



# Gene Bio COVID-19 Qualitative Real Time PCR Kit Ver. 1.0

For Detection of COVID-19 RNA (RdRp gene)  
from Body Fluid  
100 Tests



Product No.: 28511



100 rxns



Store at -25°C ... -15°C



Ver. 1



Gene Biosystems , Block 140, 3<sup>rd</sup>. Industrial Zone, 6<sup>th</sup>. Of October  
City, Egypt

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The GENE BIO COVID-19 Qualitative Real Time PCR Kit is a reagent system, based on real-time PCR technology, for the detection of t RNA.

## 1. Kit Components

Component	Master A-I	Master A-II	Master B	Internal Control	Positive Control
Number of Vials	1	1	1	1	1
Volume [µl/ Vial]	1250	250	100	1200	50

## 2. Storage

- The GENE BIO COVID-19 Qualitative Real Time PCR Kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt or if tubes have been compromised during shipment, contact Gene Biosystems for assistance.
- All components should be stored at -20°C upon arrival.
- Protect Master Mix (A-II) from light.

## 3. Product Description

The GENE BIO Kit for COVID-19 detection is designed for the in vitro quantification of COVID-19 genomes. The primers and probe are designed to have 100% homology with the 16 genome sequences available on the GISAID database as of 23 January 2020, some of which were subsequently available on NCBI. The primers and probe target the RdRp gene which has previously been used in the identification of the SARS coronavirus, however there is no cross reactivity with this or any other coronavirus sequenced thus far. The GENE BIO Kit for COVID-19 (2019-nCoV) genomes is designed for the in vitro quantification of COVID-19 genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at


the time of design. The dynamics of genetic variation means that new sequence information may become available after the initial design. GENE BIO periodically reviews the detection profiles of our kits and when required releases new versions. Probes specific for COVID-19 RNA are labelled with the fluorophore FAM. The probe specific for the target of the Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of COVID-19 specific RNA and Internal Control in the corresponding detector channels of the real-time PCR instrument.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to [info@gene-biosystems.com](mailto:info@gene-biosystems.com) and our bioinformatics team will answer your question.

The GENE BIO COVID-19 Qualitative Real Time PCR Kit can be used with the following real-time PCR instruments:

- m2000rt (Abbott Diagnostics)
- Mx 3005P™ QPCR System (Stratagene)
- VERSANT™ kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene™ 3000/6000 (Corbett Research)
- Rotor-Gene Q 5/6 plex Platform (QIAGEN)

#### NOTE

 ***Please ensure that instruments have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.***

## 4. Sample Preparation

Extracted RNA is the starting material for GENE BIO COVID-19 Qualitative Real Time PCR Kit. The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following nucleic acid extraction systems and kits are recommended:

- VERSANT™ Molecular System SP (Siemens)
- HighPure® Viral Nucleic Acid Kit (Roche)
- QIAamp® Viral RNA Mini Kit (QIAGEN)
- Gene Extract Viral RNA (Gene Biosystems)
- Gene Extract Viral Nucleic Acid Dx. (Gene Biosystems)

If using a spin column based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid, is highly recommended.

### NOTE

**⚠ *The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

- ⚠ *Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.***

## 5. Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The GENE BIO COVID-19 Qualitative Real Time PCR Kit contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

- If the IC is used as a RT-PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1 SAMPLE
Master A-I	12.5 µl
Master A-II	2.0 µl
Master B	1.0 µl
Internal Control	1.0 µl
Volume Master Mix	<b>16.5 ul</b>

If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added depends always and only on the elution volume. It represents 8-10% of the elution volume. For instance, if the nucleic acid is going to be eluted in

60 µl of elution buffer or water, 5 - 6 µl of IC per sample must be added to the specimen/lysis buffer mixture.

## NOTE

**⚠ Never add the Internal Control directly to the specimen!**

- If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions (rxns)	1 SAMPLE
Master A-I	12.5 µl
Master A-II	2.0 µl
Master B	1.0 µl
Volume Master Mix	15.5 µl

## 6. Reaction Setup

- Pipette 15 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the control (Positive or Negative Control).
- Thoroughly mix the samples and controls with the Master Mix by up and down pipetting.
- Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate lids.
- Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~3000 rpm).

