Instruction Manual
Research Use Only

Myco-Visible

Mycoplasma LAMP Detection Kit

Sensitive and Rapid Detection of Mycoplasma contamination in cell cultures

Size: 40 preps
Storage: -20 °C
Cat. No.: 093050601
Content Version: August 2022

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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*, and *Mycoplasma orale*. 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.
Master Mix	1 vial (510 µL)	093050602
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 to work at speed of ≥14,000 x g
- Heat block or dry bath at 65 °C and 95 °C
- Nuclease-free 0.5 mL and 1.5 mL sterile microcentrifuge tubes
- Sterile PBS
- Pipettes with corresponding filter tips

3. Storage and Kit Stability

All Myco-Visible Mycoplasma LAMP detection kit components are guaranteed for at least 12 months from the date of manufacture when stored appropriately.

Upon receipt, store 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.
- ❑ Incubation of LAMP reaction mix (at 65 °C) should be performed in a separate location from sample preparation and LAMP reaction setup.
- ❑ Perform the experiment using sterile, DNases, and RNases-free filter pipette tips is highly advisable.
- ❑ Do not open the tubes containing amplified LAMP product to prevent carryover contamination (air contamination of previous PCR product). Samples and amplified products are biohazards that must be sterilized before discarding.

5. Protocol

5.1 Sample Preparation

1. Confluency of test cell culture should be >80 %. Detach the cells using scraper (avoid trypsin and EDTA).
2. Count cell numbers using standard counting methods. Transfer 1 mL of cell culture samples with a minimum of $5x10^4$ cells to a 1.5 mL microcentrifuge tube.
3. Centrifuge at 14,000 x g for 2 mins and carefully decant the supernatant.
4. Resuspend cell pellets in 100 µL of sterile PBS.
5. Incubate the sample at 95 °C for 5 mins.
6. Centrifuge at 14,000 x g for 2 mins and transfer the supernatant to a new 0.5 mL tube. This supernatant will be used as template for LAMP reaction in Section 5.2.

5.2 LAMP Protocol

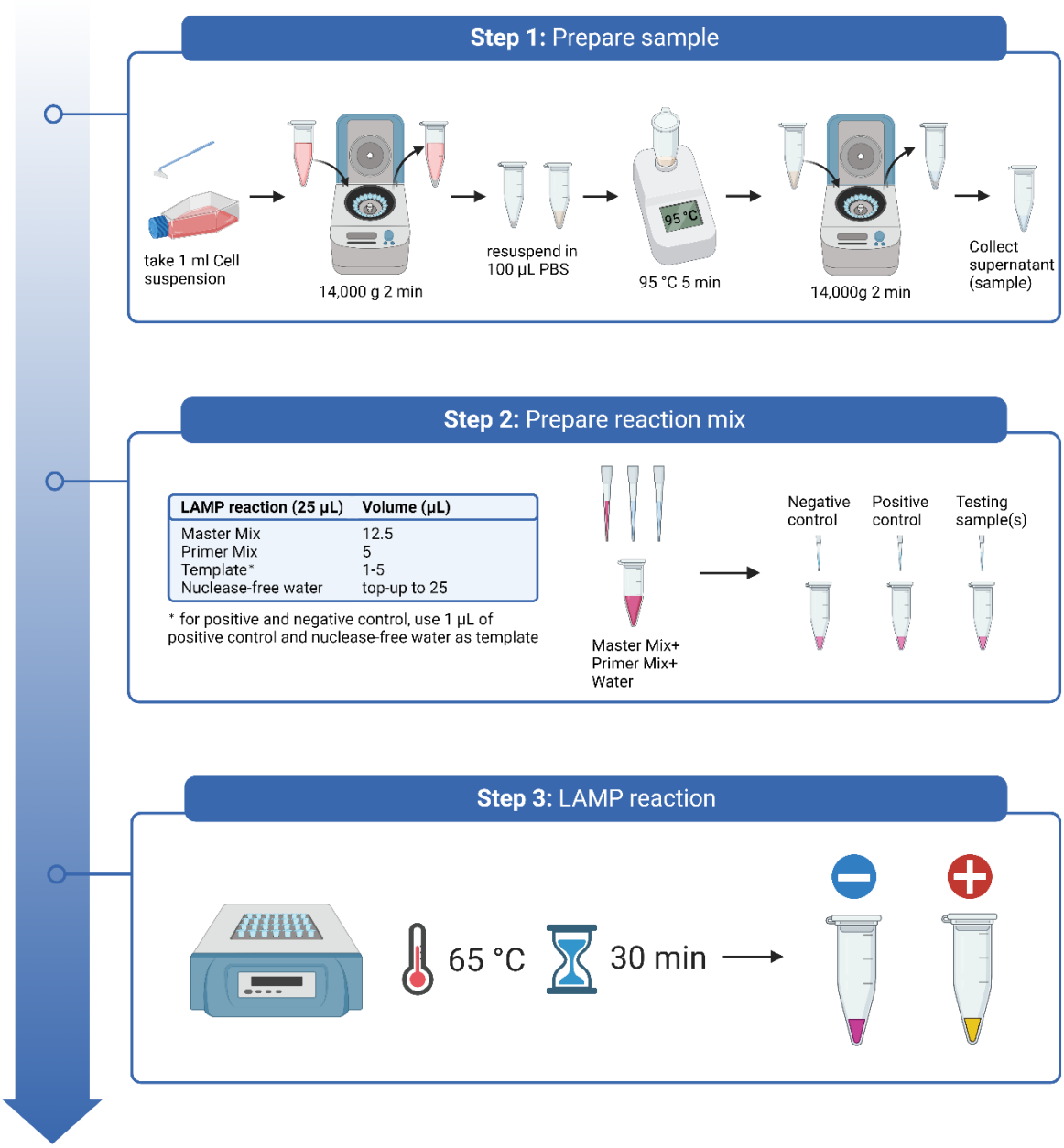
1. Setup LAMP-PCR reactions (including 1X positive control (PC) and negative control (NC)) with 0.5 mL tubes and incubate at 65°C for 30 mins (incubation should be performed in a separate location from assay setup)

