Mycoplasma qPCR Detection Kit

Introduction

Mycoplasma is the smallest prokaryotic microorganism, only 0.1-0.3 μm in size. Due to their small size, mycoplasmas can penetrate rated filters (0.22~0.45 μm). Mycoplasma contamination remains a major problem in cell culture. Mycoplasmas can alter the DNA, RNA, and protein synthesis of culture cells, but they may not noticeably affect cell growth rates in many cases. Therefore, it is difficult to discover mycoplasma contamination with the naked eye. And mycoplasma detection should be performed regularly during cell culture.

There are many methods to detect mycoplasma contamination, such as the culture-based method, fluorescent staining method, ELISA and so on. However, most of these methods are time-consuming, complex to operate, and not highly sensitive. The qPCR method is relatively simple to operate, and the results can be determined without electrophoresis analysis after PCR amplification.

Mycoplasma qPCR Detection Kit is designed with specific primers and fluorescent probes (FAM labeled) targeting the conserved 23S rRNA encoding sequence of mycoplasma genomes. It can directly use the culture cell supernatant or serum as the template for detection. This kit specifically amplifies mycoplasma DNA and does not amplify the DNA of other bacteria, fungi, or eukaryotic cells.

This kit can quickly, efficiently, and sensitively detect a variety of mycoplasma contaminations, such as *Mycoplasma hominis, Mycoplasma hyorhinis, Mycoplasma arginini, Mycoplasma fermentans*, etc. It is also compatible with all qPCR instruments with FAM and VIC detection channels, such as ABI 7500, Roche LightCycler480, and Bio Rad CFX96. This kit provides a Positive Control to confirm whether the kit is working properly. In addition, this kit also provides an Internal Control DNA to detect the presence of substances that may inhibit PCR reactions. If mycoplasma contamination is detected with this kit, contaminated cells can be discarded directly; if the cells are valuable, mycoplasma removal reagents can be considered for cell treatment.

Components and Storage

Components	100 Tests	Storage
2× Probe qPCR Mix	1 mL	-20°C away from light
Primer Probe Mix	200 μL	-20°C away from light
Internal Control DNA	100 μL	-20°C
Positive Control	200 μL	-20°C
Nuclease-free Water	1 mL	-20°C

Shipping: Dry ice	Shelf life: 1 year

Protocol

1. **Sample preparation:** If cells are cultured with cell culture media, the culture supernatant cultured for 3-6 days can be taken directly as the detection sample. If a cell suspension is used, it is necessary to extract DNA and then perform the PCR reaction.

***Note:** Penicillin and streptomycin do not affect the test.

2. **qPCR:**

1) Thaw and mix the various solutions required for the qPCR reaction in advance and place them in an ice bath. For a 96-well plate, set up the PCR system at room temperature or in an ice bath according to the table below. Template refers to the experimental sample, Positive Control, or Negative Control. The Negative Control can use Nuclease-free Water. It is recommended to set up positive and negative controls for each experiment.

Reagents	Volume
2× Probe qPCR Mix	10 μL
Primer Probe Mix	4.5 μL
Template	2 μL
Internal Control DNA	1 μL
Nuclease-free Water	2.5 μL
Total	20 μL

***Note:** If it is determined that there are no substances inhibiting the PCR reaction, Internal Control DNA can be omitted.

2) Gently mix the system with a pipette, and centrifuge at room temperature for a few seconds to collect the liquid at the bottom of the tube.

3) Place the prepared PCR tubes or PCR plates on the qPCR instrument and start the PCR reaction.

4) The fluorescent group of the mycoplasma probe is FAM, select the FAM detection channel, the quenching group is BHQ3, if there is no BHQ3, select none; the fluorescent group of the Internal Control DNA probe is VIC, select the VIC/HEX/JOE detection channel, the quenching group is TAMRA, if there is no TAMRA, select none. At the same time, set the instrument's fluorescence reference to "None". For ABI series instruments, set the "Passive Reference" to "None".

