HBV Quantitative RT PCR Detection Kit

100 Rxns | -20°C

INTENDED USE

HBV Quantitative RT-PCR Detection Kit is an *in-vitro* RT-PCR quantitative assay for the detection and quantification of Hepatitis B Virus (HBV) DNA from human plasma, serum, or whole blood along with human endogenous control. The intended user of the product is medical/research professionals in laboratory, healthcare, etc.

PRINCIPLE OF THE TEST

HBV Quantitative RT-PCR Detection Kit works on one-step, Real-Time polymerase chain reaction (RT-PCR), where the enzymes provided in the master mix amplifies viral DNA with the help of target – specific primers and detection happens through the fluorescent probes bearing reporter and quencher. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and the standards provided in the kit will help in quantification of the HBV.

ANALYTICAL SPECIFICATION

HBV Quantitative RT-PCR Detection kit is traceable to WHO International Standard 4th WHO International Standard for HBV DNA for NAT (NIBSC code: 10/266). Limit of detection of HBV is 32 IU/mL (179.2 Copies/mL) for ≥95% of the time. The linear range of the HBV Quantitative RT-PCR Detection Kit was determined by performing assay with by diluting HBV reference standard from 318,333 IU to 31.8 IU. Specificity of the kit is 100%.

COMPATIBLE INSTRUMENTS

Real-Time PCR, instruments like, BIORAD - CFX96, THERMO -QS5, QIAGEN-ROTOR - GENE Q and other instruments which supports FAM (495 nm - 520 nm) and Texas Red (596 nm - 615 nm).

PRECAUTIONS

- Treat all the specimens as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Keep separate area for master mix and template preparation and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.

STORAGE AND HANDLING

- Store all HBV Quantitative RT-PCR Detection Kit reagents at -20°C.
- Do not repeatedly freeze-thaw reagents more than 5 times it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C. Recommended freeze thaw cycle time is 5 times.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life - 12 Months from date of manufacture.

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables

PCR Plates/tubes

PCR plate covers/tube caps

KIT CONTENTS

Sl.No	Components	Volume	International Units (IU/µL)
1.	Master Mix	2 X 750 µL	-
2.	Primer and Probe mix	150 µL	-
3.	Nuclease Free Water	1000 µL	-
4.	Positive Test Control	100 µL	-
5.	Quantitation Standard 1 (QS 1)	750 µL	10,000
6.	Quantitation Standard 2 (QS 2)	750 µL	1000
7.	Quantitation Standard 3 (QS 3)	750 µL	100
8.	Quantitation Standard 4 (QS 4)	750 µL	10
9.	Quantitation Standard 5 (QS 5)	750 µL	1

Table 1:- Kit Content

FLUORESCENT PROBE DETAILS

Target	Reporter	Quencher
HBV Spike protein Gene	FAM	BHQ1
Human Beta-Actin	TEXAS RED/ROX	BHQ2

Table 2:- Target,Reporter and Quencher

***Note:** In some instruments where the BHQ option is unavailable, please set the quencher to "NONE."

REACTION MIXTURE 35 µL

Reagents	1 Rxn	20 Rxns	50 Rxns	100 Rxns
Master Mix	15 µL	300 µL	750 µL	1500 µL
Primer & Probe Mix	1.5 µL	30 µL	75 µL	150 µL
Nuclease Free Water	3.5 µL	70 µL	175 µL	350 µL
Total	20 µL	400 µL	1000 µL	2000 µL

Use 15 µL of the test DNA / Standards per reaction

Table 3:- Reaction Mixture

REACTION SET-UP

- Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
- Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1).
- Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples referring the table no. 3, in Master Mix Preparation room.
- Assay should be run along with positive controls and negative controls.
- Refer to table no. 3, to prepare the reaction mixture of 35 µL.
- Calculate the number of the samples to be tested along with the controls and multiply with 1 reaction volume extra to get the final reaction mixture.
- Spin down the tubes and dispense 20 µL reaction mix in each tube strips or 96 well plate.
- Before moving to template adding area, add 15 µL of nuclease free water in NTC well.
- Carefully add 15 µL DNA samples kept on ice in the designated wells in template addition room.
- Add 15 µL of Standards (QS) in the designated wells.
- Add 2 µL of PTC in a separate hood and make up the volume by adding 13 µL of nuclease free water.
- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLING CONDITION

