GeneProof
Molecular diagnostics for your routine

GeneProof Hepatitis B Virus (HBV) PCR Kit

CE 1023 IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

ISEX Version
IS supplied in a separate tube
Nucleic acid extraction and PCR inhibition control

REF	HBV/ISEX/025 25 rxn	HBV/ISEX/050 50 rxn	HBV/ISEX/100 100 rxn
MasterMix HBV	1x750 µl	2x750 µl	4x750 µl
Calibrator HBV 10^4 IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HBV 10^3 IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HBV 10^2 IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HBV 10^1 IU/µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard HBV	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 15 repeated freezing/thawing cycles.

TECHNICAL SPECIFICATION

Target Sequence	DNA conservative sequence of open reading frame X (ORFx)
Specificity	HBV genotype A-H, pre-core mutants HBV (HBeAg negative), 100%
Sensitivity (LoD)	Reaches up to 36.9792 IU/ml with probability of 95 % (on HBV NIBSC 05/148) using manual extraction GeneProof Pathogen Free DNA Isolation Kit)
Linear Range	10^{10} - $10^{2.5}$ IU/ml with precision of ± 0.5 log
Dynamic Range	10^{10} - 36.9792 IU/ml
Reporting Units	IU/µl (1 IU = 4.84 cp)
Validated Specimen	plasma, serum
External Quality Assessment	Regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

Quality management system is certified in compliance with the requirements of the standard ISO 13485:2016

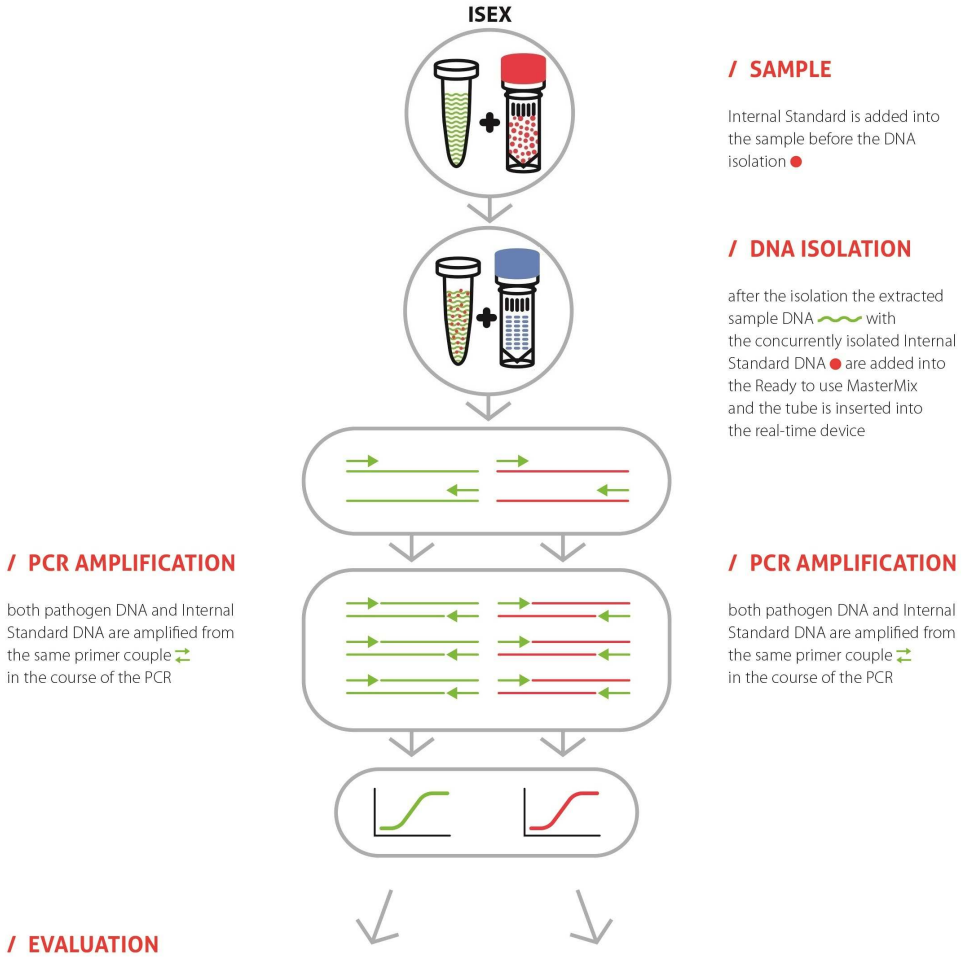
METHOD PRINCIPLES

The PCR kit is designed for detection of Hepatitis B virus (HBV) by the real-time Polymerase Chain Reaction (PCR) method. The HBV detection consists in amplification of a specific conservative DNA sequence of an open reading frame X (ORFx) and in measurement of fluorescence increase. The HBV presence is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the PCR kit, controlling the possible inhibition of the PCR and the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit uses the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detection.

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Samples of plasma and serum are required for the HBV virus DNA demonstration. Sampling of plasma and serum should be performed into sterile tubes without any transportation media. The material should be transported to the laboratory at the temperature between +2 °C and + 8 °C within 24 hours. In case of longer storage keep all samples frozen at the temperatures below -20 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid extraction should be performed by extraction kits available at the market according to protocols for the particular clinical material extraction. The manufacturer recommends the following extraction kits:

croBEE NA16 Nucleic Acid Extraction System
GeneProof PathogenFree DNA Isolation Kit

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of theextraction process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution volume	**25 µl**	**50 µl**	**100 µl**	**200 µl**
Internal Standard	2,5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl. *It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation.*

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	37 °C	2 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM+HEX	45
	60 °C	40 s		
	72 °C	20 s		

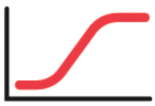
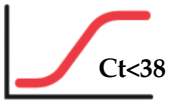
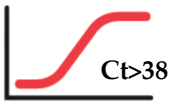
VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

croBEE Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
DT lite Real-Time PCR System
LightCycler® 2.0 / 480
LineGene 9600 / 9600 Plus
Rotor-Gene 3000 / 6000 / Q
SLAN® Real-Time PCR System

Required detection channels: FAM, HEX
GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.

CLINICAL SAMPLE ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation	
		**Valid**	HBV	**positive**
		**Valid**	HBV	**positive**
		**Valid**	HBV	**negative**
		**Invalid**		
		**Invalid**		

QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in IU/ml while taking into account the volume of material entering the extraction:

$$\text{IU/ml} = \frac{\text{SC x EV}}{\text{IV}}$$

SC - Sample concentration (IU/µl)
EV - Elution volume (µl)
IV - Isolation volume (ml)

You can use the calculator for pathogen concentration conversion at www.geneproof.com to make the calculation easier.

WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

Customer care and technical support
Tel.: +420543211679
Fax: +420516770824
email: support@geneproof.com

Orders
Tel.: +420543211679
Fax: +420516770824
email: sales@geneproof.com