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

Product Number: DTK144

Shipping and Storage

1. -20℃±5℃, stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.
2. The collected or processed samples should be stored at 2℃~8℃ for no more than 24 hours; If long-term storage is required, it should be stored at -70℃ or below, with no more than 3 freeze-thaw cycles.

Component

Component	50T
BP reaction solution	500μL×2
Enzyme solution	50μL
BP positive control product	250μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses TaqMan probe method for real-time fluorescence PCR technology, designs a pair of Burkholderia mallei specific primers, and combines them with a specific probe to perform in vitro amplification and detection of Burkholderia mallei DNA using fluorescence PCR technology, which is used for pathogenic diagnosis of suspected infectious materials in clinical practice.

Application

Melioidosis is a tropical zoonotic disease caused by Burkholderia pseudomallei (BP) infection. Infection occurs through mucosal contact, inhalation, and ingestion of BP. Clinical manifestations include multiple abscesses, refractory pneumonia, and fatal sepsis, with a mortality rate of 20-60%. Melioidosis is commonly found in Southeast Asia, with cases mainly concentrated in Vietnam, Myanmar, and Malaysia. The northern part of Australia is also a popular area. The epidemic areas of glanders in China are mainly concentrated in Hainan, Guangdong and other places. Sheep, goats, horses, pigs, cows, dogs, cats and other animals also carry glanders and can transmit similar diseases to humans. Wounds or abrasions on human skin may become infected if they come into contact with contaminated soil or water. People may also be infected by inhaling contaminated soil dust and swallowing or inhaling contaminated water.

This kit is suitable for detecting clinical specimens suspected of Burkholderia mallei infection, such as blood, sputum, urine, feces, local lesions and purulent exudate, as well as environmental contaminated specimens such as water samples and soil, for auxiliary diagnosis of Burkholderia mallei infection.

Applicable instruments

ABI7500, Agilent MX3000P/3005P, LightCycler, Bio-Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

Specimen collection

The patient's blood, sputum, urine, feces, local lesions, and purulent exudate; Environmental pollution samples such as water samples and soil.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Fecal samples: Pick rice sized feces, place them in a centrifuge tube with 0.5mL of physiological saline, shake and mix well, centrifuge 16200g for 2 minutes. Remove the supernatant and precipitate for nucleic acid extraction.

Blood samples: can be directly used for nucleic acid extraction without processing. Sputum and viscous pus samples: Add 4 times the volume of 4% NaOH to the sample, shake well, and let it liquefy at room temperature for 30 minutes. Take 0.5mL of the sample into a 1.5mL centrifuge tube, add 0.5mL of 4% NaOH and let it sit at room temperature for 10 minutes, then centrifuge at 16200g for 5 minutes. Add 1mL of sterile physiological saline to the precipitate, mix well, centrifuge at 16200g for 5 minutes, and repeat washing once more. Remove the supernatant and precipitate for nucleic acid extraction. Urine, water sample pus, environmental pollution samples, take 3mL of sample or prepared sample suspension, 16200g, 2mn, remove the supernatant, precipitate for nucleic acid extraction.

1.2. Nucleic acid extraction

We recommend using our company's nucleic acid extraction or purification reagents (magnetic bead method or centrifugal column method) for nucleic acid extraction. Please follow the reagent instructions for operation.

2. Reagent preparation (reagent preparation area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1 negative control tube+1 positive control tube); For every 10 parts of the reaction tube, an additional 1 part is prepared. The preparation of each test reaction system is shown in the table below:

reagent	BP reaction solution	Enzyme solution
Dosage (sample size N)	19μL	1μL

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20uL per tube.

3. Sample addition (sample processing area)

Take 5μL of the nucleic acid, positive control sample, and negative control sample extracted in step 1, and add them to the corresponding reaction tubes. Cover the tubes, mix well, and briefly centrifuge.

4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, and set the reaction system to 25μL;

Fluorescence channel selection: Detection channel (Reporter Dye) FAM, Quencher Dye NONE, please do not select ROX reference fluorescence for ABI series instruments, select None.

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	95℃	2min	No
2	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

(Please refer to the user manuals of each instrument for setting up, taking the ABI7500 instrument as an example)

After the reaction is complete, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End value, and Threshold value of the baseline (users can adjust them according to their actual situation, with Start value set between 3-15 and End value set between 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of negative quality control products is flat or below the threshold line). Click Analyze to automatically obtain the analysis results.

5.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 40, and the curve shows a significant exponential growth curve;

Negative: The sample test result shows no Ct value and no specific amplification curve.

Suspicious: If the sample test result is 40<Ct value ≤ 45, it is recommended to repeat the test. If the detection channel is still 40<Ct value ≤ 45 and the curve has a clear exponential growth curve, it is judged as positive. Otherwise, it is judged

as negative.

Quality control standards

Negative quality control product: no specific amplification curve or Ct value display;

Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤32;

The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

Limitations of detection methods

1. The results of sample testing are related to the quality of sample collection, processing, transportation, and preservation;
2. Failure to control cross contamination during sample extraction can result in false positive results;
3. Leakage of positive controls and amplification products can lead to false positive results;
4. Genetic mutations and recombination of pathogens during epidemics can lead to false negative results;
5. Different extraction methods have differences in extraction efficiency, which can lead to false negative results;
6. Improper transportation, storage, or preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;
7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.

Note

1. All operations must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent box should be treated as contaminants and disposed of in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".