INSTRUCTION FOR USE

GeneProof®
Molecular diagnostics for your routine

GeneProof Varicella-Zoster Virus (VZV) PCR Kit

CE IVD

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof Varicella-Zoster Virus (VZV) PCR Kit	25 reactions	VZV/ISEX/025
GeneProof Varicella-Zoster Virus (VZV) PCR Kit	100 reactions	VZV/ISEX/100

2. INTENDED PURPOSE AND USE

Indication	*In vitro* diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics, aid to diagnosis and monitoring test
What is Detected / Target	Varicella-Zoster Virus (VZV)
Automated / Manual Detection	Manual
Type of Analysis	Qualitative and quantitative
Validated Specimen	CSF, plasma, serum, whole blood (EDTA), vesicular swab*
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 SPECIFICATION

Target Sequence	*ORF62* gene
Analytical Specificity	Varicella-Zoster Virus (VZV), 100 %
Analytical Sensitivity (LoD with 95% probability)	

Sample processing	Plasma	Serum	Whole blood	CSF
GeneProof PathogenFree DNA Isolation Kit	113.1 cp/ml	66.1 cp/ml	119.9 cp/ml	66.4 cp/ml
croBEE 201A Nucleic Acid Extraction Kit	218.8 cp/ml	166.5 cp/ml	170.4 cp/ml	175.1 cp/ml

Diagnostic Specificity	97.44 % ($CI_{95\%}$: 84.92 % - 99.87 %)
Diagnostic Sensitivity	100.00 % ($CI_{95\%}$: 97.52 % - 100.00 %)
Positive Predictive Value	99.47 % ($CI_{95\%}$: 96.65 % - 99.97 %)
Negative Predictive Value	100.00 % ($CI_{95\%}$: 88.57 % - 100.00 %)
Linear Range	10^{10} – $10^{2.5}$ cp/ml with precision of ± 0.8 log
Dynamic Range	10^{10} – LoD cp/ml (LoD varying according to the extraction and material used)
Trueness (of expected concentration)	-0.01 log ($CI_{95\%}$: -0.15 – 0.14) using manual extraction GeneProof PathogenFree DNA Isolation Kit -0.07 log ($CI_{95\%}$: -0.15 – 0.00) using automatic extraction croBEE 201A Nucleic Acid Extraction Kit
Precision - Repeatability	• Intra-assay SD of log concentration = 0.076 ($CI_{95\%}$: 0.061 – 0.099)
Precision - Reproducibility	• Inter-assay SD of log concentration = 0.079 ($CI_{95\%}$: 0.051 – 0.174) • Inter-lot SD of log concentration = 0.081 ($CI_{95\%}$: 0.052 – 0.177) • Total SD of log concentration = 0.081 ($CI_{95\%}$: 0.052 – 0.179)
Reporting Units	cp/ml
Metrological Traceability	AcroMetrix™ VZV Plasma Panel (cat. no. 954530)
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	VZV	Internal Standard (IS)
croBEE Real-Time PCR System	FAM	HEX
AMPLilab Real-Time PCR System	FAM	HEX
Applied Biosystems 7300 / 7500 Real-Time PCR System	FAM	JOE
AriaMx Real-Time PCR System	FAM	HEX
BioQuant-96 Real-Time PCR System	FAM	HEX
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System	FAM	HEX
Gentier 96E/96R Real-Time PCR System	FAM	HEX
LightCycler® 2.0 / 480	FAM	HEX
LineGene 9600 / 9600 Plus	FAM	HEX
Mic qPCR Cycler	FAM	HEX
QuantStudio™ 3 / 5 Real-Time PCR System	FAM	VIC
Rotor-Gene 3000 / Q	FAM	JOE
SLAN® Real-Time PCR System	FAM	HEX
StepOne™ / StepOne Plus™ Real-Time PCR System	FAM	HEX

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

4. INTERFERENCES

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

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
PLASMA					
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
Caffeine	308 µmol/l	Partial	Prednisone	0.84 µmol/l	None
Ibuprofen	2425 µmol/l	None	Vancomycin	69 µmol/l	Partial
Fluconazole	245 µmol/l	None	Citrate	19 g/l	None
CSF					
Albumin	60 g/l	None	Lactic acid I	16.5 mmol/l	None
Glucose	55 mmol/l	None	Lactic acid II	3.8 mmol/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	
					VZV/ISEX/025 – for 25 rxn	VZV/ISEX/100 – for 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	Master Mix VZV	Blue	750	1	4
Calibrator	DNA oligonucleotide in buffer	Calibrator VZV 10^4 cp/µl	Black	200	1	1
		Calibrator VZV 10^3 cp/µl	Brown	200	1	1
		Calibrator VZV 10^2 cp/µl	White	200	1	1
		Calibrator VZV 10^1 cp/µl	Transparent	200	1	1
Internal standard	DNA oligonucleotide in buffer	Internal Standard VZV	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 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 Instruction 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
	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
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (VZV)	Channel HEX/JOE/VIC (IS)	Result	Interpretation
+	+	Valid	VZV positive
+	-	Valid	VZV positive
-	+ (C_t < 38)	Valid	VZV 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	Valid	-
Calibrator 10^2 (qualitative detection) or Calibrator Set (quantitative detection)	-	FAM	Invalid	Repeat PCR run
Negative control	-	FAM	Valid	-
Negative control	+	FAM	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 concentration 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]

$$VLC = \frac{\text{SC x EV x } 10^3}{\text{IV}}$$

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)	++++	+++	++	+	Valid exact quantification	-
Internal Standard channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	≥0.98					
Target specific channel (FAM)	++++	+++	++	+	Reduced quantification accuracy	Repeat PCR run
Internal Standard channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	<0.98					
Target specific channel (FAM)	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 Instruction 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. MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

CONSUMABLE MATERIAL

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 Specification), nucleic acid extraction system or kit (see chapter 3. Technical Specification), 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, PRECAUSIONS 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 Instruction 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 Instruction 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 Specification.

Clinical Limitations:

- Detection of VZV 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.

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 Instruction for Use		Date of Manufacture (for selected territories only)

Customer Support
Tel.: +420 730 176 222
E-mail: support@geneproof.com

Orders
Tel.: +420 543 211 679
E-mail: sales@geneproof.com

GeneProof a.s.
Vídeňská 101/119 /Dolní Heršpice/ CZ-619 00 Brno / +420 543 211 679
info@geneproof.com