INSTRUCTION FOR USE

GeneProof®
Molecular diagnostics for your routine

CE IVD

GeneProof SARS-CoV-2 PCR Kit

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof SARS-CoV-2 PCR Kit	25 reactions	COV2/GP/025
GeneProof SARS-CoV-2 PCR Kit	100 reactions	COV2/GP/100

2. INTENDED PURPOSE AND USE

Indication	*in vitro* diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics of SARS-CoV-2 and aid to diagnosis of COVID-19
What is Detected / Target	SARS-CoV-2
Automated / Manual detection	Manual
Type of Analysis	Qualitative
Validated Specimen	Nasopharyngeal swab in transport media UTM (Copan), PBS or physiological saline solution Anterior nasal swab, saliva in Bi-CoV® Anterior nasal swab in nuclease-free water (NFW)
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

3. TECHNICAL SPECIFICATION

Target Sequence	*RdRp, E* and *N* genes
Analytical Specificity	SARS-CoV-2
Analytical Sensitivity (LoD with 95% probability)	

Sample Processing	Channel	Sensitivity	Material
GeneProof PathogenFree RNA Isolation Kit	FAM	381.28 IU/ml	PBS
croBEE 201A Nucleic Acid Extraction Kit		1600.41 IU/ml	PBS
Direct detection (Bi-CoV®)		2589.8 IU/ml	Anterior nasal swab in Bi-CoV®

Diagnostic Specificity	100.00 % ($CI_{95\%}$: 95.68 % - 100.00 %)
Diagnostic Sensitivity	100.00 % ($CI_{95\%}$: 91.58 % - 100.00 %)
Positive Predictive Value	100.00 % ($CI_{95\%}$: 91.58 % - 100.00 %)
Negative Predictive Value	100.00 % ($CI_{95\%}$: 95.68 % - 100.00 %)
Reporting Units	IU/ml
Metrological Traceability	1st WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)
Extraction/Inhibition Control	PCR inhibition and RNA extraction efficiency control by Internal Control (IC)
Validated Extraction Methods	croBEE 201A Nucleic Acid Extraction Kit GeneProof PathogenFree RNA Isolation Kit
Applied Instruments	

Instrument Name	*RdRp/E*	*N*	Internal Control (IC)
croBEE Real-Time PCR System	FAM	Cy5	HEX
AMPLilab Real-Time PCR System	FAM	Cy5	HEX
Applied Biosystems 7500 Real-Time PCR System	FAM	Cy5	JOE
AriaMx Real-Time PCR System	FAM	Cy5	HEX
BioQuant-96 Real-Time PCR System	FAM	Cy5	HEX
CFX96™/ Dx Real-Time PCR Detection System	FAM	Cy5	HEX
Gentier 96E/96R Real-Time PCR System	FAM	Cy5	HEX
LightCycler® 480 *	FAM	Cy5	HEX
LineGene 9600 Plus	FAM	Cy5	HEX
QuantStudio™ 5 Real-Time PCR System	FAM	Cy5	VIC
Rotor-Gene 3000 / Q	FAM	Cy5	HEX
SLAN® Real-Time PCR System	FAM	Cy5	HEX

Detection Channels	FAM (*RdRp/E*), Cy5 (*N),* HEX/JOE/VIC (IC)
External Quality Assessment	Regularly tested in QCMD and INSTAND e.V. External Quality Assessment Panels - results at *www.geneproof.com*

** NOTE: Verified only with the Universal PCR Profile.*

4. INTERFERENCES

The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2.

