Mycoplasma Titer qPCR (TBS42030C)

DESCRIPTION

Mycoplasma Titer qPCR is designed to quantitatively detect the mycoplasma levels in test samples using real-time quantitative polymerase chain reaction (qPCR) and probe fluorescence labels. It provides a sensitive, accurate and simple method to titer mycoplasma concentration in testing samples (See Fig.1, and Table 4).

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.

Tribioscience's Mycoplasma Titer 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

- ❖ Quantitate mycoplasma concentration.
- ❖ High sensitivity and specificity for mycoplasma species.
- ❖ High efficiency: the optimal systemic conditions for PCR amplifications.
- ❖ Streamlined protocol: Just add DNA Template and water.

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
Myco qPCR Mix	0.8mL
Myco Primer-probe Mix	0.6mL
Myco Positive Control DNA (10 ^8 GC)	50µL
Negative Control	50µL

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

STORAGE CONDITION

The kit is shipped on ice and stored at -20°C for long-term storage. Shelf life of 12 months after receipt.

SAMPLE DNA PREPARATION

Four sample DNA preparation methods are recommended as below. These methods are validated with cultured mycoplasma medium. The typical data is shown in Fig. 3. We recommend clients optimize a suitable method for a specific sample.

DNA Extraction 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.

DNA Extraction Method B: Enrichment plus Boiling

Add 2mL of the Mycoplasma Cell Culture into a 2 mL screw capped microtube. Then, spin it at 13500rpm 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 10000rpm for 1min. This is the enriched DNA sample for qPCR amplification or stored at -20°C for further use.

DNA Extraction 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 (TBS6008), 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.

DNA Extraction Method D: Microbial DNA Magnetic Extraction (Recommend)

Microbial DNA Magnetic Extraction method is widely used for DNA extraction from different microorganisms. The kit catalog is TBS6025.

PCR PROTOCOL

1. **Standard Control DNA Preparation**: The mycoplasma Control DNA is $1x10^8$ GC/mL. Perform 5 serial dilutions of the Standard Control DNA at 10-fold manner by diluting 2 µL Standard DNA into 18 µL nuclease-free water in each concentration. Dilutions 1/10 to 1/10,000,000 will be used for generating the standard curve. The standard preparation is listed in table 1:

Table 1: Standard Preparation

Dilution	Mycoplasma (GC/mL)
1/10	$1x10^7$
1/100	$1x10^6$
1/1000	$1x10^5$
1/10000	$1x10^4$
1/100000	$1x10^3$
1/1000000	$1x10^2$
1/10000000	$1x10^1$

2. Set up PCR reaction for each sample in 20µL as Table 2

Table. 2: PCR Reaction Preparation

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

3. Set up PCR amplification conditions as Table 3:

Table 3: PCR Coditions

Step	Amplification	PCR	
	Pre-denature	CYCLE (40x cycles)	
		Denature	Anneal/ Extend
Temperature	94°C	94°C	60°C
Time	1 min	10 sec	60 sec

DATA ANALYSIS

The mycoplasma concentrations in samples can be calculated in accordance with the standard curve of DNA amplification standard curve.

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

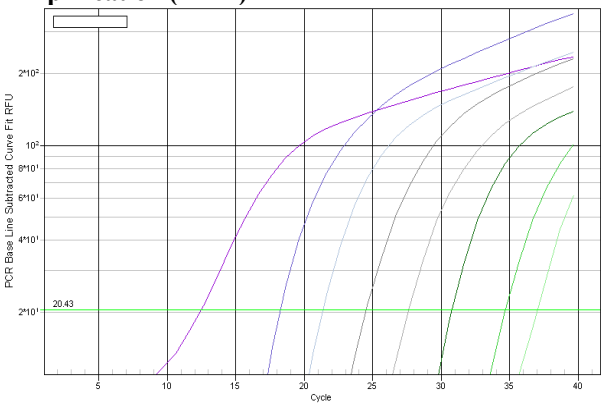

Fig.2. Internal Control Gene qPCR Amplification (Hex)

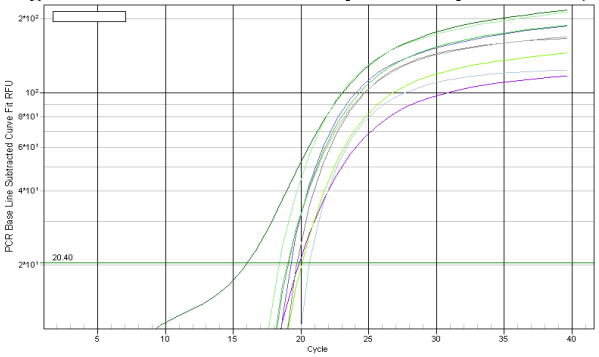

Table 4: Mycoplasma DNA qPCR Sensitivity

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

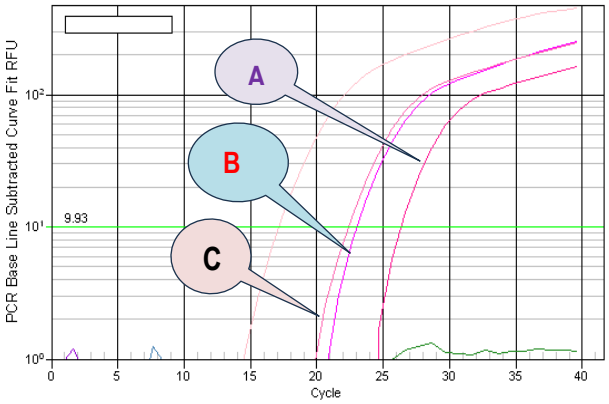

Table 5: Mycoplasma DNA amplification comparison from 3 DNA extraction methods

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

TBS6025: Microbial DNA Magnetic Extraction
TBS42020: Universal Aspergillus qPCR
TBS42021: Aspergillus Flavus qPCR
TBS42022: Aspergillus Fumigatus qPCR
TBS42023: Aspergillus Niger qPCR
TBS42024: Aspergillus Terreus qPCR
TBS42025: 4-In-1 Aspergillus qPCR
TBS42026: O157H7 E. Coli qPCR
TBS42027: STEC qPCR
TBS42028: Salmonella qPCR
TBS42029: STEC and Salmonella Multiple qPCR
TBS42031: Listeria Monocytogenes qPCR
TBS42033: Bacillus Cereus qPCR
TBS42033: Bacillus Species qPCR

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