Instruction Manual
Research Use Only

Myco-Visible Mycoplasma Rapid Test Kit

For Simple, Sensitive, and Rapid Detection of Mycoplasma Contamination in Cell Cultures

Pack Size: 24 tests
Storage: -20°C
Cat. No.: 09305090S (5 Tests)
093050901 (24 Tests)
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1. Introduction to Myco-Visible Mycoplasma Rapid Test Kit

Mycoplasmas are commonly found in research laboratories and are unaffected by many antibiotics used to control bacterial contamination in cell cultures, particularly those targeting cell wall formation, such as penicillin. Their lack of a cell wall also allows some cells to pass through 0.2 µm filters. Several studies have investigated the prevalence of mycoplasma contamination in cell cultures, with estimates ranging as high as 80%. In the USA, contamination rates are lower, around 10-15%. A global average contamination rate between 25% and 50% seems plausible. This significant issue can lead to serious economic losses for the industry. Most cases of cell culture contamination are caused by about six to eight species with human, porcine, or bovine hosts, with *Mycoplasma orale* and *Mycoplasma hyorhinis* being the most common.

Myco-Visible Mycoplasma Rapid Test Kit has been demonstrated to be a high sensitivity, specific and rapid assay for the detection of mycoplasma contamination in cell cultures. Myco-Visible Mycoplasma Rapid Test Kit achieves this by targeting and amplifying unique regions 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 salivarium and Mycoplasma pneumoniae*. The detection procedure can be completed within 1 hour and the detection limit is as low as 10 Colony-forming Units/mL (CFU/mL) or 10 fg of Mycoplasma genomic DNA per reaction. Specificity test showed no cross-reactivity with other bacterial, fungal and mammalian DNA. The test assay is simple to perform with no special technical expertise or lab equipment. Positive results can be visualized on an immunochromatographic test device in less than 5 minutes after buffer loading.

2. Kit Components and User Supplied Materials

2.1 Myco-Visible Mycoplasma Rapid Test Kit Components

	24 Tests (Cat. No. 093050901)		5 Tests (Cat. No. 09305090S)	
Components	Package	Cat. No.	Package	Cat. No.
Reaction Beads	3 x 8 ea	093050902	5 ea	0930509OS2
Sample Buffer	1 vial (2.5 mL)	093050903	1 vial (0.6 mL)	0930509OS3
Primer Mix	1 vial (110 µL)	093050904	1 vial (22 µL)	0930509OS4
Nuclease-free Water	1 vial (400 µL)	093050905	1 vial (80 µL)	0930509OS5
Positive Control	1 vial (50 µL)	093050906	1 vial (10 µL)	0930509OS6
Running Buffer	1 bottle (5 mL)	093050907	1 bottle (1 mL)	0930509OS7
Detection Device	24 x 1 ea	093050908	5 x 1 ea	093050908
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 working at speed of 20,000 x g; if not available, use the maximum speed (>14,000 x g).
- Heat block
- Thermocycler (optional, recommended to be used for 70 °C incubation)
- Sterile, Nuclease-free 0.2 mL and 1.5 mL microcentrifuge tubes
- Pipettes with corresponding filter tips

3. Storage and Kit Stability

All Myco-Visible Mycoplasma Rapid Test Kit components are guaranteed until the expiration date printed on the kit label. Upon receipt, store the unopened kit at -20 °C until first use. Once opened, it is recommended to keep the Running Buffer and Detection Device at room temperature for the ease of use. The stability of the two components will not be affected if they are stored at a lower temperature (e.g. 4 °C, -20 °C), but it is required to equilibrate them to room temperature for every use. Always store the other components: Reaction Beads, Primer mix, Nuclease-free Water and Positive Control at -20 °C when not in use.

