RTA®

HBV Real-Time PCRKit Handbook

Date of Issue –– 10.11.2017

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Quantitative detection of Hepatitis B virus DNA

For *research* use only

REF 09060025 - 25tests
09060100 -100 tests

Kit Contents

	CapColor	Reagents	25 Tests	100 Tests
1	BROWN	HBV Mix A	160 µl	610 µl
2	YELLOW	HBV Mix B	120 µl	450 µl
3	BLUE	HBV Internal Control	75 µl	250 µl
4	RED	HBV Quantification Standart 1 (10^7 IU/ml)	100 µl	100 µl
5	RED	HBV Quantification Standart 2 (10^6 IU/ml)	100 µl	100 µl
6	RED	HBV Quantification Standart 3 (10^5 IU/ml)	100 µl	100 µl
7	RED	HBV Quantification Standart 4 (10^4 IU/ml	100 µl	100 µl
8	WHITE	PCR Grade Water	100 µl	100 µl

Storage

All reagents of RTA HBV Real-Time PCR Kit should be stored at -20°C. Storage at higher temperatures should be avoided (e.g. +4°C). Under these conditions, kit contents should be stable through the expiration date printed on the label. The reagents should not be freeze-thawed more than 2 times; otherwise the shelf of the kit will reduce. During the working steps all reagents should be kept on ice.

Intended Use

RTA HBV Real-Time PCR Kit is an *in vitro* nucleic acid amplification assay for quantification of Hepatitis B virus (HBV) DNA in human serum or plasma (EDTA) using RTA Viral DNA Isolation Kit or RTA Viral Nucleic Acid Isolation Kit and Stratagene Mx3000p/Mx3005p instrument or LightCycler 2.0 or INCEPTRA Cycler 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD or Bioneer Exicycler 96 Real-Time PCR Detection System for amplification, detection and analysis. RTA HBV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings. RTA HBV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of HBV DNA or confirmation of the diagnosis of infection with HBV.

Product Use Limitations

- All reagents of the kit is for research use only.
- RTA HBV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of HBV DNA or confirmation of the diagnosis of infection with HBV.
- This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Test with other sample types may result in inaccurate results.
- This kit has been validated for use with RTA Viral DNA Isolation Kit or RTA Viral Nucleic Acid Isolation Kit. Using other isolation kits may adversely affect the performance characteristics of the kit.
- This kit has been validated for use with Stratagene Mx3000p/Mx3005p instrument or LightCycler 2.0 or INCEPTRA Cycler 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD or Bioneer Exicycler 96 Real-Time PCR Detection System. Using other instruments may adversely affect the performance characteristics of the kit.
- This kit has been optimized for use with specific PCR plastic consumables listed under Additional Materials Required section of the Handbook. Using other PCR plastic consumables may adversely affect the performance characteristics of the kit.
- Trustworthy results depends on proper sample collection, transport, storage and processing methods.
- It is intended for professional use by properly trained personnel.
- RTA HBV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings.
- The instructions in user manual should be followed strictly for optimum PCR results.
- The expired kits should not be used. Kit components from different lots should not be mixed.

Product Description

RTA HBV Real-Time PCR assay is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the Thermus aquaticus (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line. Quantification of the amount of target in unknown samples is accomplished by measuring Ct and using the standard curve to determine starting copy number. RTA HBV real-time PCR assay utilizes external standards to gather quantitative results and includes an internal control, which controls for target isolation and amplification. The target region is situated in S gene region of HBV genome and is 104-bases long. HBV DNA concentration is noted in International Units/ml (IU/ml).

If it is needed to convert to copies/ml, our conversion factor for RTA HBV Real-Time PCR Kit is 8.16 copies/IU. In other words, **1 IU/ml = 8.16 copies/ml.**

