



# **GS<sup>TM</sup> COVID-19 Real-Time PCR Kit**

**For Emergency Use Authorization Only**

Instructions for Use (IFU) *Issue 1.0*

For *In-vitro* Diagnostic (IVD) Use

Rx Only

# GS™ COVID-19 RT-PCR KIT

*In vitro* Real-Time PCR diagnostic test for Coronavirus COVID-19

## For Use with:

Sample Type	RNA Extraction	PCR Platform
nasopharyngeal/ oropharyngeal swabs	QIAamp DSP Viral RNA Mini Kit	Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument



96 and 384 tests

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Rx Only



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## Table of Contents

1. Intended Use .....	<u>4</u>
2. Summary and Explanation .....	<u>4</u>
3. Principles of the Procedure .....	<u>5</u>
4. Materials Provided .....	<u>6</u>
5. Summary of Preparation and Testing Process .....	<u>7</u>
6. Required Equipment and Consumables (Not Provided) .....	<u>8</u>
7. Real-Time PCR Instruments .....	<u>8</u>
8. Extraction Kits / Instruments .....	<u>9</u>
9. Facilities/Training Requirements.....	<u>9</u>
10. Warnings and Precautions.....	<u>9</u>
11. Reagent Storage, Handling and Stability Conditions .....	<u>11</u>
12. Specimen Collection, Handling and Storage .....	<u>11</u>
13. Reagent and Controls Preparation .....	<u>12</u>
14. General Preparation .....	<u>13</u>
15. Assay Set Up .....	<u>13</u>
16. Interpretation of Results .....	<u>17</u>
17. Limitations of The Procedure .....	<u>21</u>
18. Conditions of Authorization for the Laboratory .....	<u>21</u>
19. Performance Evaluation .....	<u>22</u>
20. Disposal .....	<u>24</u>
21. GenoSensor Quality Control .....	<u>24</u>
22. Verification Requirements for GS <sup>TM</sup> COVID-19 real-time PCR assay .....	<u>25</u>
23. Technical Support .....	<u>25</u>
24. Trademarks and Disclaimers .....	<u>25</u>
25. Explanation of Symbols .....	<u>26</u>

## 1. Intended Use

GS™ COVID-19 RT-PCR KIT is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs, nasal swabs and mid-turbinate swabs from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The GS™ COVID-19 RT-PCR KIT is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The GS™ COVID-19 RT-PCR KIT is only for use under the Food and Drug Administration's Emergency Use Authorization.

## 2. Summary and Explanation

The recent COVID-19 outbreak has a significant impact to healthcare and the economy in the U.S. and throughout the world. The secretary of U.S. Health and Human Services announced on January 31, 2020 that a Public Health Emergency Exists for the SARS-CoV-2. The GenoSensor COVID-19 assay kit uses rRT-PCR to detect SARS-CoV-2 viral RNA. The product includes an all-in-one RT-PCR master mix containing optimized oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) specifically targeting three genes, and control material used in rRT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in respiratory specimens. In this document, we have provided pertinent and timely information about the GenoSensor COVID-19 RT-PCR KIT.

### 3. Principles of the Procedure

The GS™ COVID-19 RT-PCR KIT is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The primer and probe set(s) are designed to detect RNA from the SARS-CoV-2 virus in nasopharyngeal/oropharyngeal swabs from patients suspected of COVID-19 by their healthcare provider.

SARS-CoV-2 is an RNA virus. Due to the nature of RNA susceptibility to degradation, three genes from different regions of the genome were selected. Theoretically this design should lower the false negative rate compared to assays incorporating one or two genes for detection. The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from the regions of the ORF1ab, E, and N genes. The panel is designed for the specific detection of the SARS-CoV-2 virus.

The GS™ COVID-19 RT-PCR KIT consists of a 6 RT-PCR all-in-one master mix tubes. Each tube contains all the RT-PCR required reagents, enzymes, single target primers and probes, for the 3 gene targets and 3 controls. This will not only significantly increase clinical testing throughput, but also reduce the possibility of handling errors and potential contamination compared to preparation procedures with separated reagents from multiple vendors and sources.

