

**RTA<sup>®</sup>**

# HBV Qualitative Real-Time PCR Kit

## Handbook

Date of Issue — 03.08.2017

Qualitative detection of Hepatitis B virus DNA

For *research* use only.

09026025 – 25tests

09026100 – 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 Positive Control	200 µl	200 µl
5	WHITE	PCR Grade Water	200 µl	200 µl

### Storage

All reagents of RTA HBV Qualitative 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 Qualitative Real-Time PCR Kit is an *in vitro* nucleic acid amplification assay for qualitative detection 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 Cyclor 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD Real-Time PCR Detection System for amplification, detection and analysis. RTA HBV Real-Time PCR Kit is not intended for diagnostic use or 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 *in vitro* diagnostic use only.
- RTA HBV Qualitative Real-Time PCR Kit is not intended for diagnostic use or 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 Cyclor 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD 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.
- 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 Qualitative 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. RTA HBV Qualitative real-time PCR assay utilizes 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.

### 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

- Gerlich W, Robinson WS. Hepatitis B virus contains protein attached to the 5' end of its complete strand. *Cell*. 1980;21:801–811.
- McMahon The natural history of chronic hepatitis B virus infection. *Hepatology*. 2009;49(suppl):S45–S55.
- 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.
- Custer B et al. Global epidemiology of hepatitis B virus. *Journal of Clinical Gastroenterology*. 2004, 38(10 Suppl):S158–S168.
- 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.
- 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 Qualitative Real-Time PCR Kit is 22.1 IU/ml for STRATAGENE, 25.1 IU/ml for LightCycler, 11.8 IU/ml for INCEPTRA Cyclor and 27 IU/ml for Rotor-Gene.

	INCEPTRA Cyclor	STRATAGENE	LightCycler	BIO-RAD CFX96-IVD	Rotor-Gene
RTA Viral Nucleic Acid Isolation Kit	11.8 IU/ml	22.1 IU/ml	25.1 IU/ml	10 IU/ml*	27 IU/ml

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

#### Precision

For each experiment, 24 replicates of 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	32,5329	,43500	,189	1,33
Inter_assay	24	32,6117	,28756	,083	0,88
Inter_batch	24	31,7504	,42992	,185	1,35
STRATAGENE_3005	24	32,5175	,63597	,404	1,96
INCEPTRA_Cyclor_9660	24	32,4942	,35985	,129	1,10
BIO-RAD CFX96	24	31,2446	,18111	,033	0,58
Rotor-Gene	24	32,0133	,47921	,230	1,50
Total	168	32,1664	,63553	,404	1,98

#### Genotype Detectability

The performance of RTA HBV Qualitative 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 Qualitative 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 Qualitative 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 Qualitative 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 Qualitative 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 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. Whole system failure rate of RTA HBV Qualitative Real-Time PCR Kit is  $\leq 1\%$

### Clinical Comparative Study

Total 118 clinical samples were tested. According to the results, the data gathered by RTA HBV Qualitative Real-Time PCR Kit is compatible with the results of other CE-marked devices.

### 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  $\mu$ l – 1000  $\mu$ l),
- 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 Capillaries (20  $\mu$ l) (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)

### 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 microorganisms 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  $\mu$ l 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  $\mu$ l IC/1  $\mu$ l 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  $33 \pm 5$ , otherwise, it denotes a problem during purification.

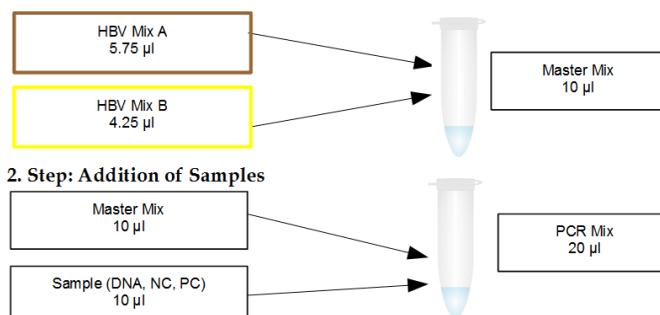
#### Positive Control

Positive Control includes plasmids containing an insert. The insert contains primer and probe binding sequences for HBV oligonucleotides. The Ct value of Positive Control should be equal to  $27 \pm 4$ , otherwise, it denotes a problem during amplification.

### 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 control, positive control 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  $\mu$ l of HBV Mix A (brown tube) and 4.25  $\mu$ l 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  $\mu$ l of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 10  $\mu$ l DNA of each sample, negative control and positive control into the tubes. Spin down briefly.
4. Perform the following protocol for LightCycler: 95°C for 10 min, 1 cycle; 95°C for 30 sec, 65°C for 60 sec, 72°C for 30 sec, 45 cycles; 40°C for 30 sec, 1 cycle.
5. Perform the following protocol for Stratagene MX3000p/MX3005p and INCEPTRA Cycler 4840/9620/9640/9660/9680: 95°C for 10 min, 1 cycle; 95°C for 30 sec, 60°C for 60 sec, 72°C for 30 sec, 45 cycles.
6. Perform the following protocol for BIO-RAD CFX96-IVD: 95°C for 10 min, 1 cycle; 95°C for 30 sec, 57°C for 60 sec, 72°C for 30 sec, 45 cycles.
7. Perform the following protocol for Rotor-Gene 3000/6000: 95°C for 10 min, 1 cycle; 95°C for 30 sec, 58°C for 60 sec, 72°C for 30 sec, 45 cycles.
8. Fluorescence is measured at 72°C; FAM and HEX channels should be chosen (See the schema in the next page).
9. Refer to the Operator's Manual of the related instruments to program and analyze the results.

### Preparation of Master Mix



Program Name	Cycles	Program for LightCycler	Program for Stratagene and INCEPTRA Cycler	Program for BIO-RAD CFX96-IVD	Program for Rotor-Gene 3000/6000
Hot Start	1	95°C, 10 min	95°C, 10 min	95°C, 10 min	95°C, 10 min
Amplification*	45	95°C, 30 sec	95°C, 30 sec	95°C, 30 sec	95°C, 30 sec
		65°C, 60 sec	60°C, 60 sec	57°C, 60 sec	58°C, 60 sec
		72°C, 30 sec	72°C, 30 sec	72°C, 30 sec	72°C, 30 sec
Cooling	1	40°C, 30 sec	-	-	-

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

### Data Analysis

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.

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

Signal detected in FAM channel	Signal detected in HEX channel	Conclusion
+	+/-	The result is valid. HBV DNA is detected
-	+	The result is valid. Target (HBV DNA) is not detected.
-	-	The result is invalid. No interpretation can be done.



RTA Laboratories  
 Biyolojik Ürünler İlaç ve Makine San. Tic. A.Ş.  
 Plastikçiler Organize Sanayi Bölgesi Cumhuriyet Cad. No:3 41400  
 Gebze /Kocaeli /Turkey Phone: +90 262 648 5300  
 Fax: +90 262 751 0677 E-mail: [rtalabs.com.tr](mailto:rtalabs.com.tr)  
 Web: [www.rtalabs.com.tr](http://www.rtalabs.com.tr)