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Mycoplasma mycoides subsp. mycoides SC Type Probe-Based Quantitative PCR Kit

Product Number: DTK563

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of one year. The positive control should be placed separately and should not contaminate other reagents.

Component

Component	50T
2 × Probe qPCR Mix	550μL
DEPC-H_2O	1mL
Fluorescent template diluent	1mL
Mixed Primer Probe Solution for qPCR of Mycoplasma fimbriatum subsp. fimbriatum SC Type	260μL
Positive control of qPCR for Mycoplasma fimbriae subsp. fimbriae SC type (1 × 10E8 copy/μL)	50μL

Note: Different batches of reagents cannot be mixed.

Description

This reagent kit can be used to detect Mycoplasma paraphylum subsp. mycorrhizae SC type. Mycoplasma mycoidessubsp. Mycoides SC (MmmSC) is the pathogen of bovine infectious pleuropneumonia, which is classified as a Class A infectious disease by the World Organization for Animal Health (OIE).

Application

This product is a detection kit for Mycoplasma fimbriae subsp. fimbriae SC type developed based on the principle of probe based fluorescence quantitative PCR. It has the following characteristics:

1. Ready to use, users only need to provide a sample DNA template.
2. Primers and other components have been optimized for high sensitivity.
3. Provide positive controls to distinguish false negative samples.
4. High specificity, the primers are designed based on the highly conserved region of the SC type DNA sequence of the filamentous subspecies of Mycoplasma, and will not cross react with the DNA of other viruses.
5. It can be used for both qualitative and quantitative testing. When used for quantitative detection, the linear range should be at least 5 orders of magnitude.
6. This product is sufficient for 50 fluorescent quantitative PCR reactions using a 20 μ L probe system.
7. This product can only be used for scientific research.

Protocol

1. DNA extraction (sample preparation area)

1.1. If there are N samples to be extracted, it is best to set N+2 extractions, with the additional being PC (positive control for sample preparation) and NC (negative control for sample preparation). You can take 10μL of 1000 fold dilution of the positive control and add a certain amount of water to make the total volume consistent with the specified volume of the sample to be extracted, and use it as PC. Additionally, use water as NC.

1.2. Extract and purify sample DNA using a self selected method, and this kit is compatible with most nucleic acid extraction

kits on the market. We recommend using our company's DNA extraction kit for extraction.

2. Dilute standard curve sample (sample preparation area)

Due to the high concentration of positive control, the following dilution operations must be performed in a separate area to avoid contaminating the sample or other components of this kit.

2.1. Mark 6 centrifuge tubes, namely 7, 6, 5, 4, 3, and 2.

2.2. Add 45μL of fluorescent template diluent separately using a core gun tip (preferably using a core gun tip, the same below).

2.3. Add 5μL of 1 × 10E8 copy/μL positive control (provided by the reagent kit) to tube 7, shake thoroughly for 1 minute, and obtain 1 × 10E7 copy/μL standard curve sample. Put it on ice for later use.

2.4. Change the gun head and add 5μL of 1 × 10E7 copy/μL positive control (obtained from the previous dilution) to tube 6. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E6 copy/μL. Put it on ice for later use

2.5. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (obtained from the previous dilution) to tube 5. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E5 copy/μL. Put it on ice for later use.

2.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use. If no standard curve is required, dilute the positive control to 1 × 10E5 copies/μL.

3. Reagent Preparation (Reagent Preparation Area)

Prepare sufficient PCR tubes (sample tube, negative control tube, positive control tube) and add the following components to each qPCR tube.

Component	N sample tubes to be tested	Time	Collect fluorescence signals
2 × Probe qPCR Mix	10μL each	10μL	10μL
Mixed Primer Probe Solution for qPCR of Mycoplasma fimbriatum subsp. fimbriatum SC Type	5μL each	5μL	5μL

Transfer to the template addition area.

4. Add Template (Template Add Area)

Add 5μL of template to each qPCR tube, in the order of negative control (DEPC-H2O), test sample template, and positive qPCR control for Mycoplasma fimbriatum subsp. fimbriatum SC type. Centrifuge for 30 seconds and immediately perform amplification reaction.

5. Amplification reaction (amplification and product analysis area)

Place the qPCR tube in the corresponding position of the qPCR amplification instrument sample slot for amplification. The amplification procedure is as follows:

Process	Temperature	Time
Pre denaturation	95℃	3min
qPCR reaction (45 cycles)	95℃	15sec
	60℃	20sec
Channel		FAM channel collects fluorescence signals

6. Result analysis

6.1. If creating a standard curve, plot the standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the DNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and determine its concentration.

6.2. If no standard curve has been created, determine the result according to the following criteria:

Positive control (1×10E5 copies/μL) result: Ct value<30, with significant exponential growth, showing a typical S-shaped curve.

Negative control result: Ct value>40 or no Ct value, no significant exponential growth period or plateau period.

Sample testing results: Ct value<38, with a significant exponential increase, indicating the detection of Mycoplasma paraphimosis SC type in the sample, and the result is positive; If the Ct value is greater than 40 or there is no Ct value, it indicates that Mycoplasma paraphimosis SC type was not detected in the sample, and the result is negative; If the Ct value is within the range of 38-40, the sample should be retested. If the Ct value of the repeated experiment is still within the range of 38-40 and there is a significant exponential increase, it is judged as positive. Otherwise, it is judged as negative.