HBV DNA Quantitative Detection Kit (Fluorescence PCR) Instruction for use (V1.0)

[REF] HWTS-HP008A

[Specification] 50 tests/kit

[Research Use Only]

The HBV PCR Kit is an nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) in human serum. Hepatitis B is an infectious disease with liver and multiple organ lesion caused by the hepatitis B virus (HBV). Most people experience symptoms such as extreme fatigue, appetite loss, lower limbs or whole-body edema, hepatomegaly, etc. 5% of adult patients and 95% of children patients infected from their mother cannot clean the HBV virus efficiently in persistent infection and progress to liver cirrhosis or primary liver cell carcinoma[1-5].

[Test Principle]

A pair of primers and a sequence-specific fluorescence probe were designed. The fluorophore FAM is attached at the 5' end of the probe and the quencher moiety TAMRA is located at the 3' end. During the PCR procedures, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye. The detectable fluorescence is proportional to the amount of accumulated PCR product, which is used to detect the HBV quantitatively.

[Kit Contents]

No.	Name	Specification	Description
1	HBV Reaction Buffer	750 µL/vial×2	HBV premier, probe, Taq DNA polymerase, dNTPs, MgCl2, and UNG at optimal concentrations
2	HBV High positive control	1 mL/vial×1	Inactivated serum from HBV positive patient
3	HBV Low positive control	1 mL/vial×1	Inactivated serum from HBV positive patient
4	HBV Negative control	1 mL/vial×1	Inactivated negative human serum.
5	HBV QS 1	1 mL/vial×1	Noninfectious HBV virus at defined concentrations in Human serum. Refer to the instruction in the package insert for the exact concentrations of QS.
6	HBV QS 2	1 mL/vial×1	
7	HBV QS 3	1 mL/vial×1	
8	HBV QS 4	1 mL/vial×1	
9	HBV Internal Control	50 µL/vial×1	In-house DNA plasmid in a buffer solution

Note: The contents in the kits of different batches are not interchangeable.

Reagents required but not provided:

DNA Extraction kit TIA Namn Virus DNA/RNA kit (DP315), 1.5 mL DNase/RNase-free microcentrifuge tubes, 0.2 mL PCR Tubes, DNase/RNase-free Tips for pipettes, Bench-top centrifuge, Vortex mixer, Water bath.

[Storage Conditions and Shelf Life]

The components of the HBV PCR Kit should be stored at temperature lower than -18°C and are stable with self-life about 12 months. Repeated thawing and freezing should be avoided more than 5 times.

The dates of manufacturing and expiry stated on the label.

[Applicable Equipment]

Applied Biosystems™ Real time PCR system 7500, ABI 7500FAST real-time PCR system, ABI QuantStudio™5 Real-time PCR system, LightCycler® 480 fluorescence quantitative PCR system, Bio-Rad CFX96 real-time fluorescence quantitative PCR instrument, Ultrassay™ XP96 Real Time qPCR System. etc.

[Acceptable Specimens]

Collection:

A volume of 2mL venous blood should be withdrawn using sterile syringe needle and standard specimen collection tubes (Sterile centrifuge tube). Freshly specimens (whole blood) may be held at ambient temperature (2 to 30°C) for up to 4 hours prior to centrifugation. After the blood is centrifuged at 1,600rpm for 20 minutes, the isolated serum should be transferred into another sterile centrifuge tube for future test.

Storage:

Serum should be stored at 2-8°C for up to 24 hours, at -18°C no more than 3 months, and at -70°C for long term. The frozen specimens should be thawed completely before the test. Multiple freeze-thaw cycles should be avoided (<5 x).

Transportation:

Ship the specimens in sealed frozen or foam box on dry ice.

[Test Procedures]

REAGENT PREPARATION (REAGENT PREPARATION AREA)

Take out all the reagents from the package and thaw them completely at room temperature.

Mix and centrifuge the reaction buffer for 10 seconds at 2000rpm. Prepare PCR tubes based on estimation of tests in the run and add 30ul reaction buffer in each tube. The tests in the run should include a Negative control, a High positive control, a low positive control and 4 Quantitative Standards in addition to the testing samples. Transfer all the tubes to the sample processing area.

SAMPLE PROCESSING (SAMPLE PROCESSING AREA)

Add 200ul samples, negative control, low positive control, high positive control and QS1-4 separately into 1.5mL RNase/ DNase free centrifuge tubes and mark the codes/names of samples and controls.

Add 1µL internal control into each tube, mix them completely and spin down briefly in the centrifuge. It's suggested using the recommended kits to extract HBV DNA according to the manufacturer's instructions. The extracted DNA should be tested immediately or stored at -18°C for up to 3 months. Multiple freeze-thaw cycles (<5 x) should be avoided.

ADD SAMPLE.

