

MBPCR181 Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit

Description

Hepatitis B is an infectious disease of liver caused by the Hepatitis B virus (HBV), which is a small DNA virus. It belongs to Hepadnaviridae family and is classified into 10 genotypes, A to J. Each genotype has a distinct geographic distribution. This virus can cause both acute and chronic infections of the liver. It is transmitted when blood or mucous membranes are exposed to infectious blood and body fluids. Other risk factors include working in healthcare, blood transfusions, dialysis, etc. Many people have no symptoms during the initial infection but some develop a rapid onset of sickness with vomiting, yellowish skin, tiredness, dark urine and abdominal pain. It may take 30 to 180 days for symptoms to begin. Early symptoms of the infection include appetite loss, vomiting and abdominal symptoms, with approximately 10-20% of those patients developing fever as well as rheumatoid joint and muscle pain. Jaundice, which may be accompanied by itching, will then develop within 2-14 days. Fulminant hepatitis then occurs in about 1% of all infected patients, which in severe cases may be fatal. Of those individuals infected by HBV, 5-10% are likely to develop chronic liver inflammation which may progress to cirrhosis of the liver or, in the worst case, primary liver cell carcinoma. Chronic Hepatitis may progress to scarring of the liver, liver failure, or liver cancer. Hence, early diagnosis and treatment of Hepatitis is important which can also contribute to reducing Hepatitis transmission.

NOTE: HiMedia's Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is for *in vitro* use only.

Intended Use

Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The kit is recommended for sensitive and specific detection and quantitation of HBV DNA in human plasma samples. The Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit targets 100% of the known HBV genotypes (HBV genotype A-J).

Product Description:

Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is based on real-time PCR technology for the detection and quantitation of Hepatitis B Virus (HBV) specific DNA encompassing all major HBV genotypes (HBV genotype A-J). The kit contains primer-probe mixture specific for detection of HBV DNA. In addition, the kit can identify the DNA extraction efficiency and ensure successful PCR reaction with the help of an endogenous internal control. The quantitative standards (HBV quantitative standards QS1-QS4) are supplied in the kit which allow the determination of viral DNA load. The assay principle is based on hydrolysis probe chemistry which confers higher specificity and sensitivity.

Positive control (or Quantitative standards)

This is a control reaction used to test for the presence of inhibitors in the sample or the efficiency of the polymerase chain reaction itself using a pre-dispensed DNA sequence and the primer set that detects it. It is usually used to ensure proper and intended functioning of all the reagents and is recommended to be used in every run to assess optimal performance.

Endogenous Internal control

This is a control sequence that should amplify in all clinical samples which indicates the presence of sufficient DNA from human gene indicating the specimen is of acceptable quality. An internal control also allows to check



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for possible PCR inhibition or reagent failure.

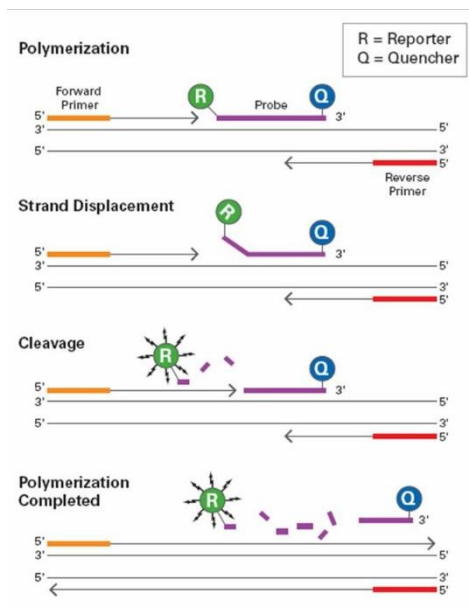
Negative Template Control

A negative template control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated with target DNA. In this reaction, Nuclease free water is used as the template. It is recommended to have a minimum of one reaction of negative template control per run.

Principle

Real-Time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. HiMedia's Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is designed to specifically detect **HBV viral DNA from subtypes A to J in FAM channel with Internal Control (IC) in ROX channel.**

Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

Strand displacement: When the probe is intact, the reporter dye emission is quenched.