4. Important Consideration Before Use

- ❑ Thaw the reagents at 4 °C or room temperature. Make sure the Detection Device and Running Buffer are at room temperature before use.
- ❑ It is highly recommended to prepare aliquots for Primer Mix (e.g. 5 x 22 µL) and Positive Control (e.g. 5 x 10 µL) upon first use to prevent frequent freeze-thaw and contamination.
- ❑ Clean the work area with 70 % alcohol or 10 % household bleach before the assay setup.
- ❑ Preparation of reaction mix should be performed in a dedicated clean area that is separate from LAMP incubation and sample loading of Detection Device. Do not bring incubated reaction tubes back into the reaction preparation area.
- ❑ Always use filter pipette tips that are sterile and nuclease free.
- ❑ Samples, amplified products and used cassettes are biohazards that must be sterilized before discarding.

5. Protocol

5.1 Sample Preparation

1. Transfer 1 mL of cell culture supernatant to a 1.5 mL microcentrifuge tube.

   Note: Test cell culture should be at more than 80% confluency. The supernatant can be stored at 4 °C for up to 3 days or long term at -20 °C before use.
2. Centrifuge at maximum speed (20,000 x g) for 2 mins and carefully decant the supernatant.

   Note: The pellet may be invisible. The presence of the pellet will not affect the result.
3. Add 100 µL of **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 at 14,000 x g for 2 mins and transfer the supernatant to a fresh 1.5 mL centrifuge tube.
6. Use the supernatant immediately as the sample in Section 5.2 or store at -20°C until required.

5.2 Reaction Mix preparation

1. Take an appropriate number of **Reaction Beads** provided in individual tubes. The number of **Reaction 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 **Reaction Beads** tube (see table below). Add X µL of **Nuclease-free water** and gently pipette up and down to dissolve the **Reaction Beads**. Add 4 µL of **Primer Mix** and up to 4 µL of sample (or 1 µL control*) to each tube.

   Note: This step should be performed in a clean and separate location to prevent carry-over contamination.

Reaction Mix	Volume
Primer Mix	4 µL
Sample / Control*	up to 4 µL / 1 µL
Nuclease-free water	X (to a total of 20 µL)

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

5.3 Mycoplasma detection

1. Incubate the reaction mix tube at **70°C** for **30 min** using a thermocycler or heat block.

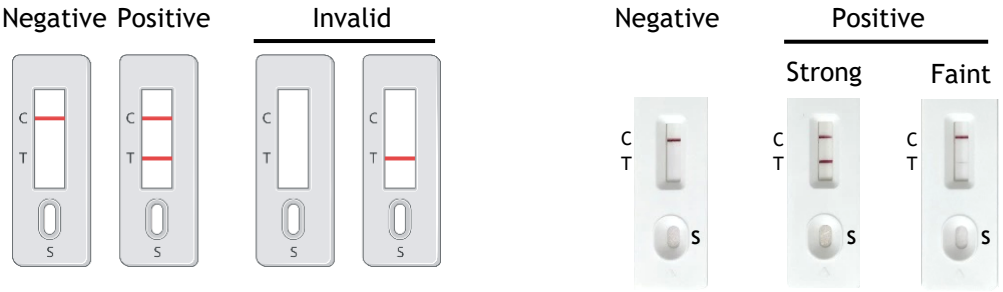
   Note: Do not incubate for more than 40 min as it will significantly affect the results.
2. Allow the reaction tube to equilibrate to room temperature, then briefly spin down the mix.
3. Pipette 5 µL of the reaction mix and load to the sample well of **Detection Device**. Add 3 drops of **Running Buffer** to the sample well and wait for 2 to 5 min for the results.