If the extracted DNA and processed controls are stored at -18°C, thaw them completely at room temperature and centrifuge at 2000rpm for 10 seconds.

Add 20µL of the extractions from samples, negative/positive controls and QS1-4 separately into the PCR tubes prepared with reaction buffer and immediately close the tubes to avoid contamination. Centrifuge them at 2000rpm for 10 seconds. Load the PCR tubes into the PCR thermal cycler and record well the exact positions of each sample, control and QS in the machine.

PCR AMPLIFICATION (AMPLIFICATION AREA)

Transfer all prepared PCR tubes to the amplification area and load them into the thermocycler. Set up the thermocycler the negative control, detecting samples, QS1-4, high positive control and low positive control with sample names and the concentration of QS.

Set the fluorescent channels (ABI7500): Please refer to the manufacturer's instruction of the thermal cycler for detailed information.

HBV-DNA detection: FAM for Reporter, None for Quencher.

Internal Control: VIC for Reporter; TAMRA for Quencher.

Reference Dye: (ABI7500, None)

Sample Volume: 50 µL

Step	Temperature	Duration	Cycles
1	50℃	2 minutes	1
	95℃	5 minutes	
2	95℃	15 seconds	5
	55℃	30 seconds	
	72℃	30 seconds	
3	95℃	15 seconds	40
	55℃	45 seconds (Fluorescence measured)	

RESULTS ANALYSIS

The amplification results will be saved automatically after the predefined protocol is done. The Amplification Plot will display the amplification results of samples in the selected well. Through setting the threshold and the baseline values starting at 3-15 and ending at 5-20, the user can calibrate the amplification plot of Negative control paralleled or lower than the threshold plot.

Click [Analyze] and view the result at Report or Plate tab for HBV DNA of detecting samples.

QUALITY CONTROL

The threshold cycles (Ct) value of Negative control should be 0 or default while Ct value of internal control should be between 25 and 33.

The Ct value of high positive control should be not higher than 21 while the Ct value of low positive control is between 25 and 33.

HBV DNA detection of 4 QS are all positive and their Ct values are all lower than 33. The correlation coefficient (R2) of standard curve should not be lower than 0.97.

All above mentioned should be met in a single run. Otherwise, the test is not valid. Tests of all samples in the run should be repeated.

[Reference Range]

The lower limit of quantitation of the kit was determined as 25 IU/mL. The internal control should be positive of HBV DNA with Ct not higher than 33.

[Explanation of Test Results]

If the amplification plot of channel FAM is in Sigmoidal shape, the concentration of HBV DNA in testing samples should be estimated quantitively as following Table.

Quantitative	Internal Control	Interpretation and suggestion
Value (IU/mL)	Ct ≤ 33	HBV DNA is Positive at a concentration lower than 25 IU/mL or lower than the lower limit of quantitation
No value	Ct > 33	The test is invalid. The whole experiment procedures should be reviewed to identify the causative factors. All the specimens and controls in the run should be reprocessed, beginning from sample preparation.
0-25	Ct ≤ 33	HBV DNA concentration is very lower, lower than 25 IU/mL or the lower limit of quantitation. The test result is just for reference.
50-1*10^9	/	HBV DNA is positive, and the testing results can be reported quantitively.
1*10^9 -	/	Directly report the concentration of HBV DNA as higher than 1*109 IU/mL. Or, dilute the original specimen to 50~1*109 IU/mL of HBV DNA by 10 folds gradient dilution with HBV-negative human serum, and repeat the test. Multiply the reported result by the dilution factor to obtain the quantitative result.

A specimen with result of “Not Detected” cannot be presumed negative for HBV DNA. The final decision should be made combining other laboratory information. The follows can lead to the negative testing results of HBV DNA.

Incorrect operations in sample collection, transportation or processing;

Very low concentration of HBV in the samples;

Mutations within the highly conserved regions of the viral genome covered by the kit’s primers and/or probe may result in under-quantitation or failure to detect the presence of the virus;

Unproved external interference factors;

Patients infected by virus or bacterium rather than HBV.

[Limitations of Test Method]

The Results of HBV PCR Kit is just for information in practices to assess infection situation of patients combining with manifestation, other laboratory markers and their viral response to antiviral therapy.

Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site.

The instruments and assay procedures can reduce the risk of false positive or cross-contamination in Nucleic acid extraction and amplification product. Good laboratory practice and the procedures specified in this package insert should be carefully adhered.

The PCR Kit is just applicable for the above-mentioned specimen types and thermal-cyclers.

[Product Performance]

1. The coincidence rate of the Kit is 100% to test the national positive and negative reference panel of HBV nucleic acid.

2. The Kit can detect HBV DNA in all L0-L6 of quantitative and qualitative standard samples conforming to the calibration value.