Assay processes: Isolated and purified RNA from nasopharyngeal/oropharyngeal swabs, nasal swabs or mid-turbinate swabs is reverse transcribed to cDNA and subsequently amplified using Applied Biosystems™ 7500 Fast Dx Real-Time PCR system with SDS version 1.4 software. In the process, the probe for each gene reaction mixture anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems 7500 Fast Dx System and analyzed with SDS version 1.4 software.

#### 4. Materials Provided

The GS™ COVID-19 RT-PCR KIT provides:

##### Kit components

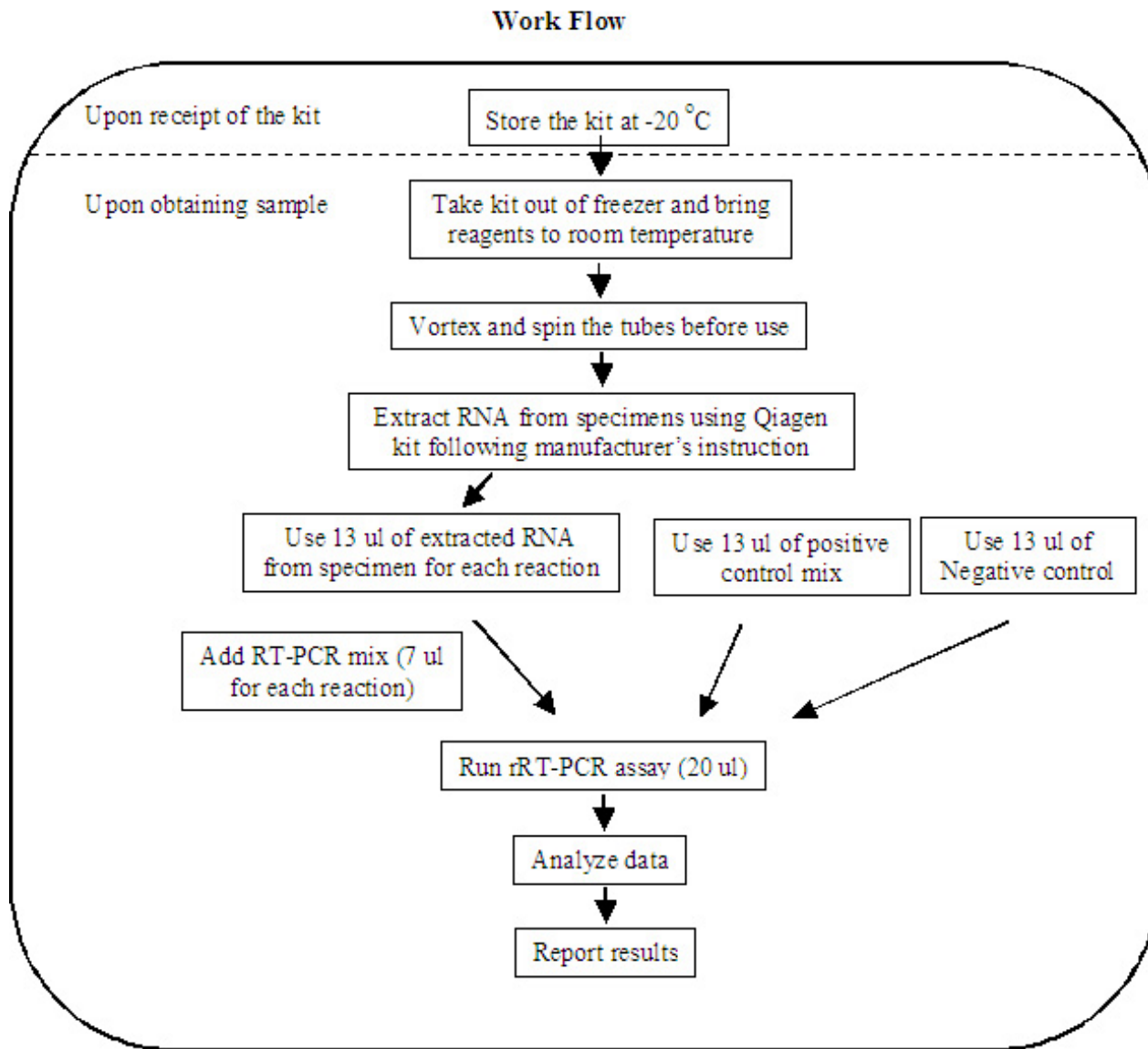
For 96 well plate (22 clinical samples)