Pathogen Information

The Hepatitis B virus (HBV) with a 3.2 kbp long and partially double stranded DNA genome is a member of the Hepadnaviridae family (1). The HBV infection can cause acute or chronic liver disease and in the chronic case, the liver infection may become life threatening by developing into cirrhosis or hepatocellular carcinoma (HCC) (2). Its transmission is similar to but much more infectious than HIV. The transmission modes are mother-to-child, parenteral and sexual through percutaneous contact with infected blood or body fluids. Once the virus enters the body, it targets the hepatocytes of liver via its surface antigen (HBsAg). HBV infection is often acquired during childhood and is generally asymptomatic. The HBV infection in this stage mostly leads to development of the chronic infection (3). Approximately 400 million people worldwide are chronically infected with the hepatitis B virus (HBV), and approximately 1 million die annually of HBV-related disease. The worldwide prevalence of hepatitis B virus ranges from 0.1% to 20%. This wide range is largely due to differences in age at the time of infection. Following acute HBV infection, the risk of developing chronic infection varies inversely with age: 90% for perinatal infection, 25–50% for infection at age 1–5 years and 1–5% for all others (4). As being in an intermediate endemic region, the prevalence of HBsAg seropositivity is 2 to 10% in Turkey (5).

References

1. Gerlich W, Robinson WS. Hepatitis B virus contains protein attached to the 5′ end of its complete strand. Cell. 1980;21:801–811.
2. McMahon The natural history of chronic hepaatitis B virusinfection. Hepatology. 2009;49(suppl):S45–S55.
3. Thiers V, Nakajima E, Kremsdorf D, Mack D, Schellekens H, Driss F, et al. Transmission of hepatitis B from hepatitis-B-seronegative subjects. Lancet. 1988;2:1273–1276.
4. Custer B et al. Global epidemiology of hepatitis B virus. Journal of Clinical Gastroenterology, 2004, 38(10 Suppl):S158–S168.
5. Toy M, Onder FO, Wormann T, Bozdayi AM, Schalm SW, Borsboom GJ, et al. Age- and region-specific hepatitis B prevalence in Turkey estimated using generalized linear mixed models: a systematic review. BMC Infect Dis. 2011;11:337. doi: 10.1186/1471-2334-11-337.

Warnings and Precautions

- All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.
- Before and after work all surfaces should be disinfected with a freshly prepared solution of 10% bleach or antiviral agents. Dispose of unused reagents, waste and specimens in accordance with country or local regulations.
- Do not pipette by mouth. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye-wear when handling clinical specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents. Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water,
- The procedures should preferably be performed in four separated areas (i.e. for DNA extraction, PCR setup, sample addition, amplification) to aid in preventing contamination. All supplies for a particular procedure should be stored in the area where that procedure is performed and should not be moved between areas Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to an area and never worn outside of that area.
- The work should flow in one direction, beginning in the extraction area, moving to the PCR setup area in which PCR Master Mix is prepared, then moving to the third area in which samples, negative control and quantification standards are added, finally moving to amplification area in which real-time PCR equipment is run.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control; to prevent sample contamination, use new, sterile aerosol barrier or positive displacement Rnase/DNase-free pipette tips and sterile pipettes.
- Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls. Store the kit away from any source of contaminating DNA, especially amplified nucleic acid.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- A single type of HBV DNA assay should be used for monitoring a patient. If RTA HBV Real-Time PCR Kit substitutes another HBV DNA assay, both tests should be used in parallel for at least two subsequent samples.
- Do not use a kit after its expiration date.

Performance Characteristics

Analytical Sensitivity Analytical sensitivity was analyzed by use of a dilution series of WHO standard, and the cutoff value of the kit was determined by probit analysis. A dilution series of a WHO International HBV standard was prepared to give the final concentrations of 160, 80, 40, 20 and 10 IU/ml. Dilutions were extracted by RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029) according to

RTA Viral Nucleic Acid Isolation Kit Handbook. Each dilution was tested in 24 replicates for each instrument. Lower limit was calculated by probit analysis done by PASW Statistics 18 program. The 95 % cutoff concentration of RTA HBV Real-Time PCR Kit is 22.1 IU/ml for STRATAGENE, 25.1 IU/ml for LightCycler, 11.8 IU/ml for INCEPTRA Cycler, 27 IU/ml for Rotor-Gene and 12.8 IU/ml for Applied Biosystems 7500.

