Mycoplasma Detection Kit (PCR)

Product Instruction Manual

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Mycoplasma Detection Kit Instruction Manual

► Product Information

Catalog Number	Specification
EDMD-01	50T
EDMD-02	100T

► Product Overview

Mycoplasma is a group of the smallest and simplest prokaryotic organisms. Mycoplasma infection alters the DNA, RNA, and protein expression of cells, leading to slow cell growth rates, morphological changes, and other pathological alterations, which significantly affect experimental results. Detection and prevention of Mycoplasma contamination is an essential step in cell experiments. This kit works by amplifying the conserved region of the Mycoplasma genome using specific primers. After PCR amplification, the presence of Mycoplasma contamination can be confirmed through electrophoresis. This method is characterized by high sensitivity, strong specificity, and rapid detection.

► Kit Components

Reagent	Storage Conditions	50T	100T
MycoD Primer MIX	-20℃	100 µL	200 µL
MycoD Control template	-20℃	100 µL	200 µL
MycoD Master Mix	-20℃	750 µL	1.5 mL
MycoD Proteinase K Solution	-20℃	50 µL	100 µL

► Transportation and Storage

Shipped with ice packs; store at -20°C with a shelf life of 12 months.

► Other Materials Required for Experiment

a. PCR Thermal Cycler;

b. Agarose Gel Electrophoresis System;

c. Microcentrifuge;

d. Sterile PCR tubes, centrifuge tubes, pipette tips, and pipettes;

e. Agarose gel, electrophoresis buffer, DNA molecular weight marker, electrophoresis dye;

f. Samples: The samples for Mycoplasma detection should be cell suspensions that have been cultured for at least 48 hours after cell inoculation, or culture medium and serum.

► Experimental Workflow

Under sterile conditions, treat the cells to be tested with MycoD Proteinase K Solution to obtain the sample. Perform PCR reactions with the test sample, positive control, and negative control. After PCR amplification, analyze the products using agarose gel electrophoresis to check for the presence of bands and their brightness. When the positive control shows a clear band and the negative control shows no bands, determine whether the test sample is contaminated with Mycoplasma and assess the level of infection based on the presence and brightness of the bands.

1. Workflow Diagram

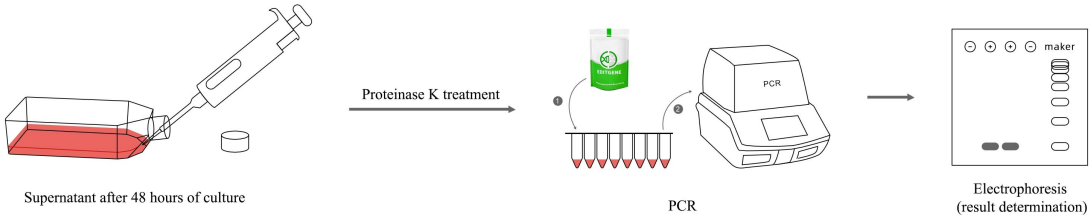

Figure 1. Schematic Diagram of the Mycoplasma Detection Workflow

2. Work Areas

The PCR reaction used in this kit is a highly sensitive amplification method. To prevent cross-contamination between PCR products and control templates, it is recommended to divide the workspace into separate areas for the experiment. The workspace division can refer to Figure 2.

