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Staphylococcus aureus (SA) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK261

Shipping and Storage

1. Store in the dark at -20±5℃, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; Avoid repeated freeze-thaw cycles, as six freeze-thaw cycles will not affect the detection results.

Component

Component	Specification/Volume μL/Quantity		Main components
	25T	50T	
qPCR premix (containing enzymes)	400μL	800μL	Tris, KCl, $MgCl_2$, dNTPs, Taq enzyme, UNG enzyme, etc
Primer probe Staphylococcus aureus	100μL	200μL	Primer probe
Positive control Staphylococcus aureus	500μL		Plasmids containing target detection gene fragments
Negative control	500μL		Normal saline

Description

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

Application

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

Applicable instruments

Suitable for ABI 7500, Bio-Rad CFX96, Roche480, Real time fluorescence PCR instruments such as Hongshi SLAN-96S and SLAN-96P.

Specimen collection

1. Sample types: nasal/pharyngeal swabs, sputum, bronchoalveolar lavage fluid; Samples such as culture materials.
2. Storage conditions: The collected specimens should be sent for testing in a timely manner. Those tested within 24 hours should be stored at 4℃, and those tested for more than 24 hours should be stored at -70℃ (if there is no -70℃ storage condition, the test sample can be stored in a -20℃ refrigerator for 10 days), and repeated freezing and thawing should be avoided.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

Melt the components of the reagent kit at room temperature, mix thoroughly, and centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1<positive control>+1<negative control>), configure the reaction system per person according to the table below, add each component to an appropriate volume centrifuge tube, mix thoroughly, and centrifuge immediately. Divide into 20μL PCR reaction tubes/plates and transfer to the sample processing area.

Component	Volume (μL)

qPCR premix (containing enzymes)	16μL
Primer probe XX	4μL
Total volume (reaction system mixture)	20μL

2. Sample Processing (Sample Processing Area)

2.1. Nucleic acid extraction:

Select the appropriate nucleic acid extraction kit to extract viral nucleic acid, and follow the instructions of the corresponding kit for specific operations.

2.2. Sampling:

Add 5μL of processed nucleic acid, negative control, and positive control to the PCR reaction tube/plate that has been added to the reaction system mix, resulting in a final volume of 25μL. Cover the tube tightly or seal the membrane, centrifuge at low speed instantly, and amplify with a fluorescence PCR detector.

3. Amplification testing (nucleic acid amplification area)

step	Temperature	Time	cycles
Pre denaturation	95℃	5min	1 cycle
Denaturation	95℃	10sec	40 cycle
Annealing/extension/fluorescence detection*	55℃	40sec	

Note: Fluorescence detection at 55℃ in step 2, using FAM as the detection channel.

Roche480 series instruments need to complete color compensation experiments before starting the experiment (when using VIC/HEX and ROX/TEXS RED simultaneously)

The ABI series fluorescence PCR instrument does not select ROX calibration, and the quenching group is selected as None.

4. Result analysis

According to the analysis of the image, adjust the start and end values (it is recommended to start from 3-15 and end from 5-20, and adjust the amplification curve of the negative control to be flat or below the threshold line). Click on the analysis button and view the results on the report interface.

Quality control standards

1. Negative control: Ct value>38 or not detected.
2. Positive control: The amplification curve is S-shaped and the Ct value is ≤ 35.
3. The above requirements must be met simultaneously for the same experiment, otherwise this 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.

Result interpretation

1. FAM channel detects Staphylococcus aureus.
2. Negative: Ct value>38 or not detected.
3. Positive: The amplification curve is S-shaped and the Ct value is ≤ 35.
4. Suspected positive: The amplification curve shows an S-shaped pattern 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.

Limitations of detection methods

1. Improper sample collection, transportation, and storage, as well as improper transportation, storage, and configuration of reagents, can all affect experimental results and even lead to false negative results.
2. If there is laboratory contamination, reagent contamination, or sample cross contamination, false positive results may occur.

Performance indicators of reagent kit

1. Minimum detection limit: 400 copies/mL.
2. Specificity: No cross reactivity with other pathogenic pathogens.

Note

1. Each stage of PCR operation should be strictly partitioned to avoid cross contamination.
2. The components of the reagent kit should be thoroughly melted and mixed before use, and centrifuged for a few seconds before use.
3. Each component shall not be interchanged with other products or corresponding ingredients of different batch numbers.
4. If the test specimen is not tested in a timely manner, it should be stored at -70℃.
5. The processing of samples should strictly follow biosafety regulations.
6. PCR operators should have experience and receive professional training.
7. This kit is only for scientific research use and is not intended for clinical diagnosis.