GenieColor Mycoplasma Detection Kit

Code: MORV0013-25 (25 Tests) / MORV0013-50 (50 Tests)

The GenieColor Mycoplasma Detection kit enables the rapid detection of Mycoplasma in cell culture by a visual pink-to-yellow color change in 1 hour without the need for specialized instrumentation.

- **Visual color change**: Identify mycoplasma contamination by eye with a pink-to-yellow color change
- **1-Step protocol**: Incubate 1ul cell culture supernatant with the reaction system for 1 hour at 60℃
- **No specialized equipment needed**: Simple set-up with no need for advanced instruments
- **Detect up to 40 mycoplasma species**: Broad detection capability including 8 strains commonly found in cell cultures

GenieColor utilizes an advanced isothermal amplification technology to detect mycoplasma contamination in cell cultures with a pink-to-yellow visual color change. It offers several benefits including ease of use, clear results, high sensitivity, strong specificity, and anti-contamination features. It also comes with a positive control meaning absolute confidence in your results.

By adding 1 µl of cell culture supernatant to the reaction system and incubating at 65°C for one hour, results can be visually observed. If mycoplasma contamination is present, the reaction solution changes from pink to yellow, providing a clear color contrast determined by eye. Additionally, the reagent incorporates an anti-contamination system to prevent false positives from amplification product aerosols, ensuring accurate and reliable results.

The GenieColor Mycoplasma Detection kit can identify 40 mycoplasma species, including 8 commonly found in cell cultures, and is compatible with a wide range of adherent cells, suspension cells, media, and serum.

Components

	Components	MORV0013-25 (25 rxns)	MORV0013-50 (50 rxns)
	GenieColor LAMP Mix	500 µl	2 × 500 µl
	Positive Control	15 µl	2 × 15 µl
	Mineral Oil	500 µl	2 × 500 µl

Storage

Store at -30°-15°C and transport at ≤0°C.

Materials Not Provided

PCR machine or water bath.

Protocol

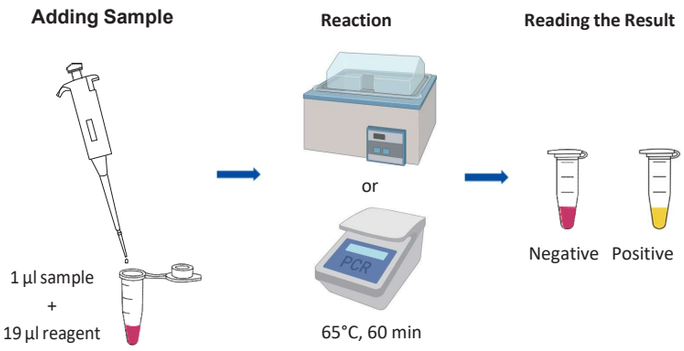

Fig 1. Workflow of GenieColor Mycoplasma Detection kit

1. Collect the cell culture supernatant

- Adherent cells: directly collect the supernatant. Cells should remain in culture for at least 72 h undisturbed prior to screening and reach 90% confluence. After 72 h, mycoplasma content in supernatant is relatively high and can be detected easily.
- Suspension cells: collect the supernatant after centrifugation at 2,300 rpm (500 × g) for 5 min. Cells should remain in culture for at least 72 h undisturbed prior to screening. After 72 h, mycoplasma content in supernatant is relatively high and can be detected easily.

2. Preparation of reaction system

- Thaw the GenieColor LAMP Mix from -30°-15°C and mix thoroughly.
- Prepare the reaction system according to the following table:

Components	Test Sample	Negative Control*	Positive Control
GenieColor LAMP Mix	19 µl	19 µl	19 µl
Test Sample	1 µl	-	-
Nuclease-free ddH_2O	-	1 µl	-
Positive Control	-	-	1 µl
Total	20 µl	20 µl	20 µl

* Negative Control: No sample added or add 1 µl of Nuclease-free ddH_2O.

