

GeneProof®

Cytomegalovirus (CMV)

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		
	CMV/ISIN/025 25 reactions	CMV/ISIN/050 50 reactions	CMV/ISIN/100 100 reactions	CMV/ISEX/025 25 reactions	CMV/ISEX/050 50 reactions	CMV/ISEX/100 100 reactions
MasterMix CMV	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 CMV 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 CMV 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 CMV 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 CMV 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 CMV	-	-	-	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

Human cytomegalovirus infections are very frequent in human population and they are usually quite unapparent; yet there are cases when they are connected with many serious clinical syndromes. Primary infection usually occurs in childhood and it proceeds mostly asymptotically – yet it may also show in the form of the EBV-negative infectious mononucleosis (about 8% of inf. mononucleosis). Then the infection usually persists for the whole life in a latent form and it may reactivate in case of hypo-immunity. “Post-perfusion syndrome” belongs among the less known manifestations and it appears after large-scale blood transfers and after heart surgeries. Also short-term decreases in immunocompetence after large-scale surgeries and after ventral vein venous access may result in the symptomatic reactivation of the infection. Congenital CMV infections are quite frequent (up to 2% of live born children) and the degree of clinical manifestation depends on the infection dose. Active cytomegalovirus infection development occurs in up to 90% of AIDS patients – the infection is symptomatic in up to 40% of them and it affects virtually all body organs, including CNS, lungs and GIT. CMV infections are still a very serious complication in patients after transplantations and in oncology juvenile patients treated with cytostatics. Endogenous latent infection may reactivate in immunosuppressed patients or seronegative patients may get infected by the transplanted body organs of seropositive donors. The infection manifests many symptoms involving various body organs: leucopenia with high fevers, thrombocytopenia, hepatitis, pneumonia, glomerulonephritis, etc. Complications with secondary viral (HHV6, EBV), bacterial and fungal infections (yeasts, aspergilla, pneumocystis) frequently occur in patients with serious CMV infection manifestations. Correct and quick diagnosis is very important since cytomegalovirus infections may be rather successfully treated if diagnosed in time. Serological diagnostics is based mostly in the determination of specific antibodies of the IgM, IgG and IgA types. Interpretation for immunosuppressed patients is frequently complicated due to the persisting serum levels of the specific IgM. It is necessary to concurrently evaluate the results of the indirect serological diagnostics and direct diagnostics, in connection with the clinical state of the patient, to determine the diagnosis of the symptomatic cytomegalovirus infection. PCR methods provide for sensitive detection of even very small amounts of the virus and they are therefore well-suited for testing biopsy samples (transplanted body organ biopsies) and for testing leukocytic fractions of bone marrow transplant patients. CMV infection detection itself is of therapeutic significance in case of bone-marrow transplant patients, to detect first infection in juvenile oncology patients, to detect the CMV in the cerebrospinal fluid, etc. Quantitative PCR (qPCR) provides the opportunity to early warn about the danger of the “CMV disease” due to the quantitative measurement of the viremia level (virus amount establishment). qPCR methods make it possible to monitor both the disease development and the reaction to the treatment.

Princip metody

CMV infection demonstration is based on the detection of a specific conservative DNA sequence of a single-copy gene for the exon 4 IE antigen by the Polymerase Chain Reaction (PCR) method. CMV positive sample results in FAM fluorescence grooving. 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 CMV laboratory detection in body fluid 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 Cytomegalovirus (CMV) PCR Kit was validated:

Rotor-Gene™ 3000 (Corbett Life Science)
Rotor-Gene™ 6000 (Corbett Life Science)
7500 Real-Time PCR System (Applied Biosystems)
7300 Real-Time PCR System (Applied Biosystems)
LightCycler® 2.0 (Roche)
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, 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 (serum, plasma, amniotic fluid, cerebrospinal fluid, saliva, urine, tears, etc.); 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

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. CMV/ISIN/...)