	INCEPTRA Cycler	STRATAGENE	LightCycler	BIO-RAD CFX96-IVD*	Rotor-Gene	ABI 7500	Bioneer Exicycler 96
RTA Viral Nucleic Acid Isolation Kit	11.8 IU/ml	22.1 IU/ml	25.1 IU/ml	10 IU/ml*	27 IU/ml*	12.8 IU/ml	29 IU/ml

*: Data taken from RTA Automated DNA/RNA Preparation and PCR Setup System

Linear Range To determine the upper limit, a dilution series of RTA HBV Reference Material ranging from 1 x 10^3 IU/ml to 1 x 10^6 IU/ml were prepared. High concentration samples (1 x 10^7, 1 x 10^8 and 1 x 10^9 IU/ml) were prepared by using calibrated plasmid DNA bearing HBV external standard. Within this range, the relationship between log of target DNA and Ct values is linear. Linear regression analyses comparing the Ct values-versus- log of target DNA were asfollows:
For STRATAGENE, Ct value = -3.190 (log of target DNA) + 42.20; with a correlation coefficient (R^2) of 0.998.
For INCEPTRA Cycler, Ct value = -3.30 (log of target DNA) + 44.61; with a correlation coefficient (R^2) of 0.990.
For Rotor-Gene, Ct value = -3.42(log of target DNA) + 42.19; with a correlation coefficient (R^2) of 0.990.
For ABI 7500, Ct value = -3.49(log of target DNA) + 14.19; with a correlation coefficient (R^2) of 0.999.
Upper limit is at least 1 x 10^9 IU/ml for STRATAGENE, INCEPTRA Cycler, Rotor-Gene, ABI 7500 and Bioneer Exicycler 96.

Lower limit was calculated by probit analysis done by PASW Statistics 18 program according to the quantification results of HBV Analytical Sensitivity Studies. 95 % lower confidence limit is 24 IU/ml for STRATAGENE, 21 IU/ml for INCEPTRA Cycler, 27 IU/ml for Rotor-Gene, 15.2 IU/ml for ABI 7500. Dynamic ranges of RTA HBV Real-Time PCR Kit:

For STRATAGENE **24 - 1 x 10^9 IU/ml**
For INCEPTRA Cycler **21 - 1 x 10^9 IU/ml**
For Rotor-Gene **27 - 1 x 10^9 IU/ml**
For ABI 7500 **15.2 - 1 x 10^9 IU/ml**
For BIO-RAD CFX96-IVD **9.9 - 1 x 10^9 IU/ml***
For Bioneer Exicycler 96 **29 - 1 x 10^9 IU/ml**

*: Data taken from RTA Automated DNA/RNA Preparation and PCR Setup System

Precision For each experiment, 24 replicates of 10^4 IU/ml WHO International Standard for HBV DNA assays were used. The results on basis of Ct values are shown in the following table:

Descriptive Statistics					
	N	Mean	Std.Deviation	Variance	Coefficient of variation (%)
Intra_assay	24	32.53	0.43	0.189	1.34
Inter_assay	24	32.61	0.28	0.083	0.88
Inter_batch	24	31.75	0.42	0.185	1.35
STRATAGENE	24	32.51	0.63	0.404	1.96
INCEPTRA_Cycler	24	32.49	0.35	0.129	1.11
BIORAD	24	31.24	0.18	0.033	0.58
RotorGene	24	32.01	0.47	0.230	1.50
ABI7500_Intra_assay	24	32.19	0.26	0.073	0.84
ABI7500_Inter_assay	24	31.72	0.45	0.211	1.45
TOTAL	216	32.12	0.60	0.363	1.88

Genotype Detectability The performance of RTA HBV Real-Time PCR Kit was evaluated with Teragenix HBV Genotype Performance Panel and 1st WHO International Reference Panel for HBV Genotypes for nucleic acid amplification technique (NAT)-based assays. The Panels contain HBV genotypes A, B, C, D, E, F, G and H. As a result of this study, RTA HBV Real-Time PCR Kit can detect and quantify all of eight HBV genotypes.

Diagnostic Specificity HBV negative clinical specimens were analyzed to determine the diagnostic specificity of RTA HBV Real-Time PCR Kit. 56 Hepatitis B virus DNA negative clinical **serum** specimens and 62 Hepatitis B virus DNA negative clinical **EDTA plasma** specimens were used. Viral DNA was extracted from HBV negative clinical specimens by RTA Viral DNA Isolation Kit according to RTA Viral DNA Isolation Kit Handbook. None of the HBV negative clinical specimens gave positive test result for Hepatitis B DNA. Diagnostic specificity of RTA HBV Real-Time PCR Kit is 100 %. All of the Internal Controls of tests gave positive result.

