

GeneProof Borrelia PCR Kit



1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof Borrelia PCR Kit	25 reactions	BOR/GP/025
GeneProof Borrelia PCR Kit	100 reactions	BOR/GP/100



2. INTENDED PURPOSE AND USE

Indication	<i>in vitro</i> diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics and aid to diagnosis
What is Detected / Target	Borrelia species from the <i>Borrelia burgdorferi sensu lato</i> group and <i>Borrelia miyamotoi</i> *
Automated / Manual detection	Manual
Type of Analysis	Qualitative
Validated Specimen	CSF, plasma, serum, tick, urine, whole blood (EDTA)
Testing Population	EU population
Intended User	For professional use in laboratories with trained staff
Test Principle	Real-time polymerase chain reaction (PCR) – amplification of the specific Target Sequence and detection using TaqMan probes with fluorophore-based detection

*NOTE: See Analytical Specificity in chapter 3. Technical Specification.

3. TECHNICAL SPECIFICATION

Target Sequence	Gene encoding 16S rRNA		
Analytical Specificity	<i>Borrelia burgdorferi sensu stricto</i> , <i>B. garinii</i> , <i>B. afzelii</i> , <i>B. andersonii</i> , <i>B. bissettii</i> , <i>B. valaisiana</i> , <i>B. lusitanae</i> , <i>B. japonica</i> , <i>B. tanukii</i> , <i>B. turdi</i> , <i>B. sinica</i> , <i>B. mayonii</i> , <i>B. spielmanii</i> , <i>B. bavariensis</i> , <i>B. kurtenbachii</i> , <i>B. miyamotoi</i> (Asian genotype), (BOR), 100 %		
Analytical Sensitivity (LoD with 95% probability)	0.568 cp/μl (performed on AmpliRun [®] <i>Borrelia burgdorferi</i> DNA control, Vircell)		
Diagnostic Specificity	98.15 % (CI _{95%} : 88.82 % - 99.90 %)		
Diagnostic Sensitivity	100.00 % (CI _{95%} : 96.93 % - 100.00 %)		
Positive Predictive Value	99.35 % (CI _{95%} : 95.86 % - 99.97 %)		
Negative Predictive Value	100.00 % (CI _{95%} : 91.58 % - 100.00 %)		
Extraction / Inhibition Control	PCR inhibition and DNA extraction efficiency control by Internal Control (IC)		
Validated Extraction Methods	croBEE 201A Nucleic Acid Extraction Kit GeneProof PathogenFree DNA Isolation Kit		
Applied Instruments	Instrument Name	BOR	Internal Control (IC)
	croBEE Real-Time PCR System	FAM	HEX
	AMPLIlib Real-Time PCR System	FAM	HEX
	Applied Biosystems 7300 / 7500 Real-Time PCR System	FAM	JOE
	AriaMx Real-Time PCR System	FAM	HEX
	BioQuant-96 Real-Time PCR System	FAM	HEX
	CFX Connect™ / CFX96™ / Dx Real-Time PCR Detection System	FAM	HEX
	Gentier 96E/96R Real-Time PCR System	FAM	HEX
	LightCycler [®] 2.0 / 480	FAM	HEX
	LineGene 9600 / 9600 Plus	FAM	HEX
	Mic qPCR Cyclor	FAM	HEX
	QuantStudio™ 3 / 5 Real-Time PCR System	FAM	VIC
	Rotor-Gene 3000 / 6000 / Q	FAM	HEX
	SLAN [®] Real-Time PCR System	FAM	HEX
StepOne™ / StepOne Plus™ Real-Time PCR System	FAM	HEX	
Detection Channels	FAM (BOR), HEX/JOE/VIC (IC)		
External Quality Assessment	Regularly tested in QCMD and INSTAND e.V. External Quality Assessment Panels - results at www.geneproof.com		

4. INTERFERENCES

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.