LAMP reaction (25 µL)	Volume (µL)
Master Mix	12.5
Primer Mix	5.0
Template*	1.0 – 5.0
Nuclease-free water	Top up to 25

* For PC and NC, use 1 uL of positive control and nuclease-free water respectively (supplied in the kit) as template.

2. Result color interpretation: Negative results are indicated in pink and positive results are indicated by a change to yellow.

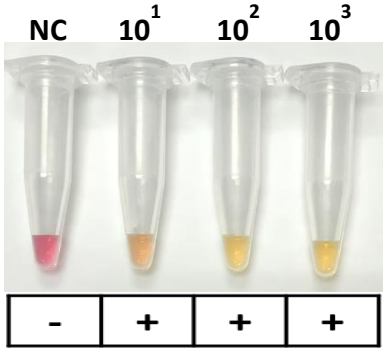
6. Flow Chart


Step 1: Prepare sample
take 1 ml Cell suspension
14,000 g 2 min
resuspend in 100 µL PBS
95 °C
95 °C 5 min
14,000g 2 min
Collect supernatant (sample)
Step 2: Prepare reaction mix
LAMP reaction (25 µL) Volume (µL)
Master Mix 12.5
Primer Mix 5
Template* 1-5
Nuclease-free water top-up to 25
* for positive and negative control, use 1 µL of positive control and nuclease-free water as template
Master Mix+ Primer Mix+ Water
Negative control
Positive control
Testing sample(s)
Step 3: LAMP reaction
65 °C
30 min

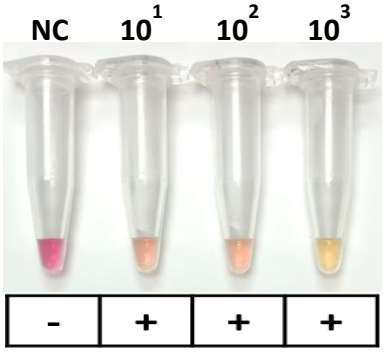
7. Data

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 6 *Mycoplasma* spp. commonly found in the laboratory cell cultures.

