Mycoplasma DNA (qPCR) Detection Kit

Catalog Number：M0311B-50

【Introduction to Kit】

Mycoplasma DNA (qPCR) Detection Kit are used to qualitatively detect the presence of mycoplasma contamination in master cell banks, working cell banks, viral seed lots, control cells, and cells for clinical treatment.This kit uses TaqMan fluorescence probe principle to quantitatively detect Mycoplasmal DNA in samples. The detection is fast, specific and reliable. This kit can cover more than 150 mycoplasma species.

【Components of the kit】

Table 1　Composition and storage conditions of the kit

Components	Total amount	Storage Conditions
Mycoplasma Primer&Probe MIX	120 μL×1 tube	-20℃ (keep in dark)
2×qPCR Reaction MIX	700 μL×1 tube	-20℃
Internal Control	220 μL×1 tube	
Positive Control	500 μL×1 tube	
DNA diluent	1.5 mL×3 tubes	

【Specifications】

50 Reactions

【Period of validity】

Valid for 12 months under the prescribed storage conditions

【Self provided equipment required for test】

Fluorescence quantitative PCR instrument

1.5mL centrifuge tube with sterile, and low retention

Optical 8-tube strip or 96-well qPCR plate with sterile, DNase/RNase-free and low retention

1000 μL, 200 μL,100 μL, 10 μL ,2.5 μLpipette gun

1000 μL, 200 μL,100 μL, 10 μL ,2.5 μL pipette tip with sterile, low retention and filter element

【Applicable models (including but not limited to)】

ABI QuantStudio 5 Real-Time PCR System

LightCycler 480 Real-Time PCR System

ABI 7500 Real-Time PCR System

Bio-Rad CFX Opus96 Real-Time PCR System

Archimed™ X Real-Time PCR System

【Testing procedures】

- **Preparation and sample adding of qPCR reaction solution**

1. Calculate the number of holes required for reaction according to the number of Positive Control and samples to be tested (generally 2 or 3 groups of multiple holes are made):

Number of reaction holes=(Positive Control+ NTC+sample to be tested)×2 or 3

2. Calculate the total amount of Mycoplasma qPCR MIX required according to the number of reaction wells: Mycoplasma 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 operating loss)

3. Put all reagents on ice to melt, shake slightly and mix well, and prepare Mycoplasma qPCR MIX as shown in Table 2.

Table 2 Preparation of Mycoplasma qPCR Mix

Components	Single reaction amount
2×qPCR Reaction MIX	12.5 μL
Mycoplasma Primer&Probe MIX	2.1 μL
Internal Control	0.4 μL

If IC was added at the time of extraction, an equal volume of DNA dilutions was replaced by IC when preparing qPCR MIX.

4. Put the required reagent on ice to melt, shake slightly and mix well, and add samples as shown in Table 3 (total volume 25μL).

Table 3 Sample adding examples of each reaction hole

Template	Template amount	Mycoplasma qPCR MIX amount
Positive	10 μL of Positive Control	15 μL
Template-free NTC	10 μL of each DNA diluent	15 μL
Sample to be tested	10 μL of each sample to be tested	15 μL

5. The test can be performed by using optical 8-tube strip or 96-well qPCR plate. Bubbles in the reaction system should be removed and centrifuged to be bottom of the tube.

- **qPCR reaction procedure and parameter setting**

Take American ABI 7500 Real-Time PCR System and software version 2.06 as an example.

1. Create a blank new program. Experiment type: Standard curve, Chemistry: TaqMan® Reagents, Run mode: Standard.

2. Use Add New Targets to add new probes and edit the probe names in Target Name, naming them Mycoplasma-DNA and Mycoplasma-DNA-IC respectively. Select the desired fluorescent and quenching groups in Reporter and Quencher.The fluorescent group of Mycoplasma-DNA is selected as FAM, the quenching group is selected as NONE, the fluorescent group of Mycoplasma-DNA-IC is selected as VIC, the quenching group is selected as NONE, and the reference fluorescence is ROX. Use Add New Samples to add new samples and name them in the corresponding Sample name column.

3. Set the reaction procedure

Stage1	Pollution to digest	Reps:1	37℃	2min
Stage2	Predenaturation	Reps:1	95℃	30s
Stage3	Cyclic reaction	Reps:45	95℃	10s
			58℃	34s

Stage3 is set to fluorescence collection at 58℃ for 34s.

4. Click the“Start Run”button in the Run interface to perform PCR measurement.

- **qPCR result analysis**

Take American ABI 7500 Real-Time PCR System and software version 2.06 as an example.

1. In the Plate interface, set the Task column of NTC (no template comparison) as “N”,set the Task column of Positive as “S”, set the Task column of samples to be tested as “U”, and name the samples in the corresponding Sample name column.

2. Click Analyze, and in the Amplification Plot panel of Results, preliminarily check whether the shape of the amplification curve is normal.

3. In the View panel of Results, you can read the Ct detection value of NTC 、Positive and the samples to be tested in the column .

4. The NTC and positive PC results to be tested

Sample	**FAM channel**	**VIC channel**
NTC	Ct ≥40 or no typical amplification curves	Ct < 40and typical amplification curves
PC	Ct < 40 and typical amplification curves	

5. Results of the sample to be tested

FAM channel	**VIC channel**	**Interpretation**

Ct < 40 and typical amplification curves	Ct < 40 and typical amplification curves	positive
	Ct ≥40 or no typical amplification curves	PCR inhibition
Ct ≥40 or no typical amplification curves	Ct < 40 and typical amplification curves	negative
	Ct ≥40 or no typical amplification curves	PCR inhibition

- The parameter setting of the analysis results depends on the specific model and the software version used, and it can also be automatically interpreted by the instrument.

【Precautions】

1. This kit has passed the verification of stability (freeze-thaw and other factors), and does not need to be divided into multiple tubes.
2. The preparation environment of negative reagents and positive reagents should be divided into different areas, and cannot be operated in one area.
3. Pay attention to replacing the tips in time between different sampling steps to avoid cross contamination, and avoid opening the tube cover for a long time.
4. The kit must be used within the validity period. All components in the kit are recommended to be used after melting in a low temperature environment.
5. Only by strictly observing the operating methods of the instructions and using all the reagents matched with this kit can the best detection effect be ensured.
6. The final test result is closely related to the effectiveness of the reagent, the operator's operation method and the test environment.
7. The company is only responsible for the kit itself, and is not responsible for the sample consumption caused by the use of the kit. Please fully consider the possible usage of samples and reserve enough samples before use.
8. This kit is only for in study, not for clinical diagnosis.

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Name：Shanxi Junyan Bioscitech Co., Ltd
Address：No.96,Tanghuai St. Xiaodian Dist., Taiyuan, Shanxi, P.R. China
Net：www.junyanbios.cn
Order：15234188795
Support：19503412257
Email：sales@junyanbios.cn