GeneProof
Molecular diagnostics for your routine

GeneProof Human Herpesvirus 8 (HHV-8) PCR Kit

CE 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

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
REF	**HHV8/ISIN/025** 25 rxn	**HHV8/ISIN/050** 50 rxn	**HHV8/ISIN/100** 100 rxn	**HHV8/ISEX/025** 25 rxn	**HHV8/ISEX/050** 50 rxn	**HHV8/ISEX/100** 100 rxn
MasterMix HHV8	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Calibrator HHV8 10^1 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HHV8 10^2 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HHV8 10^3 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator HHV8 10^4 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard HHV8	-	-	-	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	Specific conservative DNA sequence of the ORF26 gene encoding the minor capsid protein (mCP) Specific conservative DNA sequence of the ORF73 gene encoding the latency-associated nuclear antigen (LANA)
Specificity	*Human herpesvirus 8*, 100%
Sensitivity (LoD)	Reaches up to 2.225 cp/µl with the probability of 95%
Linear Range	10^9 - 10^3 cp/ml with precision of ± 0.5 log
Reporting Units	cp/µl
Validated specimens	CSF, plasma, saliva,whole blood
Quality Control	Quality management system is certified in compliance with the requirements of the standard ISO 13485

METHOD PRINCIPLES

The PCR kit is designed for human herpesviru 8 detection by the real-time Polymerase Chain Reaction (PCR) method. The HHV-8 detection consist in amplification of the specific conservative DNA sequence of the *ORF26* gene encoding the minor capsid protein (mCP) and *ORF73* gene encoding the latency-associated nuclear antigen (LANA) and in measurement of fluorescence increase. The HHV-8 presence is indicated by FAM fluorophore fluorescence growth. An Internal Standard (IS) is either included in the reaction mix, controlling the possible inhibition of the PCR (ISIN version) or excluded, controlling also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage 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 with amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detection.

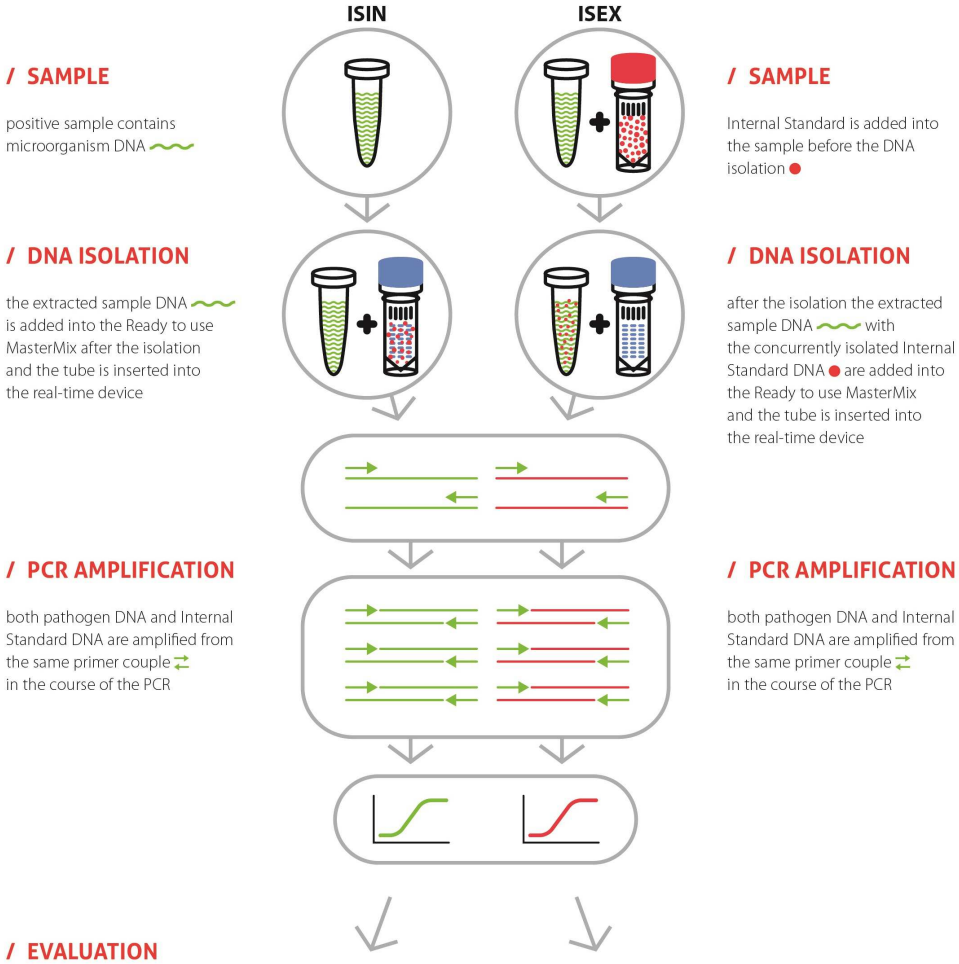
ISIN version

Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

ISEX version

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

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Sampling of all types of the samples should be performed into sterile tubes without any transportation media and the samples should be transported within 12 hours at the temperature between +2 °C and +8 °C. Non-coagulating peripheral blood should be sampled into EDTA and transported to the laboratory at the temperature between +2 and +8 °C within 24 hours. In case of longer storage keep all samples frozen at the temperature -20 ± 5 °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:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleic Acid Extraction System

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		

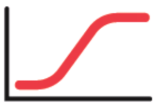
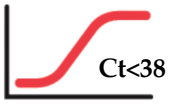
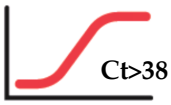
VALIDATED INSTRUMENTS

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
CFX Connect™ / CFX 96™/ Dx Real-Time PCR Detection System
LightCycler® 480
LineGene 9600 / 9600 Plus
Rotor-Gene 3000 /6000 / Q
SLAN® Real-Time PCR System
StepOne™ / StepOne Plus™ Real-Time PCR System
Mic qPCR Cycler

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**	HHV8	**Positive**
		**Valid**	HHV8	**Positive**
		**Valid**	HHV8	**Negative**
		**Invalid**		
		**Invalid**		

QUANTITATIVE DETECTION EVALUATION

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

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

SC - (cp/µl)
EV - (µl)
IV - (ml)

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

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