Instruction Manual
Research Use Only

Myco-Visible

Mycoplasma LAMP Detection Kit

Sensitive and Rapid Detection of Mycoplasma Contamination in Cell Cultures

Size: 40 tests
Storage: -20 °C
Cat. No.: 093050601
Content Version: May 2025

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1. Introduction to Myco-Visible Mycoplasma LAMP Detection Kit

Mycoplasmas are commonly found in research laboratories and unaffected by many of the antibiotics that have been used to control bacterial contamination in cell cultures, especially those that affect cell wall formation, such as penicillin. The lack of a cell wall also means that some cells may be able to pass through 0.2 μm filters. There have been several studies to determine 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 is a very significant problem for the industry and could lead 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, of which *Mycoplasma orale* and *Mycoplasma hyorrhinis* are the most common.

Myco-Visible Mycoplasma LAMP Detection Kit is highly sensitive, specific, and rapid assay for the detection of *mycoplasma* contaminations in cell cultures. Myco-Visible Mycoplasma LAMP Detection Kit achieves this by targeting and amplifying the 16S rRNA coding region 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 Pneumoniae and Mycoplasma Salivarium*. The detection procedure can be completed within 45 mins and requires as little as 10 16S rRNA copies or 10 fg of *Mycoplasma* genomic DNA per reaction. No cross-reactivity with other bacterial, fungal and mammalian DNA by Primer-BLAST. The test assay is easy to perform with no thermal cycler required. Positive results can be visualized by color changes from pink to yellow.

2. Kit Components and User Supplied Materials

2.1 Myco-Visible Mycoplasma LAMP Detection Kit Component

Components	Package	Cat. No.
Sample Buffer	2 vial (2.5 mL)	093050903
LAMP Beads	5 x 8 ea	093050606
Primer Mix	1 vial (220 µL)	093050603
Nuclease-free water	1 vial (510 µL)	093050604
Positive Control	1 vial (50 µL)	093050605
Quick-start Protocol	1 ea	
Instruction Manual	Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- Microcentrifuge capable of operating at ≥14,000 x g
- Heat block or at 65 °C and 95 °C
- Thermocycler (optional)
- Sterile and nuclease-free 1.5 mL microcentrifuge tubes
- Pipettes with corresponding filter tips

3. Storage and Kit Stability

All components of the Myco-Visible Mycoplasma LAMP Detection Kit are guaranteed until the expiration date indicated on the packaging, provided they are stored as recommended. Upon receipt, store the kit at –20 °C.

4. Important Consideration Before Use

- ❑ Thaw the kit at 4 °C or room temperature. Do not leave it at room temperature for more than 1 hour.
- ❑ Use clean, disposable gloves when performing the assay and make sure that the work area is cleaned with 70 % alcohol or 10 % household bleach before the assay setup.
- ❑ Performing the experiment using sterile, DNases, and RNases-free filter pipette tips is highly advisable.
- ❑ It is recommended to prepare aliquots for Primer Mix (e.g. 5 x 41 µL) and Positive Control (e.g. 5 x 10 µL) upon first use to prevent frequent freeze-thaw and contamination.
- ❑ Incubation of LAMP reaction mix (at 65 °C) should be performed in a separate location from sample preparation and LAMP reaction setup.
- ❑ Do not open the tubes containing amplified LAMP product or return the tubes back to the sample preparation and LAMP reaction setup areas. This is to prevent carryover contamination from aerosolized amplified material. Samples and amplified products are considered biohazards and must be sterilized properly before disposal.

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.

   Note: Cell suspension can also be used, but total cell number should not exceed 10^7 cells/mL.
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.

   Note: If 20,000 g is not available, centrifuge at ≥14,000 g for 5 min.
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 LAMP Protocol

1. Take an appropriate number of **LAMP Beads** provided in individual tubes. The number of LAMP Beads includes your **samples**, a **positive control (PC)** and a **no template control (NTC)**.
2. Determine the volume of **Nuclease-free water** (**X µL**) to add to each LAMP Beads tube (see table below). Add X µL of Nuclease-free water and gently pipette up and down to dissolve the LAMP Beads. Add **5 µL** of **Primer Mix** and up to **5 µL** of sample (or **1 µL** control*) to each tube.

