INSTRUCTIONS FOR USE

GeneProof®
Molecular diagnostics for your routine

GeneProof Herpes Simplex Virus (HSV-1/2) PCR Kit

CE IVD

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof Herpes Simplex Virus (HSV-1/2) PCR Kit	25 reactions	HSV/ISEX/025
GeneProof Herpes Simplex Virus (HSV-1/2) PCR Kit	100 reactions	HSV/ISEX/100

2. INTENDED PURPOSE AND USE

Indication	*In vitro* diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics and aid to diagnosis or monitoring test
What is Detected / Target	Herpes Simplex Virus type 1 and type 2
Automated / Manual Detection	Manual
Type of Analysis	Qualitative and quantitative
Validated Specimen	CSF, plasma, serum, urine, swab (genital, vesicular)*, whole blood (EDTA)
Testing Population	EU population
Intended User	For professional use in laboratories with trained staff
Test Principle	Real-time polymerase chain reaction (PCR) – amplification of the specific Target Sequence and detection using TaqMan probes with fluorophore-based detection

**NOTE: Qualitative evaluation only.*

3. TECHNICAL SPECIFICATIONS

Target Sequence	gene encoding B glycoprotein (gB)
Analytical Specificity	Herpes simplex virus type 1 (HSV-1), 100 % Herpes simplex virus type 2 (HSV-2), 100 %

Analytical Sensitivity (LoD with 95% probability)

Sample Processing	Type	Plasma	Serum	Whole blood	CSF
GeneProof PathogenFree DNA Isolation Kit	HSV-1	122.1 cp/ml	196.2 cp/ml	272.6 cp/ml	82.8 cp/ml
	HSV-2	194.5 cp/ml	70.0 cp/ml	160.9 cp/ml	60.2 cp/ml
croBEE 201A Nucleic Acid Extraction Kit	HSV-1	673.7 cp/ml	302.6 cp/ml	219.2 cp/ml	642.7 cp/ml
	HSV-2	440.4 cp/ml	308.4 cp/ml	219.6 cp/ml	432.6 cp/ml

Diagnostic Specificity	97.62 % ($CI_{95\%}$: 93.63 % – 99.23 %)
Diagnostic Sensitivity	100.00 % ($CI_{95\%}$: 98.24 % – 100.00 %)
Positive Predictive Value	98.53 % ($CI_{95\%}$: 96.04 % – 99.53 %)
Negative Predictive Value	100.00 % ($CI_{95\%}$: 97.15 % – 100.00 %)
Linear Range	(10^{10} – 10^{2}) cp/ml with precision of ± 0.5 log (GeneProof PathogenFree DNA Isolation Kit) (10^{10} – $10^{2.5}$) cp/ml with precision of ± 0.5 log (croBEE 201A Nucleic Acid Extraction Kit)
Dynamic Range	10^{10} cp/ml – LoD (LoD varying according to the extraction,material and channel used)
Reporting Units	cp/ml
Metrological Traceability	AcroMetrix™ HSV-1 Plasma Panel (AcroMetrix HSV-1) AcroMetrix™ HSV-2 Plasma Panel (AcroMetrix HSV-2)
Extraction/Inhibition Control	PCR inhibition and DNA extraction efficiency control by Internal Standard (IS)
Validated Extraction Methods	croBEE 201A Nucleic Acid Extraction Kit GeneProof PathogenFree DNA Isolation Kit