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
SWAB					
Whole blood	2 % (v/v)	Partial	Xylometazoline	0.25 mg/ml	Partial
Mucin	60 µg/ml	Partial	Phenylephrine	0.375 mg/ml	Partial
Sodium chloride	6.5 mg/ml	Partial	Neomycin, bacitracin	330 I.U. (neomycin) 25 I.U. (bacitracin)	Partial
Oxymetazoline	0.0625 mg/ml	Partial	Budesonide	100 µg	Partial

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

5. KIT CONTENT

Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
			COV2/GP/025 – 25 rxn	COV2/GP/100 – 100 rxn
Master Mix SARS CoV2	Blue	375	1	4
Positive Control SARS CoV2 10^4 cp/µl	White	200	1	2
Internal Control SARS CoV2	Red	250	1	4

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 3 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 must be collected and transported following professional guidelines, at temperature (2 – 8) °C.
- Samples must be transported and processed by the laboratory in the shortest possible time (preferably within 24 hours).

If a transport media is used (see chapter 2. Intended Purpose and Use), proceed to the NUCLEIC ACID PURIFICATION step.

DIRECT DETECTION (NFW)

- Add (1.5 – 3) ml of NFW to the plastic tube with a swab. The head of the swab needs to be fully submerged. Vortex the sample.
- Transfer 90 µl of the clinical sample into a new plastic tube.
- Add 10 µl of the Internal Control (IC).
- Incubate the new plastic tube in a dry heat block for 10 minutes at 90 °C (short spin on vortex after 5 minutes and continue with incubation).
- Centrifuge the sample for 1 minute at 11 000 g.
- Place the sample in a cooling rack for 10 minutes at (2 – 8) °C.
- Use the prepared sample (supernatant) directly for PCR detection.

NOTE:

Direct detection with NFW may perform lower sensitivity in comparison with the procedure requiring nucleic acid extraction.

Direct detection from a saliva sample may perform lower sensitivity in comparison with the direct detection from an anterior nasal swab due to the possibility of lower virus concentration present in the saliva sample.

DIRECT DETECTION (BI-COV®)

- Follow the Instruction for Use provided with the Bi-CoV® set.
- Continue the procedure directly to the PCR setup.

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.

  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.*
- 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.

PCR SETUP PROTOCOL

- Thaw required vials and reagents completely.
- Gently vortex and briefly centrifuge all vials before setting up the PCR run.
  NOTE:
  Keep the reagents at (2 – 8) °C for the shortest time possible until the PCR reaction is set up.
- Add 15 µl of Master Mix into PCR tubes.
- 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 25 µl.
- 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.

FAST PCR Profile

NOTE: FAST PCR Profile is designed for a specific single SARS-CoV-2 detection.

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination/ Reverse transcription	50	5 min	1 cycle	
2	Initial denaturation	95	2 min	1 cycle	
3	Denaturation	95	3 s	45 cycles	
	Annealing/Extension	60	30 s		FAM, Cy5, HEX/JOE/VIC

Universal PCR Profile

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

WARNING! For the Universal PCR Profile, the reaction volume must be set to 40 µl.

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, Cy5, HEX/JOE/VIC
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (*RdRp/E*)	Channel Cy5 (*N*)	Channel HEX/JOE/VIC (IC)	Result	Interpretation
+	+	+/-	Valid	SARS-CoV-2 positive
+	-	+/-	Valid	SARS-CoV-2 positive
-	+	+/-	Valid	SARS-CoV-2 positive*
-	-	+	Valid	SARS-CoV-2 negative
-	-	-	Invalid	-

**It is recommended to prove the results by new examination. 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, Cy5	Valid	-
Positive Control	-	FAM, Cy5	Invalid	Repeat PCR run
Negative control	-	FAM, Cy5	Valid	-
Negative control	+	FAM, Cy5	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, dry heat block, freezer (-20 ± 5) °C, refrigerator (5 ± 3) °C, cooling rack

14. WARNINGS, PRECAUSIONS AND 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!
- 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.
- 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.
- 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 kit after the expiry date.
- Do not use reagents from damaged or leaking vials.
- Dispose of unused reagents and waste in accordance with national, federal, state or local regulations.
- Use only with validated specimens (see chapter 2. Intended Purpose and Use) otherwise incorrect results could occur.
- 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.
- 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.
- This product is designed for use with the applied PCR instruments and validated extraction methods mentioned in chapter 3. Technical Specification.

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.

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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