Instructions for Use of Mycoplasma DNA Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-ZY002

Introduction

The Mycoplasma DNA detection kit is used to qualitatively detect the presence of mycoplasma contamination in master cell banks, working cell banks, virus seed lots, control cells, and cells for clinical therapy.

The kit uses qPCR-fluorescent probe technology to verify with reference to mycoplasma detection-related requirements in EP2.6.7 and JPXVII. It can cover more than 100 mycoplasmas and has no cross reaction with closely related strains. The detection is rapid which can be completed within 2 hours, with strong specificity.

Specification

50 Reactions.

Kit components

No.	Components	Specification	Storage conditions
1	Mycoplasma Primer&Probe MIX	120 μL × 1 vial	Stored at -20℃ and below away from light
2	2×qPCR Reaction MIX	700 μL × 1 vial	Stored at -20℃ and below
3	Internal control	220 μL × 1 vial	
4	Positive control	500 μL × 1 vial	
5	DNA diluent	1.5 mL × 3 vials	

Storage conditions and shelf life

The kit should be stored at -20℃ and below away from light. The shelf life is 12 months. After opening, unused kits should be kept under the specified storage conditions. See the kit label for the date of manufacture and shelf life.

Operating methods

I. Preparation

- Kit preparation

Place all kit components on ice or at 4°C to ensure adequate dissolution before operations.

- Consumables and equipment to be self-prepared:

1. Quantitative fluorescence PCR instrument, vortex mixer and centrifuge
2. High-precision pipettes with disposable sterile enzyme-free low-adsorption filter tips (0.1-2.5 μL, 0.5-10 μL, 10-100 μL, 20-200 μL, 100-1000 μL)
3. 1.5 mL sterile low-adsorption centrifuge tubes
4. Sterile enzyme-free 8-tube strips or 96-well qPCR plates

II. Operation procedures

- Detailed operating steps

1. Extraction of samples to be tested:

It is recommended to extract sample DNA using "Mycoplasma DNA Sample Preprocessing Kit (HG-CL200)" from Hillgene. Add 4 μL of internal control while adding samples. It is recommended to extract DNA diluent as the negative control while extracting the sample.

2. Preparation of qPCR reaction liquid:

1) Calculate the number of wells required by reaction according to the number of reference standards to be tested and the number of samples to be detected (generally 2-3 replicate wells per sample).

Number of reaction wells = (1 NTC + 1 PC + number of samples to be tested) × number of replicate wells

2) Calculate the total amount of qPCR MIX required this time based on the number of reaction wells:

qPCR MIX = (number of reaction wells + 2 or 3) × 12.5 μL (2×qPCR Reaction MIX) + (number of reaction wells + 2 or 3) × 2.1 μL (mycoplasma Primer&Probe MIX) + (number of reaction wells + 2 or 3) × 0.4 μL (Internal Control) (2 or 3 is the amount of operation loss)

3) Place the reagents used on ice to completely dissolve, gently shake to mix well, centrifuge instantaneously and prepare according to the following table:

Table 2 qPCR MIX Preparation Table

Components	Volume for single reaction
2×qPCR Reaction MIX	12.5 μL
Mycoplasma Primer&Probe MIX	2.1 μL
Internal control	0.4 μL
Total volume	15 μL

[Note]: If the internal control has been added at the time of extraction, an equal volume of DNA diluent should be used instead for preparation of qPCR MIX.

4) Gently shake to mix well the prepared qPCR MIX centrifuge instantaneously and dispense into 8-tube strips or 96-well plates at 15 μL/well.

5) Add the DNA diluent to the 8-tube strips or 96-well plates with dispensed qPCR MIX at 10 μL/well. See Table 4 for details. Melted unused DNA diluent may be stored temporarily at 2-8℃ or at -20℃ if not used for a long time.

3. Loading of qPCR:

1) Place the reagents required on ice, gently shake to mix well, centrifuge instantaneously and load samples (total volume: 25 μL)

Table 4 Examples of Sample Loading in Each MIX Reaction Well

Positive group	10 μL of each positive template + 15 μL of qPCR MIX
Negative group	10 μL of each DNA diluent + 15 μL of qPCR MIX
Experimental group	10 μL of each sample to be tested + 15 μL of qPCR MIX

2) Sterile enzyme-free 8-tube strips or 96-well plates can be used for the reaction in the experiment. With bubbles removed, the reaction system should be centrifuged to the bottom of the tube to prepare the reaction.

Setting of PCR procedure:

Select FAM as fluorophore and None as quencher for mycoplasma detection, select CY5 as fluorophore and None as quencher for internal control, and select ROX as reference fluorescence (based on the instrument requirements).

Reaction procedure:

Stage1	Contamination digestion	Reps: 1	37℃	2 min
Stage2	Pre-denaturation	Reps: 1	95℃	30 s
Stage3	Circular reaction	Reps: 45	95℃	10 s
			58℃	34 s

Set the program at 58°C for 34 s for fluorescence collection; the reaction volume is 25 μL.

Result analysis

	FAM signal	CY5 signal	Result determination
Negative group	CT ≥ 40 or no "S" amplification curve	CT <40 with "S" amplification curve	Negative
Positive group	CT <40 with "S" amplification curve	CT <40 with "S" amplification curve	Positive
Experimental group	CT ≥ 40 or no "S" amplification curve	CT <40 with "S" amplification curve	Negative
		CT ≥ 40 or no "S" amplification curve	With inhibition
	CT <40 with "S" amplification curve	CT <40 with "S" amplification curve	Positive
		CT ≥ 40 or no "S" amplification curve	With inhibition

Precautions

1. The kit has been validated for the stability (freezing-thawing and other factors) and does not require dispensing.
2. The preparation and sample loading for negative samples (2×qPCR Reaction MIX, Primer&Probe MIX and DNA diluent, etc.) and positive samples (PC and samples to be tested, etc.) should be separated into different environments and should not be operated in one area. The preparation personnel should wear neat masks, gloves and cleanroom garment.
3. Tips shall be changed between different loading steps in time to avoid cross-contamination and long-time opening. And liquid wall-hanging shall be avoided during the use of a pipette.
4. The kit must be used within the shelf life. It is not recommended to mix different batches of relevant reagents for use.
5. It is recommended that all components of the kit should be thawed at a low temperature before use and should be completely dissolved. And all components should be fully mixed before use so as to ensure the homogeneity of reagents. And the reagents mixed up should be centrifuged for a short time in order to let liquid on the tube wall or cap gather at tube bottom. If any precipitate is found in DNA diluent, incubation at 37°C is recommended to completely dissolve the DNA diluent.
6. The best detection effect can be ensured only by strictly following the instructions and using all the reagents provided with this kit
7. The final test results are closely related to the efficacy of reagents, operator's operation methods and test environment.
8. Our company is only responsible for the kit itself and not for the sample consumption caused by the use of the kit. Users should fully consider the possible usage amount of sample before use and reserve sufficient samples.
9. The kit is for in vitro research use only and is not used for clinical diagnosis.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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