DaAnGene

Instructions for Use of Diagnostic Kit for Quantification of Hepatitis B Virus DNA (PCR-Fluorescence Probing)

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CE 2934

Diagnostic Kit for Quantification of Hepatitis B Virus DNA (PCR-Fluorescence Probing)	**DA0041**	**Large package, 48 tests/kit**

Table of Contents

1. Product Name

Generic name: Diagnostic Kit for Quantification of Hepatitis B Virus DNA (PCR-Fluorescence Probing)

2. Package Specification

Large package, 48 tests/kit

3. Storage and Shelf Life

The shelf life of the kit is 9 months. The components of the PCR detection reagents, the quality controls, and the positive quantitative references shall be stored at -20±5°C.

During storage, repeated freeze-thaw shall be avoided (the repeated freeze-thaw cycles shall not exceed 4 times). After the kit is open, the reagents can stay stable for 8 hours at 10-30°C. The PCR reagents should be stored with dry ice or ice bags during transportation, and the transportation duration should not exceed 4 days.

Please refer to the package label for production date and expiry date of the kit .

4. Intended Use

This kit is intended for the quantitative detection of HBV DNA in human serum or plasma specimen.

HBV, which is a hepadnavirus, is mostly transmitted via blood. Globally, approximately 2 billion people have been infected HBV, about 350 million~ 400 million people worldwide are chronic carriers of the virus, and tens of millions of new infections annual.About 10-20% of acute hepatitis B patients may develop chronic hepatitis B, and the relative risk of developing primary liver cancer in people who are suffering from HBV chronic infection is at least 100 times higher than that of normal people. HBV has been classified into 9 genotypes(A-I), of which the genotype B, C, and D are common in Asia, while of which the genotype A is common in Europe.The HBV genome contains four open reading frames (ORF), called region S, C, P, and X respectively, and each region has variations at different levels.

The test result is for clinical reference only and should not be used as a sole basis for definite diagnosis or exclusion of infection.

5. Test Principle

This kit adopts the fluorescence PCR technology, designs specific primers and fluorescence probes

with relatively conserved regions in the HBV genome as target regions.

Rapid quantitative detection of HBV DNA by PCR is performed after specimen nucleic acid purification. In addition, the kit also contains an internal control for monitoring the whole process of nucleic acid extraction, so as to reduce false-negative results.

Use the nucleic acid extraction reagents recommended by this kit to process clinical specimens and extract nucleic acids, prepare PCR reaction tubes with the PCR detection reagents provided in the kit, add the extracted nucleic acids to the PCR reaction tubes, and a fluorescent quantitative PCR instrument is used for PCR amplification and the fluorescent signal detection. The instrument software system automatically draws a real-time amplification curve, and achieves the quantitative detection of unknown specimens according to the threshold cycle value (Ct value).

6. Main Components

Table 1: Main Components of the kit

Component Name		Specification	Quantity	Main Constituents
Quality controls and positive quantitative references	Negative control	600 μL/tube	1	Negative plasma
	HBV high positive control	600 μL/tube	1	Inactivated serum or plasma of hepatitis B patient
	HBV borderline positive control	600 μL/tube	1	Inactivated serum or plasma of hepatitis B patient
	HBV positive quantitative reference 1(5.0×10^6 IU/mL)	600 μL/tube	1	Inactivated serum or plasma of hepatitis B patient
	HBV positive quantitative reference 2(5.0×10^5 IU/ mL)	600 μL/tube	1	Inactivated serum or plasma of hepatitis B patient
	HBV positive quantitative reference 3(5.0×10^4 IU/ mL)	600 μL/tube	1	Inactivated serum or plasma of hepatitis B patient
	HBV positive quantitative reference 4(5.0×10^3 IU/ mL)	600 μL/tube	1	Inactivated serum or plasma of hepatitis B patient
PCR detection reagents	HBV internal control solution	250 μL/tube	1	Internal control plasmid and stabilizer
	HBV reaction solution A	96 μL/tube	1	HBV specific primer and probe
	HBV reaction solution B	144 μL/tube	1	Hot start Taq polymerase, UNG enzyme
	HBV reaction solution C	720 μL/tube	1	$MgCl_2$, Brij-58, Tris-hydrochloric acid buffer

Note: Components of different batches shall not be used interchangeably.

