GeneProof
Molecular diagnostics for your routine

# GeneProof Herpes Simplex Virus (HSV-1/2) 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<br>IS included in the MasterMix | | | ISEX Version<br>IS supplied in a separate tube<br>Nucleic acid extraction and PCR inhibition control | | |
|---|---|---|---|---|---|---|
| **REF** | **HSV/ISIN/025**<br>25 rxn | **HSV/ISIN/050**<br>50 rxn | **HSV/ISIN/100**<br>100 rxn | **HSV/ISEX/025**<br>25 rxn | **HSV/ISEX/050**<br>50 rxn | **HSV/ISEX/100**<br>100 rxn |
| MasterMix<br>HSV | 1x750 µl | 2x750 µl | 4x750 µl | 1x750 µl | 2x750 µl | 4x750 µl |
| **Calibrator**<br>HSV<br>$10^4$ cp/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| **Calibrator**<br>HSV<br>$10^3$ cp/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| **Calibrator**<br>HSV<br>$10^2$ cp/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| **Calibrator**<br>HSV<br>$10^1$ cp/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| Internal Standard<br>HSV | - | - | - | 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 a single-copy gene encoding glycoprotein B (gB) |
| **Analytical Specificity** | Herpes simplex virus type 1 (HSV-1), 100 %, Herpes simplex virus type 2 (HSV-2), 100 % |
| **Analytical Sensitivity (LoD)** (with the probability of 95 %) | for HSV-1 reaches up to 122.124 cp/ml (on AcroMetrix™ HSV-1 Plasma Panel using manual extraction GeneProof PathogenFree DNA Isolation Kit)<br>for HSV-2 reaches up to 194.49 cp/ml (on AcroMetrix™ HSV-2 Plasma Panel using manual extraction GeneProof PathogenFree DNA Isolation Kit) |
| **Diagnostic Specificity** | 99.24% ($CI_{95\%}$: 95.23% - 99.96%) |
| **Diagnostic Sensitivity** | 100% ($CI_{95\%}$: 97.08% - 100%) |
| **Linear Range** (with precision of ± 0.5 log) | for HSV-1: $10^{10}$ - $10^{2.5}$ cp/ml, for HSV-2: $10^{10}$ - $10^{2.5}$ cp/ml |
| **Dynamic Range** | HSV-1: $10^{10}$ - 122.124 cp/ml, HSV-2: $10^{10}$ - 194.49 cp/ml |
| **Metrological Traceability** | AcroMetrix HSV-1 Plasma Panel (AcroMetrix HSV-1)<br>AcroMetrix HSV-2 Plasma Panel (AcroMetrix HSV-2) |
| **Validated Specimen** | CSF, plasma, urine, 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 and differentiation of both types of Herpes simplex viruses (HSV-1 and HSV-2) by the real-time Polymerase Chain Reaction (duplex PCR) method. The HSV detection and differentiation consists in amplification of a specific conservative DNA sequence of a single-copy gene encoding the glycoprotein B (gB) and in measurement of fluorescence increase. The HSV-1 presence is indicated by the FAM fluorophore fluorescence growth and the HSV-2 presence is indicated by the Cy5 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.

