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Hepatitis B Virus (HBV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK308

Shipping and Storage

1. Store in dark at -20℃, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; After opening, store in the dark at -20℃.The expiration date has no impact. Avoid repeated freezing and thawing, freezing and thawing 6 times will not affect the detection effect.

Component

Component	Specification/Volume μL/Quantity		Main components
	25T	50T	
qPCR premix (containing enzymes)	400μL	800μL	Tris, KCl, $MgCl_2$, dNTPs, Taq enzymes, etc
Primer probe HBV	100μL	200μL	Primer probe
Positive control HBV	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 for hepatitis B virus. It is detected by a fluorescence PCR detector to achieve the detection of hepatitis B virus nucleic acid.

Application

This kit is used for qualitative detection of hepatitis B virus (HBV) nucleic acid, as well as for auxiliary diagnosis and epidemiological monitoring of HBV infection.

Applicable instruments

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

Specimen collection

1. Sample types: Blood and other samples.
2. Storage conditions: The collected specimens should be sent for testing in a timely manner, and those tested within 24 hours should be at 4℃. Store at -70℃ for more than 24 hours and avoid repeated freeze-thaw cycles.

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)	16μL
Primer probe HBV	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 mixed with the reaction system mixture, resulting in a final volume of 25μL. Cover the tube tightly or seal it with a membrane, and perform transient centrifugation followed by detection on a fluorescence PCR amplification instrument.

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.

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 ≤ 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 detection of HBV.
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 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 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 ~1×10^8copies/mL.
3. Specificity: Hepatitis B virus can be detected in all specimens and does not cross with other types.

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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 specimen to be tested 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.