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
PLASMA					
Albumin	60 g/l	None	Haemoglobin	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
Caffeine	308 µmol/l	None	Prednisone	0.84 µmol/l	None
Ibuprofen	2425 µmol/l	None	Vancomycin	69 µmol/l	None
Fluconazole	245 µmol/l	None	Citrate	19 g/l	None
WHOLE BLOOD					
EDTA	2.2 g/l	None	Heparin	30 IU/ml	None
CSF					
Albumin	60 g/l	None	Lactic acid	16.5 mmol/l	None
Glucose	55 mmol/l	None	-	-	-
URINE					
Bilirubin	20 mg/dl	None	Urea	600 mmol/l	None
pH	pH 9	None	Glucose	55 mmol/l	None
Uric acid	1.4 mmol/l	None	Albumin	5 %	None

Summary: The tested interferences were shown not to interfere with the GeneProof PCR Kit.

5. KIT CONTENT

Reagent	Content	Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
					BOR/GP/025 – for 25 rxn	BOR/GP/100 – for 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	Master Mix <i>Borrelia</i>	Blue	750	1	4
Positive control	DNA oligonucleotide in buffer	Positive Control <i>Borrelia</i> 10 ^{^2} cp/µl	White	200	1	2
Internal control	Plasmid DNA in buffer	Internal Control <i>Borrelia</i>	Red	1000	1	2

6. CALBRATOR INFORMATION

No calibrators - qualitative detection only.

7. TRANSPORT AND STORAGE

Storage Conditions	(-20 ± 5) °C
Transport Conditions	-20 °C and below
In-use Stability	thaw a maximum of 5 times or use within 30 days after the first use of a particular vial, whichever comes first

8. ASSAY PROCEDURE

SPECIMEN COLLECTION, TRANSPORTATION AND HANDLING

- Samples for DNA extraction must be collected and transported following professional guidelines, preferably at (5 ± 3) °C.
- Samples for DNA extraction must be transported and processed by the laboratory in the shortest possible time (preferably within 24 hour).

NUCLEIC ACID PURIFICATION

- Prepare specimens for the assay according to the corresponding extraction kit manual.
- Thaw required amount of Internal Control (IC or UNIC*) vials, mix and briefly centrifuge.
- Add Internal Control (IC or UNIC) directly into the sample at the beginning of the extraction process so that 1 µl of the resulting elution volume contains 0.1 µl of the IC:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Control (IC or UNIC)	2.5 µl	5 µl	10 µl	20 µl

- Continue extraction according to the appropriate protocol.

NOTE:

In case of using *UNIC - GeneProof Universal Internal Control (more information in chapter 12. Additional Products), see Instruction for Use of GeneProof Universal Internal Control.

PCR SETUP PROTOCOL

7. Thaw required vials and reagents completely.
8. Gently vortex and briefly centrifuge all vials before setting up the PCR run.
NOTE:
Keep the reagents at (5 ± 3) °C for the shortest time possible until the PCR reaction is set up.
9. Add 30 µl of Master Mix into PCR tubes.
10. Add 10 µl of the extracted nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes and mix by pipetting. The total reaction mix volume is 40 µl.
11. Close the tubes, centrifuge shortly, insert them into the real-time PCR device and amplify according to the following PCR profile.
NOTE:
It is recommended to perform at least 1 negative control and at least 1 positive control (for a qualitative kit) for each individual PCR run. Use your own negative control (not provided) in the form of nuclease-free water. For more information see chapter 10. Run Validity.

AMPLIFICATION PROFILE

Follow the thermocycler manufacturer's manual for setting the instrument and for analysis.

Universal PCR Profile

NOTE: The Universal PCR Profile is designed for parallel detection with other GeneProof PCR Kits.

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination/ Reverse Transcription	42	15 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	FAM, HEX/JOE/VIC
	Annealing	60	40 s		
	Extension	72	20 s		

DNA PCR Profile

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination	37	2 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	FAM, HEX/JOE/VIC
	Annealing	60	40 s		
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (BOR)	Channel HEX/JOE/VIC (IC)	Result	Interpretation
+	+	Valid	<i>Borrelia</i> positive
+	-	Valid	<i>Borrelia</i> positive
-	+ (Ct < 38)	Valid	<i>Borrelia</i> negative
-	+ (Ct > 38)	Invalid	-
-	-	Invalid	-

NOTE: For interpretation of PCR run see chapter 10. Run Validity.

