

GeneProof®

Cytomegalovirus (CMV)

PCR Kit



in vitro Diagnostics

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

Method principles

CMV infection demonstration is based on the detection of a specific conservative DNA sequence at the length of 249 bp of a single-copy gene for the exon 4 IE antigen by the quantitative competitive Polymerase Chain Reaction method (PCR) with the subsequent amplification product detection by the agarose gel electrophoresis. The kit provides for a qualitative and semi-qualitative detection of the virus in clinical materials. 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 and enabling a semi-quantitative description of the CMV infection.

Kit composition

	Cat. No. CMV/E/ISIN/025 25 reactions	Cat. No. CMV/E/ISIN/050 reactions	Cat. No. CMV/E/ISIN/100 reactions
MasterMix CMV	1 x 900 µl	2 x 900 µl	4 x 900 µl
Positive control CMV 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, 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. For the purposes of the PCR detection it is necessary to sample from 50µl to 2ml of body fluid samples (**serum, plasma, amniotic fluid, cerebrospinal fluid, saliva, urine, tears**, etc.) or at least 1x1x1mm of tissue. Swab or scraping on a swab "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 CMV hepatitis suspicion it is suitable to test the liver biopsy; urine samples are tested in case of glomerulonephritis symptoms; in patients with viral interstitial pneumonia the virus is detected in the BAL. 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 No. IDNA050 (GeneProof); QIAamp DNA Blood Mini Kit No. 51104 (QIAGEN); NucleoSpin Blood No. 740 951.50 (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: **

initial denaturation	96 °C/15 min.
denaturation	96 °C/10 sec.
annealing	64 °C/10 sec.
extension	72 °C/20 sec.
number of cycles	50
final extension	72 °C/2 min.

*Tubes should be maintained in cold environment when handled

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

Agarose 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

Qualitative evaluation

Positive sample – is the detected CMV amplification product with the length of 249 bp, there may also be the detected amplification product of the internal standard (IS) with the length of 300 bp (Fig. 1).

Negative sample – only the amplification product of the internal standard with the length of 300 bp is detected.

Inhibiting sample – should inhibition of the reaction components occur or should the reaction fail to proceed, none of the above-listed amplification products would be detected and the examination should be repeated.

Semi-quantitative evaluation

10⁰ of virus particles / PCR reaction - CMV (249 bp) amplification signal intensity *is at the detection sensitivity threshold*

10¹ of virus particles / PCR reaction - CMV (249 bp) amplification signal intensity *is clearly lower than the IS signal (300bp)*

10² of virus particles / PCR reaction - CMV (249 bp) amp. signal intensity *is approximately equimolar to the IS signal (300bp)*

10³ of virus particles / PCR reaction - CMV (249 bp) amplification signal intensity *is clearly higher than the IS signal (300bp)*

10⁴ and more virus particles / PCR reaction – only the amplification product **CMV (249 bp)** could be detected **and it is impossible to detect the amplification product IS (300bp)**

Notes on the semi-quantitative evaluation interpretation:

- The semi-quantitative evaluation is of indicative value only and it takes an advantage of the **CMV (249bp)** and **Internal Standard (IS 300bp)** electrophoretic signal density ratio at low concentrations of the virus particles in a sample, almost independently from the reaction efficiency. Therefore it is possible to perform the quantification even if the isolates partially inhibit the PCR – this inhibition is to be distinguished by a relative decrease of the **Internal Standard** signal density in the inhibiting sample in comparison to the negative control.
- To achieve a more precise semi-quantitative evaluation of the positive sample by means of the quantitative competitive PCR (QCPCR) the manufacturer recommends to amplify, along with the sample, also a calibration serie prepared by a sequential diluting of the Positive Control (included in the kit).
- It is recommended to express the CMV infection quantitative description in the following terms: "number of virus particles / 10⁵ of peripheral blood leucocytes" or "number of virus particles / peripheral blood plasma (serum)". Described "symptomatically critical" virus concentrations exist for these clinical materials. For example, viraemia levels from 10³ of virus particles / 10⁵ peripheral blood leucocytes or 10³ virus particles / 50µl of peripheral blood plasma (serum) seem to be clinically significant for patients after organ transplantations. Other clinical materials should be rather examined qualitatively (positive / negative). The results should be clinically interpreted only in connection with the results of other laboratory examinations (serology) and taking into account the state of the patient.

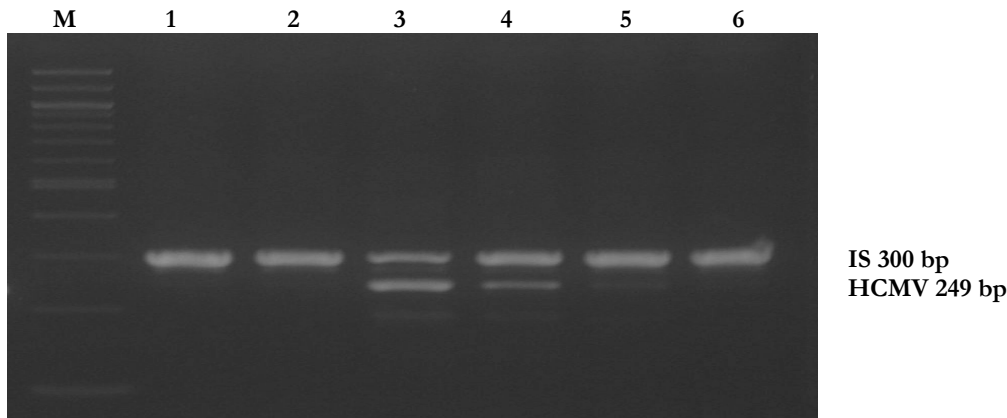


Fig. 1. Electrophoretical detection of amplification products on 2% agarose gel. 249 bp fragment results from the amplification of the single-copy gene for exon 4 IE of the CMV virus antigen, 300 bp fragment results from the amplification of the internal standard (IS). **Track No. 1, 2 and 6** – negative sample amplification result, **track No. 3** – amplification result of 10² copies of the CMV genome, **track No. 4** – amplification result of 10¹ copies, **track No. 5** – amplification result of 10⁰ copies of the CMV genome, **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.

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