Reaction Setup		
<b>Master Mix</b>		15 $\mu$ l
<b>Positive Control</b>		10 $\mu$ l
<b>Total Volume</b>		<b>25 <math>\mu</math>l</b>

## 7. Programming the Real-Time PCR Instruments

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the manual of the respective instrument. For detailed programming instructions regarding the use of the GENE BIO COVID-19 Qualitative Real Time PCR Kit. on specific real-time PCR instruments please contact our Technical Support.

### 7.1 Settings

- Define the following settings:

Settings		
<b>Reaction Volume</b>		25 $\mu$ l
<b>Ramp Rate</b>		Default
<b>Passive Reference</b>		ROX



## 7.2 Fluorescent Detectors (Dyes)

- Define the fluorescent detectors (dyes):

Detection	Detector Name	Reporter	Quencher
<b>COVID-19 specific RNA</b>	2019-NCOV	FAM	(None)
<b>Internal Control</b>	IC	HEX	(None)

## 7.3 Temperature Profile and Dye Acquisition

- Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
<b>Reverse Transcription</b>	Hold	1	-	45 °C	20:00 min
<b>Denaturation</b>	Hold	1	-	95 °C	10:00 min
<b>Amplification</b>	Quantification	50	-	95 °C	15 sec.
			√	60 °C	45 sec.
			-	72 °C	15 sec.

## 8. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument.

For detailed instructions regarding data analysis of the GENE BIO COVID-19 RT-PCR 1.0 on different real-time PCR instruments please contact our Technical Support.

## 9. Interpretation of Results

### 9.1 Qualitative Analysis

Sample ID	FAM Detection Channels	HEX Detection Channels	Result Interpretation
<b>A</b>	POSITIVE	POSITIVE*	COVID-19 specific RNA detected.
<b>B</b>	NEGATIVE	POSITIVE	COVID-19 specific RNA not detected. Sample does not contain detectable amounts of COVID-19 specific RNA.
<b>C</b>	NEGATIVE	NEGATIVE	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

### 9.2 Analytical Specificity

The analytical specificity of the GENE BIO COVID-19 Qualitative Real Time PCR Kit. is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligo- nucleotides were checked by sequence comparison analysis against public available sequences to ensure that all relevant COVID-19 genotypes will be detected.

Over a hundred different COVID-19 negative specimens were analyzed with the GENE BIO COVID-19 Qualitative Real Time PCR Kit. None of these showed a positive COVID-19 specific signal. But all showed a valid IC signal.

In addition, the specificity of the GENE BIO COVID-19 Qualitative Real Time PCR Kit. was evaluated by testing a panel of genomic DNA/RNA extracted from other herpesviruses or other pathogens significant in immunocompromised patients.

Table 6: Organisms tested to demonstrate the analytical specificity of the GENE BIO COVID-19 Qualitative Real Time PCR Kit.

Organisms	FAM Channel (2019-NCOV)	HEX Channel (Internal Control)
<b>Herpes Simplex Virus 1</b>	Negative	Valid
<b>Herpes Simplex Virus 2</b>	Negative	Valid
<b>Varicella-Zoster Virus</b>	Negative	Valid
<b>Epstein-Barr Virus</b>	Negative	Valid
<b>Human Herpesvirus 6A</b>	Negative	Valid
<b>Human Herpesvirus 6B</b>	Negative	Valid
<b>Human Herpesvirus 7</b>	Negative	Valid
<b>Human Herpesvirus 8</b>	Negative	Valid
<b>Parvovirus B19</b>	Negative	Valid
<b>BK Virus</b>	Negative	Valid
<b>JC Virus</b>	Negative	Valid
<b>Simian Virus 40</b>	Negative	Valid
<b>Hepatitis A Virus</b>	Negative	Valid
<b>Hepatitis B Virus</b>	Negative	Valid
<b>SARS coronavirus</b>	Negative	Valid
<b>MERS coronavirus</b>	Negative	Valid
<b>Coronavirus like virus</b>	Negative	Valid

## 10. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: [support@gene-biosystems.com](mailto:support@gene-biosystems.com)

## 11. Trademarks and Disclaimers

GENE BIO (Gene Biosystems - Egypt); Mx 3005P™ (Stratagene); ABI Prism® (Applied Biosystems); HighPure®, LightCycler® (Roche); Rotor-Gene™, QIAamp® (QIAGEN); VERSANT™ (Siemens).

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## 12. Explanation of Symbols



Product number



Batch code



Contains sufficient for “n” tests/reactions (rxns)



Temperature limitation



Version



Use until



Caution



Consult instructions for use



Manufacture

