpful. If there is still no internal control and target bands, please contact our technical support staff.
	Degradation of Master Mix / Primer Mix components	Always maintain appropriate preservation conditions. Keep the reagents on ice while using.
	PCR machine setting	The issue may be due to incorrect PCR machine setting. Make sure the machine is programmed correctly.
Presence of internal control band but no target band in positive reaction	Degradation of Positive Control or positive samples	Keep Positive Control/samples at -20 °C and avoid frequent freeze-thaw.
	PCR machine setting	Check the annealing temperature and ensure the machine is programmed correctly.
Presence of target band in the negative control	Contamination of reagents	Repeat experiment with fresh sterile water as negative control. Check the contamination of kit components.
	Contamination of lab instruments and lab environments	Use filter tips to reduce contamination and to sterilize pipettes prior to use. All procedures should be performed in dedicated location free of contaminants and PCR amplicons.
Poor resolution on agarose gel	Low gel concentration	Use a 1.5% agarose gel for electrophoresis. Verify resolution by comparing the band patterns with a DNA marker.
	Short running time	Perform electrophoresis using a 1.5% agarose gel at 100 V for 25–30 minutes.

10. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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