Cross Reactivity To examine the specificity of an assay, cross-reactivity studies should be performed for potential cross-reactive markers. In this study, the specificity of the assay was evaluated by testing 7 reference organism and 8 clinical specimens which were positive.

RTA HBV Real-Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the table below:

Organism	Source	Test Result
Cytomegalovirus (HBV)	Acrometrix (Cat. No: 94-2014)	Negative
Human Herpes Simplex virus type 1 (HSV-1)	NIBSC (Cat. No: 08/224)	Negative
Human Herpes Simplex virus type 2 (HSV-2)	NIBSC (Cat. No: 08/226)	Negative
Epstein-Barr Virus (EBV)	NIBSC (Cat. No: 08/316)	Negative
Hepatitis C virus (HCV)	NIBSC (Cat. No: 06/100)	Negative
Human Immunodeficiency Virus 1 (HIV-1)	NIBSC (Cat. No: 97/650)	Negative
Mycobacterium tuberculosis	ATCC (25177)	Negative
Cytomegalovirus (HBV) (2 samples)	Clinical specimens	Negative
HPV 16 and HPV 6	Clinical specimens	Negative
Parvovirus B19	Clinical specimens	Negative
Epstein-Barr Virus (EBV)	Clinical specimens	Negative
Hepatitis C virus (HCV)	Clinical specimens	Negative
Human Immunodeficiency Virus 1 (HIV-1)	Clinical specimens	Negative

Cross-Contamination In this study, cross-contamination between samples was evaluated. To do this, five different runs were performed. In every run, 4 high positive HBV sample and 4 HBV negative samples were used. Then, the kit was evaluated accordingly whether or not any cross-contamination was observed.No cross-contamination was observed during the whole process, and none of the human serum samples exhibited evidence of containing PCR inhibitors as indicated by the amplification of internal control.

Whole System Failure 60 Hepatitis B virus DNA negative clinical **serum** specimens and 60 Hepatitis B virus DNA negative clinical **EDTA plasma** specimens were spiked with WHO International HBV standard (NIBSC code: 10/264) to give a final concentrations of 30 IU/ml in the elution volume which is 3 times the 95% positive cutoff value determined by analytical sensitivity study. Spikes were extracted by RTA Automated DNA/RNA Preparation and PCR Setup System according to the instructions written in the handbook. Whole system failure rate of RTA HBV Real-Time PCR Kit is ≤1 %

Clinical Comparative Study Total 118 clinical samples were tested. According to the results, the data gathered by RTA HBV Real-Time PCR Kit is compatible with the results of other CE-marked devices. Log concentrations of all of 118 positive clinical samples are between ±1 log concentrations of the result of comparative device.

Additional Materials Required

- RTA Viral DNA Isolation Kit (Cat No: 09006; RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey),
- Real-Time PCR system,
- Disposable powder-free gloves
- Micropipettes (0.5 µl – 1000 µl),
- Sterile micropipette tips with filters,
- Microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for 2.0 ml tubes and for PCR strip tubes,
- PCR Workstation,

Real-Time PCR reaction tubes/plates/capillaries

For BIO-RAD CFX96-IVD:

- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates with Bar Codes (BIO-RAD, Cat#: HSP-9955)
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001),
- Individual PCR Tubes, Low Tube Strips, 8-tubes strip, 0.2 ml Low Profile, White (BIO-RAD, Cat#: TLS0851)
- Flat Cap Strips, Optically Clear, 8-cap strip, 0.2 ml (BIO-RAD, Cat#: TCS0803)

For STRATAGENE MX 3000p/MX 3005p:

- Optical tube strips (8x Strip) (Agilent Technologies, Cat#: 401428),
- Strip caps for PCR and qPCR applications (Agilent Technologies, Cat#: 401425)

For LightCycler 2.0:

- LightCycler Capilleries (20 µl) (ROCHE, Cat#: 4929292001)

For Rotor-Gene 3000/6000:

- Strip Tubes and Caps, 0.1 ml (Qiagen, Cat#: 981103)

For INCEPTRA Cyclers:

- 96x0,2 ml plate (Bioplastics, Cat#: B70501). EU flat cap plate (Bioplastics, Cat#: B57601),EU 0.1ml 8-tube strip attached Optical wide area cap (Bioplastics, Cat#: K72810B)