5.4 Result Interpretation

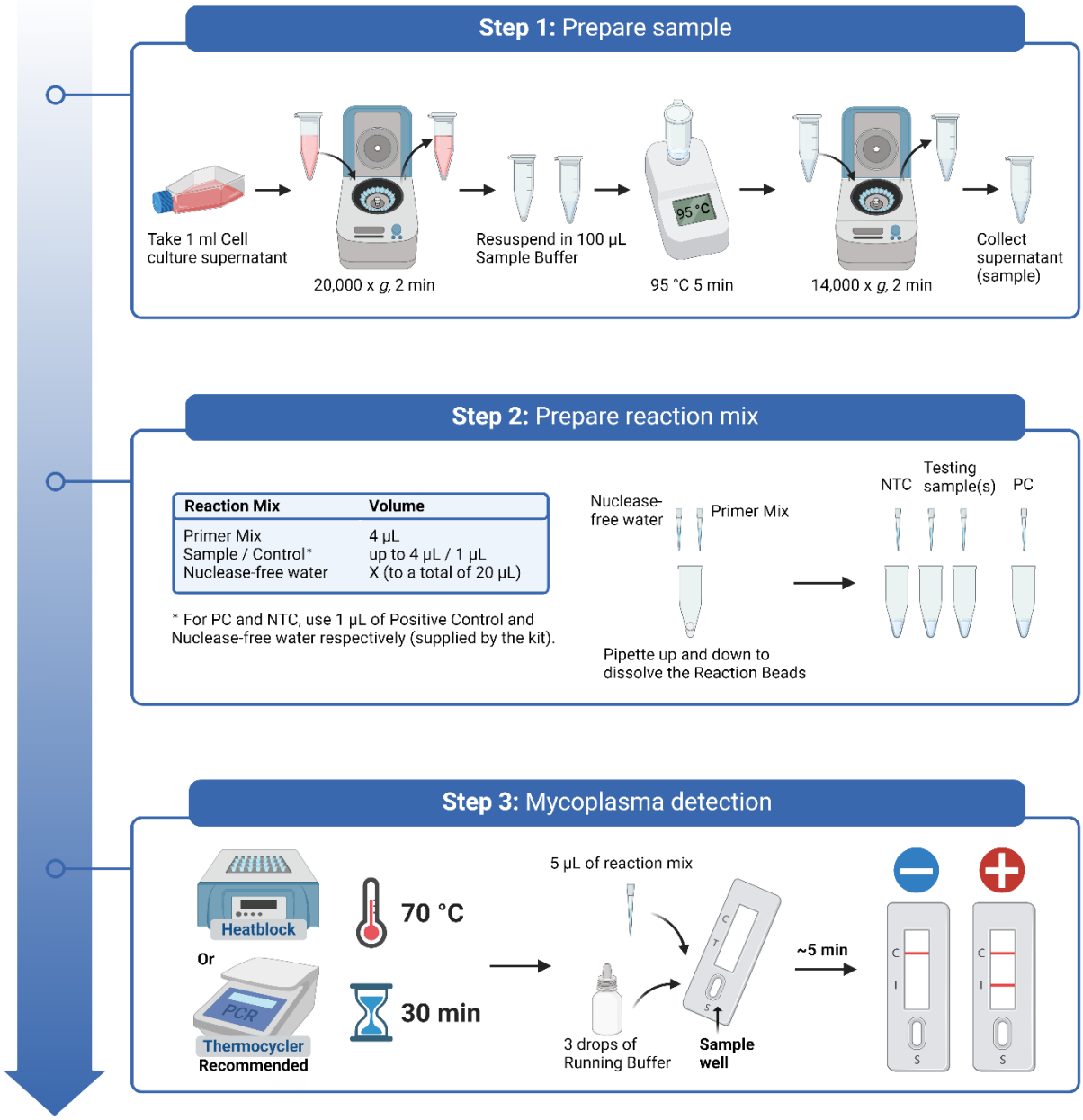
- One band (C-band only): Negative for Mycoplasma.
- Two bands (C-band and T-band): Positive for Mycoplasma.

Note: A faint T-band is also considered positive for mycoplasma. No control band indicates the test is invalid.

Note: The control band will appear within 1-2 min. If positive, the test band will appear within 5 min, test is considered negative if no T-band appeared after 5 min. Any results read after 20 min should be considered invalid since the bands may not be stable after prolonged air exposure.