Applied Instruments

Instrument Name	HSV-1	Internal Standard (IS)	HSV-2
croBEE Real-Time PCR System	FAM	HEX	Cy5
AMPLilab Real-Time PCR System	FAM	HEX	Cy5
Applied Biosystems 7500 Real-Time PCR System	FAM	JOE	Cy5
AriaMx Real-Time PCR System	FAM	HEX	Cy5
BioQuant-96 Real-Time PCR System	FAM	HEX	Cy5
CFX96™/ Dx Real-Time PCR Detection System	FAM	HEX	Cy5
CFX Opus 96 Real-Time PCR System	FAM	HEX	Cy5
Gentier 96E/96R Real-Time PCR System	FAM	HEX	Cy5
LightCycler® 480	FAM	HEX	Cy5
LineGene 9600 / 9600 Plus	FAM	HEX	Cy5
Mic qPCR Cycler	FAM	HEX	Cy5
QuantStudio™ 5 Real-Time PCR System	FAM	VIC	Cy5
Rotor-Gene 3000 / Q	FAM	JOE	Cy5
SLAN® Real-Time PCR System	FAM	HEX	Cy5

Detection Channels	FAM (HSV-1), HEX/JOE/VIC (IS), Cy5 (HSV-2)
External Quality Assessment	Regularly tested using QCMD and INSTAND e.V. External Quality Assessment Panels - results at *www.geneproof.com*

4. INTERFERENCES

The evaluation and setting of pathological values for interference testing was performed according to CLSI guidelines EP7-A2 and guidelines and recommendations of Czech Society of Clinical Biochemistry.

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
PLASMA					
Bilirubin	342 µmol/l	None	Uric acid	1.4 mmol/l	None
Haemoglobin	2 g/l	None	Albumin	60 g/l	None
Urea	42.9 mmol/l	None	Citrate	19 g/l	None
Glucose	55 mmol/l	Partial	Caffeine	308 µmol/l	None
Ibuprofen	2425 µmol/l	None	Prednisone	0.84 µmol/l	None
Fluconazole	245 µmol/l	None	Vancomycin	69 µmol/l	None
CSF					
Glucose	55 mmol/l	None	Lactate II	3.8 mmol/l	None
Lactate I	16.5 mmol/l	None	Albumin	60 g/l	None

NOTE: In the case of partial interference, inhibition may occur with the risk of a false negative result at a given concentration of interferent.

5. KIT CONTENT

Reagent	Content	Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
					HSV/ISEX/025 – 25 rxn	HSV/ISEX/100 – 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	MasterMix HSV	Blue	750	1	4
Calibrator	DNA oligonucleotide in buffer	Calibrator HSV 10^4 cp/µl	Black	200	1	1
		Calibrator HSV 10^3 cp/µl	Brown	200	1	1
		Calibrator HSV 10^2 cp/µl	White	200	1	1
		Calibrator HSV 10^1 cp/µl	Transparent	200	1	1
Internal standard	DNA oligonucleotide in buffer	Internal Standard HSV	Red	1000	1	2

6. CALIBRATOR INFORMATION

The use of all 4 calibrators is necessary for correct sample quantification. The automatic quantification based on the analysis of calibrators is generated automatically as a part of analytical process performed in the PCR instrument. Each calibrator consists of target specific DNA. Each calibrator must be designated as „standard“ in the PCR instrument. The concentration of each calibrator must be entered when samples are defined in the PCR plate set up in the data analysis software.

NOTE: In the case of qualitative detection, the Calibrator 10^2 cp/µl serves as a positive control.

7. TRANSPORT AND STORAGE

Storage Conditions	(-20 ± 5) °C
Transport Conditions	-20 °C and below
In-use Stability	Thaw a maximum of 5 times or use within 30 days after the first use of a particular vial, whichever comes first

8. ASSAY PROCEDURE

SPECIMEN COLLECTION, TRANSPORTATION AND HANDLING

1. Samples for DNA extraction must be collected and transported following professional and local guidelines, at (5 ± 3) °C.
2. Samples for DNA extraction must be transported and treated by the laboratory in the shortest possible time (preferably within 24 hours).