7. Applicable Instruments

ABI Prism 7500

8. Materials and Instruments Required But Not Provided (including but not limited to)

- Disposable gloves and mask, powder-free
- Centrifuge tube
- Adjustable micropipettes and pipette tips with filters
- Timer
- Real-time PCR amplification instrument
- PCR reaction tube
- Desktop high speed centrifuge
- Nucleic Acid Isolation or Purification Reagent（Cat.# DA-451）produced by Daan Gene Co., Ltd. are recommended.
- Nucleic Acid Isolation or Purification Reagent（Cat.# DA0900~DA0902）produced by Daan Gene Co., Ltd. are recommended.
- Nucleic Acid Isolation or Purification Reagent（Cat.# DA0620~DA0626）produced by Daan Gene Co., Ltd. are recommended.

9. Specimen Requirements

9.1. **Applicable specimen type: serum or plasma.**

9.2. **Specimen collection, storage and transportation**

9.2.1. Specimen collection

For serum, draw 2 mL of venous blood from a subject with a disposable sterile syringe and inject it into a sterile dry glass tube. After standing for 30-60 minutes at room temperature (15-25°C), the blood specimen will spontaneously coagulate to separate out serum, or directly centrifuge the blood specimen for 5 minutes at 1,500 rpm with a horizontal centrifuge, draw the upper serum and transfer it into a 1.5 mL sterilized centrifugal tube.

For plasma, draw 2 mL of venous blood from a subject with a disposable sterile syringe and inject it into a glass tube containing EDTA-2Na (Ethylenediamine Tetraacetic Acid Disodium Salt) or sodium citrate anticoagulant, immediately reverse the glass tube slightly 5-10 times so as to fully mix the

anticoagulant and the venous blood, after 5-10 minutes the plasma is separated out, transfer it into a 1.5 mL sterilized centrifugal tube.

9.2.2. Specimen storage and transportation

The collected specimen can be used for testing immediately or stored at -20°C for 6 months. 0°C is recommended for specimen transportation.

10. Test Method

10.1. **Specimen processing and nucleic acid extraction (specimen processing area)**

Specimen processing and nucleic acid extraction can be performed by using the Nucleic Acid Isolation or Purification Reagent (Catalogue No. of recommended products: DA-451,DA0900~DA0902,DA0620~DA0626) produced by the Daan Gene Co., Ltd. The internal control solution in the kit is involved in the extraction process. If the use method of internal control is not available in the extraction kit, add the internal control solution to the specimen at the ratio of specimen volume: internal control solution volume = 50 : 1 for nucleic acid extraction.

All of the quality controls and positive quantitative references in the kit need to be extracted for environment monitoring as well as quality control of PCR detection reagents.

After nucleic acid extraction of the specimen, it is recommended to proceed to the next step immediately; or store the nucleic acid at -20±5°C,for later use (within 24 hours).

10.2. **Preparation of PCR reagents (reagent preparation area)**

Take HBV reaction solution A, HBV reaction solution B, and HBV reaction solution C from the kit; thaw at room temperature, mix well by vortexing, and briefly centrifuge at 8,000 rpm for later use.

Take N PCR reaction tubes (N=number of to-be-detected specimens + negative controls + HBV high positive controls + HBV borderline positive controls + 4 tubes of HBV positive quantitative references); the table 2 below shows the preparation of single-test amplification system of HBV:

Table2:Preparation of single-test amplification system of HBV

Components	HBV reaction solution A	HBV reaction solution B	HBV reaction solution C	Total volume
Dosage	2 μL	3 μL	15 μL	20 μL

Well mix the components, then centrifuge shortly so that liquid on the tube wall completely goes to the tube bottom, and allocate 20 μL of amplification system into PCR tube.

10.3. **Sample loading (specimen preparation area)**

Add 40 μL of the extracted nucleic acid of specimen to be detected, HBV negative controls, HBV high positive controls, HBV borderline positive controls, and HBV positive quantitative references respectively into the HBV reaction tubes using pipette tips equipped with filter. Cover the tubes tightly, briefly centrifuge at 8,000 rpm, and then transfer to the amplification detection area.

