

## Quantiplus® HBV FAST RTPCR Kit (Real-Time Quantitative PCR Kit)



QTF-HBV-25 : 25 rxns  
 QTF-HBV-50 : 50 rxns  
 QTF-HBV-100 : 100 rxns



PI/QTFHBV-06

### Intended Use

Quantiplus® HBV FAST RT-PCR Kit is a Real-Time PCR based *in vitro* diagnostic assay for quantitation of Hepatitis B Virus in human plasma. The test is used to monitor the HBV DNA levels in chronic HBV infections and response to antiviral therapy. The kit contains qPCR mix with UDG/UNG, Primer Probe Mix (PPM), Standards (HBVFQS1-HBVFQS4), and Internal Control (IC-B mix). This advanced formulation enables performance of **fast PCR in shorter run time (≤60 min)**, and UDG/UNG helps in controlling PCR carryover contamination.

**This kit is not to be used for screening of blood/blood products from blood donors.**

### Background Information

Hepatitis B causes viral infection of the liver. It can cause both acute and chronic disease. The virus is transmitted through contact with the blood or other body fluids of an infected person. It is difficult to differentiate Hepatitis B from hepatitis caused by other viral agents clinically, laboratory confirmation of the diagnosis is thus essential.

### Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QTF-HBV-25)	50 rxns (QTF-HBV-50)	100 rxns (QTF-HBV-100)
Blue	DNA Fast qPCR Mix with UDG/UNG (2X)	PCR Amplification Mix	1 x 325 µL	1 x 650 µL	2 x 650 µL
Amber	HBV Fast PPM	Target specific Primer Probe Mix	1 x 50 µL	1 x 100 µL	2 x 100 µL
Natural	IC-B Mix	Exogenous Internal Control-B mix	1 x 300 µL	1 x 600 µL	2 x 600 µL
Pink	HBVFQS1	1 X 10 <sup>5</sup> IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HBVFQS2	1 X 10 <sup>4</sup> IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HBVFQS3	1 X 10 <sup>3</sup> IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HBVFQS4	1 X 10 <sup>2</sup> IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
White	MBGPW	Purified water	1 x 500 µL	1 x 500 µL	2 x 500 µL

**Note: Please pay attention to the cap color coding and the tube contents.**

**MBGPW: Molecular Biology Grade Purified Water**

### Storage and Transportation Conditions

The kit should be transported at temperature below -20 °C. The kit is stable until the expiry date mentioned on the package, if the storage temperature is within -20 ±5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

### Technical specifications

Target Sequence	Conserved DNA sequence of S gene
Specificity	HBV genotype A-H, 100% specificity
Sensitivity	3.3x 10 <sup>-2</sup> IU/µL (16.4 IU/mL or 2.1 x 10 <sup>1</sup> copies/mL)
Linear Range	1.4 x 10 <sup>6</sup> – 7.2 x 10 <sup>-2</sup> IU/µL (7 x 10 <sup>8</sup> – 3.6 x 10 <sup>1</sup> IU/mL or 9 x 10 <sup>8</sup> – 5 x 10 <sup>1</sup> copies/mL)
Reporting Units	IU/µL (1 IU = 1.28 copies)
Validated Specimen	Plasma

External Quality Assessment	QCMD EQA Panels
Standards	Calibrated against to HBV NIBSC 10/266

