DfCell Mycoplasma RT-qPCR Detection Kit

Product description

DfCell Mycoplasma RT-qPCR Detection Kit is a rapid qualitative test based on Nucleic Acid Amplification Techniques (NAT) designed to detect potential Mycoplasma contamination in raw materials, cell banks, virus seeds, virus or cell harvests, and therapeutic cell products. This assay kit utilizes quantitative PCR technology employing a multiplex PCR approach with two fluorescent probes, FAM and VIC, to respectively detect the target sequence and internal reference. It covers over 100 Mycoplasma DNA sequences and has undergone rigorous validation for specificity, detection limit, and durability according to EP 2.6.7 standards. The detection limit meets the requirement of ⩽10 CFU/mL, demonstrating high sensitivity, excellent specificity, and safety.

Specifications

Product Number	C230106E / C230106S
Specifications	25 T / 100 T

Components

Component Number	Component Name	C230106E	C230106S
C230106-A	4×qPCR Reaction Buffer	250 μL	1 mL
C230106-B	Primer & Probe Mix	25 μL	100 μL
C230106-C	Internal Control (IC)	25 μL	100 μL
C230106-D*	Positive Control (PC)	500 μL	2 mL
C230106-E**	DNA Dilution Buffer	1 mL	4×1 mL
C230106-F***	ddH_2O	500 μL	2×1 mL

*PC：Positive control solution, with a concentration of 1, 000 copies/μL;

**DNA Dilution Buffer: Used for sample and IC dilution, as well as template for NTC and NC;

***ddH_2O: Used for preparing qPCR Mix system.

Storage

Store at temperatures ranging from -15°C to -25°C with a shelf life of one year.

Please note that component C230106-B should be stored protected from light.

Notes

1. Please read this manual carefully before using this reagent. Experiments should be conducted according to standard procedures, including sample handling, preparation of reaction system, and addition of samples.

2. Sample addition and liquid handling steps should be performed on ice whenever possible.
3. Each component should be shaken well and low-speed centrifuged before use.
4. For your safety and health, wear lab coats and disposable gloves during operation.
5. For research use only.

Instructions

1. Experimental Preparation

1) Prepare the required reagents and consumables in advance.

2) Confirm instrument compatibility:

The qPCR machines compatible with this assay kit include but are not limited to the following models:

A: Bio-Rad: CFX96

B: Thermo Scientific: 7500 Real-Time PCR System; QuantStudio™ 5

2. Experimental Method

1) Extraction of Test Sample DNA

The magnetic bead-based extraction kits are recommended for nucleic acid extraction. This assay kit (Cat#C230106) includes an Internal Control (IC). If the IC is added to the sample before DNA extraction, it can validate the entire process (including DNA extraction and qPCR reaction). If the IC is directly added to the qPCR Mix, it will serve only as a qPCR control.

2) Preparation of qPCR Reaction System

a. Based on the number of samples to be tested, including Positive Control Solution (PC), No Template Control (NTC), Negative Control Solution (NC), and Test Samples (TS), calculate the required number of reaction wells according to the experimental design, generally with 2 replicate wells per sample.

*PC and NTC are samples that do not require pre-extraction treatment; NCS and TS are samples that require pre-extraction treatment.

Number of reaction wells (M1) = (1×NC + N×TS) × 2

Number of reaction wells (M2) = (1×PC + 1×NTC) × 2

Number of reaction wells (M3) = (1×PC + 1×NTC + N×TS) × 2

b. According to the experimental design and the corresponding reaction system in the table below, thaw the required reagents on ice in advance.

c. Calculate the required volume of qPCR Mix based on the number of reaction wells. Note that if this product is used for GMP activities such as product release, it is recommended to configure the system according to Table 1 and Table 2. If it is only used for research experiments, users can evaluate the experimental conditions and decide whether to add IC to TS before extraction or whether NCS control is unnecessary, and configure the system according to Table 3. Not all M1, M2,

M3 systems need to be configured.