Reagent Label	Description	Volume (µl)	Storage
ORF1ab RT-PCR mix	COVID-19 ORF1ab gene real time PCR assay mix	168	-20 °C
E gene RT-PCR mix	COVID-19 E gene real time PCR assay mix	168	
N gene RT-PCR mix	COVID-19 N gene real time PCR assay mix	168	
GUSB control RT-PCR mix	GUSB internal extraction control gene real time PCR assay mix	168	
Positive control mix	Positive control mix (ORF1ab, N, E and GUSB genes)	52	
Negative control	Water (DNase RNase free)	52	

For 384 well plate (94 clinical samples)

Reagent Label	Description	Volume (µl)	Storage
ORF1ab RT-PCR mix	COVID-19 ORF1ab gene real time PCR assay mix	672	-20 °C
E gene RT-PCR mix	COVID-19 E gene real time PCR assay mix	672	
N gene RT-PCR mix	COVID-19 N gene real time PCR assay mix	672	
GUSB control RT-PCR mix	GUSB internal extraction control gene real time PCR assay mix	672	
Positive control mix	Positive control mix (ORF1ab, N, E and GUSB genes)	52	
Negative control	Water (DNase RNase free)	52	

## 5. Summary of Preparation and Testing Process



## 6. Required Equipment and Consumables (Not Provided)

The GS™ COVID-19 RT-PCR KIT does NOT provide the following materials:

- RNA extraction kit (required): QIAamp DSP Viral RNA Mini Kit (Qiagen, Cat # 61904).

Equipment and Consumables Required (But Not Provided):

- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
- Multichannel micropipettes (5-50 µl)
- Racks for 1.5 mL microcentrifuge tubes
- 2 x 96-well -20°C cold blocks
- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS 1.4 software (Applied Biosystems; catalog #446985 or #4406984)
- Molecular grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNAZap™ (Ambion, cat. #AM9890) or equivalent
- RNase Away™ (Fisher Scientific; cat. #21-236-21) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR reaction plates (Applied Biosystems; catalog #4346906 or #4366932)  
MicroAmp Optical 8-cap Strips (Applied Biosystems; catalog #4323032)

## 7. Real-Time PCR instruments

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS 1.4 software (Applied Biosystems; catalog #446985 or #4406984)

*N.B. please ensure that all instruments used have been installed, calibrated and maintained according to the manufacturer's instruction and recommendations.*



## 8. Extraction Kits / Instruments

- RNA extraction kit (required): QIAamp DSP Viral RNA Mini Kit (Qiagen, Cat # 61904).

## 9. Facilities/Training Requirements

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures:

Refer to the Centers for Disease Control and Prevention (CDC) guidelines: Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2

<https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>

In addition, refer to the World Health Organization Interim guidance on laboratory biosafety: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: interim guidance, March 2nd, 2020

<https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117>

## 10. Warnings and Precautions

### 10.1. General

- For in vitro diagnostic use (IVD) only.
- For Emergency Use only.
- For prescription use only.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 <https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) biological safety cabinet. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited) gloves, eye protection and lab coats when handling kit reagents while performing this assay and handling materials including samples,

reagents, pipettes and other equipment and reagents.

- Please consult the material safety data sheet (MSDS) before using this kit, which is available on request.

