GeneProof
Molecular diagnostics for your routine

GeneProof Varicella-Zoster Virus (VZV) 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	**VZV/ISIN/025** 25 rxn	**VZV/ISIN/050** 50 rxn	**VZV/ISIN/100** 100 rxn	**VZV/ISEX/025** 25 rxn	**VZV/ISEX/050** 50 rxn	**VZV/ISEX/100** 100 rxn
MasterMix VZV	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Calibrator VZV 10^4 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator VZV 10^3 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator VZV 10^2 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator VZV 10^1 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard VZV	-	-	-	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 single-copy gene ORF62
Analytical Specificity	Varicella-zoster virus (VZV), 100%
Analytical Sensitivity (LoD)	reaches up to 113.05 cp/ml with the probability of 95% (on AcroMetrix VZV High Plasma Control using manual extraction GeneProof PathogenFree DNA Isolation Kit)
Diagnostic Specificity	96.97% ($CI_{95\%}$: 82.49% - 99.84%)
Diagnostic Sensitivity	100% ($CI_{95\%}$: 96.50% - 100%)
Linear Range	10^{10} - 113,05 cp/ml with precision of ± 0.8 log
Dynamic Range	10^{10} - 113,05 cp/ml
Metrological Traceability	AcroMetrix VZV High Plasma Control (cat. n. 954514)
Validated Specimen	CSF, plasma, serum, whole blood
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels
Regulatory Status	CE IVD

Quality management system is certified in compliance with the requirements of the standard ISO 13485:2016.

METHOD PRINCIPLES

The PCR kit is designed for detection of Varicella-zoster virus (VZV) by the real-time Polymerase Chain Reaction (PCR) method. The VZV detection consists in amplification of a specific conservative DNA sequence of the single-copy gene *ORF62* and in measurement of fluorescence increase. The VZV presence is indicated by the 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 of 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 by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detection.

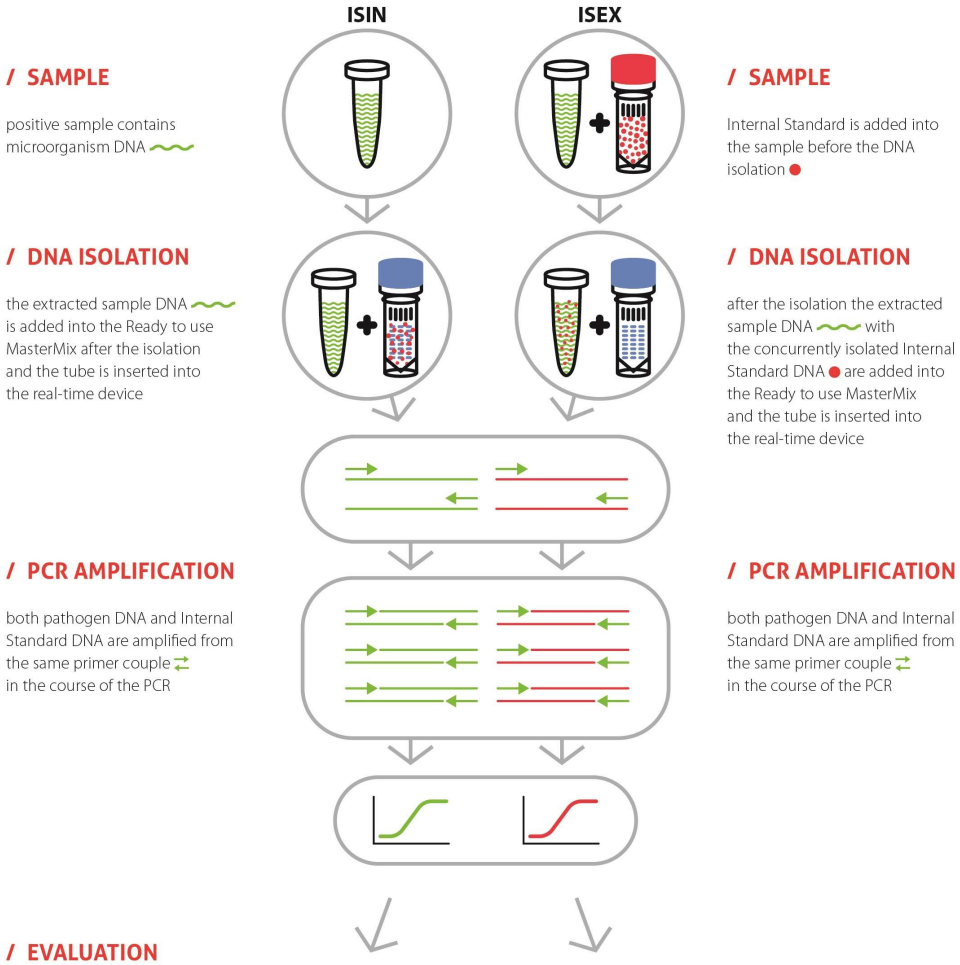
ISIN version

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 purificaion process efficiency control.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



SAMPLING AND SAMPLE STORAGE

Sampling of all sample types (plasma, serum, cerebrospinal fluid-CSF), except for blood, 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 below -10 °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 the extraction 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 Calibrator/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 Calibrator/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		

INSTRUMENTS

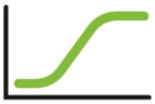
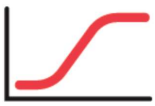
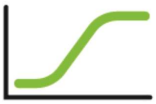
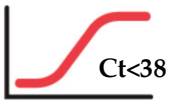
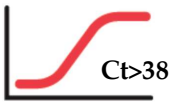
GeneProof Varicella-Zoster Virus (VZV) PCR Kit is designed for use with real-time devices from various manufacturers:

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
LightCycler® 2.0 / 480
LineGene 9600 / 9600 Plus
Mic qPCR Cycler
QuantStudio™ 3 / 5 Real-Time PCR System
Rotor-Gene 3000 / Q
SLAN® Real-Time PCR System
StepOne™/StepOne Plus™ Real-Time PCR System

Required channels: FAM, HEX

GeneProof diagnostics kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the list at support@geneproof.com.

CLINICAL SAMPLE ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation	
		**Valid**	VZV	**positive**
		**Valid**	VZV	**positive**
		**Valid**	VZV	**negative**
		**Invalid**		
		**Invalid**		

QUANTITATIVE DETECTION EVALUATION

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

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

SC - Sample concentration (cp/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 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 Service.

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