Probe qPCR Detecting All Mycoplasma Species in One Reaction Tube

Product Name: Mycoplasma Detection qPCR Kit Cat. #: T42030 Size: 100 reactions

DESCRIPTION

Mycoplasma Species qPCR Kit has been designed to specifically identify the mycoplasma genus species in a single PCR reaction using real-time quantitative polymerase chain reaction (qPCR) and probe fluorescence labels. The detection of the target DNA confirms ingredient authenticity and prevents food fraud, ethical issues, or health concerns. Mycoplasma qPCR is very sensitive, accurate, and High efficiency (See Fig.1, and Table 1).

PRINCIPLE

Authenticating ingredients utilizes real-time PCR which is based on the amplification of a specific region of the relevant target genome. The amplified product is detected using target-specific fluorescent probes that bind to the amplified product. As the PCR product accumulates, there is an increased fluorescent signal from the bound probes. Monitoring the fluorescence intensities during the PCR run allows the detection of the accumulating PCR product in real time.

Mycoplasma qPCR Kit includes mycoplasma positive and negative controls, PCR internal controls labeled with Hex, a qPCR super mix, and the primer-probe mix in which the probe has been labeled with FAM for the target gene. These aid in a straightforward interpretation of the results.

KEY FEATURES

- ❖ High sensitivity and specificity for mycoplasma species.
- ❖ High efficiency: the optimal systemic conditions for PCR amplifications.
- ❖ Streamlined protocol: Just add DNA Template and water.
- ❖ No cross reactivity with other species.

APPLICATIONS

Detect mycoplasma-target DNA in contaminated cell cultures, FBS medium, plant, cannabis, cannabis ingredients, grain, food, herbals, and animal feed.

KIT CONTENTS

Name	100x rxn	200x rxn
Myco qPCR Mix	0.8mL	1.6mL
Myco Primer-probe Mix	0.6mL	1.2mL
Myco Positive Control DNA	50µL	100µL
Negative Control	50µL	100µL
Nuclease Free Water	1mL	2x 1mL

The mycoplasma target probe has been labeled with FAM while the PCR internal control has been labeled with Hex.

STORAGE CONDITION

Ship at 4°C. Store at -20°C for long-term storage. Shelf life of 12 months after receipt.

SAMPLE DNA PREPARATION

Three sample DNA preparation methods are recommended as below. These methods are validated with cultured mycoplasma medium. The typical data is shown in the fig. 3. *Note: we recommend client to optimize a suitable method for a specific sample.*

Method A: Boiling Sample

Add 200µL of the Mycoplasma Cell Culture into a 1.5 mL screw capped microtube and place it into the boiling water for 7min. Afterwards, spin it at 13000rpm for 1min. The supernatant can be used as DNA for qPCR amplification or stored at -20°C for further use.

Method B: Enrichment plus Boiling (Recommend)

Add 1mL of the Mycoplasma Cell Culture into a 1.5 mL screw capped microtube. Then, spin it at 13000rpm for 10min to sediment the mycoplasma particles. After then, pour out the supernatant into the waste and resuspend the pellets in 50µL 1x TE buffer. Boiling it for 10min. Spin it at 13000rpm for 1min. This is the enriched DNA sample for qPCR amplification or stored at -20°C for further use.

Method C: Simple DNA Extraction (Enrichment + Extraction)

The sediment of the mycoplasma particles is as same as Method B. Afterwards, pour out the supernatant, and add 40µL of Fast DNA extract (T6008), incubate at 67°C in the water bath for 15min. Then add 160µL of DEPC water and boiling for 10min. This is the extracted DNA sample for qPCR amplification or stored at -20°C for further use.

PCR PROTOCOL

1. Set up PCR reaction for each sample in 20µL

Reaction Component	Volume (µL)
qPCR Super Mix	7.0
Primer-probe Mix	5.0
Nuclease-free Water	3.0
DNA sample	5.0
Final Volume	20µL

The Positive Control (5µL DNA/reaction) and Negative Control (5µL DNA/reaction) should be included in PCR Test. In addition, Positive control can be used for Standard curve as 10x sequentially dilution. The Copy number is labeled in the vial.