## 10.2. Preventing Contamination

- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicons).
- The GS™ COVID-19 RT-PCR KIT positive control contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
  - Maintain separate areas for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
  - Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. microcentrifuge tubes, pipette tips) for handling of specimen preparation, pre-PCR assay setup, and post-PCR amplified nucleic acids.
  - Wear a clean lab coat and disposable gloves (not previously worn) when setting up assays.
  - Change gloves between samples and whenever contamination is suspected.
  - Keep reagent and reaction tubes capped or covered as much as possible.
  - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
  - Change aerosol barrier pipette tips between all manual liquid transfers.
  - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Good aseptic technique should always be used when working with nucleic acids.
  - When mixing reagents by pipetting up and down, this should be done with a volume roughly equal to 50% of the total component volume.
  - Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g. DNA/RNA remover, ethanol, 10% bleach) to minimize risk of nucleic acid contamination.
- RNA should be maintained on a cold block or on ice during preparation and used to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken.

- Dispose of unused kit reagents and human specimens according to local, state and federal regulations.

### 10.3. Prevent DNase/RNase contamination

- Use DNase/RNase free disposable plasticware and pipettes reserved for DNA/RNA work to prevent cross-contamination with DNases/RNases from shared equipment.
- Use DNase/RNase free filter tips throughout procedure to prevent aerosol and liquid contamination.

### 10.4. Specimen nucleic acid extraction kit/system

- Please consult the relevant Instruction For Use (IFU) and Materials Safety Data Sheet (MSDS), available from the manufacturer, before using the QIAamp DSP Viral RNA Mini Kit (Qiagen, Cat # 61904).

## 11. Reagent Storage, Handling and Stability Conditions

### 11.1. Storage conditions

- The GS<sup>TM</sup> COVID-19 RT-PCR KIT is shipped on dry ice and must be stored at -20 °C upon arrival.
- If the kit's protective packaging is damaged upon receipt, please contact GenoSensor for instructions. Attention should be paid to the "use by" date specified on the pack label and individual tube labels. On this date, the kit should be discarded following the disposal instructions in **Section 18**
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic primer/probe mix from light.

### 11.2. In Use Stability

- The GS<sup>TM</sup> COVID-19 RT-PCR KIT should be stored in the original packaging and is stable for up to 6 months stored at – 20 °C.
- The kit should not be used past the "use by" date as indicated on the kit box.
- When *in use* the kit components should be returned to the freezer promptly after use to minimize the time at room temperature.
- Minimize thawing and freezing cycles, not exceeding 3 times.

## 12. Specimen Collection, Handling and Storage

### 12.1. Collecting the Specimen

- Inadequate or inappropriate specimen collection, storage and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13 (Clinical and Laboratory Standards Institute) may be referenced as an appropriate resource.
- Refer to Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) <https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html>
- Follow specimen collection devices manufacturer instructions for proper collection methods.
- Swab specimens should be collected using swabs with a synthetic tip, such as nylon or Dracon® and with an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport medium.

## 12.2. Transporting Specimens

- Specimens must be package, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8 °C and ship overnight. If a specimen is frozen at -70 °C or lower, ship overnight on dry ice.

## 12.3. Storing Specimens

- Specimens can be stored at 2-8 °C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70 °C or lower.
- Extracted nucleic acid should be stored at -70 °C or lower.
- Refer to in **Section 7** for details regarding Specimen nucleic acid extraction kits/system.

## 13. Reagent and Controls Preparation

### 13.1. GS™ COVID-19 RT-PCR KIT preparation

- Reagents are ready for use.
- Thaw reagents at room temperature.
- Don't open lid of the RT-PCR reagent tubes until ready to use (avoid light exposure).
- Immediately store any remaining reagents at -20 °C (if the entire kit is not used).
- Refer Section 14 Assay Set Up

## 14. General Preparation

### 14.1. Equipment Preparation

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use.
- Decontamination agents should be used such as 5% bleach, 70% ethanol, and DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.
- Nucleic Acid extraction performance of the GS™ COVID-19 RT-PCR KIT is dependent upon the amount and quality of template RNA purified from human specimens. The commercially available QIAamp DSP Viral RNA Mini RNA extraction kit and procedure have been qualified and validated for recovery and purity of RNA for use with this assay. Follow manufacturer's instruction.

## 15. Assay Set Up

### 15.1. Sample Preparation Procedure

Refer to the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 <https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>

### 15.2. RNA extraction

The results of the GS™ COVID-19 Real-Time PCR assay is dependent upon the amount and quality of template RNA purified from human specimens.

