Mycoplasma qPCR Detection Kit

User Guide

► Product Information

Catalog No.	Specification
EDY020-01	50 tests
EDY020-02	100 tests

► Product Overview

The Mycoplasma qPCR Detection Kit is designed for the detection of mycoplasma DNA extracted from cell suspensions using real-time quantitative PCR (qPCR) technology. To broaden the range of detectable species, the primers and probe included in the kit are specifically designed to target a conserved region of the 16S rRNA gene in mycoplasma. The kit is capable of detecting 22 common mycoplasma species found in cell culture, as well as *Escherichia coli*, *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Streptococcus pneumoniae*, while minimizing cross-reactivity. The detection limit is as low as 10 copies.

► Components

Components	50 tests	100 tests
Myco-Probe Mix	50 µL	100 µL
Myco-Primer Mix	50 µL	100 µL
Positive Control DNA (1×10^7 Copies/µL)	20 µL	40 µL
2×Probe qPCR Mix	50 µL	100 µL
Nuclease-free Water	1 µL	2×1 mL
Nuclease-free Water	50 µL	100 µL

► Transportation and Storage

Shipped with ice packs; store at –20°C. Valid for 12 months.

► Experimental Workflow

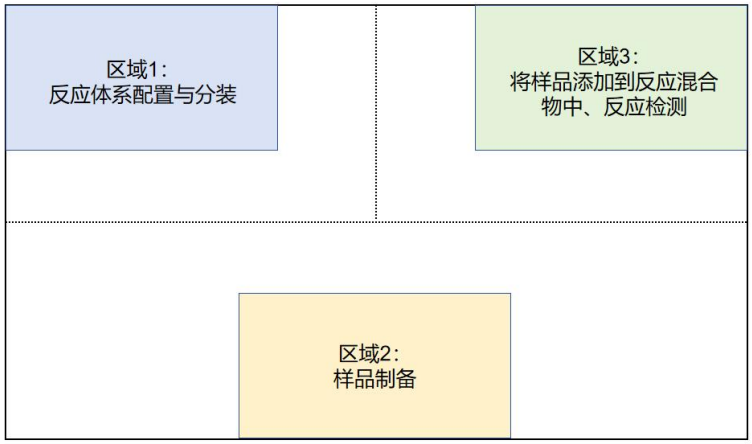
1. Precautions

(1) The probe and primers are prone to degradation by nucleases. Once degraded, they can no longer ensure accurate detection. Care must be taken to avoid contamination by nucleases introduced through perspiration or saliva during sample handling.

(2) It is strongly recommended to designate and physically separate the following three areas for reagent preparation and qPCR analysis. Avoid opening or closing any tubes containing amplification products in these areas.

2. Work Area Setup

The PCR reaction used in this kit is a highly sensitive amplification technique. To minimize the risk of cross-contamination from PCR products or template controls, it is advised to perform the experiment in physically separated work areas. Refer to Figure 1 for an example of recommended layout.



Area 1: Reaction mix preparation and aliquoting

Area 2: Sample preparation

Area 3: Addition of samples to the reaction mix and qPCR detection

Figure 1. Recommended work area division

Note: The use of a biosafety cabinet can be considered depending on the laboratory environment.

3. Workflow

3.1 Sample Preparation

1. Transfer 100 µL of cell culture supernatant (cultured for more than 24 hours) into a 1.5 mL microcentrifuge tube.

2. During cells digestion and passaging, transfer an appropriate number of cells (≥1 × 10^5) into the same 1.5 mL microcentrifuge tube. Ensure that the cell count meets the requirement.

