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Human Herpesvirus 6/7/8 Nucleic Acid Detection Kit (Triple Fluorescent PCR Method)

Product Number: DTK537

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, MgCl2, dNTPs, Taq enzymes, etc.
Primer probe HHV6/HHV7/ HHV8	100μL	200μL	Primer probe.
Positive control HHV6/HHV7/ HHV8	50μL	50μL	Plasmids containing target detection gene fragments.
Negative control	50μL	50μL	Water treated with diethyl carbonate.

Description

This kit is designed based on the principle of fluorescence PCR technology, with specific primers and Taqman probes designed for human herpesvirus 6/7/8. It is detected using a fluorescence PCR detector to achieve the detection of human herpesvirus 6/7/8 nucleic acid.

Application

This kit is used for qualitative detection of human herpesvirus 6 (HHV6), human herpesvirus 7 (HHV7), and human herpesvirus 8 (HHV8) nucleic acids, and is used for auxiliary diagnosis and epidemiological monitoring of human herpesvirus 6/7/8 infections.

Applicable instruments

Suitable for ABI 7500, Bio-Rad CFX96, Roche480 and other fully automatic fluorescence PCR detectors.

Sample requirements

1. Sample types: herpetic fluid, saliva, throat wash, corneal swabs, secretions, cerebrospinal fluid, and other samples.
2. Storage conditions: The collected specimens should be sent for testing in a timely manner. If tested within 24 hours, they should be stored for 4 hours. If tested beyond 24 hours, it is best to store them at -70℃ and avoid repeated freezing and thawing.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

Melt the components of the reagent kit at 4 ℃ in the dark, mix thoroughly, and centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1 tube of positive control+1 tube of negative control), configure the reaction system mix according to the table below, add it to an appropriate volume of centrifuge tube, mix thoroughly, and centrifuge immediately. Divide it into 20μL PCR reaction tubes/plates and transfer it to the sample processing area.

Component	Volume (μL)
qPCR premix (containing enzymes)	16

Primer probes HHV6/HHV7/HHV8	4
Total volume (reaction system mix)	20

2. Sample processing (sample processing area)

2.1. Nucleic acid extraction

Select the appropriate nucleic acid extraction kit to extract 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:*Fluorescence detection at 55℃ in step 2, using FAM as the detection channel HEX/VIC, CY5.

***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 ≤ 30.
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 HHV6, HEX/VIC channel detects HHV7, and CY5 channel detects HHV8.
2. Negative: Ct value>38 or not detected.
3. Positive: The amplification curve is S-shaped and the Ct value is ≤ 35.
4. Suspicious: 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.

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

1. Minimum detection limit: 1×10^3 copies/mL.
2. Linear detection range: 2×10^3~1×10^8 copies/mL.

3. Specificity: It can detect all specimens of human herpesvirus types 6/7/8 and has no overlap with other types.

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 -20℃ or -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.