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PCR Detection Kit for Mycoplasma suis

Cat No.:PCR-Msuis

1. Overview

Hemotropic Mycoplasma (or hemoplasma) is a group of cell wall-less bacteria that parasitize the surface of erythrocytes. Formerly named Hemobartonella and Eperythrozoon, they have been reclassified as Mycoplasma based on 16S rRNA gene sequence analysis. Three types of hemotropic Mycoplasma have been identified in pigs: *Mycoplasma suis*, *Mycoplasma parvum*, and *Candidatus Mycoplasma haemosuis*.

Mycoplasma suis, formerly known as *Eperythrozoon suis*, was first reported in 1932. It is rod-shaped and pleomorphic, and serves as the pathogen of swine eperythrozoonosis. Infected pigs exhibit diverse symptoms:

- **Acute phase**: Sows show anemia, jaundice, and anorexia for 1-3 days, often accompanied by fever, decreased milk production, and abnormal maternal behavior; piglets suffer from severe anemia and fever.
- **Chronic phase (post-acute)**: Manifested as slow growth, increased susceptibility to diseases, and reduced reproductive capacity.

Studies have indicated a direct correlation between *Mycoplasma suis* and reproductive disorders in sows.

Currently, there is no suitable in vitro culture method for *Mycoplasma suis*. Conventional detection methods include Giemsa-stained blood smears, which have low sensitivity and specificity, and are easily interfered by smear preparation methods and other blood components. Moreover, pathogens are not obvious in the acute phase, requiring disease progression for detectable levels. Serological methods are rarely used due to difficulties in obtaining sufficient antigens. In contrast, the PCR method is more convenient, with higher sensitivity and shorter detection time (results available within hours).

2. Kit Specificity & Application

This kit performs PCR identification targeting the 16S rRNA gene. The primers, verified by BLAST, are specifically targeted to *Mycoplasma suis*. They cross-react with *Candidatus Mycoplasma haematoparvum* (canine) and show weak cross-reactivity with hemotropic Mycoplasma of other animals, but no cross-reactivity with *Mycoplasma parvum*.

Due to the strict host specificity of hemotropic Mycoplasma, strict operation during blood sampling can avoid interference from these cross-reactions. Tests on blood DNA samples from 6 pigs confirmed that this kit is not interfered by *Mycoplasma parvum* or other microorganisms.

This kit is intended for the identification and detection of *Mycoplasma suis*. Note: Avoid contamination with blood products from other animals during use.

3. Kit Components

Component	Description	Volume (μL)		
		PCR-Msuis-20	PCR-Msuis-50	PCR-Msuis-100
Component A (Blue Tube)	Taq Enzyme, dNTPs, Dye	250	625	1250
Component B (Green Tube)	Forward & Reverse Primers	50	125	250
Component C (Yellow Tube)	Positive Control Sample	50	125	250
Water (White Tube)	Nuclease-Free Ultra-Pure Water	200	500	1000

4. Storage Conditions

Store at -20℃ and avoid repeated freeze-thaw cycles. The shelf life is 1 year under conditions without repeated freeze-thaw.

5. Operation Procedures

5.1 Sample Preparation

Prepare 200 μL of EDTA-treated pig blood. Extract sample DNA using a DNA extraction kit for PCR reaction.

5.2 PCR Reaction Preparation

After thawing all kit components, centrifuge briefly, then mix gently by flicking the tube (do not vortex). Prepare the reaction system in PCR tubes as shown in the table below. Include one negative control and one positive control in each experiment; multiple test reaction tubes can be set up.

Avoid vigorous operations during preparation to prevent aerosol formation and cross-contamination between samples. It is recommended to operate in a ventilated device such as a clean bench or biosafety cabinet. After preparation, centrifuge briefly to settle the liquid at the bottom of the tube.

Volume (μL)	Negative Control Tube	Positive Control Tube	Test Reaction Tube *
Component A	12.5	12.5	12.5
Component B	2.5	2.5	2.5
Component C	-	2.5	2.5 / - *
Water	10	7.5	5.5 / 8 *
Test Sample	-	-	2
Total Volume	25	25	25

* Adding Component C to the test reaction tube helps identify PCR inhibitors in the sample. When Component C is co-reacted with the sample:

- Presence of bands (684bp and/or 484bp) indicates no PCR inhibitors.
- Absence of bands indicates the presence of PCR inhibitors, and the possibility of false negatives should be noted.

5.3 PCR Reaction

- Pre-denaturation: 95℃ for 5 minutes
- Cycling (30 cycles): 95℃ for 20 seconds (denaturation), 50℃ for 30 seconds (annealing), 72℃ for 40 seconds (extension)
- Final extension: 72℃ for 5 minutes
- Hold: 4℃

5.4 Agarose Gel Electrophoresis

1. Prepare a 2% agarose gel according to standard protocols, and add EB or Gold-View for staining.
2. Take 8 μL of each reaction solution (no loading buffer needed) and load it directly into the gel wells. Reserve one well for DNA marker (preferably with visible bands in the 500-600bp range).
3. Electrophorese at 120V for approximately 30 minutes.
4. Observe the results under ultraviolet light:

- Positive control: A single band at 684bp
- Negative control: No bands
- *Mycoplasma suis*: A band at 484bp

6. Notes

1. Component A contains dye, which does not affect the PCR reaction. The reaction product can be directly used for electrophoresis, saving time.
2. When preparing the reaction system, use large-volume pipetting as much as possible. Larger volumes reduce pipetting errors.
3. PCR is highly sensitive; trace aerosols generated during operation can cause cross-contamination between samples. Therefore:
   - Operate carefully and avoid vigorous movements.
   - When adding liquids, keep the pipette tip close to the tube wall.
   - Cap all tubes immediately after use.
   - Add controls and test samples in the final step.
   - Discard pipette tips immediately after use.
   - Use sterile, disposable pipette tips (filter tips are preferred).
   - Operate in a ventilated and clean area.
   - For long-term use of this product, use filter tips and avoid environmental DNA contamination of tips.
   - Wear dust-free gloves during operation.
1. It is advisable to partition the work area and perform different steps (e.g., DNA extraction and PCR reaction mixture preparation) in separate areas at different stages.
2. This product is for **research use only** and not for diagnostic purposes.