INSTRUCTION FOR USE

GeneProof®
Molecular diagnostics for your routine

GeneProof Mycobacterium tuberculosis PCR Kit

CE IVD

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof Mycobacterium tuberculosis PCR Kit	25 reactions	MT/ISEX/025
GeneProof Mycobacterium tuberculosis PCR Kit	100 reactions	MT/ISEX/100

2. INTENDED PURPOSE AND USE

Indication	*in vitro* diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics and aid to diagnosis
What is Detected / Target	*Mycobacterium tuberculosis* complex
Automated / Manual detection	Manual
Type of Analysis	Qualitative
Validated Specimen	Sputum, BAL, swab, urine, CSF, plasma, stool*
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: Only in combination with QIAamp FAST Stool Mini Kit.*

3. TECHNICAL SPECIFICATION

Target Sequence	Multi-copy insertion sequence IS6110 and sequence for immunogenic protein MPB64
Analytical Specificity	*Mycobacterium tuberculosis* complex *(M. tuberculosis, M. africanum, M. bovis, M. microti, M. canettii, M. pinnipedi, M. caprae, M. orygis, M. mungi* and vaccination strain Bacillus Calmette–Guérin, BCG*)*, (MT) 100 %
Analytical Sensitivity (LoD with 95% probability)	0.09 cp/µl (performed on AmpliRun® *Mycobacterium tuberculosis* DNA control, Vircell)
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 myCROBE/croBEE 2.0 Universal Extraction Kit QIAamp Fast Stool Mini Kit
Applied Instruments	See table below
Detection Channels	FAM (MT), HEX/JOE/VIC (IC)
External Quality Assessment	Regularly tested in QCMD External Quality Assessment Panels - results at *www.geneproof.com*

Instrument Name	MT	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
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System	FAM	HEX
Gentier 96E/96R Real-Time PCR System	FAM	HEX
LightCycler® 480	FAM	HEX
LineGene 9600 Plus	FAM	HEX
Mic qPCR Cycler	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

4. INTERFERENCES

The evaluation and settings 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
SWAB					
Whole blood	2 % (v/v)	None	Oxymetazoline (Nasivin)	0.0625 mg/ml	None
Mucin	60 µg/ml	None	Xylometazoline (Olynth HA)	0.25 mg/ml	None
Sodium chloride (Quixx)	6.5 mg/ml	None	Phenylephrine (Vibrocil)	0.3 mg/ml	None
BAL					
Whole blood	2 % (v/v)	None	Mucin	60 µg/ml	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	
					MT/ISEX/025 – for 25 rxn	MT/ISEX/100 – for 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	Master Mix *Mycobacterium tuberculosis*	Blue	750	1	4
Positive Control	DNA oligonucleotide in buffer	Positive Control *Mycobacterium tuberculosis* 10^2 cp/µl	White	200	1	2
Internal Control	Plasmid DNA in buffer	Internal Control *Bordetella Chlamydia pneumoniae Mycobacterium tuberculosis Mycoplasma pneumoniae*	Red	1000	1	2

6. CALIBRATOR 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 9 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

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

NUCLEIC ACID PURIFICATION

3. Prepare specimens for the assay according to the corresponding extraction kit manual.
4. Thaw required amount of Internal Control (IC or UNIC*) vials, mix and briefly centrifuge.
   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.*
5. 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

6. Continue extraction according to the appropriate protocol.

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

9. INTERPRETATION OF RESULTS

Channel FAM (MT)	Channel HEX/JOE/VIC (IC)	Result	Interpretation
+	+	Valid	*M. tuberculosis* positive
+	-	Valid	*M. tuberculosis* positive
-	+	Valid	*M. tuberculosis* negative
-	-	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 Technical 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 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 in cases where multiple detection kits with a 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 the 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 amplicon contamination or by positive controls and/or clinical specimens 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.

Clinical Limitations:

- The GeneProof Mycobacterium tuberculosis PCR Kit cannot be used for differentiation of resistant strains of *M. tuberculosis*.
- Detection of pathogens' nucleic acid is dependent on the pathogen load 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
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
	Read Instruction for Use		

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