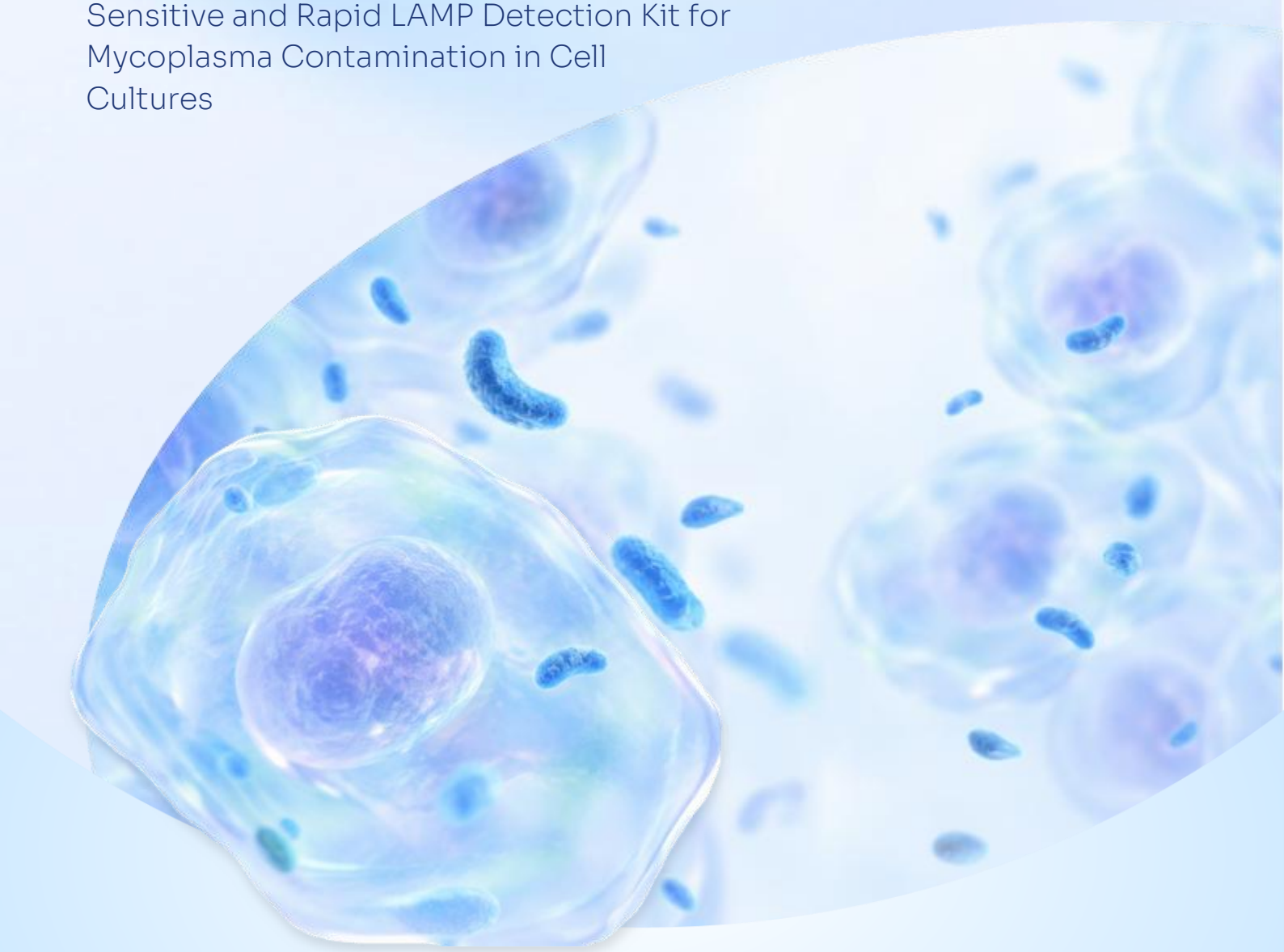
Detechgene

INSTRUCTION MANUAL

Research Use Only

DetechBeads Pro myco

Sensitive and Rapid LAMP Detection Kit for Mycoplasma Contamination in Cell Cultures