Cleavage: During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe

Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result is cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

Features

- Detection of HBV subtypes A to J.
- Sensitive and specific.
- Includes all reagents & controls for validity of the test.
- Includes quantitative standards for calculation of viral DNA load.
- Results within 2 hrs.

Types of Specimens and storage:

The internal validation of the Hi-PCR Hepatitis B Virus (HBV) detection and quantitation Probe PCR Kit was performed using DNA extracted from human EDTA plasma samples. Other sample materials are not validated. Therefore, we recommend the use of EDTA plasma sample for detection of HBV. The DNA should be extracted using a standard viral DNA extraction kit. After collection, specimens may be stored at room temperature (15-25°C) for up to 8 hours or refrigerated (2–8°C) for up to 48 hours. Specimens stored beyond these time points must be frozen at or below -20 °C. Frozen specimens should undergo no more than one freeze-thaw cycle. After extraction, store the extracted DNA samples at -20°C for short period storage and -70°C or -80°C for long period storage.

Specimen Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf life

The provided kit has a shelf-life of 12 months when stored between -10°C to -20°C. Repeated thawing and freezing of PCR reagents (< 5 freeze-thaw cycle) should be avoided, as this may reduce the sensitivity of the assay. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

Kit Contents

The provided PCR kit contains:

Components	Product code	Reagents provided for (reactions)* (μL)		
		25R	50R	100R
HBV Master Mix	DS1551	135	270	530
HBV Primer-Probe Mix	DS1304	27	54	106
Molecular Biology Grade Water for PCR	ML065	76	76	148
HBV-QS1 (1×10^5 IU/ μL)	DS0705	76	76	148
HBV-QS2 (1×10^4 IU/ μL)	DS0706	76	76	148
HBV-QS3 (1×10^3 IU/ μL)	DS0707	76	76	148
HBV-QS4 (1×10^2 IU/ μL)	DS0708	76	76	148

* For a 20 μL PCR reaction

Materials needed but not provided

All materials are available through www.himedialabs.com

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96® AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96® AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
Insta Q48® M2 Real time PCR System, 96 well block, 2 channels	LA1024
TabSpin™ Microcentrifuge	LA1089/LA1090
HiPer® Mini Plate Centrifuge	LA1099
Automated nucleic acid extraction system and materials	

Insta NX [®] Instrument - fully automated nucleic acid purification system utilizing the Innovative Super -S membrane column method	LA1056
Insta NX [®] Mag16, Insta NX [®] Mag16 ^{Plus}	LA1118, MBLA018
Insta NX [®] Mag32, Insta NX [®] Mag32 ^{Plus}	LA1096, MBLA019
Insta NX [®] Mag96, Insta NX [®] Mag96plus	LA1097, MBLA026
Extraction Kits	
HiPurA [®] Pre-filled Cartridges for Viral Nucleic Acid Purification	MB582PC16
HiPurA [®] Pre-filled Plates for Viral Nucleic Acid Purification	MB582MPF16
HiPurA [®] Pre-filled Plates for Viral Nucleic Acid Purification [For Insta NX [®] Mag32]	MB582MPF-32
HiPurA [®] Prefilled Plates for Viral Nucleic Acid Purification [For Insta NX [®] Mag96]	MB582MPF-96
HiPurA [®] Viral DNA/RNA Purification Kit	MB582
HiPurA [®] Viral DNA Purification Kit	MB575
Insta NX [®] Viral DNA Purification Kit	MBIN015
Tubes, plates, and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips, 100µl Max capacity 100 µL	LA1104A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A
8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1 mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18
1.5 ml nuclease free Micro centrifuge tubes	PW146

Kit compatibility with Real-Time PCR Systems

Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit contains fluorophores that are compatible to the following PCR systems:

Real-Time PCR system	Company	Dye 1 (HBV)	Dye 2 (IC)
Insta Q96 [®] AG/ Insta Q96 [®] AG 6.0/Insta Q96 [®] - 6.0/Insta Q96 [®] Plus/Insta Q48 [®] M4	HiMedia Laboratories Pvt. Ltd.	FAM	ROX
QuantStudio™ 3 and 5	Applied Biosystems	FAM	ROX
Applied Biosystems 7500	Applied Biosystems	FAM	ROX
BioRad CFX Opus 96/CFX96	Bio-Rad Laboratories, Inc.	FAM	ROX
Rotor-Gene [®] Q/QIAquant	QIAGEN	Green	Orange
Roche LightCycler [®] 96	Roche	FAM	ROX
AriaMx	Agilent	FAM	ROX
Alta RT-96/48	Athenese-Dx Private Limited	FAM	ROX
qTOWER ³ auto	Analytik Jena	FAM	ROX

Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufacturer's instructions and recommendations

General Preparation Instructions

- Before use all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Clear surfaces and working areas with DNA Kil™ (ML221)

Real time PCR Protocol:

Prepare the PCR mix as follows in the “Master mix Preparation” area

Components	Product code	Volume to be added for 1R (20 µL reaction volume)
HBV Master Mix	DS1551	5 µL
HBV Primer-Probe Mix	DS1304	1 µL
Total Reaction Volume		6 µL



Transfer **6µL** of the above prepared PCR reaction mix in the 0.1/0.2 mL PCR reaction plate/strips/tubes, compatible to the instrument to be used.



To the **6µL** above reaction mix, add **14 µL** quantitative standard or test DNA or molecular biology grade water to make final volume **20µL**.



Tightly cap the tubes/strips or seal the plate using an optically clear adhesive film and spin.



Place the plate/strips/tubes in Real-time PCR machine and set the PCR program.

Recommended PCR program

Step	Temperature	Time	Sampling	Cycles
1	95°C	10 minutes	---	1
2	95°C	05 seconds	---	45
3	55°C	45 seconds	Yes	
4	72°C	15 seconds	---	

Selection of channel

Target	Dye	Quencher
HBV	FAM	None
IC	ROX/Texas Red	None

Passive Reference Dye: Select “None”

Data Analysis

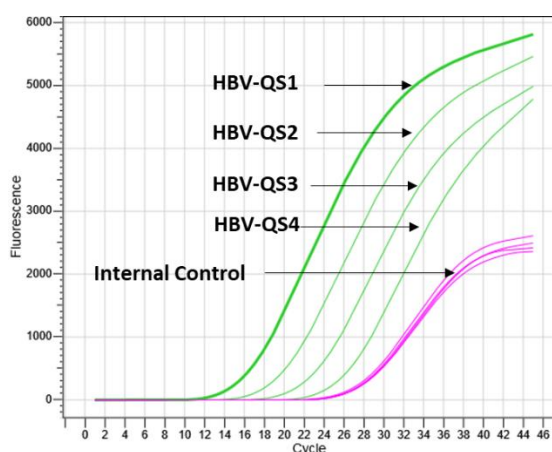
The following conditions should be met for a valid diagnostic test:

Control	Detection channel	
	FAM (HBV)	ROX (Internal Control)
HBV-QS1 (1x10 ⁵ IU/μL)	+	+
HBV-QS2 (1x10 ⁴ IU/μL)	+	+
HBV-QS3 (1x10 ³ IU/μL)	+	+
HBV-QS4 (1x10 ² IU/μL)	+	+
Negative Template Control	-	-

Standard Curve Analysis

Interpret the values for unknown samples, only if the following conditions are met:

1. $R^2 > 0.98$
2. Slope of the quantitative standards is between -3.1 to -3.7
3. PCR efficiency is between 85%-115%
4. No amplification in FAM channel of negative template control



Sr. No.	Sample	C _t
1.	Negative Template Control	N/A
2.	HBV-QS1 (1x10 ⁵ IU/μL)	15.68
3.	HBV-QS2 (1x10 ⁴ IU/μL)	18.99
4.	HBV-QS3 (1x10 ³ IU/μL)	22.40
5.	HBV-QS4 (1x10 ² IU/μL)	25.75
6.	Internal Control	27.75

Image representing amplification plot of HBV quantitative standards with Ct values using HiMedia's Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit on InstaQ 96 series of instrument (Ct values provided in table are for representation only).

Data Interpretation:

When all the standards fulfill the stated requirements, interpret the results of the specimen as follows:

Detection Channel		Result Interpretation
FAM (HBV)	ROX (Internal Control)	
+	+/-*	HBV specific DNA detected
(Ct cut off ≤ 37.5)		
-	+	HBV specific DNA is not detected. Sample does not contain detectable amounts of HBV specific DNA.
-	-	PCR Inhibition or reagent failure. Retest the sample.

