Mycoplasma PCR Detection Kit

Catalog Number：M20311A-20

【Introduction to Kit】

The Mycoplasma PCR Detection Kit utilizes PCR technology for the specific detection of conserved regions in Mycoplasma 16S and 23S rRNA fragments.This method can obtain results within two hours, which not only saves time but also improves sensitivity and accuracy compared to traditional cultivation methods.

【Components of the kit】

Table 1 Composition and storage conditions of the kit

Components	Total amount	Storage Conditions
Mycoplasma Primer MIX	60 μL×1 tube	-20℃
2×PCR Reaction MIX	300 μL×1 tube	
Positive Control	60 μL×1 tube	

【Specifications】

20 Reactions

【Period of validity】

Valid for 12 months under the prescribed storage conditions

【Self provided equipment required for test】

PCR instrument

1.5mL centrifuge tube with sterile, and low retention

200μL tube with sterile, DNase/RNase-free and low retention

1000μL, 100μL, 10μL pipette gun

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

【Self provided Reagents required for test】

1) negative control template ddH2O

2) Agarose

3) DNA Marker (2000 DNA Marker is recommended)

4) Gel-red or other nucleic acid dyes

【Testing procedures】

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

1. Calculate the number of holes required for reaction according to the number of standard samples and samples to be tested :

Number of reaction holes=Positive Control+ NTC+sample to be tested

2. Calculate the total amount of Mycoplasma PCR MIX required according to the number of reaction wells: Mycoplasma PCR MIX=(number of reaction wells+2 or 3) × 12.6 μL(2×PCR Reaction MIX)+(number of reaction wells+2 or 3) × 2.4 μL(Mycoplasma Primer MIX) (2 or 3 is the operating loss)

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

Table 2 Preparation of Mycoplasma PCR Mix

Components	Single reaction amount
2×PCR Reaction MIX	12.6 μL
Mycoplasma Primer MIX	2.4 μL

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 PCR 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 200μL tube. Bubbles in the reaction system should be removed and centrifuged to be bottom of the tube.

- **PCR reaction procedure and parameter setting**

1. Set the reaction procedure

Stage1	Pollution to digest	Reps:1	50℃	5 min
Stage2	Predenaturation	Reps:1	95℃	3 min
Stage3	Cyclic reaction	Reps:40	95℃ 60℃ 72℃	15s 15s 40s
Stage4	Extension	Reps:1	72℃	5 min
Stage5	Conserve	Reps:1	4℃	∞

2. Agarose Gel Electrophoresis

- Use 1% standard agarose gel
- Load 5 μL of each PCR reaction
- Stop electrophoresis until the dye has migrated to an appropriate distance (depending on the electrophoresis chamber used e.g., run for 25 minutes at 100 V).

- **Result analysis**

Figure 1 represents a typical result after agarose gel electrophoresis of PCR products.

1. A distinct 520 bp band should appear in positive control and no amplification of negative control indicating a successfully performed PCR

2. Samples containing Mycoplasma infection will contain a 520 bp band,Otherwise, no Mycoplasma infection.

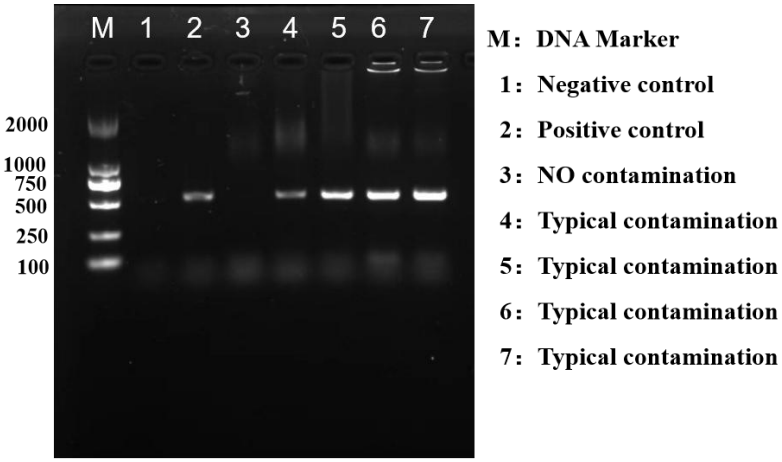

Figure 1 gel post electrophoresis

【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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