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Burkholderia pseudomallei (BP) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK144

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
Burkholderia pseudomallei qPCR primer probe mixture	160μL
qPCR positive control of Burkholderia mallei (1 × 10E8 copies/μL)	50μL

Description

This kit is designed for the detection of Burkholderia pseudomallei. Burkholderia pseudomallei is an infectious disease term introduced in 2019. It belongs to the Burkholderia genus and is a short, straight, medium-sized Gram-negative coccobacillus. It typically exists alone, is non-capsulated, non-spore-forming, and possesses more than three flagella at one end, exhibiting active motility. As an opportunistic pathogen, it serves as the etiological agent of melioidosis. Infection occurs through inhalation or contact with contaminated water or soil via damaged skin, leading to conditions such as pneumonia, lung abscess, sepsis, and soft tissue infections.

Features

This product is a Burkholderia mallei detection kit 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, primers are designed based on highly conserved regions of Burkholderia mallei DNA sequence, and will not cross react with DNA from other biological samples.
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, which can be used as PC. In addition, water can be used 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. Suggest using a bacterial genome DNA extraction kit to extract DNA .

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/μ L 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 qPCR 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	qPCR negative control	qPCR positive control
2 × Probe qPCR Mix	10μL each	10μL	10μL
Burkholderia pseudomallei qPCR primer probe mixture	3μL each	3μL	3μL

Transfer to the template addition area.

4. Add Template (Template Add Area)

Add 7μL of template to the qPCR tube, in the order of negative control (DEPC-H_2O), test sample template, and cytomegalovirus qPCR positive control. 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:

Step	Temperature	Time
Pre denaturation	95℃	10min
qPCR reaction 45 cycles	95℃	15sec
	60℃	30sec
Signal 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, the results shall be judged 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 Burkholderia mallei in the sample, and the result is positive; A Ct value greater than 40 or no Ct value indicates that Burkholderia mallei 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.