

GeneProof Cytomegalovirus (CMV) PCR Kit



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

REF	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid isolation and PCR inhibition control		
	CMV/ISIN/025 25 rxn	CMV/ISIN/050 50 rxn	CMV/ISIN/100 100 rxn	CMV/ISEX/025 25 rxn	CMV/ISEX/050 50 rxn	CMV/ISEX/100 100 rxn
MasterMix CMV	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
CALIBRATOR CMV 10 ⁴ copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR CMV 10 ³ copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR CMV 10 ² copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR CMV 10 ¹ copy/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard CMV	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

TECHNICAL SPECIFICATION

Target sequence	DNA conservative region of a single-copy gene for exon 4 of the IE antigene
Specificity	human Cytomegalovirus (CMV)
Sensitivity (LOD)	reaches 0.907 copies/µl with the probability of 95 %
Accuracy of measurement	within the range of 10 ⁴ -10 ^{1.5} copies/µl the detection accuracy is 0.5 log
Linear range of measurement	10 ⁷ -0.907 copies/µl
Sample types	whole blood EDTA, plasma in EDTA, serum, cerebrospinal fluid, bone marrow, stool, urine
Reporting units	copy/ml (1 copy/ml = 1 IU/ml)
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panel

METHOD PRINCIPLES

The PCR kit is designed for detection of human Cytomegalovirus by the real-time Polymerase Chain Reaction (PCR) method. Detection is based on the amplification of a specific conservative DNA sequence of a single-copy gene encoding the 4 IE antigen and measuring the amplification product concentration using the PCR process and fluorescence labelled probes. CMV presence is indicated by FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction (ISIN version) and possibly 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 nonspecific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit performs very sensitive CMV detection in clinical material (EDTA whole blood, EDTA plasma, cerebrospinal fluid, bone marrow, stool, urine). The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detection.

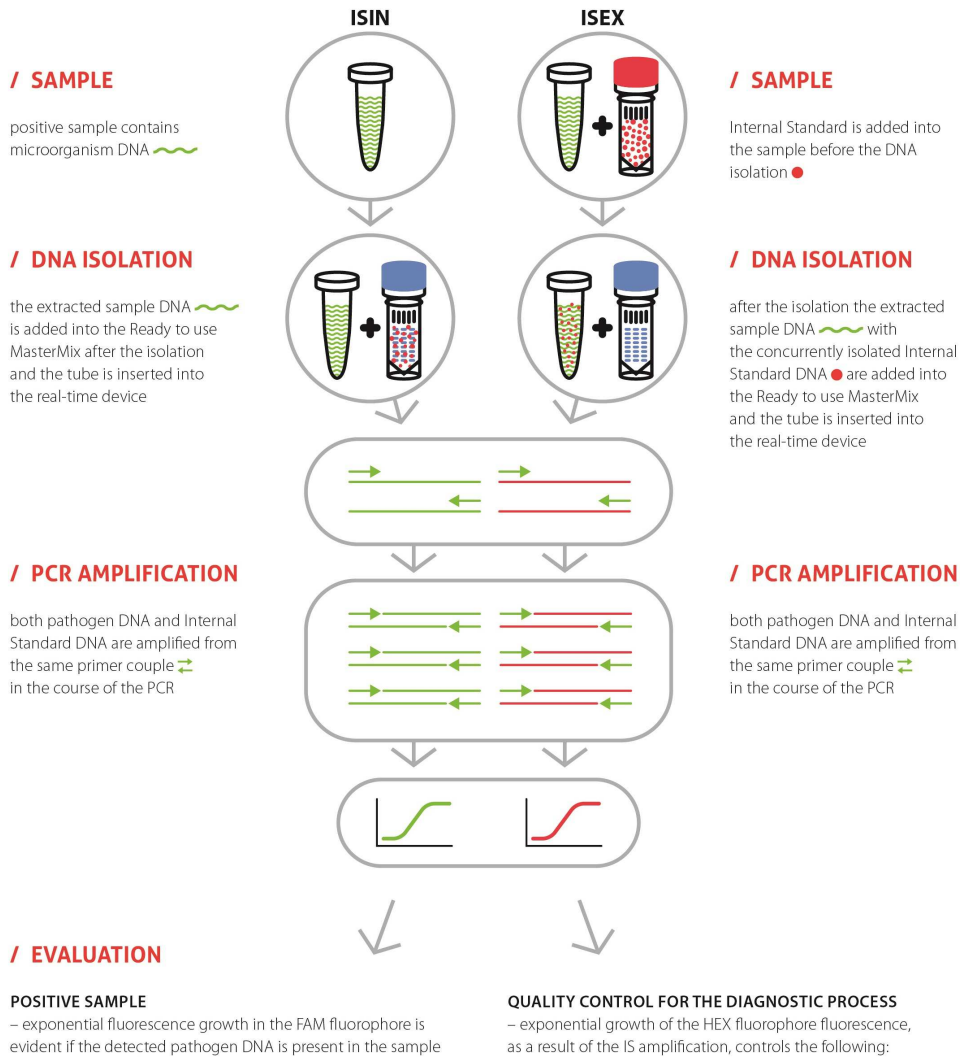
ISIN version

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 purification process efficiency control.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



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, 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. It is necessary to sample up to 2 ml of body fluid samples (plasma, serum, bone marrow, cerebrospinal fluid, urine, etc.); at least 1x1x1 mm of tissue; swab or scraping on a swab "dry". Blood sampling: a sample of incoagulable peripheral blood should be sampled into EDTA and transported into the laboratory at the temperature between +2 °C and +8 °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 keep all samples frozen at the temperature between -85 °C and -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleid 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:

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 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

Step	Temperature	Time	Data collection	Cycles
1. Hold	37 °C	2 min		1
2. Hold	95 °C	10 min		1
3. PCR	95 °C	5 s		45
	60 °C	40 s	FAM+HEX	
	72 °C	20 s		

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:

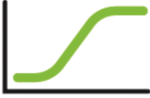
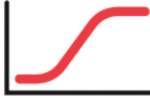
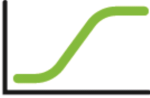


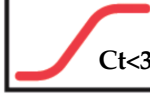




Applied Biosystems 7300/7500 Real-Time PCR System
AriaMx Real-Time PCR System
Dx/CFX96™/CFX Connect™ Real-Time PCR Detection System
LightCycler® 2.0, LightCycler® 480

LineGene 9600
Mx3000P/3005P QPCR System
Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q
SLAN® Real-Time PCR System

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



CLINICAL SAMPLE ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation
		Valid	CMV positive
		Valid	CMV positive
		Valid	CMV negative
		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 isolation:

$$\text{copy/ml} = \frac{\text{SC} \times \text{EV}}{\text{IV}}$$

SC - Sample Concentration (copy/ μ L)
 EV - Elution Volume (μ l)
 IV - Isolation Volume (ml)

$$\text{IU/ml} = \frac{\text{SC} \times \text{EV} \times 1}{\text{IV}}$$

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

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