*Detection of the IC in the ROX channel is not required for the positive results in the FAM channel. Presence of high HBV DNA load and/or PCR inhibitors in the original sample can lead to reduced or absence of internal control signal.

Note:

We recommend that the test results must be interpreted by an expert. Clinical correlation along with patient history is necessary to determine patient infection status.

Use following formula to convert concentration into IU/mL:

The quantitation standards given in the kit are defined as IU/ μ L. As the initial sample volume and elution volume will be different during isolation of viral DNA, the following formula should be used to convert the concentration values determined using the standard curve into IU/mL.

$$\text{Result (IU/mL)} = \frac{\text{Concentration of sample (IU/}\mu\text{L)} \times \text{Elution Volume (}\mu\text{L)}}{\text{Original Sample Volume (mL)}}$$

Performance Characteristics

Analytical sensitivity

The analytical sensitivity or the Limit of Detection (LOD) of the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is defined as the concentration of HBV DNA molecules that can be detected with a positivity rate of $\geq 95\%$.

The analytical detection limit in consideration with nucleic acid extraction was determined using HBV spiked plasma specimens in combination with a particular extraction method. In addition, the analytical detection limit independent of extraction was determined using a standard DNA of known concentration. The analytical sensitivity in consideration with nucleic acid extraction was determined using a dilution series of the 5th WHO International Standard for Hepatitis B virus DNA for nucleic acid amplification techniques (NIBSC code: 22/120) from 9.79×10^4 IU/mL to 19.59 IU/mL spiked into HBV negative EDTA plasma. Experiments were carried out in triplicate independent extractions (Sample volume: 200 μ L, elution volume: 60 μ L), followed by triplicate real-time PCR reactions for each extraction for three consecutive days on InstaQ 96^{®Plus} real time PCR system. The analytical detection limit in consideration of the purification of Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is ≈ 15 IU/mL.

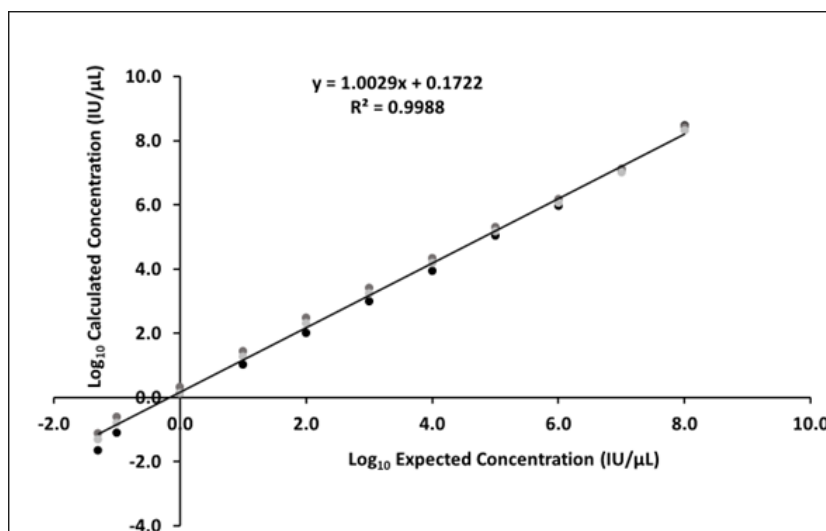
In addition, the analytical detection limit independent of extraction was determined using standard HBV DNA of known concentration. A dilution series of the standard HBV DNA was set up from 1×10^8 IU/ μ L to 1×10^{-2} IU/ μ L and analyzed in triplicates with the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit. The lowest dilution with limit of detection was then tested in 20 replicates. The analytical detection limit of the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is **0.1 IU/ μ L**. The sensitivity analysis of the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit was carried out on HiMedia's InstaQ 96 series, Biorad's CFX Series and Thermo Fisher's QuantStudio™ 5 Real-Time PCR System.

Linear Range

The linear range in consideration of the purification of the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit was determined by analyzing a logarithmic dilution series of the 5th WHO International Standard for Hepatitis B virus DNA for nucleic acid amplification techniques (NIBSC code: 22/120) and the HiMedia's HBV Quantitative Standard Set. Each dilution was extracted in independent triplicates (Sample volume: 200 μ L, elution volume: 60 μ L) and each extraction was further tested in triplicates using Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit and analyzed on InstaQ series of instruments. The linear range of the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit in consideration of the purification has been determined to cover concentration from 2.9×10^4 IU/mL to 15 IU/mL.