- Follow the instruction of QIAamp DSP Viral RNA Mini Kit.

Only clinical samples are used for RNA extraction. Don't mix positive control mix or negative control with the clinical samples.

### 15.3. Master Mix Setup

The purified nucleic acid is reverse transcribed and amplified using the GS™ COVID-19 RT-PCR KIT.

Plate set-up configuration can vary with the number of specimens. For one batch experiment preparation, four positive control and four negative control reactions should be run along with clinical RNA samples to monitor if assay is working and if a possible contamination is present. This will be in an area for handling nucleic acid and away from the negative control and any clinical specimen/samples. This is to prevent plate set-up, reagent and specimen contamination with the positive samples.

- Thaw all reagents in the kit at room temperature.
- Mix well by briefly vortexing.
- Make a following real-time RT-PCR reaction mix.  
A total of 20  $\mu\text{L}$  reaction contains 7  $\mu\text{L}$  RT-PCR mix and 13  $\mu\text{L}$  purified clinical RNA sample.

	Purified clinical RNA (13 $\mu\text{L}$ )	Total volume
ORF1ab RT-PCR mix (7 $\mu\text{L}$ )	✓	20 $\mu\text{L}$
E gene RT-PCR mix (7 $\mu\text{L}$ )	✓	
N gene RT-PCR mix (7 $\mu\text{L}$ )	✓	
GUSB control RT-PCR (7 $\mu\text{L}$ )	✓	

Similarly, a total of 20  $\mu\text{L}$  reaction contains 7  $\mu\text{L}$  RT-PCR mix and 13  $\mu\text{L}$  positive control mix in each RT-PCR gene mix, and 13  $\mu\text{L}$  negative control for each gene mix.

	Pos control mix (13 $\mu\text{L}$ )	Neg control (13 $\mu\text{L}$ )
ORF1ab RT-PCR mix (7 $\mu\text{L}$ )	✓	✓
E gene RT-PCR mix (7 $\mu\text{L}$ )	✓	✓
N gene RT-PCR mix (7 $\mu\text{L}$ )	✓	✓
GUSB control RT-PCR mix (7 $\mu\text{L}$ )	✓	✓

Sample plate layout is shown below. Test can be performed on a 96-well plate or a 384-well plate. A 20 µL volume of reaction is loaded into each well. Below is a lay-out example on a 96-well plate.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Control
ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF Pos Control
E	E	E	E	E	E	E	E	E	E	E	E gene Pos Control
N	N	N	N	N	N	N	N	N	N	N	N gene Pos Control
Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	GUSB Pos Control
ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	ORF	Neg Control (ORF1ab)
E	E	E	E	E	E	E	E	E	E	E	Neg Control (E gene)
N	N	N	N	N	N	N	N	N	N	N	Neg Control (N gene)
Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Extraction control	Neg Control (GUSB ctrl)
Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	

When running a 96-well plate, 22 clinical samples can be tested simultaneously. Each sample will be tested on ORF1ab, E, N and GUSB genes. Four positive and Four negative control assays should also be tested at the same time.

	Positive controls (including ORF1ab, E, N and GUSB)	Negative control	# of clinical specimens per plate (three reactions per specimen)	Specimen Internal Extraction Control
96-well plate	4	4	22	22
384-well plate	4	4	94	94

- Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.
- Gently vortex nucleic acid sample tubes for approximately 5 seconds.
- Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.

## 15.4. Programming the Real-Time PCR Instrument

Please refer to Applied Biosystems™ 7500 Fast Dx Real-Time PCR system Relative Standard curve and comparative Ct Experiments (Applied Biosystems manual).

- Enter the following amplification program:

Temperature	Time	Cycle
25 °C	2 min	1
50 °C	15 min	1
95 °C	2 min	1
95 °C	15 sec	45
60 °C	30 sec	

Note: Instrument used includes ABI 7500 Fast Dx Real-Time PCR System. Thermocycler for PCR uses FAM as detection color dye, Rox as Passive Reference Dye.

- Record the Ct value
- Click “Analysis” button for analysis result. Result can be viewed in “Report”. All Ct values will be automated recorded and stored.