- Vortex and briefly centrifuge to collect at the bottom of the tube, ensuring no bubbles in the reaction system. Add 20ul of mineral oil if using a water bath for incubation.

3. Reaction

- Incubate at 65°C for 60 min in a PCR instrument or water bath (make sure water bath is calibrated prior to use).

4. Results

- After the reaction is completed, observe the color in a bright environment. Pink represents negative and yellow represents positive.

Negative **Positive**

- Do not open the lid of detection tubes to prevent false positives due to aerosol contamination.

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Frequently Asked Questions (FAQs)

Q1: What is the sensitivity of the kit (minimum detection limit)?

- The kit can detect mycoplasma positive plasmid at a minimum concentration of 100 copies/µl.

Q2: How is accuracy ensured?

- The product includes both negative and positive controls to ensure accurate results. It has been tested against 40 different mycoplasma species, including 8 common mycoplasma contaminants.

Q3: What is the detection principle of this kit?

- The kit utilizes loop-mediated isothermal amplification (LAMP) technology. The reagent is pre-mixed with Bst isothermal amplification enzyme, mycoplasma-specific primers (inner and outer primers), and an optimized buffer. After adding the template, the LAMP reaction begins. If mycoplasma DNA is present, the reagent color changes from pink to yellow. A negative result remains pink, while a positive result turns yellow.

Q4: What sequence is used in the positive control?

- The positive control contains a plasmid with the M. hyorhinis sequence at a concentration of 10 ng/µl.

Q5: Does the positive control of the kit cause contamination?

- No, the positive control is a mycogenic plasmid, which does not cause contamination.

Q6: What does it mean if the reaction liquid color is between pink and yellow?

- A color between pink and yellow indicates a weak positive. It is recommended to repeat the test. If the repeated test is positive or weak positive, it is considered positive. If the repeated test is negative, it is considered negative.

Q7: Why does the reaction solution change color after adding the supernatant of the medium?

- The medium might interfere with the color change. If it does, then use the following treatment method:

Treatment method:

1. Take a small amount of culture supernatant or cell suspension, centrifuge at 500 g for 5 minutes, and collect the supernatant.

2. Centrifuge the supernatant at 12,000 g for 5 minutes. Aspirate 50 µl of supernatant and add 950 µl PBS.
3. Centrifuge again, collect the mycoplasma precipitate, discard the supernatant, retain 50 µl, and use 1 µl for detection.

Q8: What 40 Mycoplasma species are detected using this kit?

No.	Mycoplasma Name	No.	Mycoplasma Name	No.	Mycoplasma Name	No.	Mycoplasma Name
1	*M.hyorhinis**	11	*M.gallisepticum*	21	*M.testudineum*	31	*M.genitalium*
2	*M.fermentans**	12	*M.synoviae*	22	*M.columbinum*	32	*M.iguanae*
3	*M.arginini**	13	*M.auris*	23	*M.cricetuli*	33	*M.leopharyngis*
4	*M.orale**	14	*M.crocodyli*	24	*M.gallinarum*	34	*M.lipofaciens*
5	*M.hominis**	15	*M.iners*	25	*M.opalescens*	35	*M.hyosynoviae*
6	*M.salivarium**	16	*M.bovigenitalium*	26	*M.moatsii*	36	*M.falconis*
7	*M.pirum**	17	*M.simbae*	27	*M.alkalescens*	37	*M.meleagridis*
8	*A.laidlawii**	18	*M.arthritidis*	28	*M.sphenisci*	38	*M.mobile*
9	*M.pneumoniae*	19	*M.alvi*	29	*M.agalactiae*	39	*M.cloacale*
10	*M.bovis*	20	*M.primatum*	30	*M.cynos*	40	*M.spermatophilum*

* More than 95% mycoplasma contaminations in cell culture are caused by these 8 kinds of mycoplasma