   Note: This step should be performed in a dedicated clean area that is separate from the LAMP incubation area to prevent carry-over contamination.

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

* For PC and NTC, use 1 µL of Positive Control and Nuclease-free water respectively (supplied in the kit).

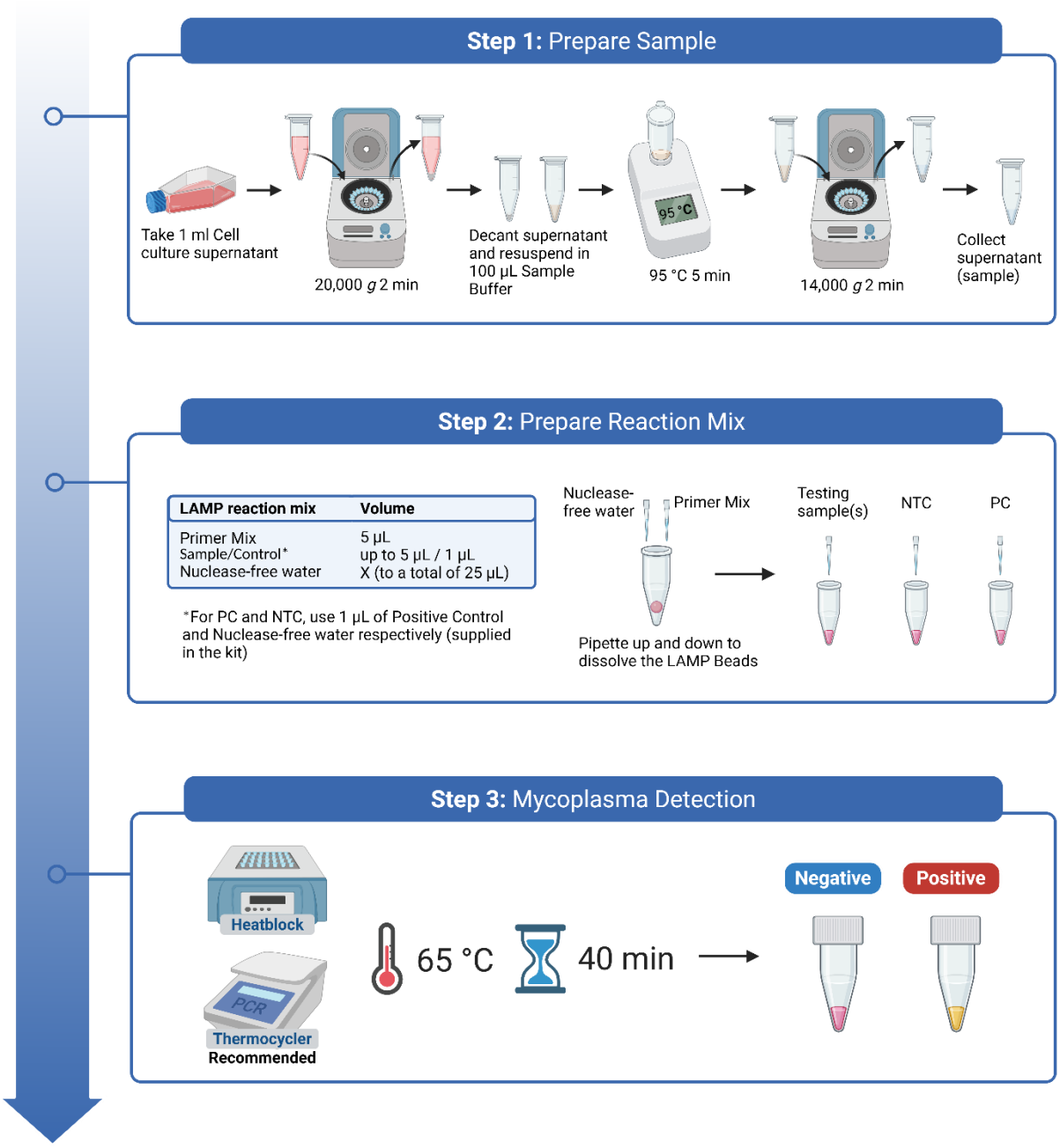
3. Incubate the reaction tube at **65°C** for **40 min** using a thermocycler (recommended if available) or heat block. The color of the reaction mix is pink at the beginning of LAMP reaction.

