

# Gnomegen

## COVID-19 RT-qPCR Detection Kit

### Instructions for Use

For Emergency Use Authorization Only

Catalog # CV0303  
500 reactions

For In-Vitro Diagnostic (IVD)  
Rx Only



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**IVD** RxOnly

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### **Intended Use**

The Gnomegen COVID-19 RT-qPCR Detection Kit is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in nasopharyngeal and oropharyngeal swab specimens collected 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. SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swab 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. The agent detected may not be the definite cause of disease. 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 Gnomegen COVID-19 RT-qPCR Detection 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 Gnomegen COVID-19 RT-qPCR Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

### **Summary and Explanation**

An outbreak of pneumonia caused by a novel coronavirus (SARS-CoV-2) in Wuhan City, Hubei Province, China was identified and reported to the WHO on December 31, 2019. The rapid spread of SARS-CoV-2 to numerous areas throughout the world necessitates preparedness and response in healthcare and lab facilities. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies, and surveillance studies.

The Gnomegen COVID-19 RT-qPCR Detection Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in rRT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

### **Principles of the Procedure**

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected

from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software. In the process, the probe 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

### Materials Required (Provided)

#### Primers and Probes:

Reagent Label	Part #	Description	Volume / Tube	Reactions / Tube
COVID-19 N1 Assay	CV03030001	An assay containing SARS-CoV-2 nucleocapsid (N gene) N1 primers and FAM labelled N1 probes.	800 µL	500
COVID-19 N2 Assay	CV03030002	An assay containing SARS-CoV-2 nucleocapsid (N gene) N2 primers and FAM labelled N2 probes.	800 µL	500
RP Assay	CV03030003	An assay containing RNase P (RP) primers and a VIC labelled reference RP probe.	800 µL	500
COVID-19 Positive Control	CV03030004	A combination of cDNA plasmids encoding the SARS-CoV-2 N gene and RP target. The COVID-19 Positive Control will yield <b>positive</b> results for both the viral SARS-CoV-2 (N1 and N2) targets and the RP.	85 µL	15

### Materials Required (But Not Provided)

#### Human Specimen Control (HSC)

- Negative human specimen material: Laboratories may prepare a volume of human specimen material (e.g., human sera or pooled leftover negative respiratory specimens) to extract and run alongside clinical samples as an extraction control. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.
- Contrived human specimen material: Laboratories may prepare contrived human specimen materials by suspending any human cell line (e.g., A549, Hela or 293) in PBS. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.

#### rRT-PCR Enzyme Master Mix Options

Reagent	Quantity	Catalog No.
TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher)	1000 reactions	A15299
	2000 reactions	A15300

#### RNA Extraction Kit and Microconcentrators

Manufacturer	Extraction Kit	Catalog No.
Qiagen	QIAamp® DSP Viral RN Mini Kit	50 reactions: 61904

Manufacturer	Microconcentrator Equipment Options	Catalog No.
Filtron	Microsep 100	OD100C40
Millipore	Ultrafree®-CL	UFC4THK 25

### QPCR Instrument

Instrument Manufacturer	Description	Catalog No.
Applied Biosystems: 7500 Fast Dx Real-Time PCR Systems with SDS 1.4 software	The Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with SDS Software is a real-time nucleic acid amplification and five-color fluorescence detection system available for <i>in vitro</i> diagnostic use.	#4406985 or #4406984

### 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
- 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)

### Warnings and Precautions

- For *in vitro* diagnostic use (IVD).
- For Emergency Use Authorization only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- 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 2019-nCoV <https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>.

- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Performance characteristics have been determined with nasopharyngeal and oropharyngeal swab specimens. Testing other types of specimens may cause inaccurate results.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) 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.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications 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 (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
  - Maintain separate areas for assay setup and handling of nucleic acids.
  - 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. Proper aseptic technique should always be used when working with nucleic acids.
  - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
  - Wear a clean lab coat and powder-free 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.
  - Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
  - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, “DNAZap™” or “RNase AWAY®” to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

### Reagent Storage, Handling, and Stability

- Store the assay and controls at -20°C.
- Store liquid HSC control materials at ≤ -20°C.

- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.
- Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

### Specimen Collection, Handling, and Storage

Adequate, appropriate specimen collection, storage, and transport are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results. CLSI MM13-A may be referenced as an appropriate resource.

- Sample acceptance criteria
  - Samples should be collected into sterile, labeled tubes, and shipped at 2°C to 8°C on frozen gel packs.
- Specimen rejection criteria
  - Samples that have not been pre-approved for testing and those that are labeled improperly will not be tested until the required information is obtained.
- Collecting the Specimen
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) <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 only swabs with a synthetic tip, such as nylon or Dacron® and 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 media.
- Transporting Specimens
  - Specimens must be packaged, 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 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to CDC on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to CDC on dry ice.
- 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.

### Reagent and Controls Preparation

#### Human Specimen Control (HSC) (not provided)

- 1) One of the acceptable Human Specimen Controls (HSC) must be extracted and processed with each specimen extraction run. Please refer to section above,

**Materials Required but not provided: Human Specimen Control (HSC)** on how to prepare the Human Specimen Control.

**No Template Control (NTC) (not provided)**

- 1) Sterile, nuclease-free water
- 2) Aliquot in small volumes
- 3) Used to check for contamination during specimen extraction and/or plate set-up

**General Preparation**

**Equipment Preparation**

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 70% ethanol, and *DNAzap*<sup>™</sup> or *RNase AWAY*<sup>®</sup> to minimize the risk of nucleic acid contamination.

**Nucleic Acid Extraction**

All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross contamination of samples.

Separate work areas should be used for:

- Nucleic acid extraction
- Reagent preparation (e.g., preparation of RT-PCR master mix; NO amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition area)
- Nucleic acid addition
- Instrumentation (e.g., thermocyclers)

**General Handling:** Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed. During the procedure, work quickly and keep everything on cold blocks when possible to avoid degradation of RNA by endogenous or residual RNases. Clean working surfaces, pipettes, etc. with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach. Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.

Performance of the Gnomegen COVID-19 RT-qPCR Detection Kit is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel:

- Qiagen QIAamp<sup>®</sup> DSP Viral RNA Mini Kit: Concentrate patient samples to a final volume of 140 µL following manufacturer's instructions. Utilize 140 µL of concentrated sample for extraction and elute with 60 µL of buffer.

- Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or at -70°C if stored longer than 4 hours.

\*Manufacturer’s recommended procedures (except as noted in recommendations above) are to be followed for sample extraction. No template control (NTC) and HSC must be included in each extraction batch.

### Assay Set Up

#### Reaction Master Mix and Plate Set Up

Note: Plate set-up configuration can vary with the number of specimens and workday organization. NTCs, HSCs and COVID-19 Positive Control must be included in each run.

- 1) In the reagent set-up room clean hood, place rRT-PCR buffer, enzyme, and primer/probes on ice or cold-block. Keep cold during preparation and use.
- 2) Thaw 4X Reaction Mix prior to use.
- 3) Mix buffer, enzyme, and primer/probes by inversion 5 times.
- 4) Centrifuge buffer and primers/probes for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
- 5) Label one 1.5 mL microcentrifuge tube for each primer/probe set.
- 6) Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the NTC, COVID-19 Positive Control, HSC and RP reactions and for pipetting error. Use the following guide to determine N:
  - If number of samples (n) including controls equals 1 through 14, then  $N = n + 1$
  - If number of samples (n) including controls is 15 or greater, then  $N = n + 2$
- 7) For each primer/probe set, calculate the amount of each reagent to be added for each reaction mixture ( $N = \#$  of reactions).

