TRIPLEX INTERNATIONAL BIOSCIENCES(CHINA) CO., LTD.

Hepatitis B Virus

Genotyping Kit Manual (Fluorescence probe PCR method)

【Product Name】

Generic name: hepatitis B virus genotyping kit (PCR- fluorescent probe method)

【Packaging Specification】

10 tests / box

【Intended Use】

This kit is suitable for genotyping of hepatitis B virus, and can detect subtype B, C and D in the serum of patients.

Studies have confirmed that the genotype of hepatitis B virus is closely related to the pathogenesis, prognosis and antiviral efficacy of hepatitis B. The clinical characteristics and pathogenicity of different genotypes are not the same. Genotypes are associated with chronic HBV carriers and the progression of primary hepatocellular carcinoma in the younger age group. Genotype C is associated with the activity of liver disease and vertical transmission between mother and infant. HBV mutations induced by lamivudine are closely related to HBV genotypes. Different HBV genotypes respond differently to lamivudine treatment and HBV infection. The different clinical prognosis may be related to genotypes. The importance of hepatitis B virus genotyping is:

1. to predict the risk of hepatocellular carcinoma (HCC) according to the hepatitis B virus genotype.
2. to predict the risk of drug resistance based on HBV genotype.
3. to provide molecular biological basis for clinical medication for asymptomatic HBV carriers.

【Detection Principle】

This kit adopts Polymerase chain reaction (PCR) combined with Taqman fluorescence probe technology to genotype hepatitis B virus in the samples, which can detect subtype b, c and d infections alone and mixed infections. Two fluorescence (FAM and HEX) probes were used, with FAM for b (or d) subtypes and HEX for c subtype. The advantage of this product is that a single experiment can detect three subtypes simultaneously, and the operation is simple. The use of dUTP and UNG enzyme in the kit can effectively eliminate possible carry-over template contamination. The disadvantage of this product is that the PCR machine is required to have two specific wavelengths.

【Kit Contents】

Contents	Amount	Number	Ingredients
DNA Concentration Solution	1mL	1 vial	EG6000, NaCl
DNA Extraction Buffer	300μL	1 vial	NaOH, Tris-HCl, NP40, Chelex100, Triton-100, EDTA
B/C Genotype Reaction Solution	440μL	1 vial	Primers, probes, dN (U) TP, buffer systems, etc.
D Genotype Reaction Solution	440μL	1 vial	
Taq/UNG	20μL	1 vial	Taq DNA polymerase, UNG enzyme
HBV Genotype Negative Control	100μL	1 vial	Over inactivated HBV subtype b positive serum
HBV Genotype Positive Control	100μL	1 vial	Inactivated HBV negative serum

disadvantage of this product is that the PCR machine is required to have two specific wavelengths.
Both positive and negative quality control samples were collected clinically and inactivated. The positive quality control samples were HBV subtype b positive serum and negative quality control samples were HBV negative serum.

【Storage conditions and validity period】

- Stored at -20±5°C， the kit is valid for 6 months. Production date and validity period are on the product label.
- After opening, valid for 5 hours at room temperature; valid if less than 5 times of repeated freezing and thawing ; valid if transportation period is within 7 days.

【Storage conditions and validity period】

Agilent Stratagene Mx3000P fluorescence PCR machine, ABI Prism 7500 fluorescence PCR machine.

【Sample Collection, Storage and Transport】

1. Specimen collection: draw 5mL venous blood with a syringe, inject into a centrifuge tube, label with patience's name and coding number. Incubate the blood sample for 1 hour at 37°C, and then centrifuge for 4-10 min at 4000rpm. Transfer 1.5mL clear light yellow serum into a clean 1.5 mL centrifuge tube and seal tightly for testing.
2. Specimen should be kept not more than 2 hours at room temperature and not more than 48 hours at 4°C , not more than 6 months at -20°C. Avoid frequent freezing and thawing.
3. Specimen transportation should be carried out at around 0°C.