   Note: Do not incubate for more than 45 min.

4. Let the reaction tube equilibrate to room temperature or 4-8 ºC (if using a thermocycler) and briefly spin down the mix.

5. Result interpretation: the color of **positive** reactions change from pink to **yellow**. The color of **negative** reactions remain **pink**.

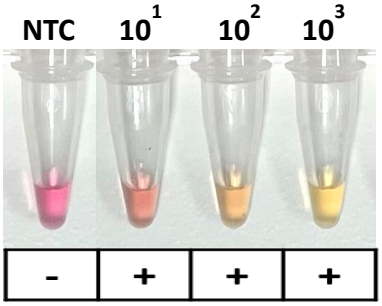
7. Flow Chart


Step 1: Prepare Sample
Take 1 ml Cell culture supernatant
20,000 g 2 min
Decant 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
LAMP reaction mix
Volume
Primer Mix
Sample/Control*
Nuclease-free water
5 µL
up to 5 µL / 1 µL
X (to a total of 25 µL)
*For PC and NTC, use 1 µL of Positive Control and Nuclease-free water respectively (supplied in the kit)
Nuclease-free water
Primer Mix
Pipette up and down to dissolve the LAMP Beads
Testing sample(s)
NTC
PC
Step 3: Mycoplasma Detection
Heatblock
PCR
Thermocycler
Recommended
65 °C
40 min
Negative
Positive

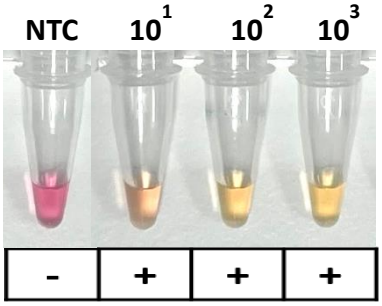
8. Data

Myco-Visible Mycoplasma LAMP detection kit demonstrates high sensitivity and specificity on detection of *Mycoplasma* spp. contamination in cell cultures. Analytical sensitivity tests were carried out using the known-copy number genomic DNA from 8 *Mycoplasma* spp. commonly found in the laboratory cell cultures.