**Table 1: One-step RTqPCR Reaction**

Reagent	Amount (µL) 1 Reaction	Amount (µL) N Reactions
Molecular Grade Nuclease Free Water	8.5	$N \times 8.5$
Combined Primer/Probe Mix	1.5	$N \times 1.5$
TaqPath™ 1-Step RT-qPCR Master Mix (4X)	5.0	$N \times 5.0$
Total	15.0	$N \times 15.0$

- 8) Dispense reagents into each respective labeled 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down. **Do not vortex.**
- 9) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
- 10) Set up reaction strip tubes or plates in a 96-well cooler rack.
- 11) Dispense 15 µL of each master mix into the appropriate wells going across the row as shown below (**Figure 1**):

**Figure 1: Example of Reaction Master Mix Plate Set-Up**

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1											
B	N2											
C	RP											
D												
E												
F												
G												
H												

- 12) Prior to moving to the nucleic acid handling area, prepare the No Template Control (NTC) reactions for column #1 in the assay preparation area.
- 13) Pipette 5  $\mu$ L of nuclease-free water into the NTC sample wells (**Figure 2**, column 1). Securely cap NTC wells before proceeding.
- 14) Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.

### **Nucleic Acid Template Addition**

- 1) Gently vortex nucleic acid sample tubes for approximately 5 seconds.
- 2) Centrifuge for 5 seconds to collect contents at the bottom of the tube.
- 3) After centrifugation, place extracted nucleic acid sample tubes in the cold rack.
- 4) Samples should be added to columns 2-11 (column 1 and 12 are for controls) to the specific assay that is being tested as illustrated in **Figure 2**. Carefully pipette 5.0  $\mu$ L of the first sample into all the wells labeled for that sample (i.e. Sample "S1" down column #2). *Keep other sample wells covered during addition. Change tips after each addition.*
- 5) Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.
- 6) Change gloves often and when necessary to avoid contamination.
- 7) Repeat steps #4 and #5 for the remaining samples.
- 8) Add 5  $\mu$ L of Human Specimen Control (HSC) extracted sample to the HSC wells (**Figure 2**, column 11). Securely cap wells after addition.
- 9) Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

### **Assay Control Addition**

- 1) Pipette 5  $\mu$ L of COVID-19 Positive Control to the sample wells of column 12 (**Figure 2**). Securely cap wells after addition of the control RNA.

**NOTE: If using 8-tube strips, label the TAB of each strip to indicate sample position. DO NOT LABEL THE TOPS OF THE REACTION TUBES!**

- 2) Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack. **NOTE:** *If using 96-well plates, centrifuge plates for 30 seconds at 500 x g, 4°C.*

**Figure 2. Gnomegen COVID-19 RT-qPCR Detection Kit Example of Sample and Control Set-up**

	1	2	3	4	5	6	7	8	9	10	11 <sup>a</sup>	12
A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	HSC	PC
B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	HSC	PC
C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	HSC	PC
D												
E												
F												
G												
H												