NUCLEIC ACID PURIFICATION

3. Prepare specimens for the assay according to the corresponding extraction kit manual.
4. Thaw required amount of Internal Standard (IS or UNIC*) vials, mix and briefly centrifuge.
5. Add Internal Standard (IS or UNIC) directly into the sample at the beginning of the extraction process so that 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 (IS or UNIC)	2.5 µl	5 µl	10 µl	20 µl

6. Continue extraction according to the appropriate protocol.
   NOTE:
   *In case of using *UNIC = GeneProof Universal Internal Control (more information in chapter 12. Additional Products), see Instructions for Use of GeneProof Universal Internal Control.*

PCR SETUP PROTOCOL

7. Thaw required vials and reagents completely.
8. Gently vortex and briefly centrifuge all vials before setting up the PCR run.
   NOTE:
   Keep the reagents at (5 ± 3) °C for the shortest time possible until the PCR reaction is set up.
9. Add 30 µl of Master Mix into PCR tubes.
10. 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 is 40 µl.
11. Close the tubes, centrifuge shortly, insert them into the real-time PCR device and amplify according to the following PCR profile.
    NOTE:
    It is recommended to perform at least 1 negative control and at least 1 full range of calibrators (for a quantitative kit) for each individual PCR run. Use your own negative control (not provided) in the form of nuclease-free water. For more information see chapter 10. Run Validity.

AMPLIFICATION PROFILE

Follow the thermocycler manufacturer`s manual for setting the instrument and for analysis.

Universal PCR Profile

NOTE: The Universal PCR Profile is designed for parallel detection with other GeneProof PCR Kits.

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination/ Reverse Transcription	42	15 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	
	Annealing	60	40 s		FAM, HEX/JOE/VIC, Cy5
	Extension	72	20 s		

DNA PCR Profile

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination	37	2 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	
	Annealing	60	40 s		FAM, HEX/JOE/VIC, Cy5
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (HSV-1)	Channel Cy5 (HSV-2)	Channel HEX/JOE/VIC (IS)	Result	Interpretation
+	-	+/-	Valid	HSV-1 positive
-	+	+/-	Valid	HSV-2 positive
+	+	+/-	Valid	HSV-1 and HSV-2 positive
-	-	+ (C_t < 38)	Valid	HSV-1 and HSV-2 negative
-	-	+ (C_t > 38)	Invalid	-
-	-	-	Invalid	-

NOTE: For interpretation of PCR run see chapter 10. Run Validity.

10. RUN VALIDITY

OVERALL VALIDITY OF DETECTION

	Signal	Channel	Run Validity	Recommendation
Calibrator 10^2 (qualitative detection) or Calibrator Set (quantitative detection)	+	FAM, Cy5	Valid	-
Calibrator 10^2 (qualitative detection) or Calibrator Set (quantitative detection)	-	FAM, Cy5	Invalid	Repeat PCR run
Negative control	-	FAM, Cy5	Valid	-
Negative control	+	FAM, Cy5	Invalid	Repeat PCR run

NOTE: If the issue persists, please contact Customer Support, see Contact information.

11. QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the viral load (**HSV-1**) in cp/ml for manual extraction (using GeneProof PathogenFree DNA Isolation Kit):

VLC – Viral load concentration [cp/ml]
SC – Sample concentration [cp/µl]
EV – Elution volume [µl]
IV – Isolation volume [µl]
CF1 – Correction factor for HSV-1, CF1 = 0.501

$$VLC = \frac{SC \times EV \times 10^3}{IV} \times CF1$$

Use the following formula to calculate the viral load (**HSV-2**) in cp/ml for manual extraction (using GeneProof PathogenFree DNA Isolation Kit):

VLC – Viral load concentration [cp/ml]
SC – Sample concentration [cp/µl]
EV – Elution volume [µl]
IV – Isolation volume [µl]
CF2 – Correction factor for HSV-2, CF2 = 1

$$VLC = \frac{SC \times EV \times 10^3}{IV} \times CF2$$

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

VALIDITY OF QUANTITATIVE DETECTION

Channel	Calibrators				Result	Recommendation
	10^4	10^3	10^2	10^1		
Target specific channel (FAM, Cy5)	++++	+++	++	+	Valid exact quantification	-
Internal Standard channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	≥0.98					
Target specific channel (FAM, Cy5)	++++	+++	++	+	Reduced quantification accuracy	Repeat PCR run
Internal Standard channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	<0.98					
Target specific channel (FAM, Cy5)	No signal of one or more calibrators				Invalid quantification	Repeat PCR run
Internal Standard channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	N/A					

R^2 – Determination coefficient – parameter evaluating the quality of standard curve
NOTE: If the issue persists, please contact Customer Support.

