MEBEP BIOSCIENCE

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Coccidioides immitis Probe-Based Quantitative PCR Kit

Product Number: DTK544

Shipping and Storage

Low temperature transportation, stored at -20°C, with a shelf life of 12 months.

Component

Component	Specification	Nine hole box packaging
2×Probe qPCR MagicMix	500μL	Red cover
Fluorescent PCR specific template diluent	1mL	Green cover
Ultrapure water	1mL	White cover
qPCR probe method for crude spore forming bacteria Primer probe mixture	150μL	Brown cover
qPCR probe method for crude spore forming bacteria Positive control (1 × 10E7 copy/μL)	50μL	Yellow cover
User Manual	YS-MP7131	1 copy

Description

Coarse ball spore fungus can cause primary skin infections in humans and animals, and cause diseases such as lung, meninges, spleen, bones, skin, and muscles. The majority of cases occur in young adults and field workers.

Application

This product is a specialized reagent kit developed based on probe based fluorescence quantitative PCR technology for detecting crude spore forming bacteria. It has the following characteristics:

1. Ready to use, users only need to provide a sample DNA template.
2. Primers and probes have been optimized for high sensitivity.
3. Provide a positive control to distinguish false negative samples.
4. High specificity, the primers are designed based on the highly conserved region of the crude spore fungus DNA, and will not cross react with the DNA of other microorganisms.
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 20μL probe system.
7. This product can only be used for scientific research.

Specimen collection

Sample DNA.

Protocol

1. **Dilute the standard curve sample (taking the 10 fold dilution of 6 copies/μL of 10E1-10E6 as an example)**

   Due to the high concentration of the standard substance, the following dilution operations must be carried out in a separate area and must not contaminate the sample or other components of this kit. To increase product stability and avoid the spread of infectious pathogens, this product does not provide live samples as positive controls, only non infectious DNA fragments are provided as positive controls.

   1.1. Mark 6 centrifuge tubes, namely 6, 5, 4, 3, 2, and 1.

   1.2. Add 45μL of fluorescent PCR specific template diluent with a core gun tip, preferably using a core gun tip, the same

below.

1.3. Add 5μL of 1 × 10E7 copy/μL positive control (provided by the reagent kit) to tube 6, shake thoroughly for 1 minute, and obtain 1 × 10E6 copy/μL standard curve sample. Put it on ice for later use.

1.4. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (diluted in the previous step) 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.

1.5. Change the gun head and add 5μL of 1 × 10E5 copies/μL positive control (obtained from the previous dilution) to tube 4. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E4 copies/μL. Put it on ice for later use.

1.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use.

2. Preparation of Sample DNA

2.1. 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.

2.2. Purify the DNA of the sample using a self selected method. This kit is compatible with most DNA extraction kits on the market. We recommend using our company's DNA extraction kit for extraction.

3. Probe qPCR reaction (20μL system, conducted in the sample preparation room)

3.1. If quantitative analysis is performed and only one replicate is performed, label N+9 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control (using water as a template), and 6 are used for the standard curve. If qualitative analysis is performed and only one repetition is made, label N+4 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control (using water as a template), and 1 is used for PCR positive control (using the positive control dilution of tube 4 as a template). Below, only quantitative analysis will be used as an example to describe the operational steps.

3.2. Add each component to the labeled tube according to the table below (this table only lists one repetition. The positive control is only set after the sample tube and negative control are set, and the positive control sample needs to be stored with all tubes covered and stored before adding):

Ingredients/per tube	Sample tube N+2	PCR negative control tube	Standard curve sample tube (1-6 tubes)
2×Probe qPCR MagicMix	10μL	10μL	10μL
QPCR Primer Probe Mixture for Coarse Sphingosporum Probe Method	3μL	3μL	3μL
N+2 DNA templates to be tested	7μL	-	-
Ultrapure water	-	7μL	-
Step 6: Standard Curve Sample Dilution Solution (1-6)	-	-	7μL (sample 1 to tube 1, sample 2 to tube 2...)

3.3. Cover the machine with the lid and perform PCR according to the following parameters:

Process	Temperature	Time
Pre denaturation	95℃	10 min
PCR reaction (45 cycles)	95℃ 60℃	15sec 60 sec (collecting fluorescence signal from FAM channel)

4. Data processing

4.1. If this reagent kit is used for quantitative detection, plot a standard curve with the log value of the positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the nucleic acid concentration of the sample from the Ct value of the test sample on the standard curve, and then calculate its concentration.

4.2. If this kit is used for qualitative testing and only determines positive or negative, the negative control Ct must be greater than or equal to 40. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct

value should be less than or equal to 37. For the test sample, if its Ct is greater than or equal to 40, it is negative; if it is less than or equal to 37, it is positive. If it is between 37-40, repeat once. If the Ct value of the repeated experiment is greater than or equal to 40, it is negative; if it is less than 40, it is positive.