NTC: No template control

HSC: Human Specimen Control

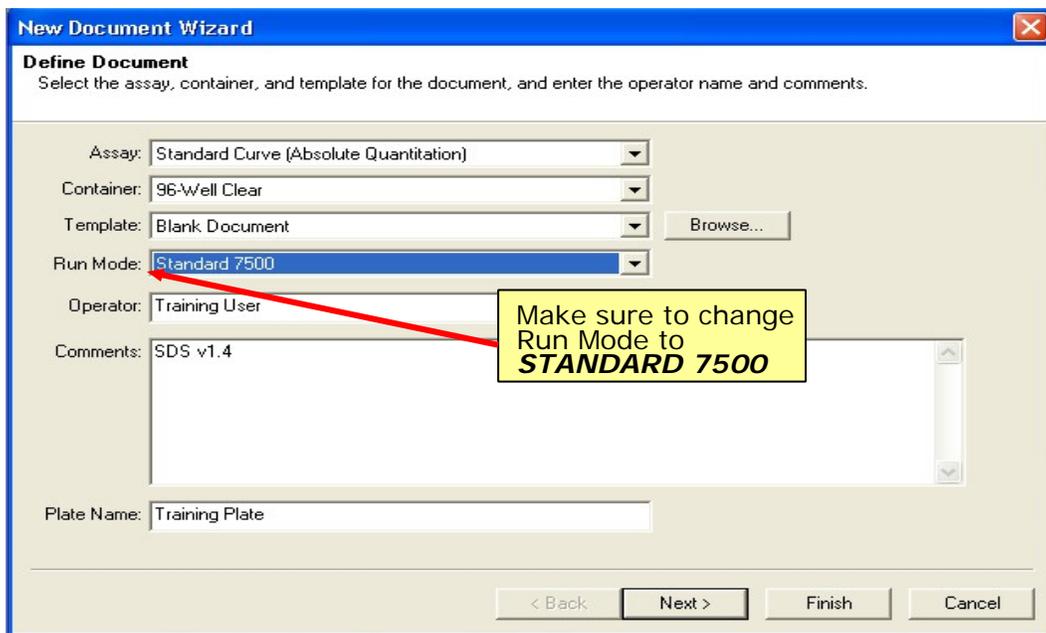
PC: Positive control

[Create a Run Template on the Applied Biosystems 7500 Fast Dx Real-time PCR Instrument \(Required if no template exists\)](#)

If the template already exists on your instrument, please proceed to the **RUNNING A TEST** section.

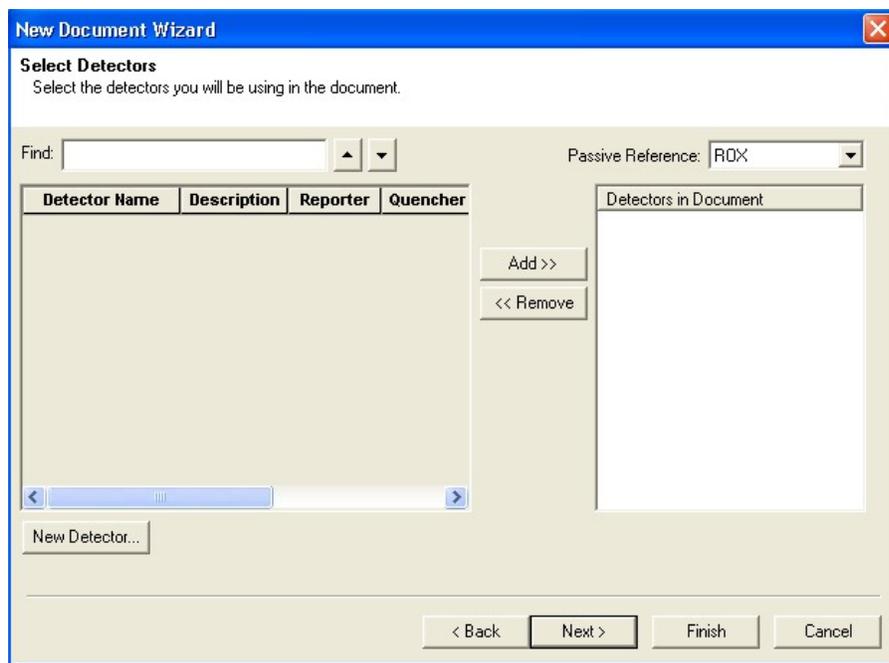
- 1) Launch the Applied Biosystems 7500 Fast Dx Real-time PCR Instrument by double clicking on the Applied Biosystems 7500 Fast Dx System icon on the desktop.
- 2) A new window should appear, select **Create New Document** from the menu.

**Figure 3. New Document Wizard Window**



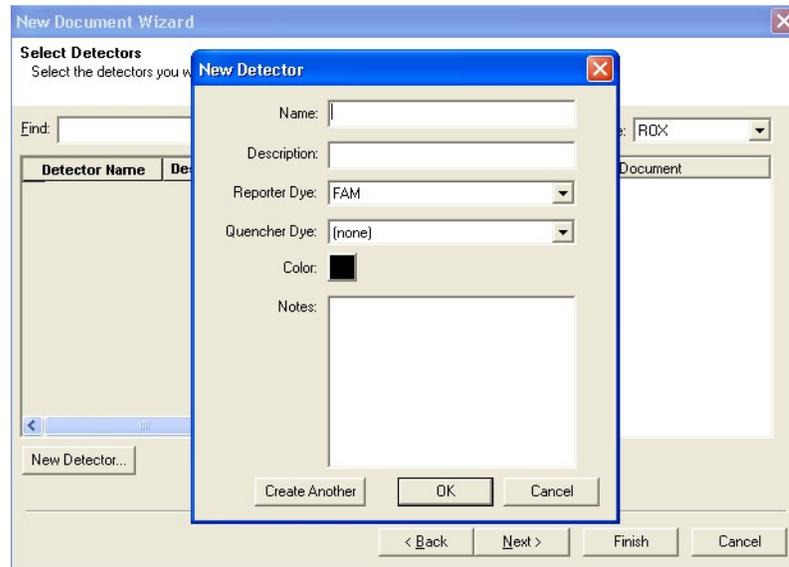
- 3) The **New Document Wizard** screen in **Figure 3** will appear. Select:
  - a. Assay: **Standard Curve (Absolute Quantitation)**
  - b. Container: **96-Well Clear**
  - c. Template: **Blank Document**
  - d. Run Mode: **Standard 7500**
  - e. Operator: **Your Name**
  - f. Comments: **SDS v1.4**
  - g. Plate Name: **Your Choice**
- 4) After making selections click **Next** at the bottom of the window.