10.4. **PCR amplification (amplification detection area)**

10.4.1. ABI Prism 7500 instrument setup

10.4.1.1. Open the "Setup" window; set the negative controls (NTC), positive controls, and unknown specimens (Unknown) and positive quantitative references (Standard) according to an order corresponding to the specimens. Set the specimen names in the column of "Sample Name"; and set the probe detection mode as Reporter Dye1: FAM, Quencher Dye1: TAMRA, Reporter Dye2: VIC, Quencher Dye2: none, Passive Reference: NONE.

10.4.1.2. Open "Instrument" window, and set the cycle conditions as follow:

2 minutes at 50°C, 1 cycle;

15 minutes at 95°C, 1 cycle;

15 seconds at 94°C to 45 seconds at 55°C (fluorescence collection), 45 cycles.

After the setting, save the file and run the program.

10.5. **Analysis of results**

After reaction, the results are automatically saved. Adjust the Start value, End value, and Threshold value of the Baseline according to the analyzed image (users can adjust the values themselves according to actual conditions, the Start value can be set at 3-15, and the End value at 5-20, the Threshold value can be set in the Log atlas window while ensuring the baseline stay in the exponential phase of the amplification curve, and the amplification curve of the negative controls is adjusted to be straight or lower than the threshold line). Click on Analysis to automatically get the analysis result, view the results on the Report interface, and record the values (C) of the unknown specimens.

10.6. **Quality control**

10.6.1. Negative controls: the amplification curve in FAM detection channel does not have an increased logarithmic phase, or the Ct value is 45; and the amplification curve in VIC detection channel has an obvious increased logarithmic phase.

10.6.2. HBV positive controls: the amplification curve in FAM detection channel has an obvious increased logarithmic phase, and the Ct value is less than 40; the determined value of the HBV high positive controls ranges from 1.0×10^6 to 4.0×10^7 IU/mL, and that of the HBV borderline positive controls ranges from 3.0×10^2 to 1.0×10^4 IU/mL.

10.6.3. HBV positive quantitative references: the amplification curve in FAM detection channel has an obvious increased logarithmic phase, the Ct value < 40, and $R^2 \geq 0.97$.

The above requirements must be met at the same time in each test run; otherwise, the test run is invalid and needs to be rerun.

10.7. **Judgement of test result**

10.7.1. If the amplification curve in FAM detection channel does not have an obvious increased logarithmic phase or the Ct value is 45, and the amplification curve in VIC detection channel has an increased logarithmic phase, the HBV DNA concentration of the specimen is determined as below the limit of detection.

10.7.2. If the amplification curve in FAM detection channel has an increased logarithmic phase and the Ct value is less than 45, judge as follows:

If the C value of the specimen satisfies $20 \leq C \leq 1.00E+009$, then the HBV DNA concentration of the specimen is C IU/mL.

If the C value of the specimen satisfies $C > 1.00E+009$, the HBV DNA concentration of the specimen > 1×10^9 IU/mL. For precise quantification of the result, dilute the specimen to a linear range before detection, then the HBV DNA concentration of the specimen = (C×dilution ratio) IU/mL.

If the C value of the specimen satisfies 10 IU/mL ≤ C < 20 IU/mL, and the amplification curve in VIC detection channel has an increased logarithmic phase, the HBV DNA concentration of the specimen is for reference only.

If the C value of the specimen satisfies C < 10 IU/mL and the amplification curve on the VIC detection channel has an increased logarithmic phase, the HBV DNA concentration of the specimen is below the detection limit of the kit.

11. Positive Judgment Value

According to the test results of clinical specimens, the reference value of the kit is determined as Ct value equals to 45.

12. Interpretation of Test Results

12.1. Negative control , HBV high positive control, and HBV borderline positive control are required to be detected for each test; only when the quality control results meet the quality control requirements can the test results be determined.

12.2. Criteria for positive results: the amplification curve in FAM detection channel has an increased logarithmic phase, and the Ct value is less than 45.

12.3. Criteria for negative results: the amplification curve in FAM detection channel does not have an increased logarithmic phase or the Ct value is equal to 45, while the amplification curve in VIC detection channel has an increased logarithmic phase.

12.4. The following report format is recommended:

Report format on negative results: HBV DNA is not detected in the specimen, and its concentration is lower than the LoD of the kit.

Report format on positive results:

1) If the test result of the specimen shows that the HBV DNA concentration satisfies 20 IU/mL ≤ C ≤ 1.00E+009 IU/mL, the report format is: HBV DNA is detected in the specimen,with a concentration of C IU/mL.

