

INSTRUCTION FOR USE



GeneProof SARS-CoV-2 PCR Kit

REF COV2/GP/100



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

#	REF	Volume	COV2/GP/100
1	MasterMix	375 µl	4
2	Positive Control	200 µl	2
3	Internal Control	250 µl	4

STORAGE AND TRANSPORTATION CONDITIONS

The kit must be transported at a temperature of -20 °C or below. The kit will remain stable 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 3 repeated freezing / thawing cycles or for a maximum of 14 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target sequence
Analytical specificity
Validated specimen
Detection channels
Sensitivity (* with the probability of 95 %)

RdRp, *N* and *E* genes
SARS-CoV-2, 100%, detection is based on the method recommended by WHO (Corman et al, 2020)
swab, transport medium, saliva
FAM, HEX, Cy5

Sample processing	Channel	Sensitivity	Material	Profile
GeneProof PathogenFree RNA Isolation Kit	FAM	75.49 cp/ml*	Qnostics SARS-CoV-2 Medium Q Control	COVID TOUCH
	Cy5	364.82 cp/ml*		
GeneProof Universal Nucleic Acid Extraction Kit	FAM	59.95 cp/ml*		
	Cy5	349.34 cp/ml*		
croBEE NA16 Nucleic Acid Extraction System	FAM	314.44 cp/ml*		
	Cy5	612.27 cp/ml*		
Direct detection (NUCLISWAB® COVID-19 VTM)	FAM	519 cp/ml	Traceability to Qnostics SARS-CoV-2 Medium Q Control	UNI PCR

Quality Control
Regulatory Status
tested in the Instand e.V. and QCMD External Quality Assessment Panels
CE IVD

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

METHOD PRINCIPLE

The PCR kit is intended for detection of SARS-CoV-2 using reverse transcription real-time Polymerase Chain Reaction (PCR) method. It detects specific sequences of the virus genome (*RdRp* gene, *E* gene and *N* gene) in a single reaction. The mechanism of multiplex targeting ensures high sensitivity of SARS-CoV-2 detection. The presence of *RdRp/E* is indicated by the increased fluorescence in FAM channel while the increased fluorescence in Cy5 channel confirms the presence of *N* gene. The kit includes an Internal Control (IC) which is used as a control for the whole diagnostic process, i.e. RNA extraction efficiency, reverse transcription step efficiency (transcription of RNA into cDNA) and PCR amplification efficiency (PCR inhibition). Positive amplification of IC is detected in fluorescence channel for HEX fluorophore. The PCR kit is designed for *in vitro* diagnostics and enables the qualitative detection. It utilizes the "hot start" technology that minimizes non-specific reactions and ensures maximum sensitivity. It is provided in a form of a Ready to Use MasterMix.

USER MANUAL

Sampling and sample storage

Swabs: Samples should be collected using sterile nylon swabs either as a dry swab or be immediately placed in a viral transport medium. Keep the samples at a temperature of 4 °C and process them before 72 hours from the collecting. For long term storage keep the samples at a temperature of -70 °C.

Nucleic acid purification

Nucleic acid extraction can be performed by extraction kits available on the market according to protocols for the particular clinical material extraction. The manufacturer recommends the following extraction kits:

Medium	Extraction Kit			Direct detection
	GeneProof PathogenFree RNA Isolation Kit	GeneProof Universal Nucleic Acid Extraction Kit	croBEE 201A Nucleic Acid Extraction Kit	
ESwab	YES	NO	NO	NO
UTM	YES	YES	YES	NO
eNAT	YES	YES	YES	NO
PBS	YES	YES	YES	NO
Nuclease-free Water	-	-	-	YES
NUCLISWAB® COVID-19 VTM	-	-	-	YES

Add IC directly to the sample at the beginning of the extraction process so that 1 µl of the resulting elution volume contains 0.1 µl of IC:

Elution volume	25 µl	50 µl	100 µl	200 µl
Internal control	2.5 µl	5 µl	10 µl	20 µl

Direct detection

Sample preparation procedure for direct detection without nucleic acid extraction:

- **10 µl of Internal Control** add to **90 µl of sample**.
NOTE: Put the IC into a cooling rack into the refrigerator, do not defrost more than 15 minutes. After the entire volume of the tube is completely thawed, gently vortex and briefly centrifuge the IC.
- Incubate clinical sample on **dry heat block 5 minutes at 90 °C**. **Vortex, short spin** and continue in incubation on **dry heat block 5 minutes at 90 °C**. Short vortex and spin.
- **Centrifuge** sample **1 minute at 11 000 g**.
- Put sample into the cooling rack and **cool to 4 °C**.
- Prepared sample (supernatant) is used directly for PCR detection.