The linear range (analytical measurement) of the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit was determined by analyzing a dilution series of HBV DNA from 1.0×10^8 IU/ μ L to 0.01 IU/ μ L. The dilution series has been calibrated against the 5th WHO International Standard for HBV DNA. Each dilution of HBV quantitative standard for concentration ranging from 1.0×10^8 IU/ μ L to 1 IU/ μ L was tested in triplicates; the concentration < 1 IU/ μ L was tested in 5 replicates using the Hi-PCR[®] Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit on InstaQ series of instruments. The linear range of the Hi-PCR[®] Hepatitis B Virus

(HBV) Detection and Quantitation Probe PCR Kit has been determined to cover concentrations from 1×10^8 IU/ μ L to 0.05 IU/ μ L.



Linear Range of the Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit: The straight line was determined by a linear regression of the log₁₀ calculated concentrations with the log₁₀ expected concentrations when using HBV DNA.

Cross-reactivity - Analytical Specificity

The specificity of the kit was ensured by performing *in silico* analysis of the HBV primers and probes using NCBI BLAST and optimizing the stringent PCR conditions. The detectability of all the relevant HBV subtypes and genotypes was ensured by analyzing the HBV primer-probes for sequence homology to the sequences available in the NCBI database using multiple sequence alignment tools. Wet testing analysis was performed against the pathogens available in the laboratory on InstaQ 96 series of instruments, CFX Maestro (Biorad), QuantStudio™ 5 (Thermo Fisher) for any potential cross-reactivity. Below mentioned table represents the list of pathogens analyzed for analytical specificity. None of the tested pathogen showed any reactivity to the HBV primers-probes except the HBV DNA.

Human immunodeficiency virus	Human parainfluenza virus
Hepatitis A virus	Plasmodium falciparum
Hepatitis E virus	Plasmodium malariae
Hepatitis B virus	Plasmodium vivax
Hepatitis C virus	<i>Klebsiella pneumoniae</i>
Dengue virus (1-4)	<i>Streptococcus pneumoniae</i>
Chikungunya virus	<i>Staphylococcus epidermidis</i>
Herpes simplex virus	<i>Candida albicans</i>
Human papillomavirus 16	<i>Escherichia coli</i>
Human papillomavirus 18	<i>Salmonella typhi</i>
Coronavirus (Covid-19)	<i>Mycobacterium tuberculosis</i>
Adenovirus	Blood DNA

Warning and Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred to in the safety data sheets of the product.

Limitations

Although rare, mutations within the highly conserved regions of the target genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target regions in these cases. Validity and performance of the assay design are revised at regular intervals. The primers-probe of the kit can detect and quantify the HBV genotypes (A–J) however, the kit cannot differentiate or identify these genotypes. Hence, the result interpretation for individual HBV genotypes is not applicable to this kit.

Evaluation

Each lot of Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit is assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. It has been functionally tested in amplification assay.

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	No amplification in test and/or control wells	Degraded samples or poor-quality DNA template	Use freshly extracted, high-quality DNA. Check DNA concentration and purity (e.g., A260/280 ratio). Avoid repeated freeze-thaw cycles for stored DNA.
		Missing or incorrect addition of reagents	Verify all reagents were added in the correct volumes and order. Recheck reaction setup steps.
		Incorrect thermal cycling conditions	Cross-check the PCR cycling profile with the IFU.
		Expired or improperly stored reagents	Confirm the expiry date and proper storage. Avoid using reagents that have undergone multiple freeze-thaw cycles
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
2.	Variability between replicates	Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
		Air bubbles in reaction mix	Briefly centrifuge PCR tubes or plate before placing in the instrument to eliminate air bubbles.
		Uneven mixing of reagents	Ensure all reagents are fully thawed and mixed by vortexing before use. Spin down before pipetting.
		Edge effect (thermal variation across plate)	Avoid using outer wells in PCR plates if not temperature-uniform; use a plate seal and consistent plate layout.
3.	Amplification of pathogen target in negative template control	Reagent contamination	Replace all critical reagents. Clean workspace, pipettes, and repeat analysis with fresh aliquots. Use filter tips and maintain a unidirectional workflow.