2) If the test result of the specimen shows that the HBV DNA concentration satisfies C > 1.00E+009 IU/mL, the report format is: HBV DNA concentration is detected in the specimen with a concentration beyond 1×10^9 IU/mL. If the detection is performed after dilution, the report format is: HBV DNA is detected in the specimen, with a concentration of (C×dilution ratio) IU/mL.

3) If the test result of the specimen shows that the HBV DNA concentration satisfies 10 IU/mL≤C<20 IU/mL while the amplification curve in VIC detection channel has an increased

logarithmic phase, the report format is: HBV DNA load in the specimen is relatively low, and the measured value is for reference only.

4) If the test result of the specimen shows that the HBV DNA concentration satisfies C<10 IU/mL while the amplification curve in VIC detection channel has an increased logarithmic phase, the report format is: HBV DNA content in the specimen is below the detection limit of the kit; if the amplification curve on the internal control detection channel does not have an increased logarithmic phase or no Ct value, the test result of the specimen is invalid. The cause should be found and eliminated, and the specimen should be tested repeatedly. (If the result is still invalid, please contact Daan Gene Co.,Ltd.)

12.5. The test result is for clinical reference only, for a definite diagnosis of HBV infection, please diagnose with clinical symptoms and other detection methods.

13. Limitations of Test Method

13.1. The specimen test results are related to the quality of specimen collection, processing, transportation, and storage, and any mistakes may lead to a false negative result.

13.2. A false positive result may occur if cross-contamination is not under good control during specimen processing.

13.3. Due to the different principles of different extraction methods, the nucleic acid extraction efficiency is different when treated with different nucleic acid extraction reagents, please use the nucleic acid extraction reagent recommended in the test method in this instructions.

14. Performance Characteristics

14.1. According to the product performance study, the linear range of the kit is from 20 IU/mL to 1.0×10^9 IU/mL, and the detection sensitivities on the specimens of HBV genotype A, B, C, D, E, F, G, H, hybrid genotype B/C and hybrid genotype C/D are all 10 IU/mL.

14.2. Analytical specificity:

14.2.1. Cross-reactivity

The kit is free of cross reactivity with the viruses that infect the same infection site or lead to similar infection symptoms, or other viruses (human cytomegalovirus, EB virus (EBV), Human immunodeficiency virus type 1 (HIV-1), Hepatitis C virus (HCV), Hepatitis A virus,

syphilis, Human herpes virus type 6, Herpes simplex virus type 1, Herpes simplex virus type 2, Influenza A virus, Propionibacterium acnes, Staphylococcus aureus, Candida albicans, Human Parvovirus B19, Varicella-Zoster Virus(VZV), Human T-lymphotropic Virus I (HTLV-I), Human T-lymphotropic Virus II (HTLV-II), Hepatitis E virus,(HEV), Human immunodeficiency virus type 1 (HIV-2).

14.2.2. Interference study

Endogenous interfering substances, including bilirubin with concentration not higher than 30 mg/dL, triglyceride with concentration not higher than 3,200 mg/dL, hemoglobin with concentration not higher than 28 g/dL, and albumin with concentration not higher than 6 g/dL, have no interference with the kit performance.

Exogenous interfering substances, including α-interferon with concentration not higher than 14.2μg/mL, lamivudine with concentration not higher than 1.1-1.5 μg/mL, adefovir with concentration not higher than 18.4 ± 6.26 ng/mL, entecavir with concentration not higher than 4.3-6.7ng/mL, telbivudine with concentration not higher than 3.69 ± 1.25μg/mL and famciclovir with concentration not higher than 3.3 ± 0.8mg/L, have no interference with the kit performance.

14.3. The CV% (coefficient of variation) of intra-batch and inter-batch precision of the kit all can achieve to less than 5%.

14.4. Clinical Performance

14.4.1. Diagnostic Sensitivity

The 104 positive samples (covering A-H genotypes) were collected from German patients and are representative for an European population and the test results were all positive. The diagnostic sensitivity (as a percent agreement with Cobas® HBV) of 100% (104/104) with a Wilson 95% CI of [96.4 – 100.0%].

14.4.2. Diagnostic Specificity

The 100 negative samples were collected from two different European blood donation centers (Ulm and Frankfurt) and are representative for an European population and the test results were negative, with diagnostic specificity of 100% (100/100), with a Wilson 95% CI of [96.3 – 100.0%].