For Applied Biosystems 7500:

- MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher, Cat#: 4306737)
- MicroAmp® Optical Adhesive Film (Thermo Fisher, Cat#: 4311971)
- MicroAmp® Optical 8-Tube Strip, 0.2 mL (Thermo Fisher, Cat#: 4316567)
- MicroAmp® Optical 8-Cap Strips (Thermo Fisher, Cat#: 4323032)

Sample Preparation

This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant.
Aseptic techniques must be employed during collection to prevent the introduction of micro-organisms into the patient's anatomical space, and to prevent the sample from being contaminated during the process of collection.
All samples should be regarded as potentially infectious and standard precautions guidelines should be followed by all healthcare workers during sample collection and handling.
Samples must be collected into appropriate containers before despatch to the laboratory.
Be careful to check for cracks in the containers and to ensure that the lids of containers are properly tightened to prevent leakage of samples during handling and transportation. This can pose infection hazards to transport and laboratory staff.
Ensure that the outer surfaces of the containers are not contaminated by the patients' samples.
Store whole blood at room temperature for no longer than 4 hours. Centrifuge blood within 4 hours of collection. Transfer serum or plasma to a screw cap cryovial tube.
Transportation of whole blood, serum or plasma must conform to country or local regulations for the transport of etiologic agents.
Serum or plasma samples may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder for long-term storage.
Avoid multiple freeze/thaw cycles of specimens.

Protocol

Viral DNA Isolation

RTA Viral DNA Isolation Kit (Cat No: 09006; RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey) should be used for viral DNA extraction from clinical samples. Please follow the manufacturer's instructions as stated in the kit manual

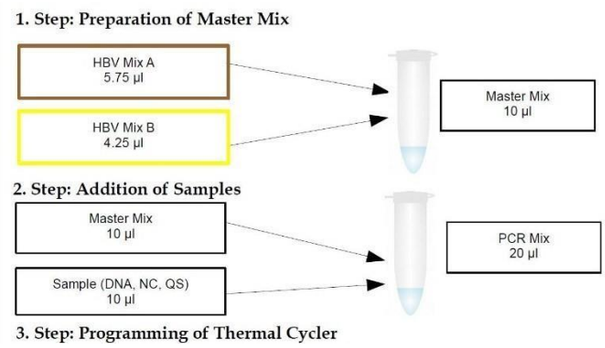
Internal Control

During DNA isolation, addition of the supplied internal control (IC) is necessary. IC allows the user to monitor DNA extraction step as well as to determine any PCR inhibition. For each sample, add 2.5 µl IC together with Solution B or Solution RL depending on the kit. Depending on your final elution volume, the volume of IC to be added can be calculated (0.05 µl IC/1 µl Elution Buffer). There will be no amplification of internal control in the tests where high positive HBV samples are amplified because there was a competition between internal control template and HBV DNA template for using PCR primers and other components. The Ct value of internal control of a negative sample should be equal to 33 ± 5, otherwise,it denotes a problem during purification.

Quantification Standards For generating a standard curve to obtain accurate quantification data on the Real-Time system, four quantification standards should be used. For each standard the corresponding concentration should be defined properly to the Real-Time PCR system before each run and the standard curve will be generated accordingly at the end of the reaction. Work with HBV Quantification Standards after preparation of clinical samples and negative control in a separate area. Caps of the tubes or capillaries of Clinical Samples SHOULD be closed in that area.

PCR Protocol

1. Thaw all components, except HBV Mix B, at room temperature. Thaw HBV Mix B on ice. Thaw HBV Mix A at 37°C for 5 min if there is a precipitate. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.
2. The final volume of Master Mix is calculated by multiplying single reaction volumes of Mix A and Mix B by the total sample size. The number of negative controls, quantification standards and the clinical samples should be included when calculating total sample size. Against possible pipetting errors, addition of an extra sample to the total sample size is recommended. PCR Grade Water should be used as the negative control.
3. To prepare master mix, add 5.75 µl of HBV Mix A (brown tube) and 4.25 µl of HBV Mix B (yellow cap) for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 10 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 10 µl DNA of each sample, negative control and quantification standards into the tubes. Spin down briefly.
4.Perform the thermal program given in the table below.
5. Fluorescence is measured at 72°C; FAM and HEX channels should be chosen (See the schema in the next page).
6. Refer to the Operator's Manual of the related instruments to program and analyze the results.