**Assay Procedure**

**DNA Extraction**

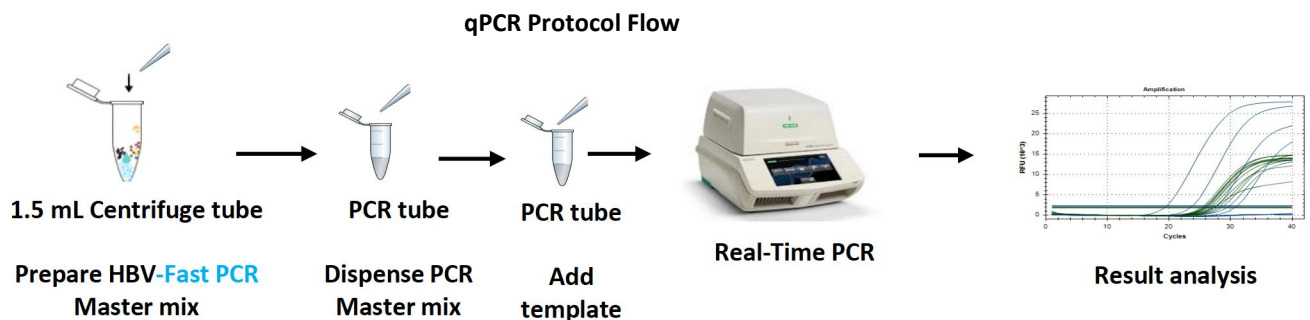
Quantiplus® HBV FAST RT-PCR Kit has been validated using the Viral DNA extraction kits mentioned below.

Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
2.	QIAamp® DNA Blood Mini Kit (Cat. No. 51104)	200 µL	100 µL

*Note: Customer can also validate their own extraction process using other Viral DNA extraction Kits.*

*IC-B mix can be added at the extraction step or while setting up the PCR*



**Preparation of Reaction Master mix**

Components	Volume per reaction (for 26µL)
DNA Fast qPCR Mix with UDG/UNG (2X)	13.0
HBV Fast PPM	2.0
IC-B Mix (if not added at extraction step)	1.0
Extracted DNA/ HBVFQS1- HBVFQS4 / MBGPW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycler.

**Cycling Conditions**

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	1 min.
2 (PCR cycling)	45	95	10 sec.
		60*	10 sec.

**\*Plate read/Data acquisition in FAM and TEXAS RED channels in Bio-Rad™ CFX 96. For Thermo Q55 Real-Time PCR System, use FAM and ROX channels. For Rotor-Gene Q 5plex, use green and orange channels.**

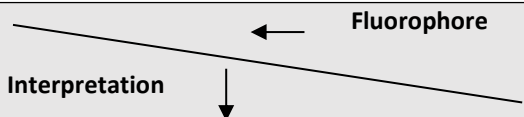
**Sample analysis and Interpretation**

The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below and also the slope of the standard curve (standards in FAM channel) is between -3.1 to -3.6, at least three standards should be included, and PCR efficiency is between 90% to 110% (0.9 to 1.1). Interpret the results of unknown samples as mentioned in Table 2

**Table 1:**

Control	FAM (HBV)	TEXAS RED (IC)
If Internal Control (IC-B Mix) is added during extraction		
Standards (HBVFQS1 to HBVFQS4)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Standards (HBVFQS1 to HBVFQS4)	√	√
Negative Control (NC)	-	√

**Table 2:**

S.No	FAM (HBV)	TEXAS RED (IC)	Interpretation	Conclusion
1	√	√		Proceed for further Analysis
2	√	-		
3	-	√		
4	-	-	Possible inhibition of PCR	Dilute the DNA sample (1:10) and repeat the Assay

**Note: All the Target channels (FAM, Texas Red) to be analyzed individually.**

**Viral load calculation (Conversion of IU/μL to IU/mL)**

$$\text{IU/mL} = \frac{\text{Obtained IU/}\mu\text{L} \times \text{Elution Volume}}{\text{Sample volume in mL}}$$

For calculating the result of diluted sample (1:10); multiply the observed IU/mL value by dilution factor, 10

$$\text{Result of 1:10 diluted sample (IU/mL)} = \text{Dilution Factor (10)} \times \frac{\text{Result (IU/}\mu\text{L)} \times \text{Elution Volume (}\mu\text{L)}}{\text{Sample Volume (mL)}}$$

**Reporting comments**

Results in IU/mL	Comments
Target not detected	HBV DNA not detected in the given sample
<36	HBV DNA detected but below the lower limit of the quantitation range of the assay. The reproducibility of the positive result is not assured
36 to $7 \times 10^8$	HBV DNA detected within the linear range of the assay
$\geq 7 \times 10^8$	HBV DNA detected but above linear range of the assay, dilute the sample for accurate result.

**Validated Instruments**

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96
- Rotor-Gene Q 5 plex



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