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Bartonella spp.(BE)Nucleic Acid Detection Kit

(Real-Time PCR Method)

Product Number:DTK294

Shipping and Storage

1. Store at -20℃ away from light, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; After opening, store in the dark at -20 ℃ without affecting the expiration date. Avoid repeated freeze-thaw cycles, as six freeze-thaw cycles will not affect the detection results.

Component

Component	25T	50T	Main components
QPCR premix (containing enzymes)	400μL	800μL	Tris, KCl, $MgCl_2$, dNTPs, Taq enzyme etc.
Han's Bartonella Primer Probe	100μL	200μL	Primer probe.
Positive control for Han's Bartonella	500μL	500μL	Plasmids containing target detection gene fragments.
Han's Bartonella negative control	500μL	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 the detection of Bartonella baumannii. The nucleic acid of Bartonella baumannii is detected using a fluorescence PCR detector.

Application

This kit is used for qualitative detection of Han's Bartonella nucleic acid and for auxiliary diagnosis and epidemiological monitoring of Han's Bartonella infection.

Applicable instruments

Suitable for real-time fluorescence quantitative PCR instruments such as ABI 7500,Bio-Rad CFX96,Roche Lightcycler480I, Lightcycler480II,cobas Z480,Hongshi SLAN-96S and SLAN-96P.

Sample requirements

Sample type: Mouse liver, spleen, and kidney tissues.

Storage conditions: The collected specimens should be tested in a timely manner, and specimens that can be tested within 24 hours can be stored at 4℃; Specimens that cannot be detected within 24 hours should be stored at -70℃ or below (if there is no -70℃ storage condition, they should 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, shake thoroughly and mix well, then centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1<positive control>+1<negative 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 20 μ L/well and transfer it to the sample processing area.

Component	Volume (μL)

QPCR premix (containing enzymes)	16
Han's Bartonella Primer Probe	4
Total volume (reaction system mixture)	20

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. Add sample

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	1cycle
Denaturation	95℃	10s	40cycles
Annealing/extension/fluorescence detection*	55℃	40s	

Note:1)*Fluorescence detection at 55 ℃ during step denaturation, using FAM as the detection channel.

2)*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

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 detection of Han's Bartonella.
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 a typical S-shaped pattern, and the Ct value is between 35 and 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 protocol

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

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1. Minimum detection limit: 500 copies/mL.
2. Specificity: No cross reactivity with other pathogens that may cross the detection target.

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 used for scientific research purposes and is not intended for clinical diagnosis.