10. RUN VALIDITY

OVERALL VALIDITY OF DETECTION

	Signal	Channel	Run Validity	Recommendation
Positive Control	+	FAM	Valid	-
Positive Control	-	FAM	Invalid	Repeat PCR run
Negative control	-	FAM	Valid	-
Negative control	+	FAM	Invalid	Repeat PCR run

NOTE: If the issue persists, please contact Customer Support, see Contact information.

11. QUANTITATIVE DETECTION EVALUATION

Qualitative detection only.

12. ADDITIONAL PRODUCTS

GeneProof Universal Internal Control

GeneProof Universal Internal Control (UNIC) is intended to be used as the Internal Control for the microbiological GeneProof PCR kits and as an alternative product to the Internal Controls included in the GeneProof microbiological PCR kits. The UNIC works only in combination with GeneProof PCR kits. It is intended to simplify the user's workflow in cases where multiple detection kits with single extract are used. For more details see the Instruction for Use for UNIC.

Product	REF
GeneProof Universal Internal Control	UNIC/GP/050

NOTE: IC is applied to the solution only once. Add UNIC instead of IC from the package of the PCR kit. Do not add IC and UNIC to the same sample at the same time.

13. MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

CONSUMABLE MATERIAL

96-well reaction plates or PCR tubes (0.2 ml volume), pipette tips with filters, powder-free gloves, biohazard waste bin, nuclease-free water

DEVICES

Real-time PCR instrument (see chapter 3. Technical Specification), nucleic acid extraction system or kit (see chapter 3. Technical Specification), desktop centrifuge (for 0.2 ml PCR tubes or 96-well plates), vortex mixer, freezer (-20 ± 5 °C), refrigerator (5 ± 3 °C), cooling rack

14. WARNINGS, PRECAUTIONS AND PROCEDURE LIMITATIONS

- Patient management decisions should never be made based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
- Any serious incident occurred in relation to the using of GeneProof PCR Kit shall be reported to the manufacturer and to the competent local authority.
- Use separate working places for sample preparation / nucleic acid extraction and amplification reactions. Never introduce an amplified product in reagent and/or nucleic acid extraction (sample preparation) area.
- Dispose of unused reagents and waste in accordance with national, federal, state or local regulations.
- Use all necessary protective equipment (protective disposable gloves, a laboratory coat and eye protection) when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of the reagents when removing aliquots from reagent vials.
- Use RNase- and DNase-free filter pipette tips only.
- Use new tips for each pipetting step.
- Close the kit components' vials immediately after use and never interchange lids.
- Do not pool reagents from different lots or from different vials within the same lot.
- Do not substitute the reagents between different lots.
- Do not use reagents from damaged or leaking vials.
- 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! The manufacturer is not responsible for the kit impairment due to incorrect handling.










Procedure Limitations:

- Read the whole Instruction for Use properly before starting the manipulation. Not following these instructions can lead to an erroneous result which can cause misdiagnosis or inappropriate treatment!
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- Do not use kit after the expiry date.
- The presence of UNG decontamination step reduces the risk of lower levels of amplicon contamination. However, contamination from very high levels of amplicons can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Instruction for Use. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This product is designed for use with the applied PCR instruments and validated extraction methods mentioned in chapter 3. Technical Specification.
- Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories.

Clinical Limitations:

- Detection of pathogen's DNA is dependent on the number of bacteria present in the specimen and may be affected by specimen collection methods and patient factors.
- Use only with validated specimens (see chapter 2. Intended Purpose and Use) otherwise incorrect results could occur.

15. EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
	this product complies with the relevant EU requirements		Lot number
	<i>in vitro</i> diagnostic medical device		Contains sufficient amount for n-tests
	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read Instruction for Use		

Customer Support

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