

GeneProof Hepatitis C Virus (HCV) PCR Kit



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

REF	ISEXplus Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
	HCV/PLUS/025 25 rxn	HCV/PLUS/050 50 rxn	HCV/PLUS/100 100 rxn
MasterMix HCV	1x1000 µl	2x1000 µl	4x1000 µl
Calibrator HCV 10 ⁴ IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HCV 10 ³ IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HCV 10 ² IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HCV 10 ¹ IU/µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard HCV	1x500 µl	1x500 µl	2x500 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	Conservative region of 5' UTR sequence
Specificity	HCV genotype 1-7, 100%
Sensitivity (LoD)	Reaches up to 7.87 IU/ml with the probability of 95% (on AcroMetrix HCV High Control using automatic extraction MagCore NA Extractor) Reaches up to 208.54 IU/ml with the probability of 95% (on AcroMetrix HCV High Control using automatic extraction croBEE NA16 Nucleic Acid Extraction System) Reaches up to 213.965 IU/ml with the probability of 95% (on HCV NIBSC 14/150 using manual extraction GeneProof PathogenFree RNA Isolation Kit)
Dynamic Range; Linear Range	10 ¹⁰ - 7.87 IU/ml; 10 ¹⁰ - 25 IU/ml with precision of ± 0.5 log
Reporting Units	IU/µl (1 IU = 5.62 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

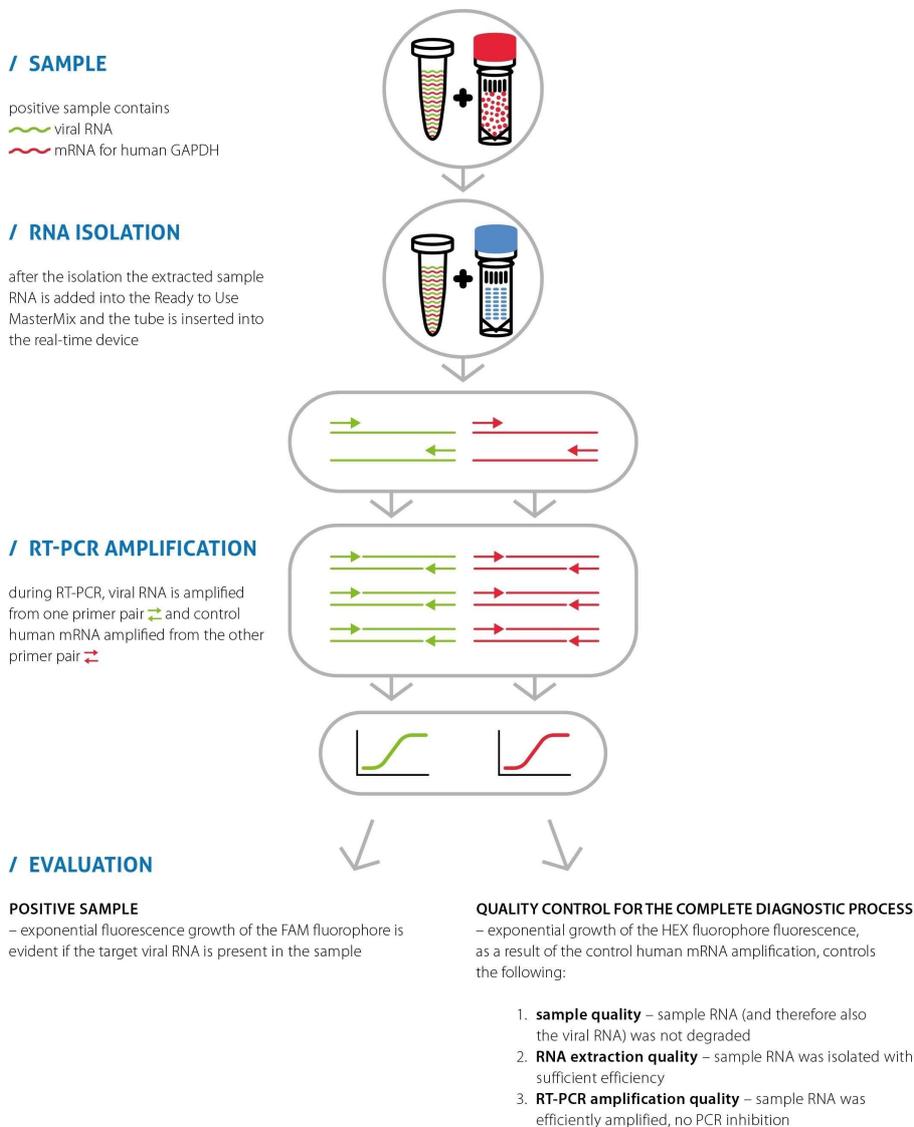
The PCR kit is designed for the Hepatitis C virus (HCV) RNA detection by the real-time Polymerase Chain Reaction (PCR) method.

The HCV detection consists in amplification of a single-copy 5' UTR RNA sequence and in measurement of fluorescence increase. The HCV presence is indicated by the FAM fluorophore fluorescence growth. For the RNA extraction and RT-PCR process control the kit uses an internal standard (IS), which is extracted from the sample together with the viral RNA and its amplification is visualised in the HEX channel (ISEX version). To boost the IS signal ISEXplus version was developed, which includes a separate tube with an external IS, which is also visualised in the HEX channel. The IS detection provides quality control of 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 detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detections.

ISEXplus version

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

MICROBIOLOGICAL RNA DIAGNOSTIC TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

To demonstrate HCV RNA there should be obtained a plasma or serum sample and it should be transported frozen according to the laboratory instructions. In case of longer storage all samples should be frozen at the 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 extraction kits:

croBEE NA16 Nucleic Acid Extraction System
GeneProof PathogenFree RNA Isolation Kit

When using the ISEXplus versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation 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 40 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 50 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	42 °C	5 min		1
Hold	95 °C	10 s		1
	95 °C	5 s		
PCR	58 °C	40 s	FAM+HEX	50
	72 °C	10 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	HCV	positive
		Valid	HCV	positive
		Valid	HCV	negative
		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:

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

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 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 Service.

Customer care and technical support

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