**Figure 4. Creating New Detectors**



- 5) After selecting next, the **Select Detectors** screen (**Figure 4**) will appear.
- 6) Click the **New Detector** button (see **Figure 4**).
- 7) The **New Detector** window will appear (**Figure 5**). A new detector will need to be defined for each primer and probe set. Creating these detectors will enable you to analyze each primer and probe set individually at the end of the reaction.

**Figure 5. New Detector Window**

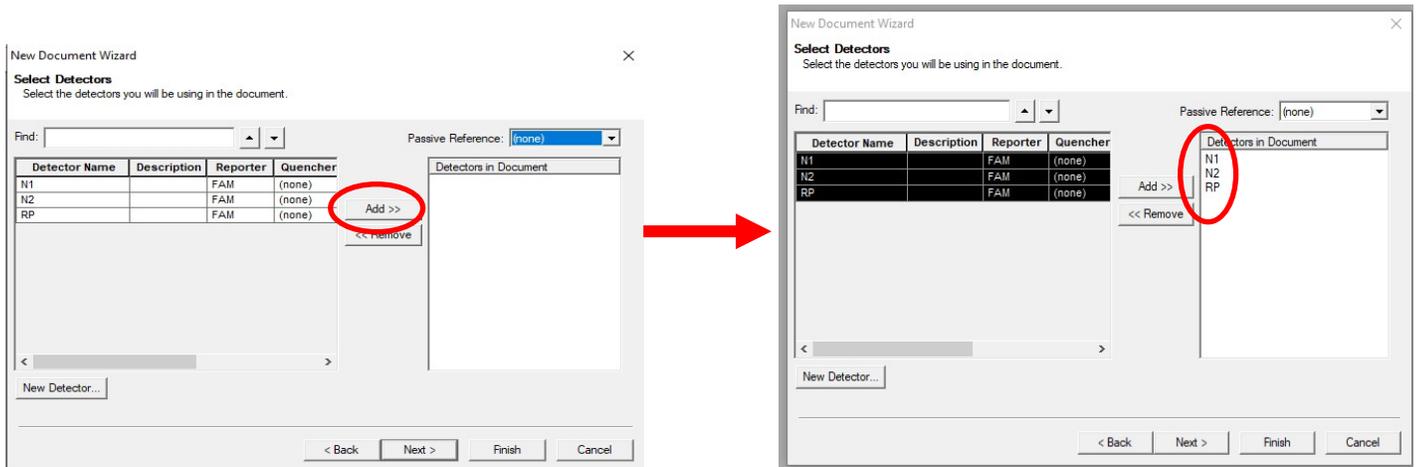


- 8) Start by creating the N1 Detector. Include the following:
  - a. Name: **N1**
  - b. Description: *leave blank*
  - c. Reporter Dye: **FAM**
  - d. Quencher Dye: **(none)**
  - e. Color: *to change the color of the detector indicator do the following:*
    - ⇒ Click on the color square to reveal the color chart
    - ⇒ Select a color by clicking on one of the squares
    - ⇒ After selecting a color click **OK** to return to the New Detector screen
  - f. Click the **OK** button of the New Detector screen to return to the screen shown in **Figure 4**.
- 9) Repeat step 6-8 for each target in the panel.