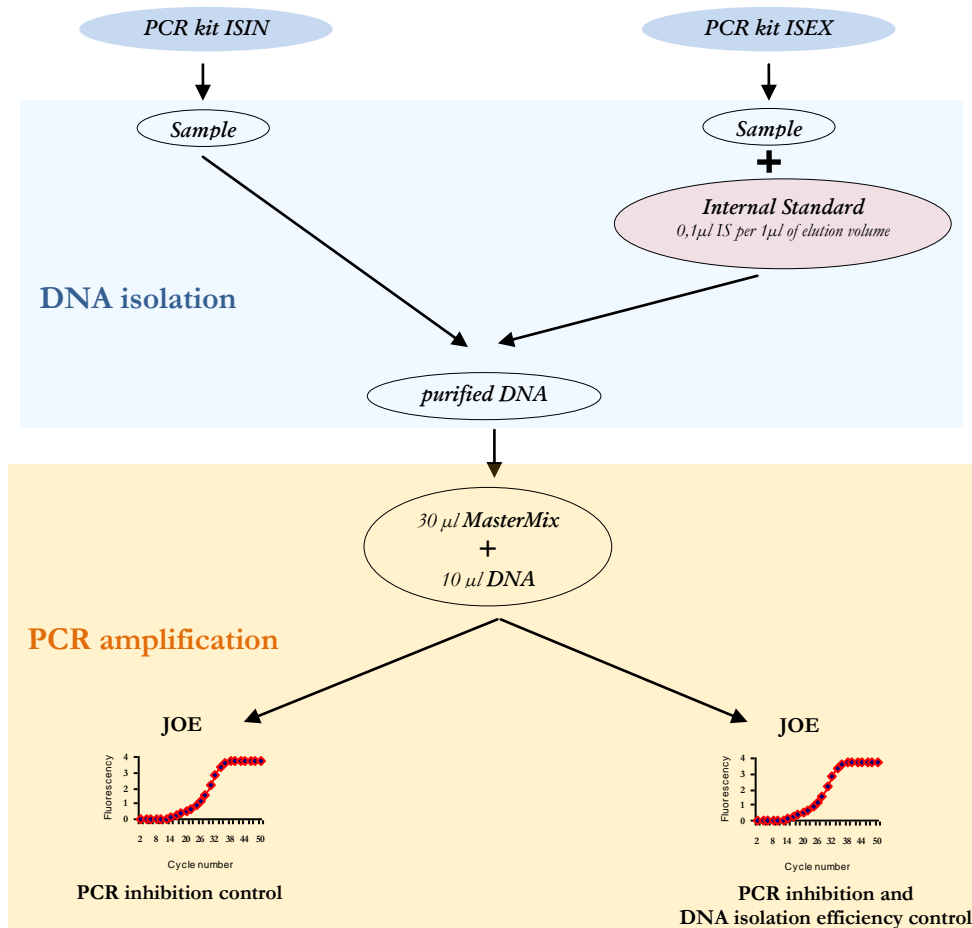
In this version of the PCR kit the Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

PCR kit ISEX (Cat. No. CMV/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



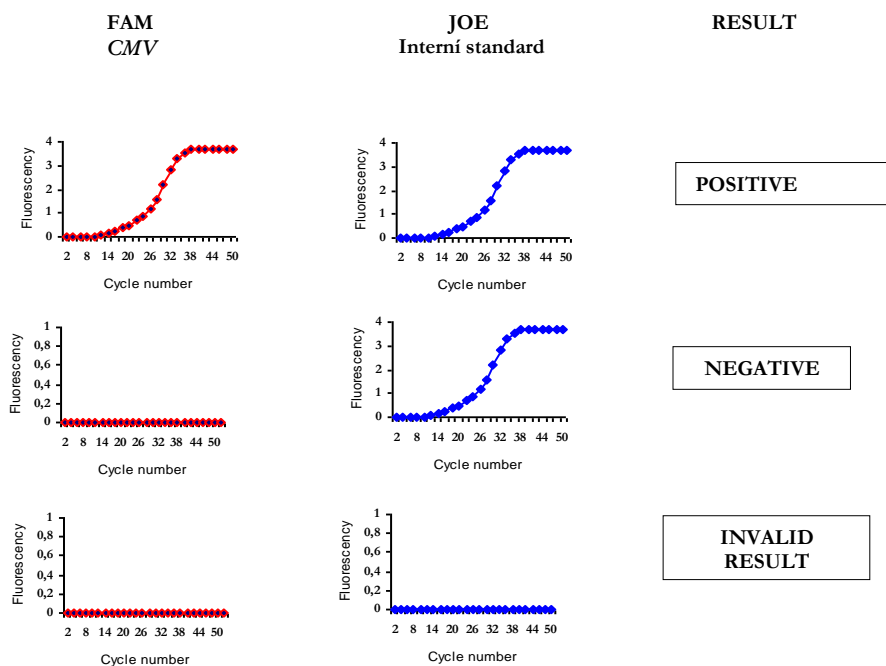
PCR amplification

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