15. Warnings and Precautions

15.1. The kit is for in-vitro diagnosis only. Please read the instructions for use carefully before test.

15.2. In order to avoid any potential biohazards in the specimen, all specimens shall be regarded as infectious, and avoid contact with skin and mucosa, prepare the specimen in a biosafety cabinet capable of preventing aerosol outflow, put the test tubes and pipette tips used in the specimen preparation area into a container with disinfectant, and sterilize them together with the wastes before disposal. The specimen handling and processing should both meet the requirements of relevant local laws and regulations.

15.3. The components in the kit should be used within the shelf life. Wrong results may be caused by not using the components provided by the kit.

15.4. Laboratory management should be in strict accordance with the practices on management of PCR gene amplification laboratories. Laboratory personnel must be trained for professional skills. The experimental process should be carried out strictly in each areas (reagent preparation area, specimen preparation area, and amplification detection area). All consumables should be sterilized for single use. Dedicated instruments and equipment are used in each stage of the test process, and supplies in different areas and stages cannot be used interchangeably.

15.5. Use autoclaved disposable centrifuge tubes and pipette tips, or purchase DNase-free / RNase-free centrifuge tubes and pipette tips.

15.6. Thaw the PCR detection reagents completely and briefly centrifuge at 8,000 rpm before use, and avoid repeated freeze-thaw.

15.7. After nucleic acid extraction of the specimen, it is recommended to proceed to the next step immediately; or store the nucleic acid at -20 °C for later use (within 24 hours).

15.8. If cross-contamination is not adequately controlled during specimen processing, a false positive result may occur.

15.9. All consumables should be sterilized for single use; dedicated instrument and equipment should be used in each stage of the test process; never exchange consumables in different stages in different areas.

15.10. Quality control must be performed over each test run.

15.11. After the test, disinfect the workbench and pipettor with 10% hypochlorous acid or 75% alcohol, then irradiate with an ultraviolet lamp for 20-30 minutes.

15.12. All positive controls in the kit and specimens shall be regarded as infectious, the specimen handling and processing should both meet the requirements of relevant local laws and regulations.

16. References

16.1. Tania M. Welzel, et al. 2006. Real-Time PCR Assay for Detection and Quantification of Hepatitis B Virus Genotypes A to G. J Clin Microbiol, 44 (9) : 3325 - 33.

16.2. Vincent Thibault, et al. 2007. Characterization of a new sensitive PCR assay for quantification of viral DNA isolated from patients with hepatitis B virus infections. J Clin Microbiol. 45 (12) : 3948 -53.

16.3. WikinsT,Sams R,Carpenter M.Hepatitis B:Screening,Prevention,Diagnosis, and Treatment [J]. Am Fam Physician,2019,99(5):314-323.

16.4. Kramvis, A. Genotypes and genetic variability of hepatitis B virus. Intervirology 2014, 57, 141–150.

16.5. Pourkarim, M.R.; Amini-Bavil-Olyaee, S.; Kurbanov, F.; Van Ranst, M.; Tacke, F. Molecular identificationof hepatitis B virus genotypes/subgenotypes: Revised classification hurdles and updated resolutions. World J. Gastroenterol. 2014, 20, 7152–7168.

17. Manufacturer

Name of manufacturer/ after-sales service unit: Daan Gene Co., Ltd.

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No.6, Lizhishan Road, Science Park, High & New Technology Development District, Guangzhou, Guangdong, P. R. China.

18. Reference standards

EN ISO 15223-1:2016 Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements

EN ISO 18113-1:2011 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements

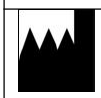
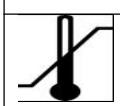
EN ISO 18113-2:2011 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use

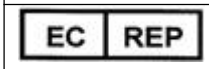
19. European Authorized Representative

MDSS GmbH

Schiffgraben 41, 30175 Hannover, Germany

20. Explanation of Symbols

	Use-by date
	Batch code
	Date of manufacture
	Manufacturer
	Catalogue number
	Temperature limit
	Contains sufficient for <n> tests
	In vitro diagnostic medical device

	Do not re-use
	CE marking
	Caution
	European Authorized Representative
	Consult Instructions for Use