12. ADDITIONAL PRODUCTS

GeneProof Universal Internal Control

GeneProof Universal Internal Control (UNIC) is intended to be used as the Internal Control/ Standard for the microbiological GeneProof PCR kits and as an alternative product to Internal Controls/ Standards included in the GeneProof microbiological PCR kits. The UNIC works only in combination with GeneProof PCR kits. It is intended to simplify the user's workflow in cases where multiple detection kits with single extract are used. For more details see the Instructions for Use for UNIC.

Product	REF
GeneProof Universal Internal Control	UNIC/GP/050

NOTE: IS is applied to the solution only once. Add UNIC instead of IS from the package of the PCR kit. Do not add IS and UNIC to the same sample at the same time.

13. MATERIALS AND DEVICES REQUIRED BUT NOT PROVIDED

CONSUMABLE MATERIALS

96-well reaction plates or PCR tubes (0.2 ml volume), pipette tips with filters, powder-free gloves, biohazard waste bin, nuclease-free water

DEVICES

Real-time PCR instrument (see chapter 3. Technical Specifications), nucleic acid extraction system or kit (see chapter 3. Technical Specifications), desktop centrifuge (for 0.2 ml PCR tubes or 96-well plates), vortex mixer, freezer (-20 ± 5) °C, refrigerator (5 ± 3) °C, cooling rack

14. WARNINGS, PRECAUTIONS AND PROCEDURE LIMITATIONS

- Patient management decisions should never be made based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
- Any serious incident occurred in relation to the using of GeneProof PCR Kit shall be reported to the manufacturer and to the competent local authority.
- Use all necessary protective equipment (protective disposable gloves, a laboratory coat and eye protection) when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of the reagents when removing aliquots from reagent vials.
- Use RNase- and DNase-free filter pipette tips only.
- Use new tips for each pipetting step.
- Use separate working places for sample preparation / nucleic acid extraction and amplification reactions. Never introduce an amplified product in reagent and/or nucleic acid extraction (sample preparation) area.
- Close the kit components vials immediately after use and never interchange lids.
- Do not pool reagents from different lots or from different vials within the same lot.
- Do not substitute the reagents between different lots.
- Do not use reagents from damaged or leaking vials.
- Dispose of unused reagents and waste in accordance with national, federal, state or local regulations.
- 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! The manufacturer is not responsible for the kit impairment due to incorrect handling.

Procedure Limitations:

- Read the whole Instructions for Use properly before starting the manipulation. Not following these instructions can lead to an erroneous result which can cause misdiagnosis or inappropriate treatment!
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- Do not use kit after the expiry date.
- The presence of UNG decontamination step reduces the risk of lower levels of amplicon contamination. However, contamination from very high levels of amplicons can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Instructions for Use. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This product is designed for use with the applied PCR instruments and validated extraction methods mentioned in chapter 3. Technical Specifications.

Clinical Limitations:

- Detection of HSV DNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors.
- Use only with validated specimens (see chapter 2. Intended Purpose and Use) otherwise incorrect results could occur.
- This test is intended for use as an aid in the management of immunocompromised patients and pregnant women. In this population, the test can be used to predict virologic response to treatment by measuring the baseline HSV DNA level and to assess the effects of antiviral therapy by measuring HSV DNA during the course of antiviral treatment.

15. EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
CE	This product complies with the relevant EU requirements	LOT	Lot number
IVD	*in vitro* diagnostic medical device	Σ	Contains sufficient amount for n-tests
REF	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read Instructions for Use		Date of Manufacture (for selected territories only)

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