Myco-Visible Mycoplasma LAMP Detection Kit

Cat. No.: 093050601 (40 Tests)

Quick-Start Manual

Revision 1.0 August 2022

Notes before starting

- ❑ Detection of mycoplasma contamination in cell culture by Myco-Visible is based on Loop-mediated isothermal amplification (LAMP) targeting 16S rRNA gene that is conserved in all mycoplasma species. The assay is rapid and specific that can give result within 40 min after sample preparation. Positive results are clearly visualized by the reaction color turning from pink to yellow.

Prepare sample

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 0.5mL tube. This supernatant will be used as template for LAMP reaction.

Prepare reaction mix

7. Setup LAMP-PCR reactions (including 1X positive control (PC) and negative control (NC)) with 0.5 mL tubes

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. For test sample, use up to 5 µL template.

LAMP reaction

8. Incubate at 65°C for 30 mins (incubation should be performed in a separate location from assay setup)
9. Result color interpretation: Negative results are indicated in pink and positive results are indicated by a change to yellow.

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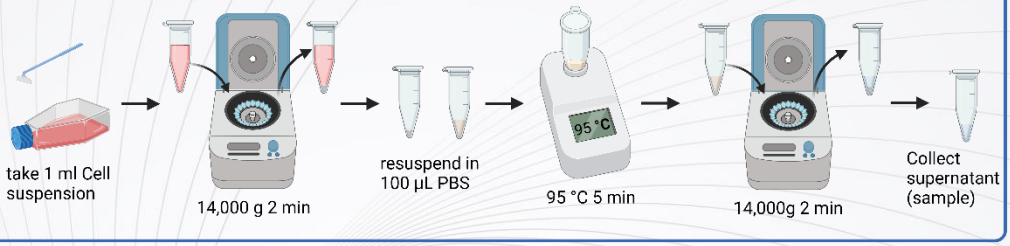
Flow Chart

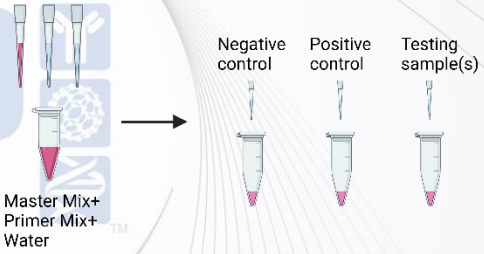
Step 1: Prepare sample



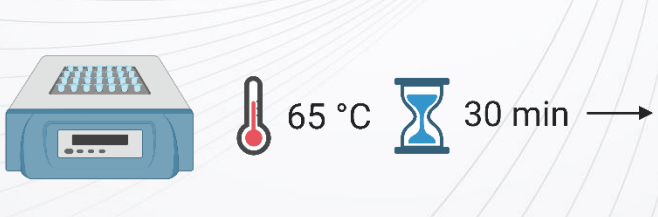
Step 2: Prepare reaction mix

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



Step 3: LAMP reaction



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