GeneProof
Molecular diagnostics for your routine

GeneProof Cytomegalovirus (CMV) PCR Kit

CE 1023 IVD

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

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
REF	**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 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator CMV 10^3 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator CMV 10^2 cp/µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl	1x200 µl
Calibrator CMV 10^1 cp/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 kit 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). The components are stable for a maximum of 5 repeated freezing / thawing cycles after the first use of a particular vial. The component must be used before the expiry date or 14 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	specific conservative DNA sequence of a single copy gene encoding the 4 IE antigen
Analytical Specificity	Human Cytomegalovirus (CMV), 100 %
Analytical Sensitivity (LoD)	reaches up to 122.594 IU/ml (on CMV NIBSC 09/162, manual extraction GeneProof PathogenFree DNA Isolation Kit),
(with the probability of 95 %)	up to 165.237 IU/ml (on CMV NIBSC 09/162, automatic extraction croBEE NA16 Nucleic Acid Extraction System)
Diagnostic Specificity	90.67% ($CI_{95\%}$: 81.15% - 95.85%)
Diagnostic Sensitivity	92.86% ($CI_{95\%}$: 64.17% - 99.63%)
Linear Range	10^{10} - $10^{2.5}$ cp/ml with precision of ± 0.5 log (using manual extraction GeneProof PathogenFree DNA Isolation Kit or automatic extractor croBEE NA16 Nucleic Acid Extraction System)
Dynamic Range	10^{10} - 122.594 cp/ml (using manual extraction GeneProof PathogenFree DNA Isolation Kit) 10^{10} - 165.237 cp/ml (using automatic extractor croBEE NA16 Nucleic Acid Extraction System)
Validated Specimen	plasma, serum, urine*, whole blood
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels
Regulatory Status	CE_{1023} IVD

Quality management system is certified in compliance with the requirements of the standard ČSN EN ISO 13485 ed.2:2016

*validated only on manual extraction GeneProof PathogenFree DNA Isolation Kit

INTERFERENCES

The interferences testing was performed using negative plasma, serum and urine with set level of biochemical markers which can be potential endogenous interferences. The negative clinical specimens were spiked with CMV positive control at 3x LoD. Elevated levels of bilirubin (342 µmol/L), albumin (60 g/L), hemoglobin (2 g/L), urea (42.9 mmol/L), uric acid (1.4 mmol/L) and D-glucose (55 mmol/L) have been tested in the presence and absence of CMV DNA in plasma and serum samples. Varying levels of bilirubin, urea, uric acid, albumin, low pH, high pH level and D-glucose have been tested in the presence and absence of CMV DNA in urine samples. The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2, hospital recommendations and guidelines (http://www.southend.nhs.uk/media/180421/pf_biochemistry_reference_intervals.pdf) and recommendations of Czech Society of Clinical Biochemistry.

PLASMA

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Albumin	60 g/L	Partial	Hemoglobin	2 g/L	None
Bilirubin	342 µmol/L	Partial	Urea	42.9 mmol/L	None
Glucose	55 mmol/L	None	Uric acid	1.4 mmol/L	None

SERUM

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Albumin	60 g/L	Partial	Hemoglobin	2 g/L	None
Bilirubin	342 µmol/L	None	Urea	42.9 mmol/L	None
Glucose	55 mmol/L	None	Uric acid	1.4 mmol/L	None

URINE

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Albumin	5 %	None	pH	Acidic condition (pH 4)	Partial
Bilirubin	1 % (w/v)	None	pH	Basic condition (pH 9)	None
Glucose	0.1% (w/v); 1% (w/v)	Partial	Uric acid	5 mmol/L	None
Hemoglobin	2 g/L	None			

The tested endogenous interferences were shown not to interfere with GeneProof CMV assay with significant level.

METHOD PRINCIPLE

The PCR kit is designed for detection of human Cytomegalovirus (CMV) by the real-time Polymerase Chain Reaction (PCR) method. The CMV detection consists in amplification of a specific conservative DNA sequence of a single-copy gene encoding the 4 IE antigen and in measurement of fluorescence increase. The CMV 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 nonspecific 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.

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, urine, whole blood); 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 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:

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 Calibrator 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. The customer has to use his own negative control in the form of water, buffer or isolate of negative clinical material in each test. All 4 Calibrators have to be used for setting the standard curve for quantitative detection.

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 Calibrators or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

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

INSTRUMENTS

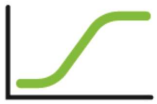
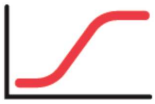
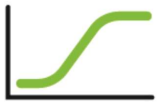
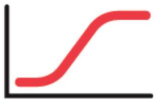
GeneProof Cytomegalovirus (CMV) PCR Kit is designed for use with real-time devices from various manufacturers:

croBEE Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 2.0 / 480
LineGene 9600 / 9600 Plus
Mic qPCR Cycler
QuantStudio™ 3/5 Real-Time PCR System
Rotor-Gene 3000 / 6000 / Q
SLAN® Real-Time PCR System
StepOne™/StepOne Plus™ Real-Time PCR System

Required channels: FAM, HEX

GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the 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**		

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:

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

SC - Sample concentration (cp/µl)
EV - Elution volume (µl)
IV - Isolation volume (ml)

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

You can use the calculator for pathogen concentration conversion at www.geneproof.com to make the calculation easier.

WARNING

A single valid Instruction For Use 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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