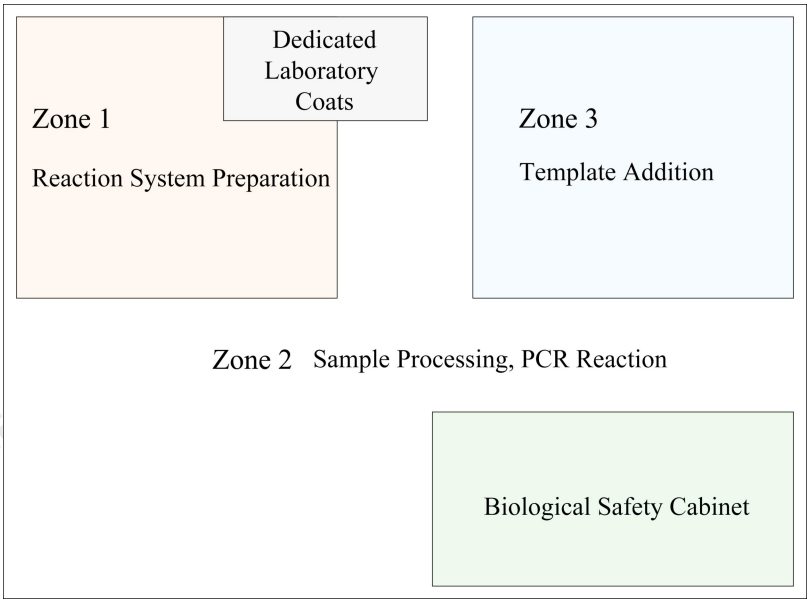

Figure 2. Suggested Division of Experimental Areas

Note: The use of a biosafety cabinet depends on the laboratory environment

3. Sample Preparation

Use cultured cells that have been continuously grown for over 48 hours after inoculation and prepare the sample for Mycoplasma detection as follows:

a. During cell passaging, collect a cell suspension containing ≥1 × 10^5 cells;

b. Centrifuge at 800 g for 5 minutes to obtain the test cell sample;

c. Add 100 µL of MycoD Proteinase K Solution to the test sample;

d. Incubate at 55°C for 15 minutes in a thermal cycler;

e. Incubate at 98°C for 2 minutes to inactivate the enzyme. The detection sample preparation is now complete.

4. PCR Reaction

a. Remove the Mycoplasma Detection Kit from the -20°C freezer and thaw it on ice;

b. Prepare the reaction system according to Table 1;

Table 1. PCR Reaction System

Component	Volume
MycoD Master Mix	15 μL
MycoD Primer Mix	2 μL
ddH2O	13 μL

c. Add 2 μL of template to the reaction system;

d. Set the reaction program and perform the PCR reaction according to Table 2;

Table 2. PCR Reaction Program Settings

Step	Temperature	Time
Pre-denaturation	95 °C	3 min
35 Cycles	95 °C	15 sec
	55 °C	15 sec
	72 °C	2 min
Final Extension	72 °C	5 min
Hold	4 ℃	∞

e. Complete the reaction to obtain the PCR product.

5. Gel Electrophoresis

a. Prepare a 1% agarose gel;

b. Load 3 μL of PCR product onto the gel;

c. Perform electrophoresis at 120 V for 30 minutes;

d. Result interpretation: Compare the results with the negative and positive controls to confirm Mycoplasma contamination. Positive bands will be approximately 500 bp in size.

► Frequently Asked Questions

1. Bands appear in the negative control

Use a freshly opened negative control for retesting. Ensure to change pipette tips during sample loading and prioritize loading the negative control first to avoid cross-contamination between samples.

2. Inconsistent results with repeated testing

PCR detection of Mycoplasma is highly sensitive, so avoid cross-contamination between samples. Ensure sterile conditions when handling each sample individually. After sample processing and PCR reaction, allow samples to cool before proceeding to the next step to avoid aerosol contamination.

3. Can the kit detect Mycoplasma in reagents?

The kit can detect Mycoplasma in common cell culture reagents, such as media and serum, without the need for sample preparation. Simply proceed directly to PCR reaction and gel loading. However, the kit cannot detect Mycoplasma in organic solvents like DMSO or ethanol.

► Precautions

1. This product is for laboratory use only and intended for research purposes. Please strictly adhere to relevant laws, regulations, and ethical guidelines. The company is not responsible for any consequences arising from misuse.
2. Follow the transportation, storage, and usage requirements for the reagents. Avoid repeated freeze-thaw cycles unless necessary. The company is not responsible for experimental failure caused by improper storage or handling.