Table 1 qPCR Mix System Corresponding to Reaction Wells (M1)

Component	Volume(1×40 μL reaction system)	Volume (M1×40 μL)
4×qPCR Reaction Buffer	10 μL	(M1+2) ×10 μL
Primer & Probe Mix	1 μL	(M1+2) ×1 μL
ROX	x μL /0 μL**	(M1+2) ×x μL /0 μL
ddH_2O	Up to 20 μL	Up to (M1+2) ×20 μL
Total	20 μL	(M1+2) ×20 μL

* The configuration system in Table 1 is based on the premise that both NC and TS have IC added before extraction, so there is no need to add IC again when configuring qPCR Mix. Method for adding IC before extraction: Firstly, dilute the IC in the kit with DNA Dilution Buffer at a 20-fold dilution. Add 1 μL of diluted IC to every 100 μL of sample to be tested before extraction.

** This assay kit does not contain ROX reference dye. If your current Real Time PCR instrument requires the addition of ROX reference dye, please refer to ROX instructions for specific ROX addition. If ROX reference dye is not required, add a volume of 0 μL.

Table 2 qPCR Mix System Corresponding to Reaction Wells (M2)

Component	Volume (1×40 μL reaction system)	Volume (M2×40 μL)
4×qPCR Reaction Buffer	10 μL	(M2+2) ×10 μL
Primer & Probe Mix	1 μL	(M2+2) ×1 μL
Internal Control (IC)	1 μL*	(M2+2) ×1 μL/0 μL
ROX	x μL/0 μL**	(M2+2) ×x μL/0 μL
ddH_2O	Up to 20 μL	Up to (M2+2) ×20 μL
Total	20 μL	(M2+2) ×20 μL

* The configuration system in Table 2 is based on the premise that both PC and NTC do not have IC added before extraction, so IC needs to be added when configuring qPCR Mix. Method for adding IC after extraction: Dilute the IC with DNA Dilution Buffer at a 100-fold dilution and add 1 μL to each reaction system.

** This assay kit does not contain ROX reference dye. If your current Real Time PCR instrument requires the addition of ROX reference dye, please refer to ROX instructions for specific ROX addition. If ROX reference dye is not required, add a volume of 0 μL.

Table 3 qPCR Mix System Corresponding to Reaction Wells (M3)

Component	Volume (1×40 μL reaction system)	Volume (M3×40 μL)
4×qPCR Reaction Buffer	10 μL	(M3+2) ×10 μL
Primer & Probe Mix	1 μL	(M3+2) ×1 μL
Internal Control (IC)	1 μL*	(M3+2) ×1 μL /0 μL
ROX	x μL /0 μL**	(M3+2) ×x μL /0 μL
ddH_2O	Up to 20 μL	Up to (M3+2) ×20 μL
Total	20 μL	(M3+2) ×20 μL

* The configuration system in Table 3 is based on the premise that IC is not added to the sample before extraction, so IC needs to be added when configuring qPCR Mix. Method for adding IC after extraction: Dilute the IC with DNA Dilution Buffer at a 100-fold dilution and add 1 μL to each reaction system.

** This assay kit does not contain ROX reference dye. If your current Real Time PCR instrument requires the addition of ROX reference dye, please refer to ROX instructions for specific ROX addition. If ROX reference dye is not required, add a volume of 0 μL.

3) Sample Addition

a. Thoroughly vortex and mix the qPCR Mix, then centrifuge at low speed to collect any residual liquid at the bottom of the tube.

b. Dispense 20 μL of the corresponding sample's qPCR Mix into each well of the reaction tube. Note that the qPCR Mix dispensed into each sample tube should match the qPCR Mix configured in the previous step of "Preparation of qPCR Reaction System" and correspond to the samples to avoid misallocation.

c. Add the sample to the reaction tube containing the previously dispensed qPCR Mix, referring to Table 4.

Table 4 Sample Addition Examples

Sample	To each tube or well, add...
TS	20 μL qPCR Mix + 20 μL purified test sample
NTC	20 μL qPCR Mix + 20 μL DNA dilution buffer
NC*	20 μL qPCR Mix + 20 μL NCS purification solution
PC	20 μL qPCR Mix + 20 μL positive control

*The final reaction volume per tube or well is 40 μL.

**NC is recommended to be pretreated using DNA dilution buffer as the sample.

***Note: Cover the reaction tube with a cap or apply an optical film. To avoid affecting fluorescence signal detection, do not make any markings on the tube cap or film, or repeatedly rub with a scraper.