Prog. Name	Cycles	Program for Bioneer Exicycler 96	Cycles	Program for LightCycler	Program for Stra- tagene and INCEPTRA Cycler	Program for BIO-RAD CFX96-IVD	Program for Rotor-Gene 3000/6000 and ABI 7500
Hot Start	1	95ºC, 10 min	1	95ºC, 10 min	95ºC, 10 min	95ºC, 10 min	95ºC, 10 min
Amp.*	50	95ºC, 30 sec	45	95ºC, 30 sec	95ºC, 30 sec	95ºC, 30 sec	95ºC, 30 sec
		55ºC, 60 sec		65ºC, 60 sec	60ºC, 60 sec	57ºC, 60 sec	58ºC, 60 sec
		72ºC, 30 sec		72ºC, 30 sec	72ºC, 30 sec	72ºC, 30 sec	72ºC, 30 sec
Cooling	1	-	1	40ºC, 30 sec	-	-	-

*** Fluorescence is measured at 72°C; FAM and HEX/JOE channels should be chosen**

Data Analysis

To be able to evaluate the experiment, PCR efficiency of the Standard Curve must be between 90%-110% and R2 value must be more than 0.98. Otherwise, the experiment should be repeated. During analysis on STRATAGENE software, adjust threshold fluorescence value manually by entering 1000 for FAM and 500 for HEX.
During analysis on BIO-RAD CFX96-IVD software, adjust threshold fluorescence value manually by entering 500 for FAM and 500 for HEX.
During analysis on INCEPTRA Cycler software, the threshold should be set empirically. At the beginning, the threshold can be set at 50 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Rotor-Gene software, the threshold should be set empirically. At the beginning, the threshold can be set at 0,04 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Applied Biosystems 7500 software, the threshold should be set empirically. At the beginning, the threshold can be set at 10,000 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Bioneer Exicycler 96 software, the threshold should be set automatically.
Concentration of each positive sample will be calculated by the software according to the standard curve as International Unit per milliliter (IU/ml) . If it is needed to convert the quantitative results from IU/ml to copies/ml, the conversion factor for RTA HBV Real-Time PCR Kit is 8.16 copies/IU.
In other words, **1 IU/ml = 8.16 copies/ml.**
Due to different starting sample volumes and elution volumes during viral DNA isolation, the following formula **SHOULD** be used to calculate the concentration of the original clinical sample:

$$\text{Concentration of the Original Sample (IU/ml)} = \frac{\text{Concentration from Software (IU/ml)} \times \text{Elution Volume (µl)}}{\text{Original Sample Volume (µl)}}$$

The interpretation on the calculated results can be done as follows:

Signal detected in FAM channel	Signal detected in HEX channel	Calculated concentration of the original clinical sample	Conclusion
+	+	<11.8 IU/ml for INCEPTRA <22.1 IU/ml for STRATAGENE <25.1 IU/ml for LightCycler <10 IU/ml for BIO-RAD <27 IU/ml for Rotor-Gene <12.8 IU/ml for ABI 7500 <29 IU/ml for Bioneer Exicycler 96	The result is valid. Quantitation is not possible since the quantitative result is below the analytical sensitivity value of the assay. Reproducibility of the positive result is not guaranteed.
+	+/-	≥11.8 IU/ml for INCEPTRA ≥22.1 IU/ml for STRATAGENE ≥25.1 IU/ml for LightCycler ≥10 IU/ml for BIO-RAD ≥27 IU/ml for Rotor-Gene ≥12.8 IU/ml for ABI 7500 ≥29 IU/ml for Bioneer Exicycler 96 and ≤1 x 10^9 IU/ml	The result is valid. HBV DNA is detected at the concentra- tion calculated by the software since the quantitative result is within the linear range of the assay.
+	+/-	>1 x 10^9 IU/ml	The result is valid. HBV DNA is detected at a concentration >1 x 10^9 IU/ml . Qu- antitation is notpossible since the quantitative result is above the linear range of the assay.
-	+	N/A	The result is valid. Target (HBV DNA) is not detected.
-	-	N/A	The result is invalid. No diagnostic interpretation can be done,

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