

GeneProof®

Herpes Simplex Virus (HSV-2)

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		
	HSV2/ISIN/025 25 reactions	HSV2/ISIN/050 50 reactions	HSV2/ISIN/100 100 reactions	HSV2/ISEX/025 25 reactions	HSV2/ISEX/050 50 reactions	HSV2/ISEX/100 100 reactions
MasterMix HSV 2	1 x 750 µl	2 x 750 µl	4 x 750 µl	1 x 750 µl	2 x 750 µl	4 x 750 µl
Calibrator HSV 2 10 ⁴ 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
Calibrator HSV 2 10 ³ 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
Calibrator HSV 2 10 ² 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
Calibrator HSV 2 10 ¹ 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
Internal standard HSV 2	-	-	-	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 human herpes simplex virus are very frequent in human population and mostly they occur without serious symptoms. All symptoms connected with the HSV infections may be caused by both virus types; nevertheless HSV-1 is more frequently found in connection with infection in the upper part of the body while HSV-2 causes mostly genital infections. Primary infection usually occurs in childhood and it is usually asymptomatic, yet it may be manifested as acute gingivostomatitis. Recurrent manifestations in the mouth region and on the lips may appear at any time. The virus is released during both primary and secondary infections or during endogenous reactivations into respiration tract secrets and into saliva. Genital infections usually recur after the primary attack and they are manifested as vulvovaginitis and cervicitis. They may be connected with lymphadenitis, rarely accompanied by meningitis. Eye infections (keratoconjunctivitis) are almost exclusively caused by the HSV-1 type and the repeated and untreated disease may result in serious eye damage. Herpesvirus encephalitis is a rather serious disease. Correct diagnosis is very important for the herpesvirus infection treatment due to the possibility to apply the rather efficient antiviral therapy. Clinical symptoms are mostly sufficient for diagnosing diseases caused by the HSV. Serologic tests may be well complemented by direct virus detections in case of recurrent infections. Virus direct demonstration methods, except for PCR, are usually time-consuming and little sensitive (virus isolation on tissue cultures, electron microscopy, immunofluorescence). On the other hand, the PCR method provides a very sensitive HSV detection. The fact that the virus is, in limited amounts, released from most members of the healthy population, complicates the interpretation of some clinical material examinations. The examinations should be always interpreted in connection with the clinical state and with the results of other laboratory investigations.

Method principles

This PCR kit is designed for the detection of Herpes Simplex virus type 2 (HSV-2) by the polymerase chain reaction (PCR) method. The HSV-2 detection is based on the amplification of a specific conservative DNA sequence of a single-copy gene for glycoprotein B (gB) and on measuring the amplification product concentration in the course of the PCR process by means of fluorescence marked probe (real-time PCR). HSV-2 positive sample results in FAM fluorescence growing. The reaction mix includes an Internal Standard (IS) controlling the possible inhibition of the PCR reaction and the efficiency of DNA isolation process. 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 very high sensitivity of the HSV-2 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 *Herpes Simplex Virus (HSV-2)* PCR t was validated:

Rotor-Gene™ 3000 (Corbett Life Science)
Rotor-Gene™ 6000 (Corbett Life Science)
7500 Real-Time PCR System (Applied Biosystems)
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

If you want use kit with other instrument mentioned above, contact please our Product Support Department at: 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.

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 2 ml 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. HSV2/ISIN/...)