Name	Reporter Dye	Quencher Dye
N1	FAM	(none)
N2	FAM	(none)
RP	FAM	(none)

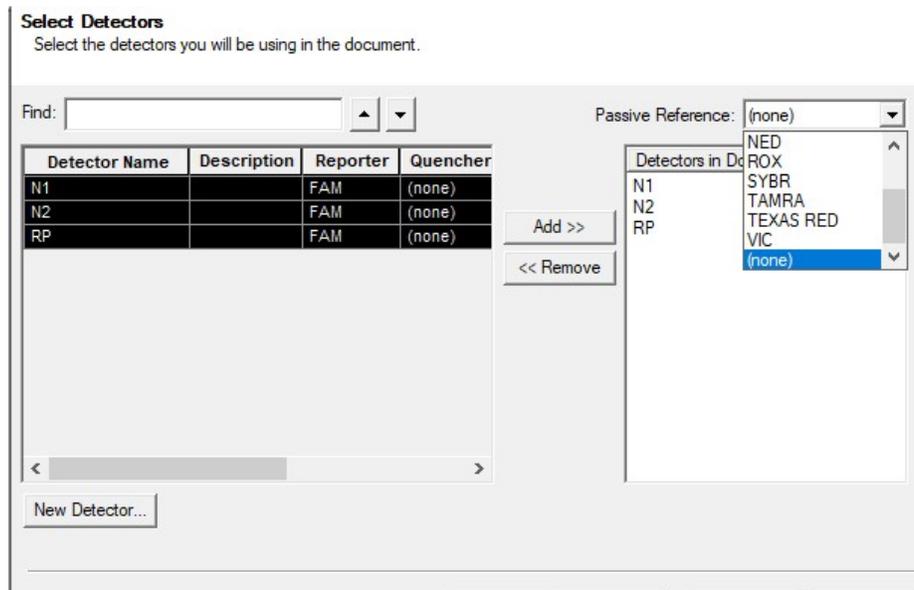
- 10) After each Detector is added, the **Detector Name**, **Description**, **Reporter** and **Quencher** fields will become populated in the **Select Detectors** screen (Figure 6).
- 11) Before proceeding, the newly created detectors must be added to the document. To add the new detectors to the document, click **ADD** (see Figure 6). Detector names will appear on the right-hand side of the **Select Detectors** window (Figure 6).

**Figure 6. Adding New Detectors to Document**



- 12) Once all detectors have been added, select **(none)** for **Passive Reference** at the top right-hand dropdown menu (Figure 7).

**Figure 7. Select Passive Reference**

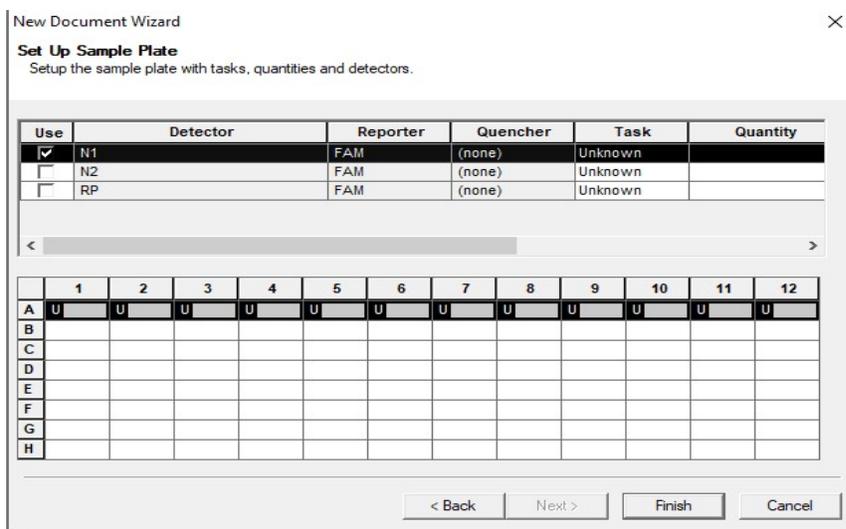


Passive reference should be set to “(none)” as described above.