【Procedure】

1. Reagent Preparation

The reaction buffer was prepared according to the number of experimental sample number N (N = serum sample number + 2 quality controls): b/c Genotyping Reaction Solution and d Genotyping Reaction Solution were taken out and thawed at room temperature, then N*44 μL b/c Genotyping Reaction Solution and N*1 μL Taq/UNG enzyme system were added into a centrifuge tube, vortexed briefly, spin down briefly, aliquoted into N tubes with 45 μL/tube, and these tubes are used as the b/c subtype PCR reaction tubes; then N*44 μL d Genotyping Reaction Solution and N*1 μL Taq/UNG enzyme system are added into another centrifuge tube, vortex mixed, spin down briefly, aliquoted into N tubes with 45 μL/tube, and these tubes are as the d subtype PCR reaction tubes. After covered with covers, all the PCR reaction tubes are moved to sample adding area, and are stored at 4 degrees and away from light for further use.

2. Sample Processing

To 0.5mL centrifugal tubes, 100 μL DNA Concentration Solution and 100 μL serum samples were added. The samples were vortexed, and centrifuged for 10 minutes at 12,000 rpm. Aspirate and discard the supernatant (be careful not to touch the bottom sediment), add 30 ☒L DNA Extract Buffer to the sediment (the DNA Extract Buffer should be fully mixed before used), boil it at 1000C for 10 minutes, centrifuge for 5 minutes at 12,000 rpm, and keep the supernatant as the PCR template. If the PCR template is not used immediately, it must be kept below – 180C. It should be thawed and spin down before reuse.

Sample adding: For each experimental sample, add 5μL PCR template into a b/c and a d subtype PCR reaction tubes. The PCR reaction tubes were vortexed briefly, spin down briefly, and loaded onto a fluorescent PCR instrument.
Note: HBV Genotype Negative Control and HBV Genotype Positive Control serum samples were processed simultaneously.

3. PCR Amplification

TAmplification is carried out according to the following temperature profile.
Stage 1, 37°C ---- 2 min
Stage 2, 94°C ---- 2 min
Stage 3 (40 cycles), 94°C ---- 15 sec
60°C ---- 45 sec
Note: fluorescence is collected at Stage 3 (600C frame). Fluorescein is set to FAM and HEX (set to JOE if HEX is unavailable).

Result analysis: The signal information will be saved automatically. After reaction, adjust the Start value, End value and Threshold value of Baseline according to the image after analysis (users can adjust themselves according to the actual situation, Start value can be set at 2-6, End value can be set at 10-15, adjust the amplification curve of the negative control straight or below the threshold line). Click Analysis to automatically get the analysis results and see the results at the Report interface

4. Quality Control

Quality Control	Requirements
HBV Genotype Positive Control	Detected as HBV B subtype: B/C FAM channel Ct≤35.1, and both HEX channel(for d subtype) Ct=40
HBV Genotype Negative Control	Detected as HBV B/C/D negative: both B/C FAM channels and D HEX channel Ct>= 40

Note: The above requirements should be met in a single experiment, otherwise the results are invalid and retest is needed.

5. Result Judge

Result analysis: The signal information will be saved automatically. After reaction, adjust the Start value, End value and Threshold value of Baseline according to the image after analysis (users can adjust themselves according to the actual situation, Start value can be set at 2-6, End value can be set at 10-15, adjust the amplification curve of the negative control straight or below the threshold line). Click Analysis to automatically get the analysis results and see the results at the Report interface. Make judgment as below:

B/C Genotype PCR Solution		D Genotype PCR Solution	Results
FAM channel	HEX channel	FAM channel	
Ct≤35.1	Ct=40	Ct=40	HBV B genotype
Ct=40	Ct≤35.1	Ct=40	HBV C genotype
Ct=40	Ct=40	Ct≤35.1	HBV D genotype
Ct≤35.1	Ct≤35.1	Ct=40	HBV B&C mixed infection
Ct≤35.1	Ct=40	Ct≤35.1	HBV B&D mixed infection
Ct=40	Ct≤35.1	Ct≤35.1	HBV C&D mixed infection
Ct≤35.1	Ct≤35.1	Ct≤35.1	HBV B&C&D infection
Ct=40	Ct=40	Ct=40	HBV B&C&D negative

Note: 35.1＜Ct＜40 is detection gray area, retest is suggested, if same result occurs, refer to Ct≤35.1 for judgment.

