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

<table>
<thead>
<tr>
<th>REF</th>
<th>HSV1/ISIN/025 25 rxn</th>
<th>HSV1/ISIN/050 50 rxn</th>
<th>HSV1/ISIN/100 100 rxn</th>
<th>HSV1/ISEX/025 25 rxn</th>
<th>HSV1/ISEX/050 50 rxn</th>
<th>HSV1/ISEX/100 100 rxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>MasterMix HSV-1</td>
<td>IS included in the MasterMix</td>
<td>IS supplied in a separate tube</td>
<td>Nucleic acid extraction and PCR inhibition control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator HSV 10^4 cp/µl</td>
<td>1x750 µl</td>
<td>2x750 µl</td>
<td>4x750 µl</td>
<td>1x750 µl</td>
<td>2x750 µl</td>
<td>4x750 µl</td>
</tr>
<tr>
<td>Calibrator HSV 10^3 cp/µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
</tr>
<tr>
<td>Calibrator HSV 10^2 cp/µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
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<td>1x200 µl</td>
</tr>
<tr>
<td>Calibrator HSV 10^1 cp/µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
<td>1x200 µl</td>
</tr>
<tr>
<td>Internal Standard HSV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1x1000 µl</td>
<td>1x1000 µl</td>
<td>2x1000 µl</td>
</tr>
</tbody>
</table>

## 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 the glycoprotein B (gB)

**Specificity**
Herpes simplex virus type 1 (HSV-1), 100%

**Sensitivity (LoD)**
Reaches up to 122.124 cp/ml with the probability of 95%

**Linear Range**
10^{10} - 10^{2.5} cp/ml with precision of ± 0.5 log

**Metrological Traceability**
AcroMetrix HSV-1 Plasma Panel (AcroMetrix HSV-1)

**Validated Specimen**
CSF, plasma, urine, whole blood

**Quality Control**
In accordance with ISO 13485, each lot of GeneProof PCR Kit is tested against predetermined specifications to ensure consistent product quality.

**External Quality Assessment**
Regularly tested by QCMD and Instand e.V. External Quality Assessment Panels
The PCR Kit is designed for detection of Herpes simplex virus type 1 (HSV-1) by the real-time Polymerase Chain Reaction (PCR) method. The HSV-1 detection 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. 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

**ISIN version**
- **SAMPLE**
  - positive sample contains microorganism DNA
- **DNA ISOLATION**
  - the extracted sample DNA is added into the Ready to use MasterMix after the isolation and the tube is inserted into the real-time device
- **PCR AMPLIFICATION**
  - both pathogen DNA and Internal Standard DNA are amplified from the same primer couple in the course of the PCR
- **EVALUATION**
  - exponential fluorescence growth in the FAM fluorophore is evident if the detected pathogen DNA is present in the sample

**ISEX version**
- **SAMPLE**
  - Internal Standard is added into the sample before the DNA isolation
- **DNA ISOLATION**
  - after the isolation the extracted sample DNA are added into the concurrently isolated Internal Standard DNA are added into the Ready to use MasterMix and the tube is inserted into the real-time device
- **PCR AMPLIFICATION**
  - both pathogen DNA and Internal Standard DNA are amplified from the same primer couple in the course of the PCR
- **QUALITY CONTROL FOR THE DIAGNOSTIC PROCESS**
  - exponential growth of the HEX fluorophore fluorescence, as a result of the IS amplification, controls the following:
    1. Inhibition and efficiency of the PCR amplification – ISIN version
    2. DNA extraction quality, inhibition and efficiency of the PCR amplification – ISEX version
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:

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 isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

<table>
<thead>
<tr>
<th>Elution volume</th>
<th>25 µl</th>
<th>50 µl</th>
<th>100 µl</th>
<th>200 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Standard</td>
<td>2.5 µl</td>
<td>5 µl</td>
<td>10 µl</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

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 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 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

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Data Collection</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold</td>
<td>37 °C</td>
<td>2 min</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>95 °C</td>
<td>10 min</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>PCR</td>
<td>95 °C</td>
<td>5 s</td>
<td>FAM+HEX</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>60 °C</td>
<td>40 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72 °C</td>
<td>20 s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

croBEE Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX ConnectTM / CFX96TM / Dx Real-Time PCR Detection System
LightCycler® 2.0 / 480

Required Detection Channels FAM, HEX

GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.
CLINICAL SAMPLE ANALYSIS EVALUATION

<table>
<thead>
<tr>
<th>Channel FAM</th>
<th>Channel HEX</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Valid</td>
<td>HSV-1 positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valid</td>
<td>HSV-1 positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valid</td>
<td>HSV-1 negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Invalid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Invalid</td>
<td></td>
</tr>
</tbody>
</table>

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:

\[
\text{cp/ml} = \frac{\text{SC} \times \text{EV}}{\text{IV}}
\]

- 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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