

MycoStrip® 2.0

Mycoplasma detection kit

Catalog code: rep-mysv2-10, rep-mysv2-20, rep-mysv2-50

www.invivogen.com/mycostrip

For research use only, not for clinical use

Version 26E28-AK

Contents

	10 Tests	20 Tests	50 Tests
• Reaction Mix	1 vial	2 vials	5 vials
• Reaction Buffer	1 vial	2 vials	4 vials
• Migration Buffer	2 vials	3 vials	1 bottle
• Positive Control	1 vial	1 vial	1 vial
• Detection strips	10	20	50

Storage and stability

- Kit is shipped at room temperature.
- Store all components (incl. the detection strips) at -20°C.
- Stable for 18 months when stored properly.

Other materials and consumables required

- Thermocycler, heat block, water/dry bath
- Pipettes with corresponding filter tips
- 0.2 ml and 1.5 ml microcentrifuge tubes
- Bench centrifuge
- Sterile phosphate buffered saline (PBS)

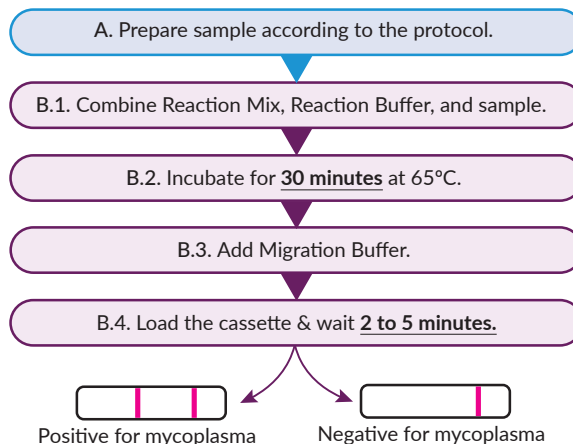
PRODUCT DESCRIPTION

MycoStrip® 2.0 is an enhanced version of our innovative mycoplasma detection assay, featuring a next-generation enzyme that boosts overall assay performance:

- **Higher sensitivity:** now detects as little as 1 genome copy (GC)/ μ l under optimal conditions
- **Increased robustness:** improved tolerance to temperature variations
- **Broader detection:** detects more mollicute genera
- **Faster turnaround:** results in 30 minutes

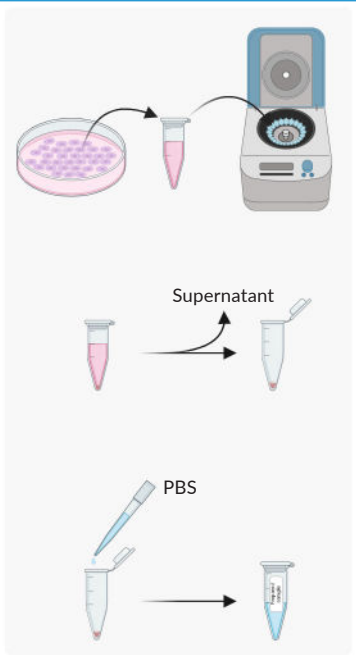
This loop-mediated isothermal amplification (LAMP) assay amplifies a region of the 16S rRNA gene, identifying both live and dead mycoplasma. Results are visible on an immunochromatographic strip in 2–5 minutes.

MycoStrip® 2.0 Protocol Outline



METHODS: Preparation of samples

- 1 Collect **1 ml** of cell culture supernatant
- 2 Centrifuge at **16,000 x g** for **5 minutes**
- 3 Using a pipette, discard the supernatant (leave $\leq 50 \mu$ l supernatant)
- 4 Add **500 μ l** of sterile PBS & vortex



MycoStrip® 2.0 can be used to test supernatant from adherent or suspension cell culture prior to passaging, as well as fetal bovine serum.

A. Test sample preparation

1. Transfer **1 ml** of the **cell culture supernatant** to a microcentrifuge tube.
Note: The supernatant can be stored short term at 4°C (one week) or long term at -20°C. Before use, mix well by vortexing.
Optional: To avoid spreading contamination, we recommend to heat the supernatant at 95°C for 5 minutes to kill the mycoplasma.
2. Centrifuge at **16,000 x g (RCF)** for **5 minutes** to pellet the mycoplasma.
Note: The pellet will be invisible except if some cells have been collected. Importantly, their presence will not affect the detection assay.
3. Using a pipette, carefully discard the supernatant without disturbing the pellet. Ensure there is no more than 50 μ l of medium remaining, as it will impact the detection assay.
4. Add **500 μ l** of **sterile PBS** and mix well by pipetting.
5. Use **prepared sample** immediately or store at -20°C until required.

General recommendations

While preparing test samples, you may be handling live mycoplasma. Thus, handle your samples with due attention and care. To avoid cross-contamination, use filter tips throughout the protocol and change tips between tubes at each step.

