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 the 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

REF:	HBV/ISEX/025 25 rxn	HBV/ISEX/100 100 rxn
MasterMix HBV	1 x750 µl	4 x750 µl
Calibrator A HBV 10^4 IU/µl	1x200 µl	1x200 µl
Calibrator B HBV 10^3 IU/µl	1x200 µl	1x200 µl
Calibrator C HBV 10^2 IU/µl	1x200 µl	1x200 µl
Calibrator D HBV 10^1 IU/µl	1x200 µl	1x200 µl
Internal Control HBV	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kit must be transported at a temperature of -20 °C or below. The kit will remain stable at least until the expiry date printed on the package if the storage temperature is kept (-20 ± 5)°C. The components are stable for a maximum of 5 repeated freezing / thawing cycles after the first use of a particular vial. The component must be used before the expiry date or 30 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	DNA conservative sequence of open reading frame X (ORFx)
Analytical Specificity	HBV genotype A-H, precore mutants HBV (HBeAg negative), 100 %
Analytical Sensitivity (LoD with the probability of 95 %)	36.9792 IU/ml (on HBV NIBSC 05/148 using GeneProof PathogenFree DNA Isolation Kit), 64.067 IU/ml (on HBV NIBSC 10/266 using croBEE 201A Nucleic Acid Extraction Kit), 13.9 IU/ml (on HBV NIBSC 10/266 using manual extraction SpinStar Viral Nucleic Acid Kit 1.0 with SpinStar Pretreatment Solution)
Diagnostic Specificity	100 % ($CI_{95\%}$: 99.06 % - 100 %)
Diagnostic Sensitivity	100 % ($CI_{95\%}$: 95.90 % - 100 %)
Linear Range	10^{10} - 10^2 IU/ml with precision of ± 0.5 log
Dynamic Range	10^{10} - 36.9792 IU/ml (using GeneProof PathogenFree DNA Isolation Kit), 10^{10} - 64.067 IU/ml (using croBEE 201A Nucleic Acid Extraction Kit)
Reporting Units	IU/ml
Metrological traceability	HBV NIBSC 10/266 (4th WHO International Standard)
Validated Specimen	plasma, serum
Quality Control	regularly tested by QCMD and INSTAND e.V. External Quality Assessment Panels
Regulatory Status	CE_{1023}IVD

Quality management system is certified in compliance with the requirements of the standard ČSN EN ISO 13485 ed.2:2016

INTERFERENCES

The interference testing has been performed using negative plasma and serum with set level of biochemical markers which can be potential endogenous interferences. The negative plasma and serum were spiked with HBV positive control at 3x LoD. Elevated levels of bilirubin (342 µmol/l), albumin (60 g/l), haemoglobin (2 g/l), urea (42.9 mmol/l), uric acid (1.4 mmol/l) and D-glucose (55 mmol/l) in samples have been tested in the presence and absence of HBV DNA. The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2. The test has been validated for use with human plasma collected in EDTA, heparin and citrate as anticoagulant substances. The validation was done according to the directive 2009/108/EC on positive and negative samples.

PLASMA

Tested substance	**Tested level(s)**	**Observed interference**	**Tested substance**	**Tested level(s)**	**Observed interference**
Albumin	60 g/l	None	Haemoglobin	2 g/l	Partial
Bilirubin	342 µmol/l	None	Urea	42.9 mmol/l	Partial
Glucose	55 mmol/l	None	Uric acid	1.4 mmol/l	Partial

SERUM

Tested substance	**Tested level(s)**	**Observed interference**	**Tested substance**	**Tested level(s)**	**Observed interference**
Albumin	60 g/l	None	Hemoglobin	2 g/l	None
Bilirubin	342 µmol/l	Partial	Urea	42.9 mmol/l	Partial
Glucose	55 mmol/l	None	Uric acid	1.4 mmol/l	Partial

The test of endogenous interferents showed partial interference of the GeneProof Hepatitis B Virus (HBV) PCR Kit with high (pathological) concentration of haemoglobin, urea and uric acid in plasma samples. Partial inhibition was observed with high (pathological) concentration of bilirubin, urea and uric acid in serum samples.

Tested anticoagulants (citrate, heparin, EDTA) were shown not to interfere with the GeneProof Hepatitis B Virus (HBV) PCR Kit.

METHOD PRINCIPLE

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 Control (IC) is included in the PCR kit, controlling the possible inhibition of the PCR and the DNA extraction process quality. IC positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring the maximum sensitivity. The Ready-to-Use Master Mix contains uracil-DNA-glycosylase (UDG) eliminating possible contamination of the PCR with amplification products. The kit is designed for *in vitro* diagnostics and provides the qualitative and quantitative detection.

USER MANUAL

SAMPLING AND SAMPLE STORAGE

Plasma and serum samples are used for HBV DNA detection. Sampling of plasma and serum should be performed into sterile tubes without any transportation media. The material should be transported to the laboratory at a temperature between (2 – 8) °C within 24 hours. In case of longer storage keep all samples frozen at a temperature 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 products:

GeneProof PathogenFree DNA Isolation Kit

croBEE 201A Nucleic Acid Extraction Kit

Add the Internal Control (IC) directly into the sample at the beginning of the extraction process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IC:

Elution volume	**25 µl**	**50 µl**	**100 µl**	**200 µl**
Internal Control	2.5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Gently vortex and briefly centrifuge the Master Mix and Calibrators' tubes.

2. Add 30 µl of Master Mix into PCR tubes.

3. Add 10 µl of the extracted nucleic acid sample or 10 µl of Calibrator into the individual PCR tubes and mix by pipetting. The total reaction mix volume will be 40 µl. *It is necessary to keep all components at (2 – 8) °C during the PCR preparation. The isolate of negative isolation control with Internal Control (IC) should be used in each test. The negative clinical material, water or buffer can be used as negative isolation control. The customer must use his own negative control. All 4 Calibrators must be used for setting the standard curve for quantitative detection.*

4. 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 Calibrators or the clinical material; incorrect handling could result in contamination and the consequent impairment of the kit components! 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		45
	60 °C	40 s	FAM + HEX	
	72 °C	20 s		

INSTRUMENTS

GeneProof Hepatitis B Virus (HBV) PCR Kit is designed for use with real-time devices from various manufacturers:

croBEE Real-Time PCR System
AMPLilab Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
BioQuant-96 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
Mic qPCR Cycler
QuantStudio™ 3 / 5 Real-Time PCR System
Rotor-Gene 3000 / 6000 / Q
SLAN® Real-Time PCR System
StepOne™ / StepOne Plus™ Real-Time PCR System

Required channels: FAM, HEX

GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at *www.geneproof.com* or can be requested at *support@geneproof.com*

CLINICAL SAMPLE ANALYSIS EVALUATION

Channel FAM (HBV)	Channel HEX (IC)	Result	Interpretation
+	+	Valid	HBV positive
+	-	Valid	HBV positive
-	+	Valid	HBV negative
-	-	Invalid	-

QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in IU/ml for **manual** extraction (using GeneProof PathogenFree DNA Isolation Kit):

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

SC - Sample concentration (IU/µl)

EV - Elution volume (µl)

IV - Isolation volume (ml)

To easily calculate pathogen concentrations using manual or automated extraction, you can use the calculator at *www.geneproof.com*

WARNING

A single valid Instruction for Use for a specific kit is included in the package or can be requested for a particular lot from the manufacturer. Use only the combination of components from the specific PCR kit. The kit should be disposed of after use according to the current legal regulations considering the fact, that the kit does not 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 care.

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