

# GeneProof®

## Varicella-Zoster Virus (VZV)

### PCR Kit



### *in vitro* Diagnostics

The kit is designed for professional use in specialized clinical and research laboratories.

#### Kit composition

Cat. No	Internal Standard is included in the MasterMix for inhibition control			Contains independent Internal Standard for inhibition and isolation process control		
	VZV/ISIN/025 25 reactions	VZV/ISIN/050 50 reactions	VZV/ISIN/100 100 reactions	VZV/ISEX/025 25 reactions	VZV/ISEX/050 50 reactions	VZV/ISEX/100 100 reactions
<b>MasterMix VZV</b>	1 x 750 µl	2 x 750 µl	4 x 750 µl	1 x 750 µl	2 x 750 µl	4 x 750 µl
<b>Calibrator VZV</b> 10 <sup>4</sup> copies/µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl
<b>Calibrator VZV</b> 10 <sup>3</sup> copies /µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl
<b>Calibrator VZV</b> 10 <sup>2</sup> copies /µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl
<b>Calibrator VZV</b> 10 <sup>1</sup> copies /µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl	1 x 200 µl
<b>Internal standard VZV</b>	-	-	-	1 x 1000 µl	1 x 1000 µl	2 x 1000 µl

#### Storage and transportation conditions

Transport the kits at temperatures ranging from -20 °C to -80 °C. The kit remains stable for 9 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix, Internal Standard and the Positive Control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 30 µl directly to PCR tubes and hold in stock at -20°C. Positive Control and the Internal Standard may be held in stock at 4°C.

## Pathogen information

Infections by the Varicella-Zoster virus are very frequent in human population and mostly they occur without serious symptoms. Primary infection mostly occurs in childhood under the appearance of the benign disease varicella. Complications in the form of encephalitis are rather rare. Primary infection may be dangerous in the first 5 months of pregnancy. In people with hypo-immunity the VZV infections cause a very serious disease (abundant and long-term occurrence of hemorrhagic efflorescences with the affection of internal body organs). After the primary infection the virus persists in nerve ganglia for the whole life and there may occur a reactivation of the endogenous infection, manifested mostly in the form of herpes zoster. In immunosuppressed persons the zoster development may be very complicated (pneumonias, encephalitis). There may occur serious affections of eyes (keratitis, iridocyclitis, panophthalmitis) or facial and eye-moving nerves. Correct and early diagnostics is important not only for differential diagnostics but mostly for early application of an efficient antivirus therapy. Clinical symptoms are mostly sufficient for diagnosing diseases caused by the VZV. Virus detection in saliva may help to detect primary infection mostly in immunodeficient patients. Serological tests, i.e. mostly the detection of specific IgM antibodies are of higher specificity value than HSV tests, yet there is the problem of the crossed-over reactivity with HSV. Classic methods of the virus direct demonstration are slow, tedious and little sensitive. PCR method is very efficient here, since it provides for a very sensitive VZV detection in any clinical materials. This method is especially important in testing samples of cerebrospinal fluid in case of neurological complications or samples of eye fluid, tears, eye scrapings, etc. To assure correct interpretation this test should be complemented by a peripheral blood test. Due to possible latent life-long infection the tests of these and other clinical materials (blood, smears, blister fluid, etc.) should be interpreted in connection with the clinical state and with the results of other laboratory tests.

## Method principles

This kit is designed for Varicella-Zoster virus (VZV) detection by the real-time polymerase chain reaction (real-time PCR). The VZV detection is based on the amplification of a specific conservative DNA sequence of a single-copy ORF62 gene and on measuring the amplification product concentration in the course of the PCR process by means of a fluorophore FAM marked probe. The reaction mix includes an Internal Standard (IS) controlling the possible inhibition of the PCR reaction and the efficiency of DNA isolation process (ISEX version). Amplification of IS results in positive signal in JOE channel. The detection kit takes an advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity and contains the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products. This provides for a very high sensitivity of the VZV laboratory detection in body fluid (liquor, serum) and blood samples. The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detections.