3. Add 1 µL of Proteinase K to the 100 µL sample.

4. Vortex thoroughly and incubate in a 55 °C water bath for 15 minutes.

5. After incubation, heat the sample at 98 °C for 2 minutes to inactivate Proteinase K.

6. Cool the sample at 4 °C for 30 minutes. Once cooled, briefly centrifuge and proceed with loading.

3.2 Preparation of qPCR Reaction

1. Prepare the reaction mix on ice according to the reagent components listed in Table 1 below. To ensure sufficient volume, prepare at least 10% extra mix. Each sample should be run in technical triplicate. *(Perform in Area 1)*

Table 1. Mycoplasma detection reaction system (20 µL per reaction)

Component	Volume per well (µL)
Probe qPCR Mix	10
Myco-Primer Mix (10 µM)	1

Myco-Probe	1
Nuclease-free Water	6
Total	**18**

2. Mix the reaction components thoroughly and aliquot 18 µL into each qPCR reaction tube. *(Perform in Area 1)*

3. Add the negative control (nuclease-free water), test samples (cell samples processed in Section 3.1), and positive control template (Positive Control Template) to the qPCR tubes. Add the negative control first, followed by the test samples, and finally the positive control to minimize the risk of contamination. *(Perform in Area 3)*

4. Briefly centrifuge the tubes, then proceed with the qPCR run according to the program shown in Table 2.

Table 2. qPCR program setup

Step	Temperature	Time
Contamination digestion	37℃	2 min
Initial denaturation	95℃	30 sec
40 Cycles	95℃	10 sec
	58.5℃	30 sec (Signal collection)

Fluorescence Detection Channel:

The mycoplasma-specific probe is labeled with the FAM fluorophore, and fluorescence should be detected in the FAM channel.

4. Result Analysis

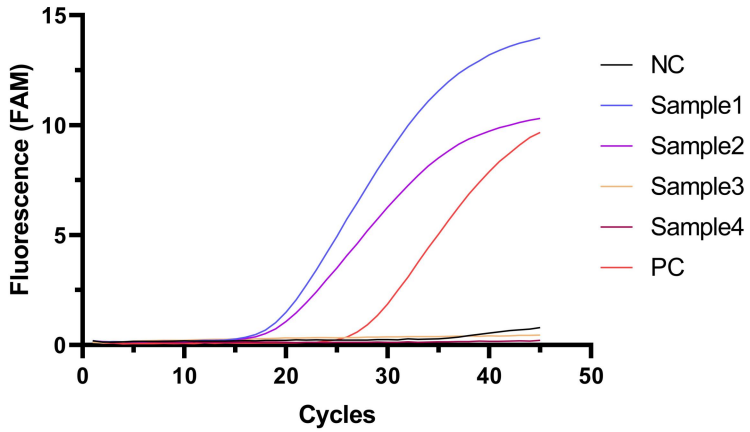
4.1 Criteria for Result Interpretation

Table 3. Criteria for detection results

Sample	FAM Signal	Interpretation
Positive Control	CT < 35 with clear amplification curve	Positive result (expected)
Negative Control	CT ≥ 35 with no significant peak	Negative result (expected)
Sample 1	CT < 35 with clear amplification curve	Mycoplasma positive

Sample 2	CT ⩾ 35 with no significant peak	Mycoplasma negative

4.2 Example Experimental Results



In the figure above, the positive control (PC) shows a CT value less than 35 with a clear amplification curve, while the negative control (NC) exhibits a CT value greater than or equal to 35 with no significant peak.

Samples 1 and 2 have CT values less than 35 with clear amplification curves, indicating mycoplasma-positive results.

Samples 3 and 4 have CT values greater than or equal to 35 with no significant peaks, indicating mycoplasma-negative results.

Precautions

1. This product is intended for laboratory research use only. Please strictly comply with all applicable laws, regulations, and ethical guidelines. Any consequences arising from improper use are the sole responsibility of the user, and EDITGENE Co., Ltd. assumes no liability.
2. Please follow the specified instructions for transportation, storage, and usage of the reagents. Avoid repeated freeze-thaw cycles unless absolutely necessary. EDITGENE Co., Ltd. is not responsible for any experimental failures resulting from improper storage or handling.