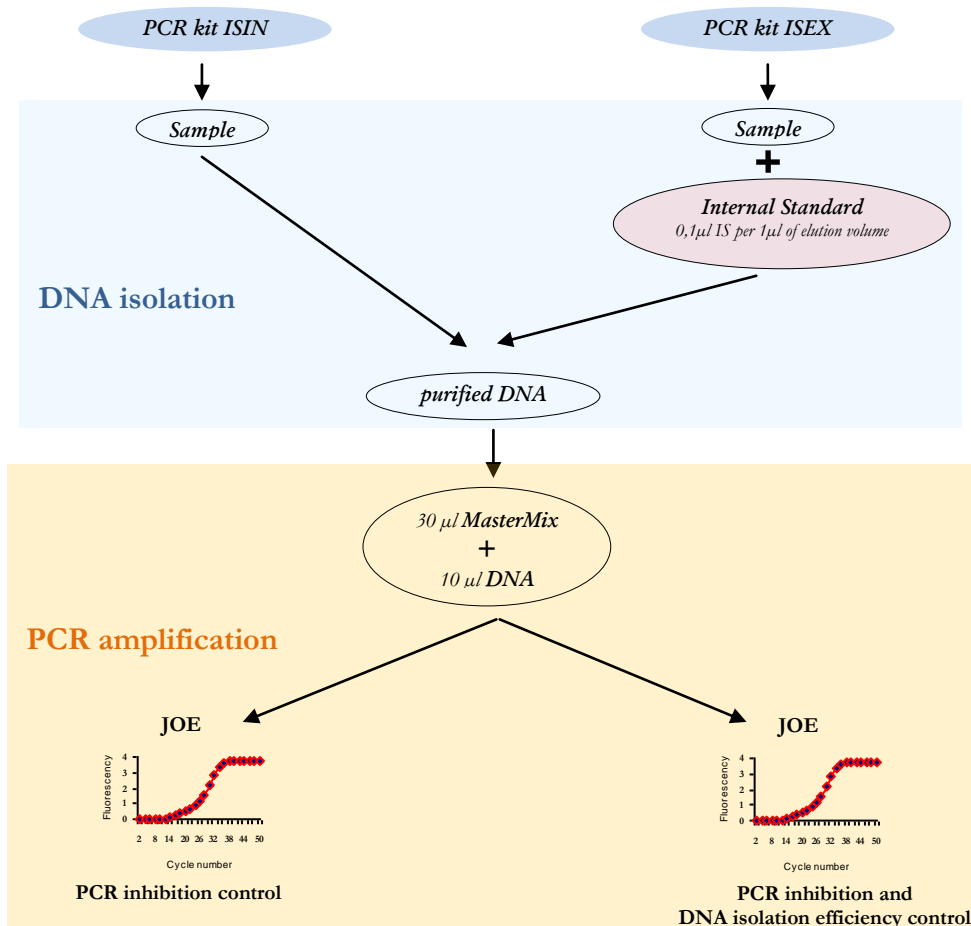
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

PCR kit ISEX (Cat. No. HSV2/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



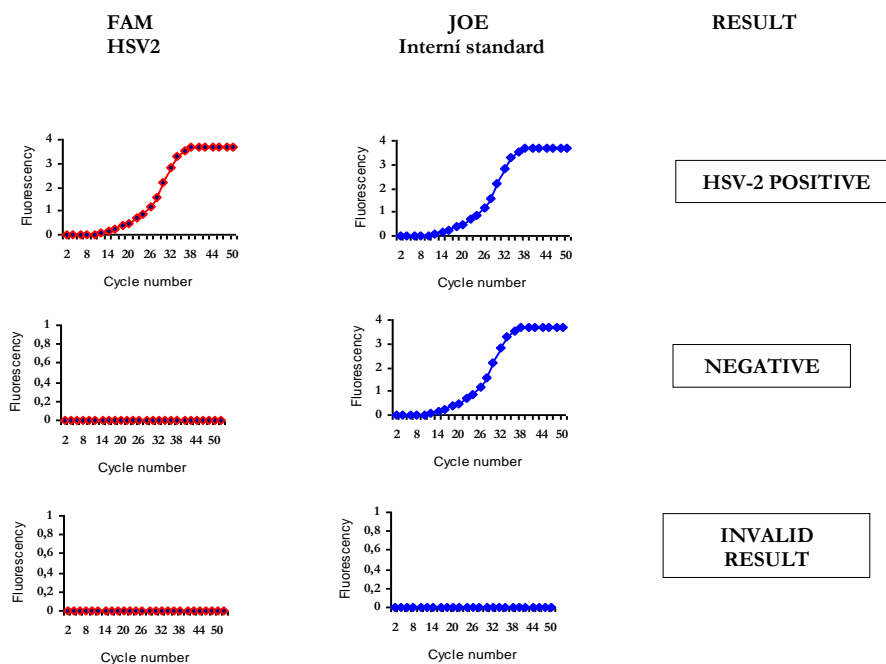
PCR amplification

1. Add 30 μl of the MasterMix and 10 μl of the DNA or 10 μl of the Positive Control into a tube. The final reaction mix volume should be 40 μl .
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 / μl
 EO = selected elution volume in μl
 I = volume of sample used for isolation in ml