**GeneProof PCR kits are designed to be performed on real-time instruments of different manufacturers.**

**With following real-time instruments Varicella-Zoster Virus (VZV) PCR Kit was validated:**

**Rotor-Gene™ 3000 (Corbett Life Science)**  
**Rotor-Gene™ 6000 (Corbett Life Science)**  
**7500 Real-Time PCR System (Applied Biosystems)**  
**LightCycler® 2.0 (Roche)**  
**LightCycler® 480 System (Roche)**  
**SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)**

Ask distributor of the kits for detailed manuals for the particular real-time devices or download them from the [www.geneproof.com](http://www.geneproof.com)

If you want use kit with other instrument mentioned above, contact please our Product Support Department at: [support@geneproof.com](mailto:support@geneproof.com)

### Warning:

- The kit has been manufactured in harmony with the EC Directive 98/79/EC as an *in vitro* medical diagnostic device.
- 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.
- 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 Product Support Department at: [support@geneproof.com](mailto:support@geneproof.com)

# User Manual

## Sampling and sample storage

Sampling of all sample types (tears, swabs and scrapings, liquor, saliva, tissues), except for blood, should be performed into sterile tubes without any transportation media and the samples should be transported within 12 hours at +4°C. It is necessary to sample up to 2ml of body fluid samples or take wad smears or swabs “dry”. **Blood sampling:** a sample of incoagulable peripheral blood should be sampled into the EDTA and transported into the laboratory at +4 °C within 24 hours. **In case of longer storage all samples should be frozen at -20°C.**

## DNA isolation

DNA isolation should be performed by isolation kits available at the market according to specific protocols for the particular microorganism isolation. The manufacturer recommends the following isolation kits:

PathogenFree DNA Isolation Kit (GeneProof); Arrow Viral NA Kit (NorDiag), Arrow Blood DNA Kit (NorDiag).

All GeneProof PCR kits include an Internal Standard providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods. **GeneProof develops and sells two basic versions of PCR kits with various compositions of the Internal Standard:**

### PCR kit ISIN (Cat. No. VZV/ISIN/...)

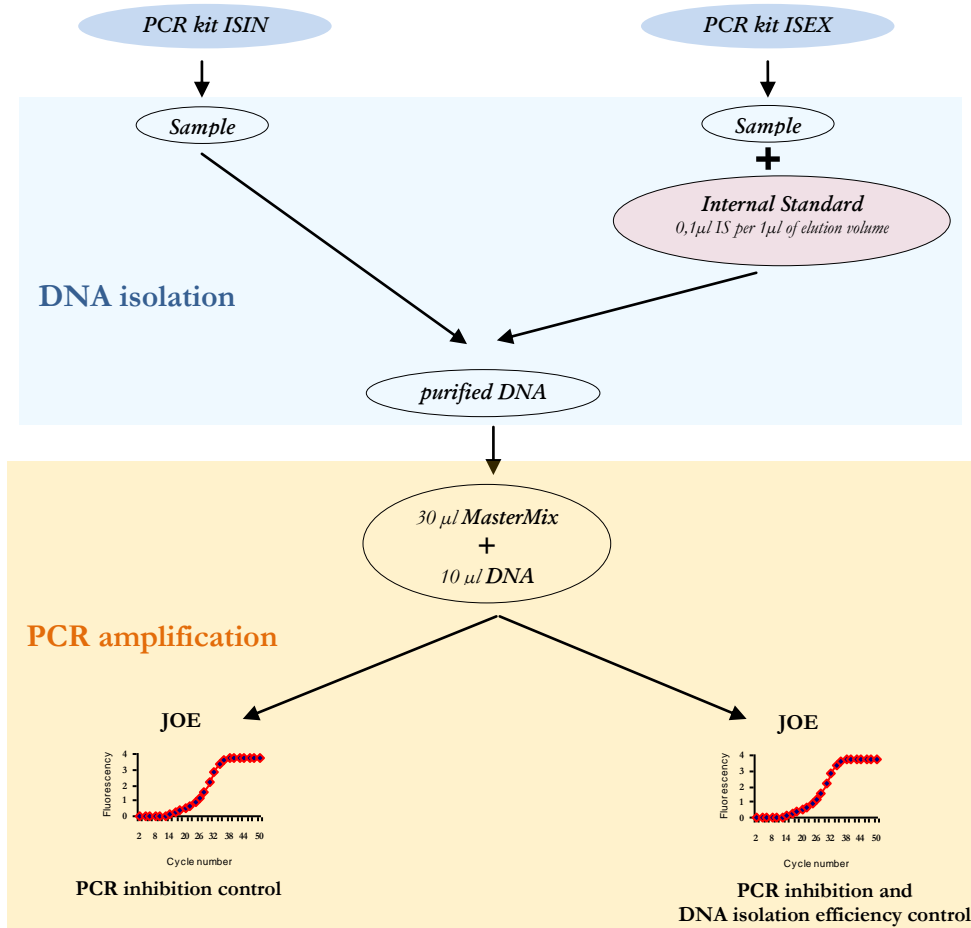
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