Sizes:
8 Tests
56 Tests

Storage:
-20 °C

REF No.:
DB-MYCP-10016
DB-MYCP-10023

Content Version:
Nov 2026

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1 INTRODUCTION

1.1 Why test cell cultures for Mycoplasma

Mycoplasmas are among the smallest of bacteria, spanning around 0.3 µm in diameter. However, its distinguishing feature is that it lacks a rigid cell or peptidoglycan, making them variable in size or shape. Moreover, the lack of a cell wall also makes it naturally resistant to cell-wall-active antibiotics. Due to these features of the Mycoplasma spp., it is quite a common contaminant in cell culture laboratories around the world.

Laboratories that actively work with cells and cell culture, often utilize expensive cell lines, cell culture media and other components to further their research. Mycoplasma, due to its tiny and flexible nature, bypasses filters found in cell culture hoods meant to prevent contamination. Moreover, mycoplasma spreads through cross-contamination of reagents and other cultures. Together with the rich nutrients found in the media and antibiotic-resistant nature, mycoplasma thrives in contaminating cell culture work.

Cells contaminated with mycoplasma alter the cells' metabolism and proliferation, thereby depleting the nutrients present in the media. However, in-spite of these major changes, there are neither visual indicators such as turbid cell culture media, nor can a mycoplasma be visualized under microscope. Detection of mycoplasma contamination is done commonly via PCR, fluorescence staining and direct culture on an agar plate. All of which are time-consuming and expensive to run.

1.2 Intended Purpose

The **DetechBeads Pro MYCO** is a colorimetric LAMP based detection kit which is designed for the qualitative detection of mycoplasma contamination in cell culture supernatants.

The kit can detect the **4 major mollicutes** that occur in mycoplasma cell culture contaminations with a limit of detection (LoD) at 25 copies per reaction.*

i) *Mycoplasma orale*

ii) *Mycoplasma hyorhinis*

iii) *Mycoplasma arginini*

iv) *Acholeplasma laidlawii*

*This kit is only intended for trained laboratory personnel. This kit is for Research Use Only (RUO) and is not intended for diagnostics procedures. Refer to section 7 for limitations. LoD is representative only for the aforementioned organisms.

1.3 Explanation of the Kit

The **DetechBeads Pro MYCO** LAMP Detection Kit uses a quick workflow involving DetechBeads, which are lyophilized LAMP beads consisting of all required components of a LAMP reaction. Only primers and water, supplied in separate tubes, are required to resuspend the DetechBeads.

The workflow consists of a short sample preparation step and loading. The LAMP results are read visually after 40 minutes at 65°C using the DetechRig (recommended) or a suitable alternative heat source (e.g. Thermocycler). Positive results are identified via a color change from pink (negative/no-amplification) to yellow (positive amplification).

2 MATERIALS

2.1 Provided Materials

Product Number	DB-MYCP-10016	DB-MYCP-10023
Product Name	DetechBeads Pro MYCO (8)	DetechBeads Pro MYCO (56)
Reactions	8	56
Sample Buffer	1 vial (1 mL)	7 vials (7 × 1 mL)
DetechBeads	1 × 8-well strip	7 × 8-well strip
Primer Mix	1 vial (25 µL)	1 vial (175 µL)
Nuclease-free water	1 vial (0.5 mL)	2 vials (2 × 1 mL)
Positive Control	1 vial (25 µL)	1 vial (150 µL)

Table 1: Kit Content

The kit contains 8 or 56 reactions in total. Every run needs one positive control (PC) and one no-template control (NTC), so the number of samples you can test depends on how you group your runs.

The Safety Data Sheet is available at www.detechgene.com.

