GeneProof
Molecular diagnostics for your routine

GeneProof Parvovirus B19 PCR Kit

CE 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

ISEX Version
IS supplied in a separate tube
Nucleic acid isolation and PCR inhibition control

REF	B19/ISEX/025 25 rxn	B19/ISEX/050 50 rxn	B19/ISEX/100 100 rxn
MasterMix B19	1x750 µl	2x750 µl	4x750 µl
Calibrator B19 10^4 IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator B19 10^3 IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator B19 10^2 IU/µl	1x200 µl	1x200 µl	1x200 µl
Calibrator B19 10^1 IU/µl	1x200 µl	1x200 µl	1x200 µl
Internal Standard B19	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 VP1 protein (capsid protein)
Analytical Specificity	Parvovirus B19, 100 %
Analytical Sensitivity (LoD)	reaches up to 90.29 IU/ml (on Parvovirus B19 NIBSC 12/208 using GeneProof PathogenFree DNA Isolation Kit)
(with the probability of 95 %)	reaches up to 156.7 IU/ml (on Parvovirus B19 NIBSC 12/208 using croBEE NA16 Nucleic Acid Extraction System)
Linear Range	10^8 - $10^{2.5}$ IU/ml with precision of ± 0.5 log (using GeneProof PathogenFree DNA Isolation Kit or croBEE NA16 Nucleic Acid Extraction System)
Dynamic Range	10^8 - 90.29 IU/ml (using manual extraction GeneProof PathogenFree DNA Isolation Kit) 10^8 - 156.7 IU/ml (using automatic extraction croBEE NA16 Nucleic Acid Extraction System)
Conversion Factor	1 IU = 1.6 cp
Validated Specimen	plasma, whole blood
External Quality Assessment	regularly tested by by QCMD and Instand e.V. External Quality Assessment Panels
Regulatory Status	CE IVD

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

INTERFERENCES

The interference testing was performed using negative plasma with set level of biochemical markers which can be potential endogenous interferences. The negative clinical specimens were spiked with Parvovirus B19 positive control at concentration 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), D-glucose (55 mmol/L) and citrate (190 g/L) have been tested in the presence and absence of Parvovirus B19 DNA in plasma samples. The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2 and guidelines 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	None	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
Citrate	190 g/L	None			

The tested endogenous interferences were shown not to interfere with GeneProof Parvovirus B19 PCR Kit with significant level.

METHOD PRINCIPLE

The PCR kit is intended for detection of the Parvovirus B19 DNA detection by the real-time Polymerase Chain Reaction (PCR) method. The method consists in the measurement of fluorophore labelled probes fluorescence increase. The Parvovirus B19 detection is based on the amplification of conservative DNA sequence of single-copy gene encoding VP1 protein (capsid protein). Parvovirus B19 presence is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is excluded, controlling also the DNA extraction process quality. IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR with amplification products. The kit is designed for in vitro diagnostics and provides qualitative and quantitative detection.

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.

SAMPLING AND SAMPLE STORAGE

Clinical material commonly used to detection Parvovirus B19 are whole blood and plasma. They should be transported according to the laboratory instructions. When isolating DNA from whole blood it is not suitable to freeze the samples; it is advisable to keep them between +2 and +8 °C (a sample from non-coagulating peripheral blood should be sampled into EDTA and transported to the laboratory within 24 hours).

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.

croBEE NA16 Nucleic Acid Extraction System
GeneProof PathogenFree DNA Isolation Kit

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 a 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		

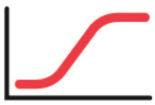
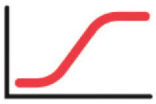
INSTRUMENTS

GeneProof Parvovirus B19 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
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 480
LineGene 9600 Plus
Mic qPCR Cycler
Rotor-Gene 3000 / 6000 / Q
SLAN® Real-Time PCR System

Required channels: FAM, HEX
GeneProof diagnostic kits are continually verified with various type 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	B19	positive
		Valid	B19	positive
		Valid	B19	negative
		Invalid		

QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in IU/ml while taking into account the volume of material entering the isolation:

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

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

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 does not 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
Tel.: +420543211679
Fax: +420516770824
email: support@geneproof.com

Orders
Tel.: +420543211679
Fax: +420516770824
email: sales@geneproof.com