### PCR kit ISEX (Cat. No. VZV/ISEX/...)

In this PCR kit version the Internal Standard is included as an independent item within the package. This PCR kit enables both, PCR inhibition control and DNA isolation process efficiency control.

**The Internal Standard should be added into the sample at the beginning of the isolation process as follows: 0.1 µl of the Internal Standard per 1 µl of elution volume:**

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard	2.5 µl	5 µl	10 µl	20 µl



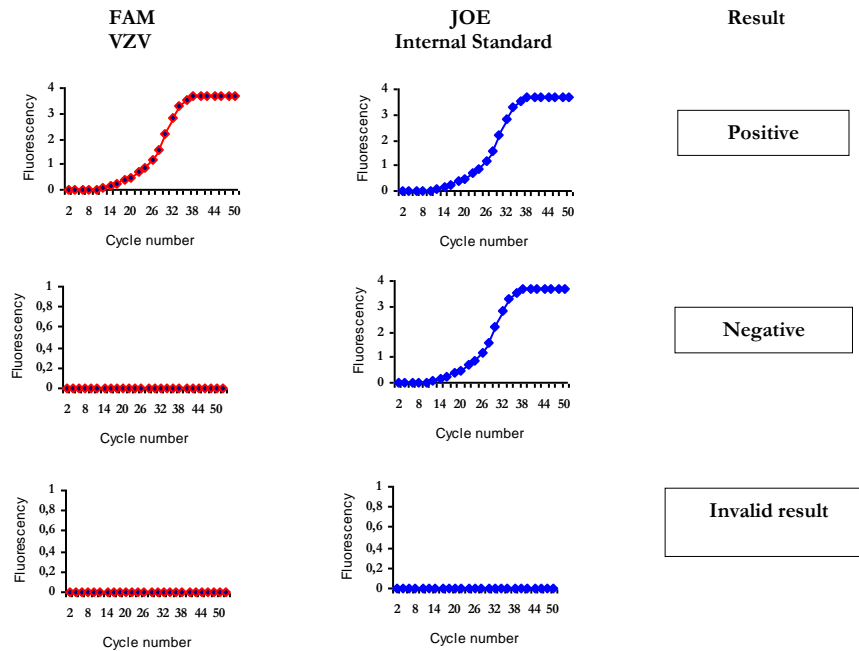
## PCR amplification

1. Add 30  $\mu\text{l}$  of the MasterMix and 10  $\mu\text{l}$  of the DNA or 10  $\mu\text{l}$  of the Positive Control into a tube. The final reaction mix volume should be 40  $\mu\text{l}$ .
2. Close the tubes, shortly centrifuge, insert into the device and program according to the following table:

### Amplification program:

UDG decontamination	37 °C/2 min.
initial denaturation	95 °C/10 min.
denaturation	95 °C/5 sec.
annealing	60 °C/40 sec. - reading of the fluorescence signal
extension	72 °C/20 sec.
number of cycles	45

## Qualitative evaluation of detection



## Quantitative evaluation of detection

Only concentrations in the range specified by the calibration curve may be measured for a quantitative evaluation of the results.

Quantification of samples out of calibration curve should be considered to be not very precise. Samples upper the highest concentrated calibrator could be diluted to achieve more precise quantification. Samples with lower concentrations than the lowest concentrated calibrator can be quantified approximately only.

The following formula can be used to convert sample concentrations to *units/ml* taking into account the isolation procedure:

$$\text{Concentration/ml} = \frac{\text{cVZ} \times \text{EO}}{\text{I}}$$

cVZ = sample concentration in units / $\mu\text{l}$   
 EO = selected elution volume in  $\mu\text{l}$   
 I = volume of sample used for isolation in ml