

GeneProof®

HSV/VZV

PCR Kit



in vitro Diagnostics

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

Method principles

This kit provides for the simultaneous detection of the *Herpes simplex virus (HSV)* and *Varicella-Zoster virus (VZV)* by the Polymerase Chain Reaction method (PCR). The VZV detection is based on the amplification of a specific conservative DNA sequence of a single-copy gene for the ORF62 (IE62 transactivator). Detection of both HSV types (HSV-1, HSV-2) is based on the PCR amplification of a specific conservative DNA sequence of a single-copy gene for glycoprotein B (gB). The procedure takes an advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. An internal standard is included in the reaction mix, controlling the possible inhibition of the PCR reaction. Sensitivity of the detection kit runs in single occurrences of each virus in a reaction. This provides high sensitivity for the laboratory detection in hundreds of viruses per ml in body fluid samples (liquor, serum) or blood. The kit is designed for in vitro diagnostics and provides qualitative detection.

Kit composition

	Cat. No. HV/E/ISIN/025 25 reactions	Cat. No. HV/E/ISIN/050 50 reactions	Cat. No. HV/E/ISIN/100 100 reactions
MasterMix <i>HV</i>	1 x 900 µl	2 x 900 µl	4 x 900 µl
Positive control <i>HV</i> 10 ² copies/µl	1 x 50 µl	1 x 50 µl	2 x 50 µl

Storage and transportation conditions

Transport the kits at temperatures ranging from -20 °C to -80 °C. The kit remains stable for at least 6 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix and the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 36 µl directly to PCR tubes and hold in stock at -20°C. Positive control may be held in stock at 4°C.

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

Isolation recommended by means of commercial DNA isolation kits according to the particular protocols of the isolation kit manufacturers. The manufacturer recommends the following isolation kits: PathogenFree DNA isolation kit (GeneProof); QIAamp DNA Blood Mini Kit (QIAGEN); NucleoSpin Blood (Macherey-Nagel).

PCR amplification

1. Add **36 µl of the MasterMix** and **4 µl of the DNA isolate** or **4 µl of the Positive control** into the PCR tube. The final reaction mix volume should be 40 µl.*
2. Insert the tubes into a thermocycler and amplify them according to the following recommended program:

Amplification program: **

UDG decontamination	37 °C/5 min.
initial denaturation	96 °C/15 min.
denaturation	96 °C/20 sec.
annealing	64°C/20 sec.
extension	72 °C/40 sec.
number of cycles	50
final extension	72 °C/2 min.

*Tubes should be maintained in cold environment when handled (at 0 – 15 °C)

**Hereby presented amplification conditions may differ for various types of thermocyclers. The above listed conditions apply for PTC 200 (MJ Research) thermocycler.

Agarose gel electrophoresis

The resulting amplification products should be separated on 2% agarose gel electrophoresis (5V/cm) containing Ethidium bromide (5 µg/1 ml) and visualized by means of UV illumination. Since the loading buffer is included in the reaction mix, there should be directly loaded at least 10 µl of the amplification product.

Detection evaluation

The resulting amplification products should be detected on 2% agarose gel electrophoresis containing Ethidium bromide (5 µg/1 ml) and visualized by means of UV illumination. Since the loading buffer is included in the reaction mix, there should be directly loaded at least 10 µl of the amplification product. In case of an **HSV positive sample there is detected an amplification product with the length of 341bp**, in case of the **VZV positivity a product with the length of 267bp**. Only amplification product of the internal standard with the length of 591 bp is detectable in the negative sample. Should inhibition of the reaction components occur or should the reaction fail to proceed, there would be detectable none of the above-listed amplification products (Fig. 1).

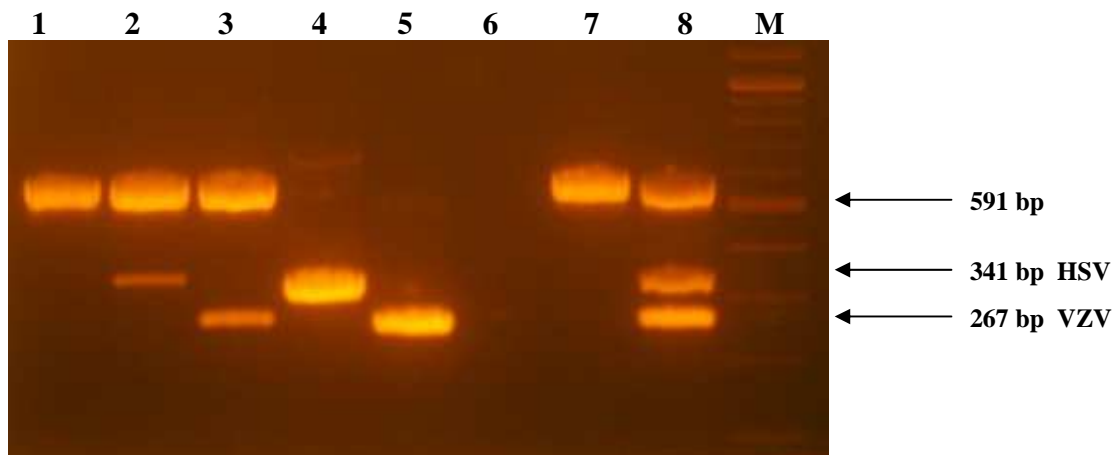


Fig. 1. Agarose electrophoresis result. In case of an **HSV positive sample there is detected an amplification product with the length of 341bp**, in case of the **VZV positivity a product with the length of 267bp**. Only amplification product of the internal standard with the length of 591 bp is detectable in the negative sample. Track No. 1: negative sample; track No. 2 and 4: HSV positive sample; track No. 3 and 5: VZV positive sample; track No. 6: reaction inhibition; track No. 7: negative control; track No. 8: positive control; M – 100 bp weight marker.

Warning:

- The kit is manufactured according to the European IVD Directive 98/79/EC.
- 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.
- Service fee assuring returnability and recycling of the waste packaging material has been paid under the identification number EK-F00041495.