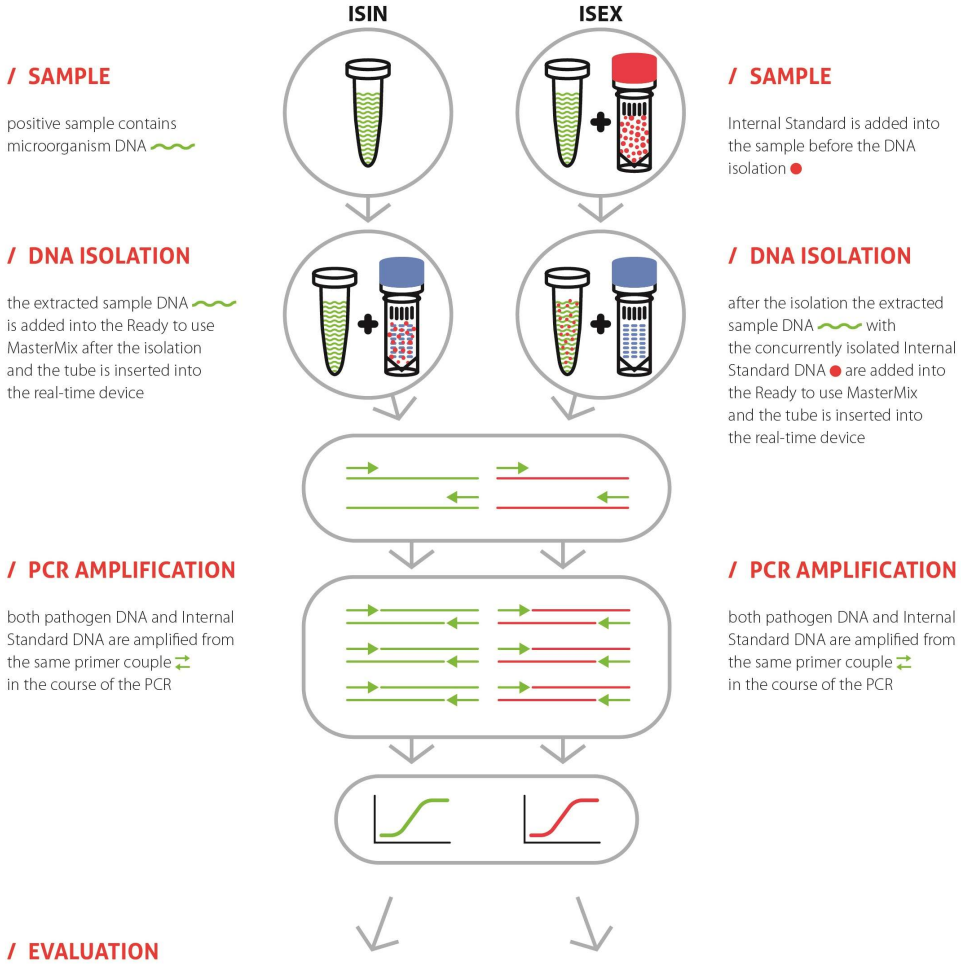
**ISIN version**

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 extraction process efficiency control.

## MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



# USER MANUAL

## SAMPLING AND SAMPLE STORAGE

Sampling of all sample types (whole blood, plasma, cerebrospinal fluid-CSF, urine), 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:

croBEE NA16 Nucleic Acid Extraction System
GeneProof PathogenFree DNA Isolation Kit

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation 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+Cy5 | 45 |
| | 60 °C | 40 s | | |
| | 72 °C | 20 s | | |

# INSTRUMENTS

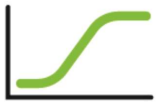
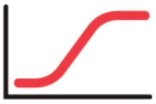
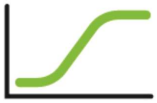
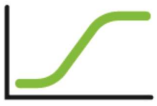
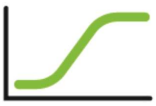
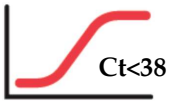
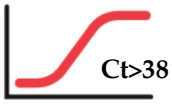
GeneProof Herpex Simplex Virus (HSV-1/2) PCR Kit is designed for use with real-time devices from various manufacturers:

**croBEE Real-Time PCR System**
Applied Biosystems 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 480
LineGene 9600 / 9600 Plus
Mic qPCR Cycler
QuantStudio™ 5 Real-Time PCR System
Rotor-Gene 3000 / Q
SLAN® Real-Time PCR System

Required channels: FAM, HEX, Cy5

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 Cy5 | Channel HEX | Result | Interpretation |
|---|---|---|---|---|
|  | |  | Valid | HSV-1 positive |
| |  | | Valid | HSV-2 positive |
|  |  | | Valid | HSV-1+HSV-2 positive |
| | |  Ct<38 | Valid | negative |
| | |  Ct>38 | 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.


Customer care and technical support
Tel.: +420543211679
Fax: +420516770824
email: support@geneproof.com

Orders
Tel.: +420543211679
Fax: +420516770824
email: sales@geneproof.com