【Positive Judgment Value】

Through the analysis of clinical test results, the reference value of the kit was determined to be 35.1 by ROC curve method.

Through analysis of clinical test results using ROC curve method, we finally determined the positive judgment Ct value for this kit is 35.1

【Results Interpretation】

1. It is difficult to detect low viral content samples by gold standard methods such as DNA sequencing analysis for subtype mix samples.
2. HBV B, C, D negative only means that the samples do not contain B, C and D hepatitis B virus, the samples may contain other types of hepatitis B virus.

【Detection Limitation】

The kit is designed for the most common hepatitis B, C and D viruses in China, and cannot detect other genotypes. Samples below the minimum detectable concentration cannot be detected.

【Kit Performance】

Minimum detectable amount: HBV-B type 1 x 103 copy/mL, HBV-C type 1 x 103 copy/mL, HBV-D type 1 x 103 copy/mL; Precision: within batch CV < 10% (n = 10), between batch CV < 10% (n = 10); Analysis specificity: upper limit of endogenous interfering factors in blood samples (direct bilirubin 515 micromol/L, cholesterol 13 mmol/L, bile acid 30 micron OI/L, triglyceride 41 mmol/L, glucose 55 mmol/L) had no effect on the detection. Serum samples with other common pathogens (HAV, HCV, HEV, HIV, CMV) were detected to be negative. Serum samples of other HBV types (HBV A, E, F, G, H) were detected to be negative. The results of clinical studies with 868 patients show that the results using this kit are consistent with that using DNA sequencing, the golden standard, analyzed using kappa test. Because the K value was above 0.9, the consistency intensity was very strong according to the reference judgment index.

【Warnings and Precaution】

1.This product is for in vitro diagnosis purpose only.
2. Please read the full text of the instruction carefully before the experiment.
3. The experimenters should be professionally trained and the experimental process should be carried out strictly in different areas. The articles and lab coats in each area are for special use. They should not be used interchangeably to avoid contamination. Clean the workbench immediately after the experiment.
4. The product should be fully melted and mixed at room temperature before use, but repeated freezing-thawing should be avoided.
5. Specimen handling should be carried out in biosafety cabinets to protect the safety of operators and prevent environmental pollution.
6. Carry out quality control for each experiment.
7. Do not interchangeably use reagents of different batches and use the kit within the validity period.
8. Samples should be completely mixed into the reaction solution, no samples should adhere to the wall of the tubes, and the caps should be tightly applied as soon as possible after the samples are added.
9. The PCR reaction tubes, centrifuge tubes and pipet-tips used for PCR reaction preparation should be autoclaved and used as disposable.
10. After amplification, the reaction tubes should be unloaded and sealed in a special plastic bag and disposed as medical wastes.
11.The pipet-tips used in the experiment should be dismounted directly into the waste tank containing 10% sodium hypochlorite and discarded after sterilized together with other waste materials.
12. The workbench and all the experimental articles should be frequently sterilized using 10% sodium hypochlorite, 75% alcohol, and ultraviolet light.

【References】

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2. Abdel-Rahman N, Mohamed M, Nahed I et a1. Hepatitis B virus (HBV) genotypes in Egyptian pediatric cancer patients with acute and chronic active HBV infection. Virology Journal 2007, 4:74.
3. Ying Liu, Munira Hussain, Stephen Wong et a1. A Genotype-Independent Real-Time PCR Assay for Quantification of Hepatitis B Virus DNA. Journal of Clinical Microbiology,Feb. 2007, p. 553–558.

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