INSTRUMENTS

This diagnostic kit is designed for use with real-time devices from various manufacturers:

croBEE Real-Time PCR System
Applied Biosystems 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 480
LineGene 9600 Plus
QuantStudio™ 3 / 5 Real-Time PCR System
Rotor-Gene 3000 / Q
SLAN® Real-Time PCR System

The Threshold must be set according to the valid manuals for the individual Real-Time PCR instrument.

GeneProof diagnostic kits are continually verified with various types of device. Current list is available at www.geneproof.com or request the list at support@geneproof.com.

PCR setup

- Gently vortex and briefly centrifuge the MasterMix and Positive Control tubes.
- Add 15 µl of the MasterMix into PCR tubes.
- Add 10 µl of the isolated nucleic acid sample or 10 µl of the Positive Control into individual PCR tubes and mix by pipetting several times. The total reaction mix volume will be 25 µl.
- Close the tubes, centrifuge them shortly, insert them into the device and let them amplify according to the following PCR profile. WARNING! For UNI PCR profile the reaction volume must be set to 40 µl.

- Plan your workflow. Put the Internal Control into a cooling rack and put the cooling rack with the Internal Control into the refrigerator. Always defrost the Internal Control between +2 and +8 °C and do not defrost the Internal Control more than 15 minutes.
- It is highly recommended that you take the rest of the kit components (MasterMix and Positive Control) out of the freezer 30 minutes before the extraction of your samples is completed, so that PCR preparation can begin immediately after the sample extraction (the less time the kit is handled at a higher temperature, the better).
- Take the Positive Control out of the box and let it defrost at laboratory temperature (between 15 °C and 25 °C). The speed of the Positive Control defrosting may vary depending on the actual laboratory temperature. At lower laboratory temperatures, you may need to take the Positive Control out of the box and let it defrost earlier than the MasterMix.
- Put the MasterMix into a cooling rack and put the cooling rack with the MasterMix into the refrigerator. Always defrost the MasterMix between +2 and +8 °C and do not defrost the MasterMix more than 30 minutes.
- Always use the kit components after the entire volume of the tube is completely thawed.
- Do not warm the MasterMix tubes by hand and do not leave them at laboratory temperatures. Always keep the MasterMix tubes in the properly cooled cooling rack while working with the kit.
- Place the extracted samples directly into the cooling rack and perform the PCR without delay in the shortest possible time. After use, store the remaining kit components at -20 ± 5 °C in the freezer immediately. The maximum proven time for keeping the kit components in the refrigerator between +2 and +8 °C is 4 hours altogether without repeated freezing or thawing. The maximum proven time for keeping the kit components at laboratory temperatures between 15 °C and 25 °C is 1 hour altogether without repeated freezing or thawing.
- The isolate of negative isolation control with Internal Control should be used in each test. PBS, physiological saline solution or buffer can be used as a negative isolation control (not included in the kit).
- 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.

Amplification profile

Please note, the COVID TOUCH PCR profile is intended for specific single SARS-CoV-2 detection. The UNI PCR profile is intended for SARS-CoV-2 detection in parallel detection with other GeneProof PCR kits.

Profile UNI PCR			
#	Temp.	T	N
PRE 1	42 °C	15 min	1x
PRE 2	95 °C	10 min	1x
PCR	95 °C	5 s	45x
	60 °C (DATA)**	40 s	
	72 °C	20 s	

Profile COVID TOUCH PCR*			
#	Temp.	T	N
PRE 1	42 °C	15 min	1x
PRE 2	95 °C	10 min	1x
PCR	95 °C	5 s	7x
	55 °C	40 s	
	72 °C	20 s	
PCR	95 °C	5 s	38x
	60 °C (DATA)**	40 s	
	72 °C	20 s	

*To obtain the actual Ct value it is necessary to add 7 more cycles.

**Data collection. Required channels: FAM, HEX, Cy5

CLINICAL SAMPLES ANALYSIS EVALUATION

FAM	Cy5	HEX	Result	Interpretation
RdRp/E	N gene	IC		
+	+	+/-	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

WARNING

The only valid Instruction for Use for a particular kit is included in the package or to be requested for a specific lot from the manufacturer. Use only the combination of components present in a particular PCR kit. 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 subjected to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions, please, contact our Customer Service.

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