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Quantitative Diagnostic Kit for Hepatitis B virus(HBV)DNA

Product Number: QDHBV01

Shipping and Storage

1. Store below 30°C. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used HBV PCR Master Mix should be stored at (-20±5)°C. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. Storage time should not exceed 21 days.
4. Date of manufacture and term of validity: see the label.

Components

Component	48Test
HBV RT-PCR Mater Mix	1 bottle
HBV quantitative standard substance 1	1 Tube
HBV quantitative standard substance 2	1 Tube
HBV quantitative standard substance 3	1 Tube
HBV quantitative standard substance 4	1 Tube
Positive Control	1 Tube
Negative Control	1mL
Redissolved Diluent	1.5mL

Note:Do not mix reagents from different batches.

Amplification Instrument:Real-time fluorescence PCR instrument with FAM, VIC channels

Description

This kit is suitable for the quantitative detection of hepatitis B virus nucleic acid (HBV DNA) in human serum samples in vitro, and the test results are used for the auxiliary diagnosis of hepatitis B. The test results are not the only indicators for the evaluation of the patient's condition, and the condition must be comprehensively analyzed in combination with the patient's clinical manifestations and other laboratory tests.

Hepatitis B is a worldwide epidemic and is a group of infectious diseases characterized by liver damage caused by Hepatitis B Virus (HBV).It has the characteristics of strong infectivity, complex transmission route, wide prevalence and high incidence. The main clinical manifestations are fatigue, loss of appetite, nausea, vomiting, hepatomegaly and abnormal liver function. After a person is infected with HBV, those who have not cleared the virus for 6 months are called chronic HBV infection. Without appropriate intervention, some infected persons may develop liver failure, cirrhosis and primary hepatocellular carcinoma.

This kit utilizes real-time fluorescent PCR detection technology, and uses specific primers and probes for HBV to achieve quantitative detection of HBV DNA in serum samples. The kit is provided with an Internal Control(IC), which can monitor whether there is PCR inhibitor in the sample to be tested by detecting whether the internal control is normal or not, so as to avoid false negative PCR.

Sample Requirements

1. Applicable sample type: Serum specimens.
2. **Sample collection：**

   Use a sterile syringe to draw 2mL of the subject's venous blood and inject it into a sterile centrifuge tube. Leave it at room temperature for no more than 4 hours. Centrifuge at 2000rpm for 5 minutes. Take the upper serum (be careful not to bring in red blood cells) and transfer it to another sterile centrifuge tube for use.

3. Sample storage and transportation:

The collection or processing sample should not exceed 24 hours under the conditions of 2℃~8℃. If long-term preservation is needed, it should be stored below -70°C, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the HBV PCR Master Mix, open each bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 60 seconds until the liquid is clear and transparent. Subpackage it into PCR reaction tubes according to 20μL/ tube.

2. Nucleic acid extraction:

This kit is not included for Nucleic Acid(NA) extraction reagent.

Commercially available extraction kits that have been shown to generate highly purified DNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted DNA is not used immediately, it should be stored below -20℃. For long-term storage, it should be stored below -80℃ and avoid repeated freezing and thawing.

Note:1.The Negative Control and the Positive Control does not require nucleic acid extraction. The Positive Control needs to be redissolved with 100μL of redissolved diluent and mixed well before use.

2.The HBV quantitative standard substances 1~4 needs to be redissolved with 100μL of Redissolved Diluent and mixed well before use separately.

3. Add sample:

The correspond substances were added to that above PCR reaction tubes according to the following table:

Type	Add sample description
Testing Sample	Add 5μL of the extract prepared in step 2 to the reaction tube, and close the tube cover.
Negative Control/Positive Control	Add 5μL of negative control and positive control to the reaction tube, and cover the tube tightly.
HBV quantitative standard substances 1,2,3,4	Add 5μL of HBV quantitative standard substances 1,2,3,4 respectively, to the PCR reaction tube, and cover the tube tightly

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be centrifuged for 15s on a palm centrifuge and then delivery to the nucleic acid amplification region. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR amplification:

Place the reaction tube in the automatic fluorescent PCR instrument, set the Negative control, Positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

Select FAM channel to detect HBV DNA, select VIC channel to detect the IC. Set the Reaction Volume per Well to 25μL.

