GeneProof®
Molecular diagnostics for your routine

GeneProof Hepatitis C Virus (HCV) Diagnostic 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:	HCVD/ISEX/025 25 rxn	HCVD/ISEX/100 100 rxn
MasterMix HCVD	1x750 µl	4x750 µl
Calibrator A HCVD 10^5 IU/µl	1x400 µl	1x400 µl
Calibrator B HCVD 10^4 IU/µl	1x400 µl	1x400 µl
Calibrator C HCVD 10^3 IU/µl	1x400 µl	1x400 µl
Calibrator D HCVD 10^2 IU/µl	1x400 µl	1x400 µl
Internal Control HCVD	1x500 µl	2x500 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 at (-20 ± 5) °C. The components are stable for a maximum of 6 repeated freezing / thawing cycles after 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	conservative region of 5' UTR sequence
Analytical Specificity	HCV genotype 1 - 8, 100 %
Analytical Sensitivity (LoD with probability of 95 %)	53.505 IU/ml (on HCV NIBSC 14/150 using GeneProof PathogenFree RNA Isolation Kit), 170.062 IU/ml (on HCV NIBSC 14/150 using croBEE 201A Nucleic Acid Extraction Kit), 33.473 IU/ml (on Acrometrix HCV-S Panel using MagCore Automated NA Extractor), 7.95 IU/ml (on Acrometrix HCV-S Panel using SpinStar Viral Nucleic Acid Kit 1.0 with SpinStar Pretreatment Solution)
Diagnostic Specificity	100 % ($CI_{95\%}$: 99.07 % - 100 %)
Diagnostic Sensitivity	100 % ($CI_{95\%}$: 95.39 % - 100 %)
Linear Range (with precision of ± 0.5 log)	$10^{8.5}$ - 10^{2} IU/ml (GeneProof PathogenFree RNA Isolation Kit) $10^{8.5}$ - 170.062 IU/ml (croBEE 201A Nucleic Acid Extraction Kit) $10^{8.5}$ - $10^{1.7}$ IU/ml (MagCore Automated NA Extractor)
Dynamic Range	$10^{8.5}$ - 53.505 IU/ml (GeneProof PathogenFree RNA Isolation Kit) $10^{8.5}$ - 170.062 IU/ml (croBEE 201A Nucleic Acid Extraction Kit) $10^{8.5}$ - 33.473 IU/ml (MagCore Automated NA Extractor)
Reporting Units	IU/ml
Validated Specimen	plasma (EDTA, citrate), serum
External Quality Assessment	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 was 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 HCV positive control at level of 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 HCV RNA. 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 or citrate as anticoagulant substances. The validation was done according to the directive 2009/108/EC.

PLASMA, SERUM

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
Albumin	60 g/l	None	Haemoglobin	2 g/l	None
Bilirubin	342 µmol/l	None	Urea	42.9 mmol/l	None
Glucose	55 mmol/l	None	Uric acid	1.4 mmol/l	None

The tested endogenous interferences were shown not to interfere with GeneProof Hepatitis C Virus (HCV) Diagnostic PCR Kit with significant level.

Citrate plasma samples were shown not to interfere with GeneProof Hepatitis C Virus (HCV) Diagnostic PCR Kit.

METHOD PRINCIPLE

The HCV detection is based on the amplification of a single-copy 5' UTR RNA sequence and the measurement of fluorescence increase. The mechanism of duplex targeting ensures maximum sensitivity and specificity and enables detection of the virus in a sample before seroconversion. The specificity was tested to all six identified virus genotypes including the in-silico analysis proving the correct detection of a genotype 7 and newly discovered genotype 8. The presence of HCV is indicated by the increased FAM fluorophore fluorescence. An Internal Control (IC), which is a part of the PCR kit, is used as a control for the whole diagnostic process, i.e. RNA extraction efficiency, reverse-transcription step efficiency (transcription of RNA into cDNA) and PCR amplification efficiency (PCR inhibition). The IC positive amplification is detected in the HEX fluorophore fluorescence channel. The PCR kit is designed for in vitro diagnostics for both qualitative and quantitative detection and it utilizes the "hot start" technology minimizing non-specific reactions and ensuring maximum sensitivity. Ready-to-Use Master Mix contains uracil-DNA-glycosylase (UDG) which eliminates possible contamination of the PCR with amplification products.

USER MANUAL

SAMPLING AND SAMPLE STORAGE

Centrifuge the whole blood samples and remove the plasma to a secondary tube within 4 hours of blood collection. If the sample cannot be processed within four hours, it is recommended to centrifuge whole blood and freeze the plasma sample at -20°C or -70°C. Serum should be shipped frozen on dry ice for either DNA or RNA analysis. For long-term storage, serum can be stored at -20°C or -70°C or lower. Serum separation should be performed within 4 hours of blood collection.

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 RNA Isolation Kit

croBEE 201A Nucleic Acid Extraction Kit

MagCore Automated NA Extractor

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 20 µl of the extracted nucleic acid sample or 20 µl of Calibrator into the individual PCR tubes and mix by pipetting. The total reaction mix volume will be 50 µl.

WARNING! Reaction volume must be set to 40 µl. *It is necessary to keep all components at (2 – 8) °C during the PCR preparation. The isolate of negative extraction control with Internal Control should be used in each test. The negative clinical material, water or buffer can be used as negative extraction 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	42 °C	15 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 C Virus (HCV) Diagnostic 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
LightCycler® 480
LineGene 9600 Plus
Mic qPCR Cycler
Rotor-Gene 3000 / 6000 / Q

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

QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in IU/ml for **manual** extraction (using GeneProof PathogenFree RNA 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 to be requested for the particular lot from the manufacturer. Use only 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 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 care.

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