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Nocardia (Noc) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK559

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 above specimens can be stored at -20℃ in the short term and -70℃ in the long term, but cannot exceed 6 months. The specimens should be transported in 2-8℃ ice packs, and repeated freezing and thawing are strictly prohibited.

Component

Component	50T
qPCR premix (containing enzymes)	800μL
Primer probe	200μL
Noc positive control	500μL
Negative control	500μL

Note: Different batches of reagents cannot be mixed.

Description

This reagent kit is designed based on the principle of fluorescence PCR technology, with specific primers and Taqman probes designed for Nocardia. It is detected using a fluorescence PCR detector to achieve the detection of Nocardia nucleic acid.

Application

This kit is used for qualitative detection of Nocardia nucleic acid and for auxiliary diagnosis and epidemiological monitoring of Nocardia infection.

Applicable instruments

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

Specimen collection

Herpes liquid, saliva, throat wash, corneal swabs, secretions, cerebrospinal fluid and other samples.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Cerebrospinal fluid: Take 1-3mL of cerebrospinal fluid from the subject in a sterile 5mL glass tube, seal and send for testing, and wait for nucleic acid extraction.

1.2. Nucleic acid extraction

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

2. Reagent preparation (reagent preparation area)

Melt the components of the reagent kit at room temperature, shake thoroughly and mix well, then centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1 tube of negative control+1 tube of positive control), configure the reaction system according to the table below, add each component to the same appropriate volume centrifuge tube, mix thoroughly, and centrifuge immediately to prepare the reaction system mixture. Transfer it to the PCR reaction tube/plate at

a rate of 20μL/well and transfer it to the sample processing area.

Composition of reaction solution	Dosage (μL)/per reaction
qPCR premix (containing enzymes)	16μL
Primer probe	4μL
Total volume (reaction system mixture)	20μL

3. Sample addition (sample processing area)

Add 5μL of processed nucleic acid, negative control, and positive control to the PCR reaction tube/plate that has been mixed with the reaction system mixture, resulting in a final volume of 25μL. Cover the tube tightly or seal the film, mix well, and centrifuge instantly.

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		Temperature	Time	Cycles
1	Pre denaturation	95℃	5min	1 cycle
2	Denaturation	95℃	10sec	40 cycles
	Annealing, extension, and fluorescence detection	55℃	40sec	

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 amplification curve is S-shaped and the Ct value is ≤35.

Negative: Ct value>38 or not detected.

Suspicious: The amplification curve shows a typical S-shape and 35<Ct value ≤38, requiring retesting; If the retest results are consistent, the judgment result is positive. If the CT value is greater than 38 or not detected, the judgment result is negative.

Quality control standards

1. Negative control: Ct value>38 or not detected.
2. Positive control: The amplification curve is typically S-shaped, and the Ct value is ≤35.
3. The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.
4. Each detection target requires a positive and negative control, and the baseline threshold is adjusted for different targets based on their corresponding negative results.

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. During the epidemic, genetic mutations and recombination of pathogens 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 inaccurate 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 kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".