Note: For ABI series instruments, select 'None' under 'Quencher',and select 'None' as the dye to use as the passive reference.

Recommended reaction program setting:

Step	Cycles	Temperature(℃)	Time	Collect fluorescence signal
1	1	95℃	2min	No
2	45	95℃	15s	No
		60℃	30s	Yes

5. Result analysis:

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3~15, the End value can be set at 5~20, the amplification curve of the negative control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis, make the parameters meet the requirements in the following '6.Quality control', and then go to the Plate window to record the Ct value.

6. **Quality control**

Negative control: FAM and VIC detection channels have no amplification curves,in the Reports interface, the Ct column displays 'Undet'.

Positive control:The FAM and VIC channels have obvious S-shaped amplification curves, and the Ct value is ≤32.00.

HBV quantitative standard substances 1~4:FAM channel has obvious amplification curve, and the standard curve correlation coefficient $|r| \geq 0.98$.

The above requirements must be met at the same time in the same experiment. Otherwise this experiment is invalid and needs to be repeated.

Positive Judgment Value

Through the study of the reference value, the minimum detection limit of this kit for HBV was determined to be 500copies/mL, and the Ct positive judgment value of VIC was 32.00.

Explanation of Test Result

1. If the Ct value of the VIC channel of the test sample is >35.00, the test result of the sample is invalid, and the cause should be found and eliminated. Samples need to be recollected and the experiment repeated.
2. For samples with measured value $<5.00\times10^2$ copies/mL or Ct value was 'Undet', and the Internal Control detection is positive and Ct value ≤32.00, it was reported as lower than the detection limit of the kit. If the Internal Control is abnormal, the test result of this sample is invalid, and the test is repeated for this sample.
3. For samples with measured values between 5.00×10^2 copies/mL~1.00×10^8 copies/mL, report the corresponding determination results. For samples with measured value $>1.00\times10^8$ copies/mL, the Internal Control detection is positive and Ct value ≤32.00, It is recommended to re-measure after diluting with 1×TE, calibrate the results according to the dilution factor, report the calibrated value, and indicate "reportable concentration",and the measurement results are for reference only.

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. There is no control of cross -pollution during the sample extraction process, and false positive results will occur.
3. Positive control and leakage of amplification products can lead to false positive results.
4. The genetic mutations and reorganizations during epidemics can lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Reagent transportation, improper preservation, or inaccurate reagent preparation reagent detection performance decreases, and the results of false negative or quantitative detection occur.
7. The results of this test are for reference only. If the diagnosis must be confirmed, please combine clinical symptoms and other test methods.

Performance Parameters

1. Minimum detection limit: The minimum detection limit of this reagent is 5.00×10^2 copies/mL.
2. Linear range: 5.00×10^2 copies/mL~1.00×10^8 copies/mL.
3. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) value of detected concentration logarithm is ≤5.00%.
4. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise

reference is 100%, and the compliance rate of positive reference products is 100%.

5. Analysis of Specificity:
   5.1. Cross-reaction: There is no cross-reaction with cytomegalovirus, Hepatitis A virus, Hepatitis B virus, Adenovirus, HSV-1, HSV-2, VZV, NG, HHV-6, Staphylococcus aureus, Candida albicans.
   5.2. Interfering substances: common interfering substances such as mucin, blood, commonly used drugs for patients with common respiratory symptoms epinephrine, dexamethasone, sulfur, gold, menthol, zanamivir, mupirocin, tobramycin are under test When the concentration in the sample is 0.1mg/mL,10%,100pg/mL,100pg/mL,4.5mg/mL,4.5mg/mL, 5mg/mL,3mg/mL,10mg/mL,0.3mg/mL respectively do not interfere with the test results of the kit.

Note

1. Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and carry out quality control for each experiment.
3. The reaction solution should be stored away from light.
4. Try to avoid bubbles in the reaction, and the tube cover needs to be tight.
5. Use disposable heads, disposable gloves and special work clothes in each district.
6. Sample processing, reagent preparation, and samples need to be performed in different areas to avoid cross -pollution.
7. After the experiment is completed, use 10% hypochloride or 75% alcohol or ultraviolet light to treat the workbench and pipette.
8. All items in the kit should be treated as pollutants and processed in accordance with the "Biological Safety General of Microbiological Biomedical Laboratory".