****After completing the sample addition, briefly centrifuge the reaction tubes or reaction plate at

low speed, then thoroughly vortex and mix. Followed by another short low-speed centrifugation, collect any residual liquid from the tube cap and tube walls to the bottom of the tube or plate. Try to avoid bubble formation during this process. This step is crucial; inadequate mixing or incomplete mixing can affect the stability of the baseline.

4) qPCR Program Parameter Settings

a. Program File Settings:

Taking the Thermo Scientific 7500 Real-Time PCR System instrument and Real-Time PCR Software v2.4 as an example:

Instrument Type: 7500 (96 Wells)

Experiment Type: Quantitation-Standard Curve

Reagent for detecting target sequences: Taqman® Reagents

Program Speed: Standard (~2 hours to complete a run)

b. Detection Channel Settings:

In "Plate Setup" under "Define Targets and Samples", create Target 1 channel (FAM) and select FAM as the reporting fluorophore, and MGB or none as the quencher fluorophore. Create Target 2 channel (VIC) and select VIC as the reporting fluorophore, and none as the quencher fluorophore. In "Plate Setup" under "Assign Targets and Samples", select "none" if no additional ROX dye is added. Select "ROX" if ROX dye is added.

c. Standard Amplification Program Settings:

Table 5 Standard Amplification Program

Number	Reaction Phase	Temperature	Time	Number of Cycles
1	Pre-denaturation	95°C	5 min	1
2	Denaturation	95°C	15 sec	45
3	Annealing/Extension (Fluorescence signal collection)	62°C	30 sec	

d. Baseline and Threshold Settings:

Baseline Adjustment Principle: The baseline is typically set automatically. If manual adjustment is required, the starting cycle for the baseline should be selected before the exponential growth phase, avoiding fluctuations in the region of initial fluorescence collection. The endpoint should be chosen within 1-2 cycles before the earliest appearance of the Ct value for samples undergoing exponential amplification.

Threshold Setting Principle: Automatic thresholding is generally employed. If manual adjustment is needed, the threshold line should be set above the negative control or baseline noise. Typically, it is set in the later stage of the exponential growth phase where sample repeatability is good. Different channels should have independently set and appropriate threshold lines.

5) Result Analysis

a. Judgment of PC, NTC, and NC Results:

If an internal control (IC) is included in the reaction system, each quality control sample must meet the conditions outlined in Table 6.

Table 6 Judgment of PC, NTC, and NC Results

Quality Control Sample	FAM Signal	VIC Signal
PCS	Ct < 40 and clear amplification curve	Ct < 40 and clear amplification curve
NTC	Ct ⩾ 40 or no clear peak	Ct < 40 and clear amplification curve
NCS	Ct ⩾ 40 or no clear peak	Ct < 40 and clear amplification curve

If no IC is added in the reaction system: Each quality control sample needs to meet the conditions in the FAM signal column of Table 6; there is no need to analyze the VIC channel.

b. Judgment of Test Sample (TS) Detection Results:

Prerequisite: Before assessing the TS detection results, it is necessary to first determine whether each quality control sample (PC, NTC, and NC) meets the standards in Table 6. If they pass, further analysis can proceed. If they fail, the TS sample results may be unreliable, requiring investigation into the cause.

If an IC is added in the reaction system: Determine the judgment of the TS sample results based on the results of both the FAM and VIC signals, as specified in Table 7.

Table 7 Judgment of Test Sample Results (with IC)

FAM Signal	VIC Signal	Result Judgment
Ct < 40 and clear amplification curve	Ct < 40 and clear amplification curve	Positive
	Ct ⩾ 40 or no clear peak	*Inhibition, repeat experiment*
Ct ⩾ 40 or no clear peak	Ct < 40 and clear amplification curve	Negative
	Ct ⩾ 40 or no clear peak	*Inhibition, repeat experiment*

*If VIC signal is inhibited, retesting is required, or appropriate sample treatment is needed to eliminate inhibitory factors.

If no IC is added in the reaction system: There is no need to analyze the VIC channel; judgment of the results should be based solely on the results of the sample's FAM signal as found in Table 8.

Table 8 Judgment of Test Sample Results (without IC)

FAM Signal	Result Judgment
Ct < 40 and clear amplification curve	Positive
Ct ⩾ 40 or no clear peak	Negative