		Cross-contamination or aerosol contamination during reaction setup	Maintain strict unidirectional workflow. Set up reactions in a PCR hood or clean bench. Use aerosol-resistant filter tips for all pipetting steps. Avoid fast, forceful pipetting. Regularly clean work surfaces and equipment with DNA-decontaminating agents. Minimize opening of positive control tubes and avoid splashing.
		Template contamination in workspace or pipettes	Decontaminate work surfaces, pipettes, and equipment using DNA/RNA decontamination solutions. Perform regular cleaning.
		Improper sealing of PCR plate/tubes	Ensure plates/tubes are properly sealed to prevent cross-well contamination during thermal cycling. Use optical-grade seals if required.
4.	No signal with positive control (Quantitative standards) or partial target amplification	Degradation of the positive control material due to improper storage or repeated freeze-thaw cycles	Use a fresh aliquot of positive control. Ensure storage conditions follow IFU and avoid repeated freeze-thaw. Discard expired or compromised controls.
		Incorrect thermal cycling conditions	Cross-verify cycler settings with IFU. Ensure annealing/extension temperature and time are as specified.
		Improper mixing of positive control (PC) before use	Vortex and briefly spin down the positive control before adding it to the PCR mix. Mix well to ensure homogeneity.
5.	Early Ct value with non-sigmoidal amplification	Instrumental noise, background fluorescence, or non-specific amplification	Visually inspect amplification plots. Disregard flat or atypical (non-sigmoidal) curves that show a sudden rise in fluorescence with very early Ct values (e.g., <6). These may not indicate true amplification. Repeat the assay if necessary. Adjust threshold setting in software within exponential phase. Refer to instrument-specific guidance.
6.	Non-specific amplification of pathogen targets in samples and/or negative control	Improper threshold setting in the real-time PCR software	Visually inspect all amplification plots. Do not rely on Ct values alone. Manually adjust the threshold as recommended in the threshold setting table. Refer to the instrument-specific guidelines or user manual for proper threshold adjustment.
		Contamination of reagents or workspace	Use fresh aliquots of reagents. Prepare reactions in a contamination-free environment using separate areas for pre- and post-PCR steps. Use aerosol-resistant filter tips and routinely decontaminate surfaces and pipettes.
7.	No Internal Control (IC) amplification in	High load of target may suppress IC amplification	If valid Ct for target is observed, it is still considered a valid result

	target-positive sample		
8.	High Ct values in positive samples (late amplification)	Low viral load or suboptimal sample	Results near the cut-off should be interpreted with caution. Repeat test using freshly extracted DNA. Confirm with orthogonal test if clinically significant.
9.	Inconclusive result (no amplification of target or IC)	Sample inhibition, extraction failure, or expired reagents	Repeat extraction or test with fresh sample. Check IC amplification to rule out PCR inhibition. Use validated extraction method and fresh reagents.
		Instrument malfunction	Verify that the real-time PCR instrument is functioning properly and calibrated.
10.	Signal in only one replicate (of duplicate or triplicate reactions)	Pipetting error or borderline positivity	Repeat the test. If consistent upon retesting, interpret cautiously in context of clinical findings. Borderline cases (Ct of 37-38) may require repeat sampling or orthogonal testing (e.g., NGS). Use calibrated pipettes and proper technique.
11.	Standard Curve does not show expected PCR parameters such as efficiency and slope	Improper Mixing of standards	Thaw and mix the standard vials properly before setting the PCR run.
		Inappropriate storage conditions	Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary.
		Inconsistent pipetting or error in reaction setup	Prepare a single master mix for all replicates to minimize variation. Vortex thoroughly and aliquot carefully. Use calibrated pipettes and consistent technique.
12.	Viral DNA detected above the linear range of the assay	High viral load sample	Dilute the DNA elute and repeat the assay. Multiply the observed IU/mL value by dilution factor.

Safety Information

HiMedia's Hi-PCR® Hepatitis B Virus (HBV) Detection and Quantitation Probe PCR Kit for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.









Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBPCR181

Rev.No.:05

Date of Issue: 2025-10

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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