Mycoplasma Rapid Detection Kit (PCR)

Cat. No.: PB180525

Size: 20Tests / 50Tests / 100Tests

Product Description

The Mycoplasma detection kit has been carefully optimized by the Procell's R&D team and has undergone long-term testing. It can be used to detect common Mycoplasma in biological products such as cell culture media and serum, including Mycoplasma arginini, Mycoplasma hyorhinis, Mycoplasma orale, Mycoplasma pulmonis, Mycoplasma bovis and Mycoplasma yeatsii. This product is designed with specific primers for conserved sequences of common mycoplasma, which can directly use cell culture medium as a detection template to amplify mycoplasma DNA specifically. This product is easy to operate, has strong specificity, and high sensitivity.

Kit Contents

Product Component	Volume (20Tests)	Volume (50Tests)	Volume (100Tests)
Taq PCR Master Mix (With Dye), 2 ×	0.2 mL	0.5 mL	1 mL
Primer Mix (10 μM)	16 μL	40 μL	80 μL
Positive Control	16 μL	40 μL	80 μL
MycoFree H_2O	1 mL	1 mL	1 mL

Stored at -5 ~ -20℃. Valid for 24 months.

Protocol

Special Note: The liquid preparation process of the PCR reaction system should be operated in a sterile and mycoplasma free environment, such as a clean bench or a biosafety cabinet. Strict aseptic operation is required to avoid false positive results caused by exogenous contamination.

1. Cells culture for 48-72 hours. It is recommended to take 10 μL of cell culture medium as the PCR template for backup when the cell density grows to over 80%. (Centrifuge the suspended cells at 1500 rpm for 3 minutes, and then take the supernatant).
2. Configure the PCR reaction system in the PCR exclusive area according to the table below, and set up a negative control (MycoFree H_2O) and a positive control (Positive Control) group.

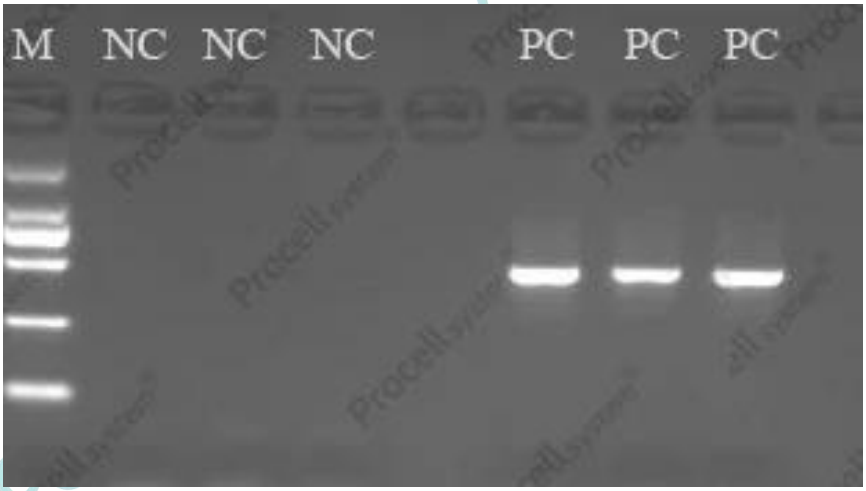
Component	Test Sample	Positive Control	Negative Control
Taq PCR Master Mix (With Dye), 2 ×	10 μL	10 μL	10 μL
Sample	Test Sample 2 μL	Positive Control 2 μL	/
Primer Mix (10 μM)	0.8 μL	0.8 μL	0.8 μL
MycoFree H_2O	7.2 μL	7.2 μL	9.2 μL

3. Gently mix the reaction system and centrifuge it briefly. Set the standard PCR reaction procedure as shown in the table below:

Step	Temperature	Duration	Cycles
1	94°C	3 min	1
2	94°C	15 s	35-40*
3	60°C	15 s	
4	72°C	15 s	
5	72°C	1 min	1
6	4°C	-	-

* The general recommended number of cycles is 35, with 40 cycles being used if a brighter band is required for retesting.

4. After PCR is completed, it should be detected as soon as possible. If it cannot be detected immediately, the PCR product can be stored at 4°C for later use and should not be stored for more than a week.
5. Gel electrophoresis: take 10 μL of product, 1-1.5% agarose gel for electrophoresis (160 V, 15 min is recommended), take photos with gel image analyzer and save the results.
6. Compare the results with the positive and negative control groups to determine whether the sample is contaminated with mycoplasma. The size of the positive band is about 500 bp, with slight fluctuations depending on the species.



Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. Ensure that all reagents are thoroughly thawed before use (refrigerate at 4°C for 2 hours or leave at room temperature for 0.5 hours). After thawing, please separate (recommended 1000 rpm, 30 s) all components and mix well before use. For components with small amounts of liquid, centrifuge to collect the liquid from the wall of the tube.
3. After the PCR system configuration is completed, it should be placed in the PCR machine for reaction as soon as possible, or temporarily stored in a refrigerator at 4°C (within 6 hours). The product is ready for use and it is not recommended to leave the pre-configured reaction system overnight.
4. Reagents should avoid repeated freezing and thawing as much as possible. If the product is stored at 4°C, please use it up within 3 months. If not in use for a long time, please freeze it at

-5~-20°C.

5. The sample to be tested should be tested as soon as possible within 48 hours after sampling. If it cannot be tested immediately, please freeze (-5~-20°C) and complete the test within one month.
6. Please wear a mask during operation to avoid contamination of the sample with mycoplasma carried in the mouth, which may result in false positive results.
7. For samples with weak bands or other suspicious situations, the samples can be concentrated 10 times and retested for confirmation. Concentration operation: Centrifuge at 12000 rpm for 3 minutes to remove the supernatant; Add mycoplasma-free water (self prepared) of 1/10 volume of the original solution and mix well again.
8. All products should be used within the shelf life. If they exceed the shelf life, they must be discarded.

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