Hepatitis–B virus [HBV] Real-time PCR Kit

(For Qualitative Detection)

REF 8001

IVD -15℃ -25℃

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INTENDED USE

The HBV Real-time PCR Kit is an *in-vitro* nucleic acid amplification kit for the detection and quantification of Hepatitis B virus [Genotype A to H] specific DNA.

KIT CONTENTS

Components	Number of vials	Volume Per vials
Probe PCR Master Mix	1	250µl
HBV Primer Probe Mix [HBV PP mix]	1	65µl
Internal control Primer Probe Mix [IC PP Mix]	1	65µl
Internal control template [IC template]	1	125µl
HBV Positive control [QS1]	1	250µl
Water, PCR grade	1	4ml

STORAGE

1. The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

PRODUCT DESCRIPTION

HBV Real-time PCR Kit constitutes a ready-to-use system for the detection of HBV specific DNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the HBV genome, and for the direct detection of the specific amplicon in FAM channel. In addition, it contains an internal control amplification system to identify possible PCR inhibition and DNA purification efficiency. External positive control is supplied, which can be used as both qualitative and quantitative to determination the amount of viral load.

SPECIFICITY

HBV primer and probe have been designed for the specific and exclusive *in-vitro* quantification of HBV. The target sequence (Core Protein gene) is highly conserved and has previously been shown to be a good genetic marker for HBV. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of DNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified HBV specific DNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.25 copies per micro liter.

DNA PURIFICATION

If you are using a spin column-based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

INTERNAL CONTROL TEMPLATE

Add 5µl of the internal control template to each test sample. Do not add directly to test sample. Add after adding lysis buffer to the test sample [sample/lysis buffer mix]. Complete purification according to the manufacturer's protocols.

Amplification of the internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 21 +/-10.

PREPARATION OF STANDARD CURVE DILUTION SERIES:

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction. Prepare fresh for every run.
8. 1IU = 1 copy

Standards	Copies per µl
QS-1	2500000
QS-1	250000
QS-1	25000
QS-1	2500

DETECTION PROTOCOL

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Make sure that internal control template is added during DNA purification. If not, pipette 2.5µl of the internal control template directly into the purified DNA.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

PRODUCT USE LIMITATIONS

Components	Volume per reaction
Probe PCR Master Mix	10µl
HBV PP Mix	2.5µl
IC PP Mix	2.5µl
Master Mix Total	15µl
Purified DNA / Positive Control / Negative Control	10µl
Final reaction volume	25µl

Negative Control setup [NTC]
Add 10µl of PCR grade water.

Qualitative Positive Control setup
Add 10µl of any one of the Positive controls [From QS1 to QS4]

Quantitative Positive controls setup
10µl of all Positive controls prepared from QS1 to QS4.

PROGRAMMING THERMAL CYCLER

Sample volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference	None
Ramping rate	Default

THERMAL PROFILE

Cycles	Step	Time	Temp
1	Taq enzyme activation / Hold	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	56ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	HBV
HEX	Internal control

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance

READING THE GRAPH

Step-1 – Internal control Validation
Select the test samples alone for the internal control analysis. Select HEX dye and view the graph of internal control amplification. A successful amplification Ct value must be within Ct 21 +/- 10.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

Step-2 – FAM - Negative and Positive control validation
Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-3 –FAM - Test Sample status
In FAM channel, select test sample well one by one, analyze the graph/amplification.

QUALITATIVE INTERPRETATION OF RESULTS

Test Sample	Negative Control	Positive Control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	HBV specific DNA detected
Negative	Negative	Positive	Positive	No HBV specific DNA Detected. Sample does not contain detectable amounts of HBV
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

Qualitative

Observation		Inerpretation
FAM-HBV	**HEX-IC**	
<37	<31	HBV detected

Recommendation:

In FAM [HBV] channel, the Ct value beyond 35 is required careful analysis. The analysis may include that the status of NTC amplification curve, threshold adjustment, linear/log scale view assessment, etc.,

STANDARD CURVE ANALYSIS

- Interpret the values for unknow samples, only if the R>0.98
- Slope of calibrators is between-3.0 to -3.7
- PCR efficiency is between 85% and no amplification in FAM channel of negative control.
- 1 copy is equal to 1 IU

Calculating copies or IU per ml

Input the machine indicated copy number into the following formula

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/}\mu\text{l) x Elution Volume (}\mu\text{l)}}{\text{Sample Volume (ml)}}$$

Note:

Elution volume: must be typed in micro liter format, example 30µl, 60µl or 100µl. Sample volume: must be typed in milliliter format, example 0.2ml or 0.5ml

Observation	Interpretation
<50 IU/ml	HBV detected, but below limit of detection
50 IU to 3 x 10⁹ IU/mL	HBV detected and quantified
<3 x 10⁹ IU/mL	HBV detected, but upper limit of quantitation

LIMITATIONS

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
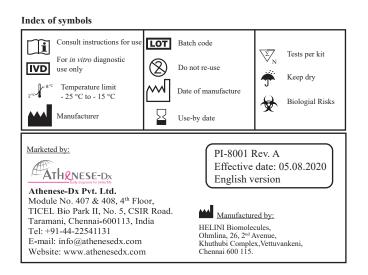
The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, HBV Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

In accordance with in house Quality Management System, each lot of HBV Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Index of symbols

	Consult instructions for use	**LOT**	Batch code	Σ N	Tests per kit
IVD	For *in vitro* diagnostic use only	⊗	Do not re-use		Keep dry
8℃ 2℃	Temperature limit - 25 °C to - 15 °C		Date of manufacture		Biologial Risks
	Manufacturer	⊠	Use-by date		