## 16. Interpretation of Results

All test controls (positive and negative) should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

### 16.1. GS™ COVID-19 RT-PCR KIT Controls – Positive, Negative and Internal

The table below is expected performance of controls included in the GS™ COVID-19 RT-PCR KIT.

Control Type	Control Name	Used to Monitor	RT-PCR mix				Expected Ct Values
			ORF1ab	E gene	N gene	GUSB Control	
Positive	Positive Control	Substantial reagent failure including primer and probe integrity	+	+	+	-	<40.00 Ct
Negative	Negative Control	Reagent and/or environmental contamination	-	-	-	-	None detected
RNA Extraction	External Control	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	-	+	<40.00 Ct

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

## 16.2. Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Control evaluation should use the following matrix to determine if assay is valid or not:

Evaluation on Controls		
Positive	Negative	Interpretation
+	-	Valid assay and read the samples
-	-	Invalid assay
+	+	Invalid assay
-	+	Invalid assay

If the assay is valid, the patient result should be read and interpreted using the table below. Two or more genes show positive, the result will be interpreted as **POSITIVE**; if one gene shows positive, the result is **INCONCLUSIVE** and testing should be repeated; if none of genes are positive, then the report will be **NOT DETECTED**.

The table below shows the examination and interpretation of patient specimen. It lists the expected results for the SARS-CoV-2 rRT-PCR and guidelines to interpret patient specimen. If results are obtained that do not follow these guidelines, re-extract and re-test the sample.

ORF1ab	E gene	N gene	GUSB	Result Interpretation	Report	Actions
+	+	+	±	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results to appropriate public health authorities and sender.
If two of the three targets are positive			±	SARS-CoV-2 detected	Positive SARS-CoV-2	Report results to appropriate public health authorities and sender.
If one of the three targets is positive			±	Inconclusive result	Inconclusive	Repeat extraction and rRT-PCR. If the repeated result remains inconclusive, collection of a new specimen is recommended.
-	-	-	+	SARS-CoV-2 not detected	Not detected	Report results to sender. Consider testing for other respiratory viruses.
-	-	-	-	Invalid result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

GUSB control is an RNA extraction control gene. All clinical samples should exhibit fluorescence growth curves in the GUSB reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human GUSB housekeeping gene. Failure to detect GUSB in any clinical specimens may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.

- Improper assay set up and execution.
- Reagent or equipment malfunction.

If the GUSB assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the ORF1ab, E gene and N gene of SARS-CoV-2 are positive even in the absence of a positive GUSB, the result should be considered valid. It is possible, that some samples may fail to exhibit GUSB growth curves due to low human cell numbers in the original clinical sample. A negative GUSB signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.
- If all SARS-CoV-2 markers AND GUSB are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

SARS-CoV-2 is represented by the ORF1ab, E gene and N gene.

- When all controls exhibit the expected performance, a specimen is considered negative (NOT DETECTED) if all three SARS-CoV-2 marker (ORF1ab, E and N gene) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the GUSB growth curve DOES cross the threshold line within 40.00 cycles (< 40.00 Ct).
- When all controls exhibit the expected performance, a specimen is considered presumptive positive for SARS-CoV-2 if all markers (ORF1ab, E and N gene) cycle threshold growth curve crosses the threshold line within 40.00 cycles (< 40.00 Ct). The GUSB may or may not be positive as described above, but the SARS-CoV-2 result is POSITIVE.
- When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (ORF1ab, E and N gene) (but not all three markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is INCONCLUSIVE. Repeat extraction and rRT-PCR.
- When all controls exhibit the expected performance and the cycle threshold growth curve for any two markers (ORF1ab, E and N gene) (but not all three markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is POSITIVE.

## 17. Limitations of The Procedure

- The GS™ COVID-19 RT-PCR KIT has been validated for use with oropharyngeal swab samples run on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR System and RNA extraction performed using the QIAamp DSP Viral RNA Mini Kit per manufacturer's instructions.
- Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the GS™ COVID-19 RT-PCR KIT but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic testing for SARS-CoV-2 for additional information
- The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results.
- Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- As with any molecular test, mutations within the target regions of the GS™ COVID-19 RT-PCR assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- False negative results may be caused by:
  - Unsuitable collection, handling and/or storage of samples.
  - Sample outside of viremic phase.
  - Failure to follow procedures in this handbook.
  - Use of unauthorized extraction kit or PCR platform.
- False positive results may be caused by:
  - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
  - Unsuitable handling of amplified product.
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.

