INSTRUCTIONS FOR USE

GeneProof®
Molecular diagnostics for your routine

GeneProof™ Epstein-Barr Virus (EBV) PCR Kit

CE 2797 IVD

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof™ Epstein-Barr Virus (EBV) PCR Kit	25 reactions	EBV/GP/025
GeneProof™ Epstein-Barr Virus (EBV) PCR Kit	100 reactions	EBV/GP/100

2. INTENDED PURPOSE AND USE

The intended purpose of the device is related to the medical context and clinical conditions under which the device may be used.

Clinical Conditions	EBV is implicated in a number of diseases, including infectious mononucleosis, Burkitt's lymphoma, Hodgkin's lymphoma, stomach cancer, nasopharyngeal carcinoma, multiple sclerosis and lymphomatoid granulomatosis; direct evidence of EBV DNA (PCR) is used primarily in the diagnosis of lymphoproliferative diseases in immunodeficient patients, chronic active EBV infections, neurological complications associated with EB viral infection, and in monitoring response to treatment
Indication	*In vitro* diagnostic medical device
Regulatory Status	CE_{2797} IVD / Regulation (EU) 2017/746
Summary of Safety and Performance	See EUDAMED – European Database on Medical Devices at *ec.europa.eu*
Function	Diagnostics, aid to diagnosis and for monitoring
Specific Information	Testing of immunocompromised patients, patients with active EBV infection or neurological complications associated with EB viral infection
What is Detected / Target	Epstein-Barr Virus (EBV; Human herpesvirus 4)
Automated / Manual detection	Manual
Type of analysis	Qualitative and quantitative
Validated Specimen	DNA extracted from whole blood (EDTA)*, plasma, CSF, BAL
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: EBV persists latently in memory B-cell at low level. Therefore, even healthy individuals can carry measurable EBV loads in their peripheral blood.*

3. TECHNICAL SPECIFICATIONS

Target Sequence	EBNA1
Analytical Specificity	Epstein-Barr virus, 100 %
Analytical Sensitivity (LoD with 95% probability)	**Sample Processing** / **Whole blood** / **Plasma** / **CSF** / **BAL** GeneProof™ PathogenFree DNA Isolation Kit: 129.2 IU/ml / 196.1 IU/ml / 110.8 IU/ml / 206.0 IU/ml croBEE™ 201A Nucleic Acid Extraction Kit: 250.2 IU/ml / 289.5 IU/ml / 586.1 IU/ml / 138.1 IU/ml
Diagnostic Specificity	94.19 % ($CI_{95\%}$: 86.35 % – 97.84 %)
Diagnostic Sensitivity	99.10 % ($CI_{95\%}$: 96.45 % – 99.84 %)
Positive Predictive Value	97.79 % ($CI_{95\%}$: 94.63 % – 99.18 %)
Negative Predictive Value	97.59 % ($CI_{95\%}$: 90.76 % – 99.58 %)
Linear Range [IU/ml]	**Extraction method** / **Whole blood** / **Plasma** / **BAL** / **CSF** (with precision of ± 0.5 log / with precision of ± 0.6 log) GeneProof™ PathogenFree DNA Isolation Kit: 10^{10} – $10^{2.5}$ / 10^{10} – $10^{2.5}$ / 10^{10} – $10^{2.5}$ / 10^{10} – $10^{2.5}$ croBEE™ 201A Nucleic Acid Extraction Kit: 10^{10} – 10^{3} / 10^{10} – 10^{3} / 10^{10} – 10^{3}
Dynamic Range	10^{10} IU/ml – LoD (LoD varying according to the extraction method and material used)
Trueness (of expected concentration)	-0.07 log ($CI_{95\%}$: -0.13 – 0.00) using manual extraction GeneProof™ PathogenFree DNA Isolation Kit -0.08 log ($CI_{95\%}$: -0.13 – 0.03) using automatic extraction croBEE™ 201A Nucleic Acid Extraction Kit
Precision – Repeatability	• Intra-assay SD of log concentration = 0.084 ($CI_{95\%}$: 0.068 – 0.110)
Precision – Reproducibility	• Inter-assay SD of log concentration = 0.092 ($CI_{95\%}$: 0.059 – 0.202) • Inter-lot SD of log concentration = 0.112 ($CI_{95\%}$: 0.072 – 0.246) • Total SD of log concentration = 0.115 ($CI_{95\%}$: 0.074 – 0.254)
Reporting Units	IU/ml
Conversion Factor	1 IU = 1 cp
Metrological Traceability	EBV NIBSC 09/260 (1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques)
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**	**EBV**	**Internal Control (IC)**
	croBEE™ Real-Time PCR System	FAM	HEX
	AMPLilab 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
	CFX Opus Real-Time PCR System	FAM	HEX
	Gentier 96E/96R Real-Time PCR System	FAM	HEX
	LightCycler® 480	FAM	HEX
	LineGene 9600 / 9600 Plus	FAM	HEX
	Mic qPCR Cycler	FAM	HEX
	Montania 4896 Real-Time PCR thermocycler	FAM	HEX
	QuantStudio™ 3 / 5 Real-Time PCR System	FAM	VIC
	Rotor-Gene™ 3000 / Q	FAM	JOE
	SLAN® Real-Time PCR System	FAM	HEX
	StepOne™ / StepOne Plus™ Real-Time PCR System	FAM	JOE
Detection Channels	FAM (EBV), HEX/JOE/VIC (IC)		
External Quality Assessment	Regularly tested using QCMD and INSTAND e.V. External Quality Assessment Panels – results at *www.geneproof.com*		