2.2 Required Materials but not provided

- Microcentrifuge for 1.5 mL tubes, ≥ 14,000 × g (20,000 × g recommended)
- Heat block at 95 °C for sample preparation
- **DetechRig** at 65 °C (highly recommended) as incubation device or Thermocycler set to 65 °C.
- Sterile, nuclease-free 1.5 mL tubes
- Pipettes (1–1000 µL) and sterile filter tips
- Disposable gloves, lab coat, eye protection
- Mini-centrifuge for 0.2 mL 8-tube strips

2.3 Storage and Stability

The **DetechBeads Pro MYCO** LAMP Detection Kit is shipped cooled. On receipt of the kit store at -20°C. Thaw the reagents at room temperature or 2–8 °C. Do not leave them at room temperature for more than 1 hour. Shelf life from manufacture is 6 months. Refer to Table 2 for optimal use of the kit components.

Component	On receipt	Important note
All components	Store at –20 °C. Use by the expiry date on the label.	Thaw all components to be used at 2-8 °C or room temperature.
DetechBeads	Keep in the sealed pouch with desiccant. Store at -20°C.	Take out only the tubes you need, then reseal the pouch

Component	On receipt	Important note
		at once. Protect from moisture! Use immediately after opening.
Primer Mix & Positive Control	Store at –20 °C.	To avoid more than 4 freeze-thaw-cycles, split into aliquots on first thaw.

Table 2: Storage conditions

3 WARNINGS AND SAFETY INSTRUCTIONS

Treat all cell culture samples as potentially infectious and follow the biosafety rules of your laboratory. Positive control is a non-infectious genomic DNA template. Treat positive control as potentially infectious and wear gloves, a lab coat and eye protection. The Safety Data Sheet (SDS) is available at www.detechgene.com.

3.1 Do's and Don'ts

Do	Don't
Wear gloves and appropriate protection.	Open reaction tubes after incubation.
Clean surfaces with 70 % ethanol or 10 % bleach before and after each run.	Use amplified product as template for a new reaction.
Use filter tips for every pipetting step.	Store reagents near amplified products.
Include a PC and an NTC in every run.	Leave reagents at room temperature.

Table 3: Do's and Don'ts

3.2 Important Notes Before Use

- Thaw the kit at 2-8 °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 and template-free before the assay setup.
- Performing the experiment using sterile, DNases-free filter pipette tips is highly advisable.
- **OPTIONAL**: It is recommended to prepare aliquots for Primer Mix and Positive Control upon first use to prevent frequent freeze-thaw cycles and contamination.
- Incubation of LAMP reaction mix (at 65 °C) should be performed in a separate location from sample preparation and LAMP reaction setup.
- **IMPORTANT NOTE:**
  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 properly disposed.

3.3 Disposal

Please follow local regulations regarding the collection and disposal of hazardous waste. Contact your waste disposal company to obtain information on laboratory waste disposal. For further information, please refer to the instructions supplied with our SDS. Dispose of samples and used reaction tubes as biological waste according to local regulations. Keep reaction tubes closed when you dispose of them.

4 PROTOCOL

Note: Read the protocol thoroughly before the experiment!

Step 1: Sample Preparation

> **NOTE**: Sample requirements
>
> The cell culture should be >80% confluent. The supernatant can be stored at 2-8 °C for up to 3 days or long term at -20 °C before use.

1. Transfer 1 mL of cell culture supernatant to a sterile 1.5 mL tube (not provided).

   NOTE: Cell suspension can also be used, but total cell number should not exceed 10^7 cells/mL.
2. Centrifuge for 2 min at 20,000 × g. If your centrifuge cannot reach 20,000 × g, centrifuge for 3 min at ≥ 14,000 × g.
3. Remove the supernatant thoroughly with a pipette. Do not disturb the pellet!

   NOTE: The pellet may be invisible. The presence of the pellet will not affect the result.
4. Add 100 µL Sample Buffer and mix by pipetting up and down 3 times.
5. Place the tube in the heat block and incubate for 5 min at 95 °C.
6. Centrifuge for 2 min at 14,000 × g.
7. Transfer the supernatant to a fresh 1.5 mL tube. The sample is ready for the LAMP reaction.