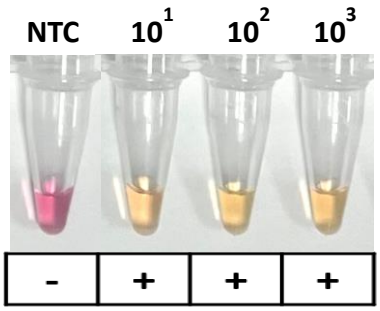
A. laidlawii



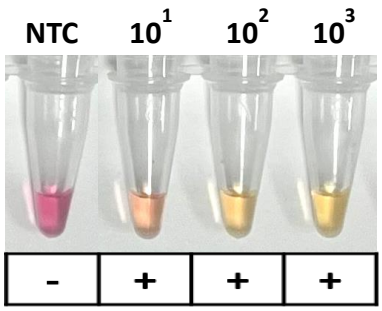
M. hyorhinis



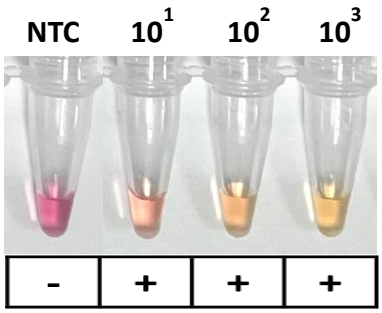
M. arginini



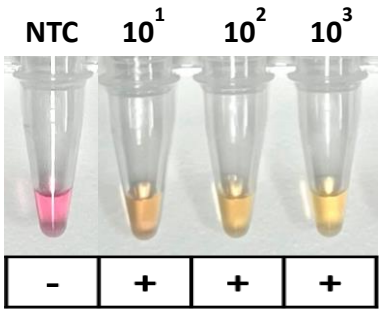
M. orale



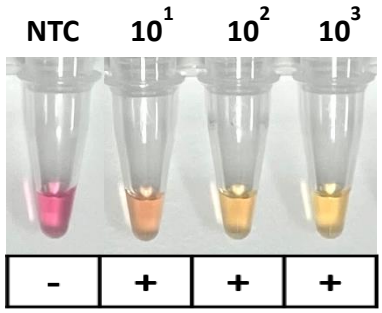
M. fermentans



M. pneumoniae



M. hominis



M. salivarium

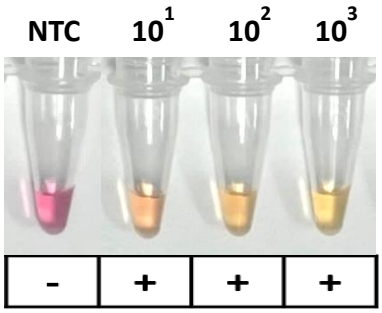
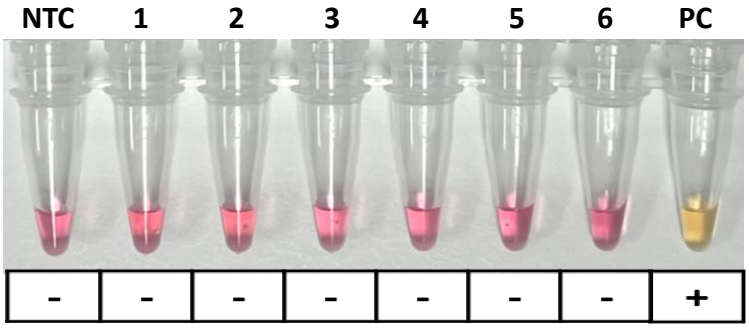


Figure 1: A 10-fold serial dilution ranging from 10^3 to 10 copies/µL was performed using six strains of Mycoplasma spp. The results demonstrated that the kit can detect as few as 10 copies of 16S rRNA or 10 fg of Mycoplasma genomic DNA.

Myco-Visible Mycoplasma LAMP detection Kit specifically detects mycoplasma without cross-reactivity to other bacterial, fungal, or mammalian DNA. In the specificity test, purified gDNA from various off-target species, at 1 x 10^5 copies per reaction, was assessed. All species tested negative for mycoplasma detection, and no interference was observed in the assay.



1: *Bacillus subtilis*
2: *Candida albicans*
3: *Escherichia coli*
4: *Staphylococcus epidermidis*
5: *Micrococcus luteus*
6: *Staphylococcus Aureus*

Figure 2: Specificity test of Myco-Visible Mycoplasma LAMP detection Kit. Purified gDNA at 1 x 10^5 copies/reaction from 6 bacteria species that are genetically related to mycoplasma were tested. All samples showed a negative result, indicating no cross-reactivity.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Color change in No template Control	Reaction tube temperature high	• Check the color again after reaction tubes equilibrated to room temperature or below.
	Incubation time too long	• Do not incubate the reaction tubes for more than 45 min as this significantly impacts the reaction
	Carry-over contamination of the reagent	• Discard current aliquot of reagent and use new one. • Reaction set up should be performed in dedicated environments free of contamination and separate from the incubation site. • Use filter tips will reduce the contamination.
No color change in Positive Control	Degradation of reagent	• Avoid frequent freeze-thaw cycles of the Primer Mix and Positive Control.
Normal color change in NTC and PC but ambiguous color change in some samples	Weak *Mycoplasma* infection of the sample	• Increase the volume of template (up to 5 µL) • Grow the cell culture for additional 48 hours and repeat the test • Use first-time LAMP product as template to repeat the test.

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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