4. INTERFERENCES

The evaluation and setting of pathological values for interference testing was performed according to CLSI guidelines EP7-A2, 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	Partial	Urea	42.9 mmol/l	Partial
Glucose	55 mmol/l	Partial	Uric acid	1.4 mmol/l	None
Caffeine	308 µmol/l	None	Fluconazole	245 µmol/l	None
Ibuprofen	2425 µmol/l	Partial	Prednisone	0.84 µmol/l	Partial
Citrate	190 g/l	None	Vancomycin	69 µmol/l	None
	19 g/l	None	Valganciclovir	20 mg/l	Partial
CSF					
Albumin	60 g/l	Partial	Lactic acid	16.5 mmol/l	Partial
Glucose	55 mmol/l	Partial		3.8 mmol/l	None
BAL					
Whole blood	2 % (v/v)	Partial	Mucin	60 µg/ml	None

NOTE: In case of partial interference, inhibition may occur with the risk of a false negative result at a given concentration of interferent.

5. KIT CONTENTS

Reagent	Content	Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
					EBV/GP/025 – 25 rxn	EBV/GP/100 – 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	MasterMix EBV	Blue	750	1	4
Calibrator	DNA oligonucleotide in buffer	Calibrator A EBV 10^4 IU/µl	Black	200	1	1
		Calibrator B EBV 10^3 IU/µl	Brown	200	1	1
		Calibrator C EBV 10^2 IU/µl	White	200	1	1
		Calibrator D EBV 10^1 IU/µl	Transparent	200	1	1
Internal control	Plasmid DNA in buffer	Internal Control EBV	Red	1000	1	2

DESCRIPTION OF REAGENTS AND LIMITATIONS

Mixtures in this product (Master Mix, Calibrators and Internal Control) are not classified as dangerous according to Regulation (EC) No 1272/2008.

6. CALIBRATOR INFORMATION

Use of all 4 calibrators is necessary for a correct sample quantification. An automatic quantification based on the analysis of calibrators is generated automatically as a part of analytical process performed in the PCR instrument. Each calibrator consists of target specific DNA. Each calibrator must be designated as „standard“ in the instrument (thermocycler). The concentration of each calibrator must be entered into the data analysis software during PCR plate setup.

NOTE: For qualitative detection, the Calibrator C 10^2 IU/µl serves as a positive control.

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.

Samples for DNA extraction must be transported and processed by the laboratory as soon as possible.

Whole blood specimens should be collected in EDTA-containing collection tubes and transported at room temperature within 24 hours. Whole blood specimens can be stored at 4 °C for up to 14 days from collection.

Plasma specimens should be collected in EDTA-containing collection tubes and transported at (5 ± 3) °C. Plasma specimens can be stored at (5 ± 3) °C for up to 7 days from blood collection.

CSF specimens should be transported at (5 ± 3) °C. If specimens cannot be processed immediately, CSF should be stored at -20 °C or at -70 °C or lower.

BAL specimens should be transported on ice and assayed within 24 hours from collection. If specimens cannot be assayed within 24 hours, BAL specimens should be stored at (5 ± 3) °C for up to 72 hours or frozen at -70 °C or lower.

NOTE:
For more information see Instructions for Use of the corresponding extraction kit.

NUCLEIC ACID PURIFICATION

1. Prepare specimens for the assay according to the corresponding extraction kit manual.
2. Thaw required number of Internal Control (IC or UNIC*) vials, mix and briefly centrifuge.
3. Add the Internal Control (IC or UNIC) directly to the sample at the beginning of the extraction process so that 1 µl of the final 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

4. Continue extraction according to the appropriate protocol.

   NOTE:
   *If using *UNIC = GeneProof™ Universal Internal Control (more information in chapter 12. Additional Products), see Instructions for Use of GeneProof™ Universal Internal Control.*

   *The samples of **DNA extracted from whole blood, plasma, CSF and BAL** can be stored for 7 days at (5 ± 3) °C, at (20 ± 5) °C or at (-20 ± 5) °C. The samples can be frozen and thawed 3 times after the extraction.*