6. Flow Chart


Step 1: Prepare sample
Take 1 ml Cell culture supernatant
20,000 x g, 2 min
Resuspend in 100 µL Sample Buffer
95 °C
95 °C 5 min
14,000 x g, 2 min
Collect supernatant (sample)
Step 2: Prepare reaction mix
Reaction Mix
Volume
Primer Mix
4 µL
Sample / Control*
up to 4 µL / 1 µL
Nuclease-free water
X (to a total of 20 µL)
* For PC and NTC, use 1 µL of Positive Control and Nuclease-free water respectively (supplied by the kit).
Nuclease-free water
Primer Mix
Pipette up and down to dissolve the Reaction Beads
NTC
Testing sample(s)
PC
Step 3: Mycoplasma detection
Heatblock
Or
PCR
Thermocycler
Recommended
70 °C
30 min
5 µL of reaction mix
3 drops of Running Buffer
Sample well
~5 min
C
T
S

7. Data

Myco-Visible Mycoplasma Rapid Test kit is designed to detect cell culture contaminating Mycoplasma and Acheloplasma species. The detection limit is as low as 10 Colony-forming Units/mL. To identify the analytical sensitivity, inactivated mycoplasma preparations titrated to 100 and 10 CFU/mL from 7 *Mycoplasma* spp in the matrix of a cell suspension of $1x10^6$ CHO cells/mL were tested in the study. The 7 Mycoplasma spp tested here contain key cell culture contaminating and human infecting mycoplasma that are listed in Pharmacopoeia (EP2.6.7/USP <63>/JP G3). The detection limit of the kit (10 CFU/mL) meets the sensitivity guidance of nucleic acid amplification technology (NAT)-based mycoplasma detection method provided in EP/USP/JP.