> **IMPORTANT NOTE**: Storage Condition
>
> Untreated supernatant: up to 3 days at 2–8 °C, or long term at –20 °C.
>
> Prepared sample: up to 2 weeks at –20 °C.

Step 2: Reaction Mix Preparation

8. Take the appropriate number of LAMP Bead tubes for your run, including tubes for each sample, one positive control (PC) and one no-template control (NTC).
9. Add 17.5 µL of Nuclease-free water and add 2.5 µL of Primer Mix to each DetechBead (see table 4 below). Add 5 µL of sample (or 5 µL of control*) to each tube. Briefly centrifuge to collect the mix.

   NOTE: Perform this step in a dedicated clean area, separate from the incubation area, to prevent carry-over contamination.

Reaction Mix (1x)	Volume
Primer Mix	2.5 µL
Sample / Control*	5 µL
Nuclease-free water	17.5 µL

Table 4: Reaction Mix Volume Specifications

***NOTE**: For PC and NTC, use 5 µL of Positive Control and Nuclease-free water respectively (supplied in the kit).

Step 3: Incubation and Readout

10. Incubate at 65°C for 40 minutes using the **DetechRig** incubation device or a suitable alternate heat source (e.g. Thermocycler). The reaction mix will appear pink at the start of the LAMP reaction.

    NOTE: Do not exceed 40 minutes of incubation.

11. Allow the reaction tubes to equilibrate 5 minutes to room temperature. Briefly centrifuge to collect the mix. **DO NOT** open the tubes.

12. Interpret the results within 15 minutes after end of incubation:

    POSITIVE: Yellow/Orange

    NEGATIVE: Pink

5 WORKFLOW CHART

01 SAMPLE PREPARATION



Collect 1 mL
cell culture **supernatant** into a microtube



Spin 20,000 × g · 2 min or 14,000 × g · 3 min
mycoplasma collects in the **pellet**

Remove supernatant **thoroughly** without disturbing the pellet!



Resuspend 100 µL Sample Buffer



Heat 95 °C · 5 min



Spin 14,000 × g · 2 min
debris goes to the bottom



Collect supernatant
use as test sample

02 REACTION MIX PREPARATION



Resuspend bead 17.5 µL water + 2.5 µL Primer Mix



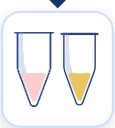
Add 5µL template Sample · NTC · PC

Controls: **5 µL** Positive Control (PC) or nuclease-free water (NTC), both supplied in the kit

03 INCUBATION & READOUT



Incubate 65 °C · 40 min
DetechRig (recommended) or **Thermocycler**



Read color
compare against the controls

READ THE RESULT



6 RESULT INTERPRETATION

Read the results at room temperature within 15 min after incubation, against a white background.

Results are read visually: a color change from pink to yellow indicates a positive result, while a reaction that remains pink indicates a negative result (see figure 1 below). Run is valid only if PC is yellow and NTC is pink. If not, run fresh aliquots.

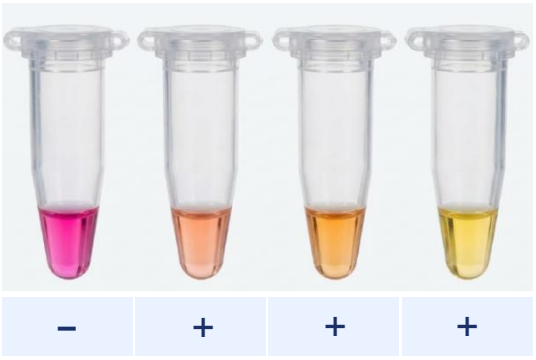


Figure 1: Visual Readout Interpretation. Color change from pink to yellow

6.1 Positive Culture

- Isolate the culture straight away and decide whether to discard or treat it.
- Test the other cultures that were handled in the same cabinet or incubator.
- Decontaminate the biosafety cabinet, incubator and water bath.
- Consider confirming the result with a second method, especially for valuable cell lines.