PCR SETUP PROTOCOL

5. Thaw required vials and reagents completely.
6. Vortex gently and centrifuge briefly 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.
7. Add 30 µl of Master Mix into PCR tubes.
8. Add 10 µl of the extracted nucleic acid sample or 10 µl of Calibrator into each individual PCR tube and mix by pipetting. The total reaction mix volume is 40 µl.
9. 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 include at least 1 negative control and at least 1 complete set of calibrators (for a quantitative kit) in each individual PCR run. Use nuclease-free water as your own negative control (not provided). 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

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	
	Annealing	60	40 s		FAM, HEX/JOE/VIC
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (EBV)	Channel HEX/JOE/VIC (IC)	Result	Interpretation
+	+	Valid	EBV positive
+	-	Valid	EBV positive
-	+	Valid	EBV negative
-	-	Invalid	-

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

10. RUN VALIDITY

OVERALL VALIDITY OF DETECTION

	Signal	Channel	Run Validity	Recommendation
Calibrator C 10^2 (qualitative detection) or Calibrator set (quantitative detection)	+	FAM	Valid	-
Calibrator C 10^2 (qualitative detection) or Calibrator set (quantitative detection)	-	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

Use the following formula to calculate the viral load concentration in IU/ml for manual extraction (using GeneProof™ PathogenFree DNA Isolation Kit):

VLC – Viral load concentration [IU/ml]
SC – Sample concentration [IU/µl]
EV – Elution volume [µl]
IV – Isolation volume [µl]

$$VLC = \frac{\mathrm{SC\ x\ EV\ x\ 10^3}}{\mathrm{IV}}$$

To easily calculate pathogen concentrations, use the calculator at *www.geneproof.com*, where manual or automated nucleic acid extraction used is considered.

VALIDITY OF QUANTITATIVE DETECTION

Channel	Calibrators				Result	Recommendation
	A 10^4	B 10^3	C 10^2	D 10^1		
Target-specific channel (FAM)	++++	+++	++	+	Valid accurate quantification	-
Internal Control channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	≥0.98					
Target-specific channel (FAM)	++++	+++	++	+	Reduced quantification accuracy	Repeat PCR run
Internal Control channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	<0.98					
Target-specific channel (FAM)	No signal of one or more calibrators				Invalid quantification	Repeat PCR run
Internal Control channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	N/A					

R^2 – Determination coefficient – parameter evaluating the quality of standard curve
NOTE: If the issue persists, please contact Customer Support.

12. ADDITIONAL PRODUCTS

GeneProof™ Universal Internal Control

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

Product	REF
GeneProof™ Universal Internal Control	UNIC/GP/050

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

13. MATERIALS AND DEVICES REQUIRED BUT NOT PROVIDED

CONSUMABLE MATERIALS

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 Specifications), nucleic acid extraction system or kit (see chapter 3. Technical Specifications), 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 based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
- Any serious incident that occurred in relation to using of the 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 Calibrators or the clinical material; incorrect handling could result in contamination and the consequent defectiveness of the kit components! The manufacturer is not responsible for the kit defectiveness due to incorrect handling.

Procedure Limitations:

- Read the whole Instructions 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 the kit after its 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 these Instructions 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 Specifications.
- Proper homogenization of used clinical material is necessary for quantitative analysis of EBV DNA. Homogenization by a short vortex and short spin is recommended.

Clinical Limitations:

- The clinical performance characteristics have been evaluated for individuals who have undergone after allogeneic hematopoietic stem cell transplantation, have been diagnosed with EBV disease. No information is available on test performance in patients undergoing other types of transplant procedures.
- The clinical performance includes also immunodeficient patients with active EBV and in monitoring response to treatment.
- Though rare, mutations within the highly conserved regions of the viral genome covered by GeneProof™ Epstein-Barr Virus (EBV) PCR Kit primers and/or probes may result in the under-quantitation or failure to detect the virus.
- Detection of EBV DNA is dependent on the number of virus particles 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.
- A limitation of whole blood is the inability to discern how much EBV DNA is present in PBMCs (Peripheral Blood Mononuclear Cells) and how much is present in plasma. Because this distinction has clinical relevance, whole blood assays may be enhanced by the additional quantification of EBV DNA in plasma in situations where the copy number in whole blood is quite elevated.
- EBV DNA detected in CSF may reflect contamination by EBV-infected B lymphocytes or reactivation by co-pathogens rather than indicate a causal role of a CNS disease.

15. EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
CE	This product complies with the relevant EU requirements	LOT	Lot number
IVD	*In vitro* diagnostic medical device	Σ	Contains sufficient amount for n-tests
REF	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
www.geneproof.com/ifu	Read electronic Instructions for Use	UDI	Unique Device Identifier

16. REFERENCES

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17. MODIFICATIONS INTRODUCED IN THE LATEST VERSION

- Correction of the Negative predictive value in Chapter 3. Technical Specifications
- Clarification of the internal control channel for StepOne™ / StepOne Plus™ Real-Time PCR System
- Correction of the description of the Internal Control composition in Chapter 5. Kit Contents
- Addition of the trademark symbol (™) to the GeneProof and croBEE trademarks throughout the IFU.

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