## 18. Conditions of Authorization for the Laboratory

The GS™ COVID-19 RT-PCR KIT Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd>

However, to assist clinical laboratories using the GS™ COVID-19 RT-PCR KIT, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories<sup>1</sup> using GS™ COVID-19 RT-PCR KIT will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using GS™ COVID-19 RT-PCR KIT will use the product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive GS™ COVID-19 RT-PCR KIT will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using GS™ COVID-19 RT-PCR KIT will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of GS™ COVID-19 RT-PCR KIT and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: [CDRH-EUA-Reporting@fda.hhs.gov](mailto:CDRH-EUA-Reporting@fda.hhs.gov)) and GenoSensor (via email: [tech\\_service@genosensorcorp.com](mailto:tech_service@genosensorcorp.com)) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the product of which they become aware.

F. All laboratory personnel using GS™ COVID-19 RT-PCR KIT must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

G. GenoSensor, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

<sup>1</sup> The letter of authorization refers to, “laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests” as “authorized laboratories.”

## 19. Performance Evaluation

The GS™ COVID-19 RT-PCR assay performance evaluation has been generated on the Applied Biosystems® 7500 Fast Dx Real-Time PCR system for analytical sensitivity.

### 19.1. Analytical Sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of the SARS-CoV-2 virus at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples.

Analytical Sensitivity Results: This data demonstrates that the GS™ COVID-19 RT-PCR assay detects 1 copy/μL of SARS-CoV-2 whole viral genome RNA ≥95% of the time. This concentration therefore is the limit of detection of the assay.

Gene	LoD (copies/μl)	Positive calls/Total	% Replicate detection	Mean Ct	Standard Deviation (Ct)
ORF1ab	1	20/20	100	35.72	0.38
E gene	1	20/20	100	36.60	0.39
N gene	1	20/20	100	35.79	0.47

## 19.2. Analytical Specificity

### Analytical Reactivity (inclusivity)

BLASTn analysis queries of the SARS-CoV-2 rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search included GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excluded EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1 and 2 HTGS sequences and sequences longer than 100Mb. The search parameters automatically adjust for short input sequences and the expect threshold is 1000.

As of March 12, 2020, the analysis was mapped to 185 complete SARS-CoV-2 genomes. Primer and probes sequences for SARS-CoV-2 ORF1ab, E gene, and N gene assays had 100% homology to all SARS-CoV-2 isolates analyzed.

### Cross-reactivity (exclusivity)

Cross-reactivity of the GS™ COVID-19 RT-PCR KIT was evaluated using *in silico* analysis.

An *in silico* analysis for possible cross-reactions with all the organisms listed in the Table below was conducted by mapping primers and probes in the GS™ COVID-19 RT-PCR KIT test individually to the sequences. No potential unintended cross reactivity with other organisms is expected based on the *in silico* analysis.

Human coronavirus 229E	Rhinovirus/Enterovirus
Human coronavirus OC43	Parechovirus
Human coronavirus HKU1	<i>Candida albicans</i>
Human coronavirus NL63	<i>Corynebacterium diphtheriae</i>
SARS-coronavirus	<i>Legionella</i> (non-pneumophila)
MERS-coronavirus	<i>Bacillus anthracis</i> (Anthrax)
Adenovirus	<i>Moraxella catarrhalis</i>
Human Metapneumovirus (hMPV)	<i>Neisseria elongata</i> and <i>Neisseria meningitidis</i>
Parainfluenza 1	<i>Pseudomonas aeruginosa</i>
Parainfluenza 2	<i>Staphylococcus epidermidis</i>

