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-ZY001

Introduction

The Mycoplasma DNA Detection Kit has been validated in accordance with criteria for mycoplasma testing in EP2.6.7 and JPXVII and can be used to detect the presence of mycoplasma contamination in master cell banks, working cell banks, cells for clinical therapy, and biological products.

The kit has been validated in accordance with criteria for mycoplasma testing in EP2.6.7 and JPXVII with fluorescence probe qPCR technology. The detection is rapid and can be completed within 2 hours, with potent specificity.

Specification

100 Reactions.

Sensitivity and Specificity

The sensitivity of this kit has been tested for 10 mycoplasma 10CFU standards mentioned in EP2.6.7 and JP XVII (purchased from MB), all of which could reach 10 CFU/mL, and the test results of 3 mycoplasma-associated bacteria mentioned in EP2.6.7 and JP XVII were negative. These results obtained met the sensitivity and specificity requirements of EP 2.6.7 and JP XVII.

Table 1. Test Results of 10 Mycoplasma Standards

Bacterial strain	Positives/total number of strains	Bacterial strain	Positives/total number of strains
Mycoplasma orale	24/24	Mycoplasma synoviae	24/24
Mycoplasma gallisepticum	23/24	Mycoplasma arginini	24/24
Acholeplasma leyeri	24/24	Mycoplasma hyorhinis	24/24
Mycoplasma fermentans	23/24	Spiroplasma limonii	24/24
Mycoplasma pneumoniae	24/24	Mycoplasma salivarius	24/24

The results showed that when each mycoplasma standard was tested at a concentration of 10 CFU/mL with this mycoplasma detection kit, the detection ratios were greater than 95%, which met the specificity and sensitivity requirements of the European and Japanese Pharmacopoeias.

Table 2. Test Results of Three Mycoplasmas Closely Related Bacteria

Bacteria name	Lactobacillus acidophilus	Streptococcus pneumoniae	Clostridium acetobutylicum
Test results	Negative	Negative	Negative

The results showed that three bacterial genomes closely related to mycoplasma were negative, which met the specificity requirements of European and Japanese pharmacopoeias.

Stability

After five freeze-thaw tests, the performance of the Mycoplasma DNA Detection Kit was not affected.

Test instrument

- CFX96 (Bio-Rad)

Kit components

Table 3. Kit Components

Components	Volume	Packaging
Buffer	2x750 μL	Clear tube with green cap
Primer/probe mix	400 μL	Brown tube
Internal control	2 x 1 mL	Clear tube with blue cap
Positive template	1 mL	Clear tube with red cap
Sterile water	2 x 1 mL	Clear tube

Storage and shelf life

Store at -20℃, with a shelf life of 24 months.

Procedure

1. Preparation of Master Mix

1.1 Thaw each reagent on ice, gently mix the Buffer by inversion and then centrifuge gently, and vortex the other reagents followed by gentle centrifugation.

1.2 Prepare Reaction Master Mix as follows:

Table 4. Master Mix Preparation

Reagent component	Amount per well
Buffer	15 μL
Primer/probe mix	4 μL
Internal control	1 μL
Total volume	20 μL

Note: This test is divided into 1 positive group, 1 negative group and N experimental groups, and each group should have 2 replicates; the total amount of master mix required should be calculated according to the number of reaction wells. If the internal control has been added to the samples in the experimental group during DNA extraction, the internal control should be replaced with sterile water when preparing the reaction master mix.

2. Dispensing and loading

2.1 Gently mix the mixed reaction master mix by pipetting, dispense into qPCR 96-well plate at 20 μL per well (when arranging negative control samples in a plate layout, it is preferable to place them in the "A" row, and add the positive group or experimental group every other hole to reduce the probability of contamination);

2.2 According to the following table, add the corresponding template to the bottom of reaction well (change the tip between samples to avoid contamination);

Table 5. Example of Loading in Each Reaction Well

Negative group	10 μL of sterile water + 20 μL of master mix
Positive group	10 μL of positive template + 20 μL of master mix
Experimental group	10 μL of test sample + 20 μL of master mix

2.3 Seal the 96-well plate with optical film, mix well by gentle shaking, quickly centrifuge and place in the qPCR instrument (avoid touching the optical film by hand).

3. QPCR program setup

3.1 Select the dual-channel hydrolysis probe method program (FAM is mycoplasma detection channel, while HEX is internal control detection channel) or set dual-channel detection according to different instruments used, FAM is mycoplasma detection channel, while HEX is internal control detection channel.

3.2 Set up the reaction program:

Table 6. Reaction Procedures

Phase	Pre-denaturation	Denaturation	Annealing/Extension
Temperature	95℃	95℃	60℃
Time	2min	5s	35s
Number of cycles	1	48	
Detection	None	None	Collected signal
Reaction volume	30 μL		

4. qPCR result analysis

Different instruments have different analytical methods, and check whether the amplification curve morphology is normal after analysis. After analysis, the test results are judged with reference to the following table:

Table 7. Criteria for Result Analysis

	FAM signal	HEX signal	Judgment results
Negative group	CT ≥ 40 or no "S"-shaped amplification curve	CT < 40 with "S"-shaped amplification curve	Negative
Positive group	CT < 40 with "S"-shaped amplification curve	CT < 40 with "S"-shaped amplification curve	Positive
Experimental group	CT ≥ 40 or no "S"-shaped amplification curve	CT < 40 with "S"-shaped amplification curve	Negative
		CT ≥ 40 or no "S"-shaped amplification curve	With inhibitory effects
	CT < 40 with "S"-shaped amplification curve	CT < 40 with "S"-shaped amplification curve	Positive
		CT ≥ 40 or no "S"-shaped amplification curve	With inhibitory effects

Note: If the HEX signal is inhibited, it is recommended to perform genome extraction followed by detection of the sample.

Disclaimer

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

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com info@eaglebio.com

Jiangsu Hillgene Biopharma Co., Ltd.
Address: Building 4, Yuewang Wisdom Valley, 1463 Wuzhong Avenue, Wuzhong District, Suzhou, China
T.400 900 1882 W.www.hillgene.com