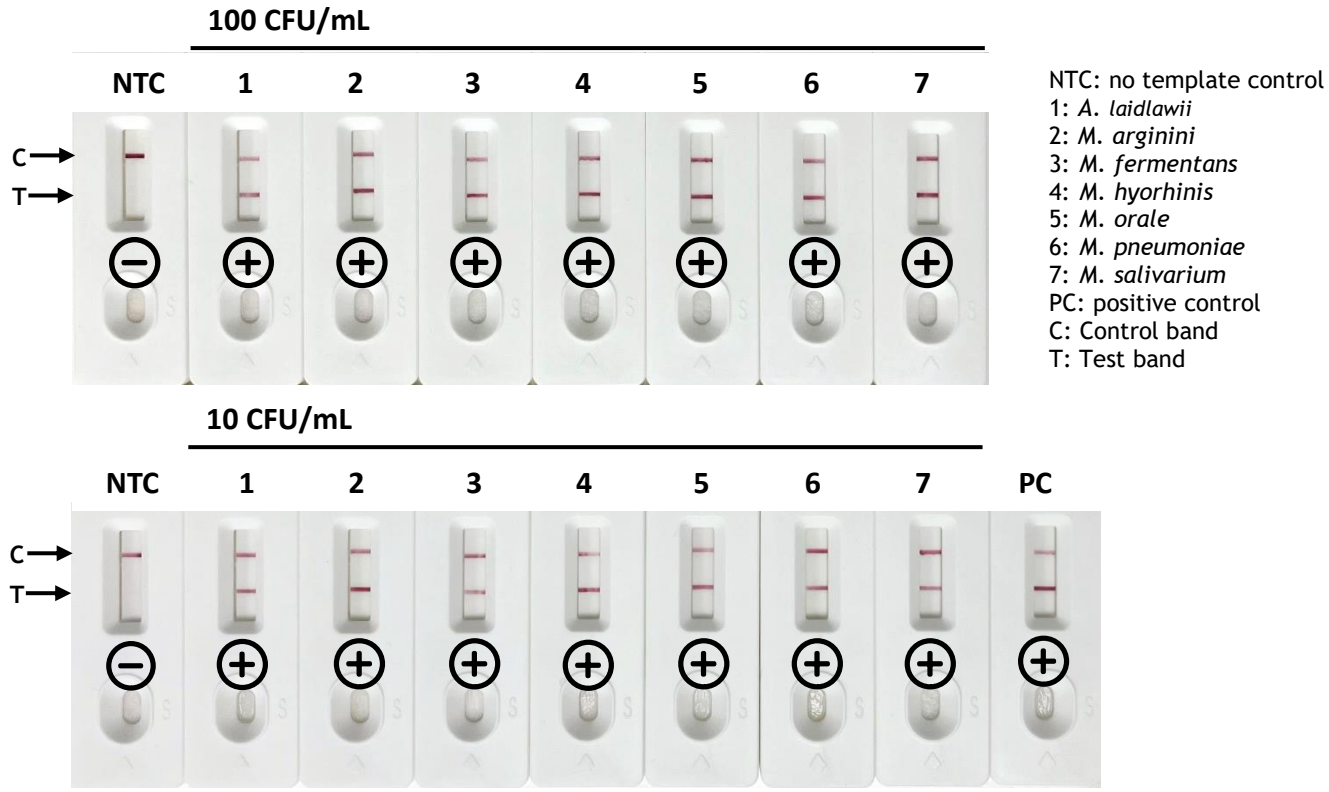


Figure 1: Analytical sensitivity test of Myco-Visible Mycoplasma Rapid Test Kit. All seven strains of mycoplasma were tested at concentrations of 100 and 10 CFU/mL. All samples yielded positive results at both concentrations. *The negative control is a no template reaction mix (nuclease-free water) incubated along with other samples.*

Myco-Visible Mycoplasma Rapid Test Kit specifically detects mycoplasma without cross-reactivity with other bacterial, fungal or mammalian DNA. In this specificity test, purified gDNA at 1 x 10^5 copies/reaction from various off-target species were assessed. All species give a negative result for mycoplasma detection and there was no interference in the test.

NTC: No template control
1: Bacillus subtilis
2: Candida albicans
3: Listeria monocytogenes
4: Salmonella typhimurium
5: Staphylococcus epidermidis
6: Micrococcus luteus
7: Bacillus cereus
8: Pseudomonas aeruginosa
9: *Staphylococcus Aureus*
10: *Escherichia coli*
11: Human blood
12: Mouse tail
13: CHO cell
14: hMSC cell
15: *Saccharomyces cerevisiae*
16: *Pichia pastoris*
PC: Positive Control

Figure 2: Specificity test of Myco-Visible Mycoplasma Rapid Test Kit. In the upper row, purified gDNA at 1 x 10^5 copies/reaction from 10 bacteria species that are genetically related to mycoplasma were tested. In the lower row, gDNA isolated from human, mouse, yeast (15,16) as well as uncontaminated cell culture (13,14) were tested. All samples showed a negative result, indicating no cross-reactivity.

8. Troubleshooting

Problem	Possible Cause	Recommendation
Two bands in no template control (NTC)	Reaction time too long	• Do not exceed incubation time (70 °C for 40 min) as this significantly impacts the reaction.
	Carryover 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 and result interpretation site. • Use of filter tips will reduce the contamination.
Only Control band is present in Positive Control	Degradation of reagent	• Avoid frequent freeze-thaw cycles of the Primer Mix and Positive Control.
	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 a template to repeat the test.
No bands	Detection Device spoiled	• Store the device under proper condition. • Equilibrate the device to room temperature before use. • Use within 1 hour after opening
	Running Buffer contamination	• Ensure the Running Buffer is stored properly and not contaminated.
Weak Test band	Mild contamination	• If a light pink test band is observed for test sample and no band observed for NTC. The results should be considered positive. • If both the test sample and NTC show weak test bands, the result should be considered invalid due to contamination.

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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