Parainfluenza 3	<i>Streptococcus salivarius</i>
Parainfluenza 4	<i>Leptospira sp.</i>
Influenza A	<i>Chlamydophila pneumoniae</i>
Influenza B	<i>Chlamydophila psittaci</i>
Influenza C	<i>Coxiella burnetii</i> (Q-Fever)
Enterovirus	<i>Staphylococcus aureus</i>
Respiratory Syncytial Virus A	<i>Haemophilus influenzae</i>
Respiratory Syncytial Virus B	<i>Legionella pneumophila</i>
<i>Bordetella pertussis</i>	<i>Mycobacterium tuberculosis</i>
<i>Mycoplasma pneumoniae</i>	<i>Streptococcus pneumoniae</i>
<i>Pneumocystis jirovecii</i> (PJP)	<i>Streptococcus pyogenes</i>

The *in silico* analysis indicates that significant amplification of non-target sequences that result in cross-reactivity or potentially interfere with detection of SARS-CoV-2 is not likely to occur.

### 19.3. Clinical Performance Evaluation

Clinical evaluation of the GS™ COVID-19 RT-PCR assay was conducted with contrived nasopharyngeal/oropharyngeal swabs (32 positive and 32 negative). Thirty-two swabs were contrived with SARS-CoV-2 whole viral genomic RNA and tested blindly to generate the Positive Percentage Agreement (PPA) and Negative Percentage Agreement (NPA):

	SARS-CoV-2 concentration	Positive calls/Total	Agreement with expected Result
ORF1ab	1X LoD	10/10	100%
	3X LoD	10/10	100%
	10-10000x LoD	12/12	100%
E gene	1X LoD	10/10	100%
	3X LoD	10/10	100%
	10-10000x LoD	12/12	100%
N gene	1X LoD	10/10	100%
	3X LoD	10/10	100%
	10-10000x LoD	12/12	100%
Negative		0/32	100%

### 20. Disposal

Dispose of unused kit reagents, human specimens and sealed post-amplification plates according to local, state and federal regulations.



## 21. GenoSensor Quality Control

Strictly following GenoSensor Standard Operating Procedure, each batch of the GS™ COVID-19 RT-PCR KIT is tested against predetermined specifications to ensure consistent product quality.

GenoSensor performs weekly *in silico* analysis of all published SARS-CoV-2 genomes (GenBank+EMBL+DDBJ+PDB+RefSeq) to identify if the virus mutates in the COVID-19 primer and probe target region.

## 22. Verification Requirements for GS™ COVID-19 RT-PCR KIT

### 22.1. SARS-CoV-2 RNA Verification Process

Purified SARS-Cov-2 viral RNA listed below is an example of a verification material. Laboratories can use other available appropriate materials to verify the GS™ COVID-19 RT-PCR KIT assay.

- SARS-CoV-2 viral RNA (SeraCare, Cat # 0505-0126) was used for this process. RNA with 10-fold serial dilutions (e.g.  $10^3$ ,  $10^2$ ,  $10^1$ ,  $10^0$  RNA copies/reaction, and 5 replicates each) was spiked into tested samples. Human Hela cells and viral transport medium were used to mimic clinical tested samples.
- Set up assays and prepare reaction mixes according to **Section 14.3**.
- Prepare a reaction plate according to **Section 14.3** and program your chosen PCR platform according to **Section 14.4**.
- Negative control is free from amplification in the FAM channel
- All SARS-Cov-2 RNA reference material tested concentrations should produce amplification through the FAM channel.

## 23. Technical Support

For Technical support, please contact our dedicated technical support team:

Email: [tech\\_service@genosensorcorp.com](mailto:tech_service@genosensorcorp.com)







Phone: 1-480-598-5378

## 24. Trademarks and Disclaimers

Trademarks: GS™ and the GenoSensor logo.

All other trademarks that appear in this IFU are the property of their respective owners.

## 25. Explanation of Symbols

<b>Rx</b>	Prescription Use Only
<b>IVD</b>	In vitro diagnostics
	Manufacturer
<b>REF</b>	Catalogue number
	Suffices for
	Use by Date
	Temperature limit
	Consult Electronic Instructions for Use
<b>LOT</b>	Batch Code
	Keep away from sunlight (primer/probe mix)
<b>CONTROL+</b>	Positive Control