2. Suggested PCR conditions

Step	Amplification	PCR	
	HOLD	CYCLE (40x cycles)	
		Denature	Anneal/ Extend
Temperature	95°C	95°C	60°C
Time	2 min	15 sec	30 sec

DATA ANALYSIS

Positive Reaction: Sample Ct ≤ 37 w/ Positive, Negative and Blank controls normal.
Negative Reaction: Sample Ct ≥ 38 w/ Positive, Negative and Blank controls normal.
PCR internal control is positive in all samples, positive and negative controls. The positive response indicates a normal PCR amplification. Otherwise, the PCR reaction may be inhibited.
Repeat Reaction: If one of the control reactions is not normal, PCR reaction is failed, and should be repeated.

Fig.1. Mycoplasma DNA concentration dependent qPCR Amplification (FAM)

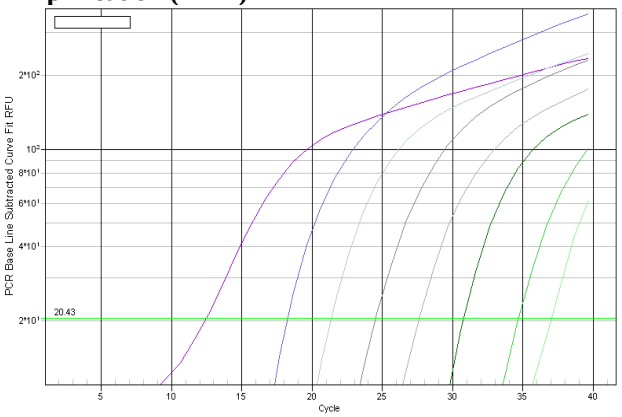

Fig.2 Internal Control Gene qPCR Amplification (Hex)

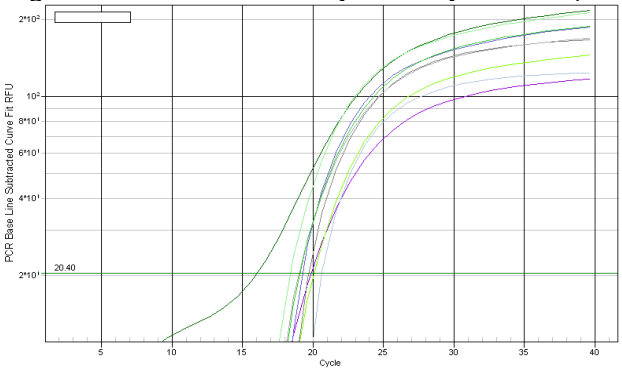

Table 1: Mycoplasma DNA qPCR Sensitivity

MycoDNA test (GC/ml)	Threshold Cycle
10^8	12.45
10^7	18.2
10^6	21.32
10^5	24.48
10^4	27.59
10^3	30.76
10^2	34.66
10^1	36.95
Negative Control	N/A

Fig.3. Mycoplasma DNA preparation method validation

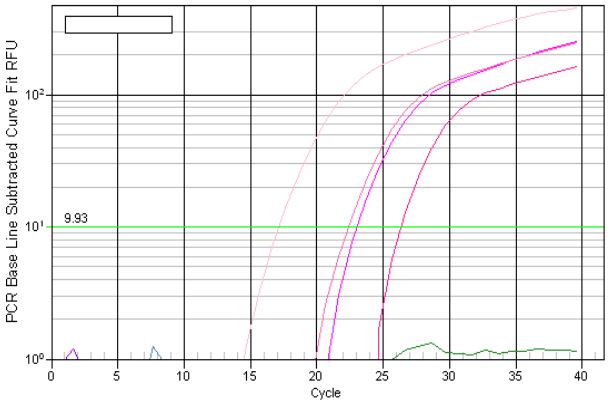

Table 2 Mycoplasma DNA efficiency comparison from 3 methods

Mycoplasma DNA	Threshold Cycle (Ct)
Method A: Boiling	26.44
Method B: Enrichment	23.11
Method C: Simple DNA extraction	22.50
Positive	17.24
Negative	N/A

RELATIVE PRODUCTS

T6025: Microbial DNA Magnetic Extraction
T 42020: Universal Aspergillus qPCR
T42021: Aspergillus Flavus qPCR
T42022: Aspergillus Fumigatus qPCR
T42023: Aspergillus Niger qPCR
T42024: Aspergillus Terreus qPCR
T42025: 4-In-1 Aspergillus qPCR
T42026: O157H7 E. Coli qPCR
T42027: STEC qPCR
T42028: Salmonella qPCR
T42029: STEC and Salmonella Multiple qPCR
T42031: Listeria Monocytogenes qPCR
T42033: Bacillus Cereus qPCR
T42033: Bacillus Species qPCR

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