Note: MycoStrip® is a registered trademark of InvivoGen.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873 / info@invivogen.com
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39 / info.eu@invivogen.com
InvivoGen Asia: +852 3622-3480 / info.hk@invivogen.com

METHODS: MycoStrip® 2.0 Detection assay



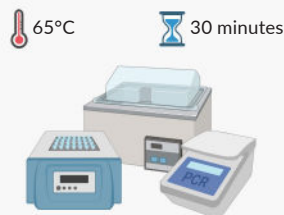
Before starting the assay, prepare aliquots of the **Reaction Mix** and **Positive Control**. Use immediately or store at -20°C



- 1 To 15 µl of Reaction Mix:
 - add 5 µl of Reaction Buffer*
 - add 5 µl of prepared sample* (or control)
 (*mix well before use)



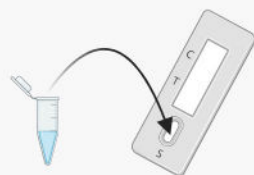
- 2 Spin down briefly and immediately incubate at 65°C for 30 minutes



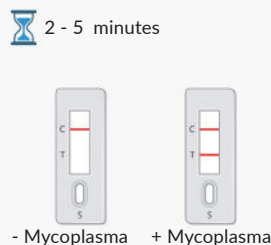
- 3 Add 200 µl of Migration Buffer and mix well



- 4 Load 100 µl to the cassette



- 5 Wait 2 - 5 minutes for the result
 - One band (C): negative
 - Two bands (C & T): positive



Before starting the assay

- Thaw all samples and reagents and use immediately or keep on ice.
- Avoid freeze-thaw cycles of the **Reaction Mix** and **Positive Control** by preparing aliquots **upon first use**:
 - Spin down and gently homogenize 1 vial of the Reaction Mix and the vial of Positive Control.
 - Prepare 15 µl aliquots of the Reaction Mix (10 - 12 vials).
 - Prepare 10 - 15 µl aliquots of the Positive Control.
 - Use aliquots immediately or store at -20°C until required.
- Set the thermocycler, heat block, or water/dry bath to 65°C.
- **IMPORTANT:** before each use, mix well the Reaction Buffer by several vortex pulses to avoid salt precipitation.

B. Detection assay

- In a 15 µl **Reaction Mix** aliquot add,
 - 5 µl of the **Reaction Buffer** (mix well before pipetting),
 - 5 µl of the **prepared sample** (or **control**) (mix well before pipetting).
 Then, mix gently and spin down briefly.

Optional: Include the provided positive control (contains DNA only, not a contamination source) and a negative control (not provided; use sterile PBS). Prepare controls after your test sample to minimize cross-contamination.

- Incubate the reaction tube(s) immediately at 65°C for 30 minutes using a thermocycler, heat block, or water/dry bath.

Note: Preparation of the reaction tube(s) should not exceed 15 minutes or work on ice.

- Briefly spin sample down and immediately add 200 µl of the **Migration Buffer** to stop the reaction.

- Mix well by pipetting and load 100 µl of the test sample into the cassette well ('S'). Wait 2 to 5 minutes to see results.

C. Data interpretation

There are two distinct results using MycoStrip® 2.0:

- **One band ('C' band only)**
Indicates that mycoplasma was not detected in the processed sample.
- **Two bands ('C' and 'T' bands)**
Indicates that the test sample is contaminated by mycoplasma.

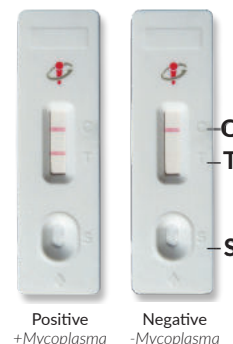
Note: The migration control band ('C') will appear within 1-2 minutes. If after 5 minutes there is no test band ('T'), the test is considered negative.

- **To confirm a 'faint' positive result:**

For increased sensitivity, incubate sample for 50 minutes at 65°C. A faint 'T' band after 50 minutes is considered negative for mycoplasma. Alternatively, you may grow the cell culture for 48h until it reaches 100% confluency and re-test a newly prepared sample.

- **If your sample is positive:** eradicate your contamination by treating your culture for 2 weeks using InvivoGen's **Plasmocin®** or **Plasmocure™**.

For more information visit: www.invivogen.com/cell-culture-contamination.



Need more information?

Please visit the FAQ section on our product page for answers to common questions and troubleshooting: www.invivogen.com/mycostrip

Illustrations were created with Biorender.com

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873 / info@invivogen.com
 InvivoGen USA (International): +1 (858) 457-5873
 InvivoGen Europe: +33 (0) 5-62-71-69-39 / info.eu@invivogen.com
 InvivoGen Asia: +852 3622-3480 / info.hk@invivogen.com