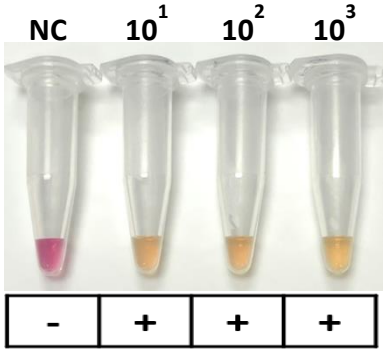
A. laidlawii



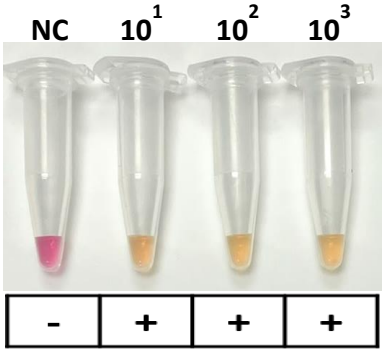
M. hyorhinis



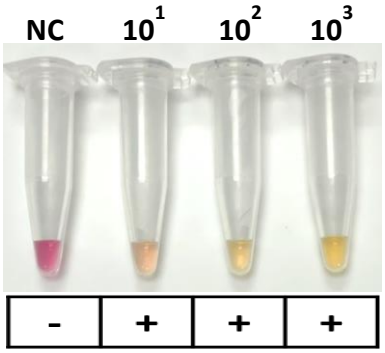
M. arginini



M. orale



M. fermentans



M. pneumoniae

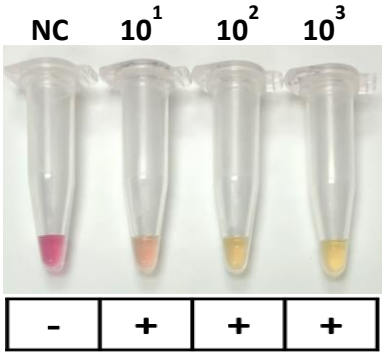


Figure 1: Analytical sensitivity test of Myco-Visible Mycoplasma LAMP detect Kit. 10-fold serial dilution from 10 to 10^3 copies/µl have been performed using six strains of *mycoplasma spp*. Results shown that the kit is ablet to detect as low as 10 16S rRNA copies or 10 fg of *Mycoplasma* genomic DNA.

8. Troubleshooting

Problem	Possible Cause	Recommendation
NC color changes intermittently	Long reaction time	Check the color again when tubes equilibrated to room temperature.
	Trace contamination of the reagent	• Do not exceed incubation time (65 °C @ 30 mins) as this significantly impacts the reaction • Discard current reagent and use new one. All procedures should be performed in a dedicated environment that is free of contamination. Use filter tips will reduce the contamination.
PC color does not change to yellow	Degradation of reagent	Avoid frequent freeze-thaw cycles of the LAMP reagent
Faint positive result for 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.

9. 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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Australia
Tel: +61 2.8824.2100
Tel: +61 1800.249.998
Email: custserv.au@mpbio.com

Austria & Germany
Tel: 0800.426.67.337
Tel: 00800.7777.9999
Email: custserv.de@mpbio.com

Belgium
Tel: 00800.7777.9999
Email: custserv.be@mpbio.com

Canada
Tel: +1 800.854.0530
Email: custserv.ca@mpbio.com

China
Tel: +86 400.150.0680
Email: custserv.cn@mpbio.com

Europe
Tel: +33 3.88.67.54.25
Tel: +33 00800.7777.9999
Email: custserv.eur@mpbio.com

France
Tel: +33 3.88.67.54.25
Email: custserv.fr@mpbio.com

India
Tel: +91 22.27636921/22/25
Email: custserv.in@mpbio.com

Italy
Tel: 00800.7777.9999
Email: custserv.it@mpbio.com

Japan
Tel: +81 3.6667.0730
Email: custserv.jp@mpbio.com

Latin America
Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.la@mpbio.com

New Zealand
Tel: +64 9.912.2460
Email: custserv.nz@mpbio.com

North America
Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.na@mpbio.com

Poland
Tel: 00800.7777.9999
Email: custserv.po@mpbio.com

Russia
Tel: +7 495 604.13.44
Email: custserv.rs@mpbio.com

Serbia
Tel: +381 11.242.1972
Email: custserv.se@mpbio.com

Singapore/ APAC
Tel: +65 6775.0008
Tel: +65 6394.7675
Email: custserv.ap@mpbio.com

South Korea
Tel: +82 2.425.5991
Email: custserv.kr@mpbio.com

Switzerland
Tel: 00800.7777.9999
Email: custserv.ch@mpbio.com

The Netherlands
Tel: 00800.7777.9999
Email: custserv.nl@mpbio.com

United Kingdom
Tel: 0800.282.474
Email: custserv.uk@mpbio.com

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