7 LIMITATIONS

All products described in this instruction manual are for Research Use Only (RUO). They are not intended for use as drugs, medical devices, food, food additives or household products, and must not be used for the diagnosis, treatment, mitigation, cure or prevention of any disease in humans or animals, whether as part of an established therapy or an experimental clinical investigation.

The test is qualitative and does not quantify the amount of Mycoplasma present. It detects DNA and cannot distinguish between viable and non-viable organisms. A sample may remain positive for some time after successful treatment. Very low levels of contamination, or growth suppressed by antibiotics, may produce a false-negative result. Mycoplasma species other than those listed in Section 1.2 may not be detected. Substances in the sample that alter pH or inhibit amplification may affect the result. Amplified-product contamination can cause false-positive results. Follow the precautions described in Section 3. This test must only be used with the sample types and according to the procedure described in this document. No internal amplification control provided, pH-based color readout is sensitive to acidic samples. Consider a spiked-sample option. In the case of color change during loading of sample, reduce the sample input up to 1 µL and run the reaction.

Intellectual Property

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Detechgene warrants that products will meet our specifications at the time of delivery. No other warranty, express or implied – including warranties of merchantability or fitness for a particular purpose – is provided. In the event of a product defect, Detechgene's sole liability, at its discretion, shall be limited to product credit, refund of the purchase price or replacement of non-conforming materials.

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Claims & Notice Period

Any claims must be submitted within 30 days of receipt of the product. Failure to notify Detechgene within this period constitutes a waiver of all claims with respect to the relevant materials.

8 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 40 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.
Color change in Sample without incubation	Carry-over contamination of the sample preparation step and/or as a result of high acidity	Reduce sample input.
No color change in Positive Control or both PC and NTC are pink	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	▪ Concentrate sample by pelleting 2 mL of cell culture supernatant. ▪ Grow the cell culture for additional 48 hours and repeat the test.
PC and NTC both have a change in color	Possible contamination of amplicons.	▪ Ensure sample preparation is carried out in a template-free environment. ▪ Ensure that the LAMP reaction takes place in separate environment than the sample preparation.
DetechRig		
Fast red blink + urgent, rapid beeping	Lid opened during heating or soaking	Close the lid within 15 seconds to resume the run (a short confirmation beep sounds on resume). If the lid stays open longer than 15 s, the run ends and a two-tone descending beep sound (see next row).

Problem	Possible Cause	Recommendation
Slow red blink, silent	Run aborted (lid left open > 15 s)	Remove the sample. Reclosing the lid does NOT restart the run. The device returns to standby only after the chamber is confirmed empty (about 2 s).
Yellow blink + periodic double-beep	Power-loss lockout	Remove the sample. The device clears once the chamber is confirmed empty (about 2 s). Do not attempt a new run until it has cleared.
Stuck on a red or yellow blink although the chamber is empty; will not return to standby	Detection fault (empty chamber read as occupied)	Confirm the chamber is genuinely empty and clean. Power-cycle once. If it persists, remove the unit from testing and return it for service. Record the unit ID.

NOTE: On the two red patterns: a fast red blink accompanied by beeping indicates the live lid alarm (recoverable); a slow red blink with no sound indicates an aborted run (not recoverable). Solid red indicates a normal soak.

9 SYMBOLS

Symbols used in this document are in accordance with EN ISO 15223-1:2021.

Symbol	Description
	Manufacturer
	Reference Number
	Batch Number
	Temperature limitation
	Do not re-use
	Manufacturing Date
	Expiration Date
	Consult instructions for use
	For Research Use Only. Not for use in diagnostic procedures (attention label)

DetechBeads Pro

myco

LAMP Detection Kit
Instruction Manual

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SIMPLE AND PRECISE DETECTION OF PATHOGENS