

Quantiplus® HBV FAST Detection Kit (Real-Time Qualitative PCR Kit)



QLF-HBV-25 : 25 rxns
 QLF-HBV-50 : 50 rxns
 QLF-HBV-100 : 100 rxns



PI/QLFHBV-04

Intended Use

Quantiplus® HBV FAST Detection Kit is a Real-Time PCR based in vitro diagnostic assay for detection of Hepatitis B Virus in human plasma. The kit contains qPCR mix with UDG/UNG, Primer Probe Mix (PPM), Positive control (HBVFPC) 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 (QLF-HBV-25)	50 rxns (QLF-HBV-50)	100 rxns (QLF-HBV-100)
Blue	DNA Fast qPCR Mix with UDG/UNG (2X)	PCR Amplification Mix	1 x 325 μ L	1 x 650 μ L	2 x 650 μ L
Amber	HBV Fast PPM	Target specific Primer Probe Mix	1 x 50 μ L	1 x 100 μ L	2 x 100 μ L
Natural	IC-B Mix	Exogenous Internal Control-B mix	1 x 300 μ L	1 x 600 μ L	2 x 600 μ L
Red	HBVFPC	Positive Control	1 x 100 μ L	1 x 100 μ L	2 x 100 μ L
White	MBGPW	Purified water	1 x 500 μ L	1 x 500 μ L	2 x 500 μ L

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

MBGPW: Molecular Biology Grade Purified Water

Storage and Transportation Conditions

The kits 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	3.3×10^{-2} IU/ μ L (16.4 IU/mL or 2.1×10^1 copies/mL)
Validated Specimen	Plasma
External Quality Assessment	QCMD EQA Panels

Assay Procedure

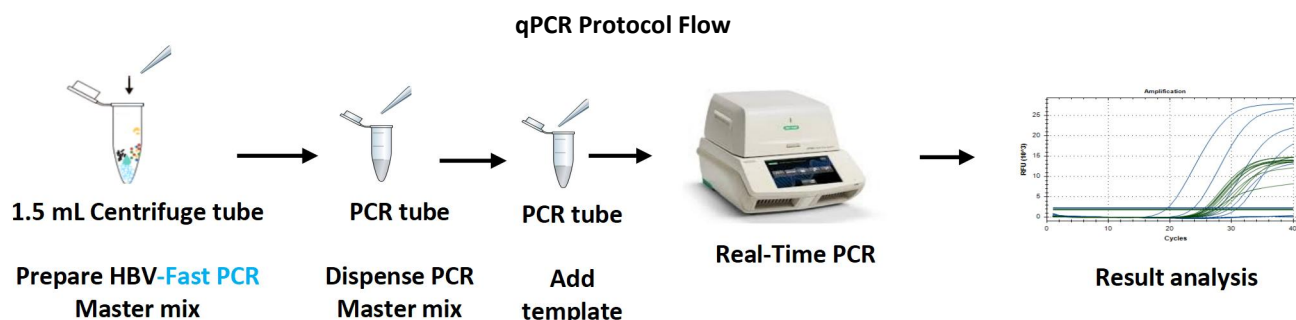
DNA Extraction

Quantiplex® HBV FAST Detection 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/ HBVFPC/ 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 QS5 Real-Time PCR System, use FAM and ROX channels. For Rotor-Gene Q 5plex, use green and orange channels.**

Sample analysis and Interpretation

For unknown sample analysis the cutoff Ct for HBV DNA (FAM) and IC (TEXAS RED) are ≤ 40 and ≤ 30 respectively. 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. Interpret the results for 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		
Positive Control (PC)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Positive Control (PC)	√	√
Negative Control (NC)	-	√

Table 2

S.No	FAM (HBV)	TEXAS RED (IC)	Interpretation	Conclusion
1	√	√	HBV DNA detected	Proceed for further Analysis
2	√	-		
3	-	√	HBV DNA not detected	Dilute the DNA sample (1:10) and repeat the Assay
4	-	-	Possible inhibition of PCR	

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

Validated Instruments

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



HLSS Manufacturing Pvt Ltd
 Plot No's M14, M15, M16, TSIIIC Medical device park
 Sultanpur village, Ameenpur Mandal,
 SangareddyDist, TS-502319