5) Perform the PCR reaction under the following conditions:

PCR Condition	Temp	Time
Step 1 (Initial denaturation)	95°C	2 min
Step 2 (Denaturation)	95°C	15 s
Step 3 (Annealing/ Extension)	60°C	20 s
Step 4	Go to Step2 for 40 cycles	

3. **Result Analysis:**

1) If positive and negative controls are performed, the results must meet the following conditions to be considered normal. If not performed, skip this step.

Template	FAM Signal	VIC Signal
Positive Control	Typical S-shaped amplification curve and Ct≤33	Typical S-shaped amplification curve and Ct≤33
Negative Control	No typical S-shaped amplification curve or Ct>35	Typical S-shaped amplification curve and Ct≤33

1) Sample Result Judgment:

FAM Signal	VIC Signal	Sample Result Judgment
Typical S-shaped amplification curve and Ct≤33	Typical S-shaped amplification curve and Ct≤33	Positive
No typical S-shaped amplification curve or Ct>35	Typical S-shaped amplification curve and Ct≤33	Negative
33<Ct≤35	Typical S-shaped amplification curve and Ct≤33	Suspicious, retest the sample culture 2-5 days later
No typical S-shaped amplification curve or Ct>35	No typical S-shaped amplification curve or Ct>35	PCR inhibition, appropriately dilute the sample or extract DNA for detection

4. **Appendix:** The types of mycoplasmas that this kit can detect

Species	Species	Species
Mycoplasma agalactiae	*Mycoplasma cloacale*	***Mycoplasma hominis***
Mycoplasma alkalescens	*Mycoplasma columbinasale*	*Mycoplasma hyopharyngis*
Mycoplasma anseris	*Mycoplasma columbinum*	*Mycoplasma hyopneumoniae*
Mycoplasma arginini	*Mycoplasma conjunctivae*	***Mycoplasma hyorhinis***
Mycoplasma arthritidis	*Mycoplasma cynos*	*Mycoplasma lagogenitalium*
Mycoplasma bovigenitalium	*Mycoplasma dispar*	*Mycoplasma leachii*
Mycoplasma bovirhinis	*Mycoplasma edwardii*	*Mycoplasma maculosum*
Mycoplasma bovis	***Mycoplasma fermentans***	***Mycoplasma mycoides***
Mycoplasma bovoculi	*Mycoplasma flocculare*	*Mycoplasma neuro/yticum*
Mycoplasma californicum	*Mycoplasma gallinaceum*	***Mycoplasma orate***
Mycoplasma canadense	*Mycoplasma gallinarum*	*Mycoplasma ovipneumoniae*
Mycoplasma capricolum	*Mycoplasma gallopavonis*	***Mycoplasma salivarium***
Mycoplasma citelli	*Mycoplasma glycophilum*	***Mycoplasma synoviae***

Note

1. Since qPCR reactions are very sensitive, it is best to perform operations in a standard PCR laboratory. The PCR reaction setup area should avoid various potential sources of amplification product contamination. It is recommended not to tear open PCR sealing films or open PCR tube caps in the PCR reaction setup area. PCR products should be treated according to the requirements for amplified products after sealing to avoid high-concentration PCR product contamination.
2. It is recommended to use a tip with a filter element to perform the PCR reaction to minimize false positives.
3. It is recommended to prepare the PCR reaction with a dedicated pipette.
4. Wear a disposable mask throughout the experiment and avoid talking as much as possible to avoid

mycoplasma contamination in saliva.

5. If mycoplasma contamination is indeed present after detection, the contaminating cells can generally be discarded directly. However, if cells are precious, consider using Mycoplasma Remover Reagents (catalog number: C7201) to eliminate contamination. Meanwhile, use Mycoplasma Prevention Reagent (catalog numbers: C7202 or C7203) in routine cell culture.
6. For your safety and health, please wear lab coats and gloves during the experiment.
7. For research use only. Not to be used in clinical diagnostic or clinical trials.

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