STEPS	TEMP (° C)	TIME	QUANTITATION	CYCLE
Denaturation	95	2 min	Off	1
PCR and Quantitation	95	15 sec	Off	45
	58	30 sec	On	

Table 4:- Thermal Cycling Condition

STANDARD CURVE CONTROL PARAMETER

Control Parameters	Valid Value
Slope	-3.00 / 3.74
PCR Efficiency	85% / 115%
R square (R^2)	0.95-1

Table 5:- Curve Control Parameter

SAMPLE TYPE

- HBV Quantitative RT-PCR Detection Kit developed by NeoDx Biotech Labs Pvt Ltd is a robust and convenient molecular diagnostic device for the Quantitation of HBV DNA in extracts from human plasma, serum and whole blood.
- The kit can be used for Quantitation of HBV viral DNA if coupled with either NucleoDX RT DNA or any other reference DNA extraction kits approved by CE-IVD, EU or FDA. If using other DNA extraction kit, elute the DNA in 30-40 µL of elution buffer for accurate results.
- Always use freshly extracted patient DNA or the DNA samples stored following proper guidelines, to get the optimal results.

RESULT VALIDATION

HBV genes	HBV Assay result
HBV DNA > 32 IU/mL	The result is within the acceptance range. The detection probability if HBV DNA is >95%. The Positive test result is statistically ensured.
HBV DNA < 32 IU/mL	The result is outside the acceptance range. The reproducibility of the positive result is not assured.
No amplification for HBV target	No HBV DNA was detected.

Table 6:- Result Validation

DATA ANALYSIS

- After completion of the run, analyze the data as per the RT-PCR manufacturer instructions.
- Analysis should be performed separately for each target using a manual threshold settings.
- In case internal control has not worked for a sample re-do the test with 2 or more dilutions.
- Negative results do not exclude infection and should not be used as the sole basis for the treatment.
- The quantitation standards are defined as IU/µL. The following equation has to be applied to convert the values determined using the standard curve into IU/ mL of sample material.

$$\text{Result (IU/mL)} = \frac{\text{Elution Volume (µL)} \times \text{Result (IU/µL)}}{\text{Sample Volume (mL)}}$$

Sample Volume is the volume of sample used for DNA extraction.

QUANTITATIVE STANDARD IN IU/µL

Standards	International Units (IU/µL)
Quantitation Standard 1 (QS 1)	10000
Quantitation Standard 2 (QS 2)	1000
Quantitation Standard 3 (QS 3)	100
Quantitation Standard 4 (QS 4)	10
Quantitation Standard 5 (QS 5)	1

Table 7:- Quantitative Standards

WARNING

Positive control showed no amplification

Inappropriate storage of reagents

- *Store the reagents at recommended temperature for their optimal performance.*
- *Avoid repetitive freezing and thawing.*
- *Check the expiry of reagents.*

Negative controls are positive

Causes - Cross-Contamination

- *Follow good laboratory practices to avoid contamination issues.*
- *Use a new batch of reagents and repeat the experiment.*

Abnormal plot and/or low ΔRn values in amplification curve

The baseline was set improperly (some samples have CT values lower than the baseline value)

- *Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample crosses the threshold).*

An amplification signal is detected in the early cycles

Dilute the sample to increase the CT value.

REFERENCES

1. Krajden M, McNabb G, Petric M. The laboratory diagnosis of hepatitis B virus. Can J Infect Dis Med Microbiol. 2005 Mar;16(2):65-72. doi: 10.1155/2005/450574. PMID: 18159530; PMCID: PMC2095015.