3. The precision analysis of the HBV PCR Kit showed that the Coefficient of Variation (CV) of intra-batch variability and the inter-batch variability are all less than 5%.

4. Quantitative concentration Interval of the Kit is between 50IU/mL and 1*109IU/mL. The lower limit of quantitation is 50IU/mL and lower limit of detection is 25IU/mL, which has been verified by HBV genotype A/B/C/D.

5. The specificity of the HBV PCR Kit was validated with 50 samples of HBV negative serum from healthy volunteers. Test of these samples did not generate any signals.

6. Interference trial shows that performance of the HBV PCR Kit is stable when the concentrations of Bilirubin, blood-lipid, hemoglobin from hemolysis, and total IgG in specimen are lower than 168.2 μ mol/mL, 65 mmol/mL, 130g/L, and 5mg/mL as well, respectively.

7. A potential cross-reactivity of the HBV PCR Kit was tested and none of the tested pathogens and human genome have been reactive. No cross-reactivities appeared with mixed infections. The tested pathogens include HCV, Cytomegalovirus, EB virus, HIV, HAV, Syphilis, Human Herpesvirus-6, HSV-1/2, Influenza A, Propionibacterium Acnes, Staphylococcus Aureus and Candida albican.

8. The HBV PCR Kit can detect the HBV DNA at a blood virus load higher than the limit of detection.

[Precautions]

The QS, internal control and other reagents in the package can contain human sourced and/or potentially infectious components. Tests have been conducted and found to be nonreactive for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered

infectious and use Universal Precautions when performing the assay.

The laboratory should be equipped with instruments and technical staff in line with the Regulation of PCR laboratory in laboratory practice. The assay should be operated strictly according to the instructions.

Separate areas are dedicated to performing predefined procedures of the assay.

1st Area: Preparation Area—Prepare testing reagent;

2nd Area: Sample processing—Process the specimen and controls;

3rd：Amplification Area—PCR conducted.

All materials used in one area, including reagents, laboratory coats, pipettes, pipette tips, and vortexes, instruments, etc., should always be remained in the area and should not be moved or used in another area. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected timely and regularly using 1.0% sodium hypochlorite,75% ethanol or UV light.

Components of the Kit should be stored at -18°C or -70°C. Thaw all components thoroughly at room temperature (15–25°C) and centrifuge briefly before starting an assay.

Ensure that no foam or bubbles are present in the reagent tubes when pipetting specimens, controls, calibrators, or amplification Reagents. The tubes should be capped fasten and move to the processing area in sealable plastic bag. When pipetting the samples into the PCR tubes, care should be taken to ensure the samples exactly pipetted into the reaction mix and avoid sticking the samples to the inside tube wall. The tubes should be capped fasten immediately after the addition.

Ensure all PCR tubes capped fasten before loading them into the thermal cycler to avoid any possible leakage and contamination.

After the protocol of amplification is done, remove PCR tubes from the thermal cycler and seal them in a sealable plastic bag, discard them to designated waste bins. All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in the waste bin with Clorox (84) disinfectant and disposed with other laboratory wastes after decontamination.

[References]

[1] Ganem D, Prince A M. Hepatitis B virus infection--natural history and clinical consequences[J]. N Engl J Med, 2004,350(11):1118-1129.

2. Zhou Bin Hou Jinlin. Hepatitis B virus genotype, subtype and clinical [J]. Journal of Clinical Internal Medicine, 2006, 23(12): 797-799.

3. Pool Cloud. Introduction and comparison of clinical detection methods of hepatitis B virus [J]. Chinese Journal of Clinicians, 2007, 35(3): 64-66.

4. Liu Yong Zhang Ying. Progress in detection technology of hepatitis B virus and its clinical application[J]. Chinese Journal of Practical Internal Medicine, 2007, 27(3): 236-238.

5. Wang Qiaoya. Epidemiological trend and prevention of hepatitis B. Chinese Journal of Nursing [J]. 2007, 42(4): 382-384.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
RUO	RESERCH USE ONLY	REF	CATALOGUE NUMBER

	MANUFACTURER		CAUTION
	USE BY DATE		TEMPERATURE LIMITATION
LOT	BATCH CODE		CONSULT INSTRUCTIONS FOR USE
	DATE OF MANUFACTURE		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	Σ	CONTAINS SUFFICIENT FOR N TESTS

[Basic Information]

Jiangsu Macro & Micro-Test Med-Tech Co., Ltd.

Manufacturer Address: No. 888, Zhujiang Road, Juegang Street, Rudong County, (Life and Health Industrial Park of Rudong High-tech Zone), 226499 Nantong City, Jiangsu Province, PEOPLE'S REPUBLIC OF CHINA

Tel：+86-513-80562880

Website：http://www.hongweitest.com