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Hantavirus nucleic acid detection PCR Kit

Product Number: DTK586

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.
3. Production date and expiration date: see outer packaging box.

Component

Component	25T	50T	Main components
qRT-PCR reaction solution	300μL	600μL	Tris, KCl, $MgCl_2$, dNTPs, Taq enzymes, etc
qRT-PCR enzyme mixture	100μL	200μL	Reverse transcriptase, RNAse inhibitor, Taq enzyme, etc.
Primer probe HV	100μL	200μL	Primer probe
Positive control HV	50μL	50μL	Plasmids containing target detection gene fragments
Negative control	50μL	50μL	Water treated with diethyl carbonate

Description

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

Application

This kit is used for qualitative detection of Hantavirus (HV) nucleic acid and for auxiliary diagnosis and epidemiological monitoring of Hantavirus infection.

Applicable instruments

Suitable for fully automatic fluorescence PCR detectors such as ABI 7500, Bio Rad CFX96, Roche480, etc.

Specimen collection

1. Sample types: serum, urine; Tissue culture and other samples.
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 beyond 24 hours should be stored at -70℃, avoiding 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)
qRT-PCR reaction solution	12
qRT-PCR enzyme mixture	4
Primer probe HV	4
Total volume	20

2. Sample processing (sample processing area)

Nucleic acid extraction: Select a suitable nucleic acid extraction kit to extract viral nucleic acid, and follow the instructions of the corresponding kit for specific operations.

Sample addition: 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	Cycles	Temperature	Time
Reverse transcription	1 cycle	50℃	10min
Pre denaturation	1 cycle	95℃	2min
Denaturation	40 cycles	95℃	10sec
Annealing/extension/fluorescence detection*		55℃	40sec

Fluorescence detection at 55 ℃ in step 3, using FAM as the detection channel.

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

4. Result analysis and judgment

4.1. 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.

4.2. Result judgment

FAM channel detects HV.

Negative: Ct value>38 or not detected.

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

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.

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.

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: 1×10^3 copies/mL.
2. Linear detection range: 2×10^3~1×10^8 copies/mL.
3. Specificity: Can detect Hantavirus in all specimens and does not cross 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 for scientific research use and is not intended for clinical diagnosis.