- 13) Click **Next** at the bottom of the **Select Detectors** window to proceed to the **Set Up Sample Plate** window (Figure 8).

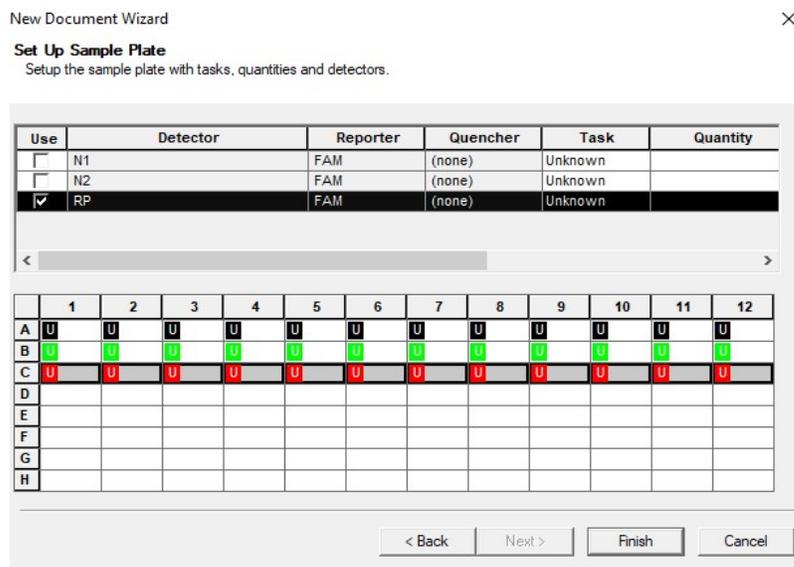
- 14) In the **Set Up Sample Plate** window (**Figure 8**), use your mouse to select row A from the lower portion of the window, in the spreadsheet (see **Figure 8**).
- 15) In the top portion of the window, select detector **N1**. A check will appear next to the detector you have selected (**Figure 8**). You will also notice the row in the spreadsheet will be populated with a colored “U” icon to indicate which detector you’ve selected.
- 16) Repeat step 14-15 for each detector that will be used in the assay.

**Figure 8. Sample Plate Set-up**



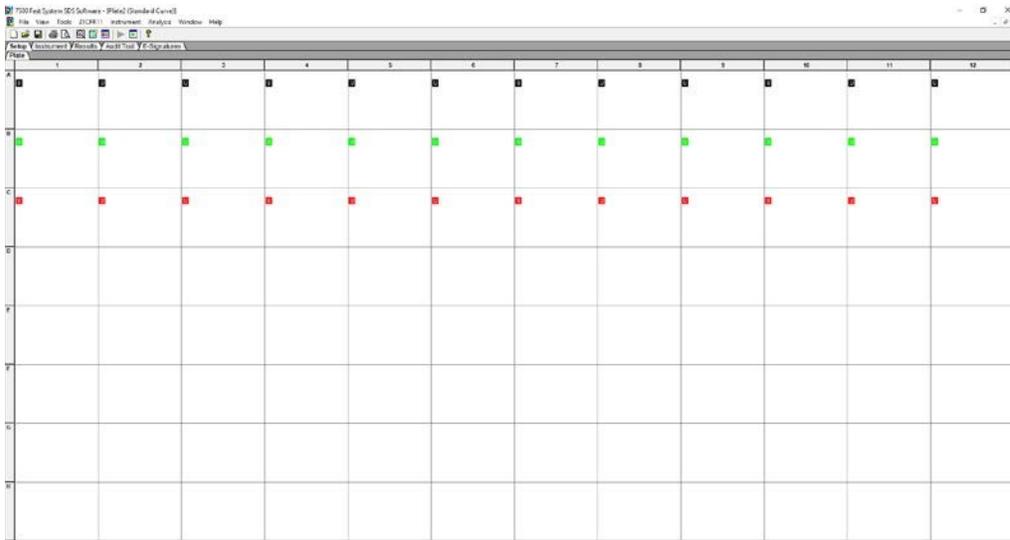
- 17) Select **Finish** after detectors have been assigned to their respective rows. (**Figure 9**).

**Figure 9. Finished Plate Set-up**



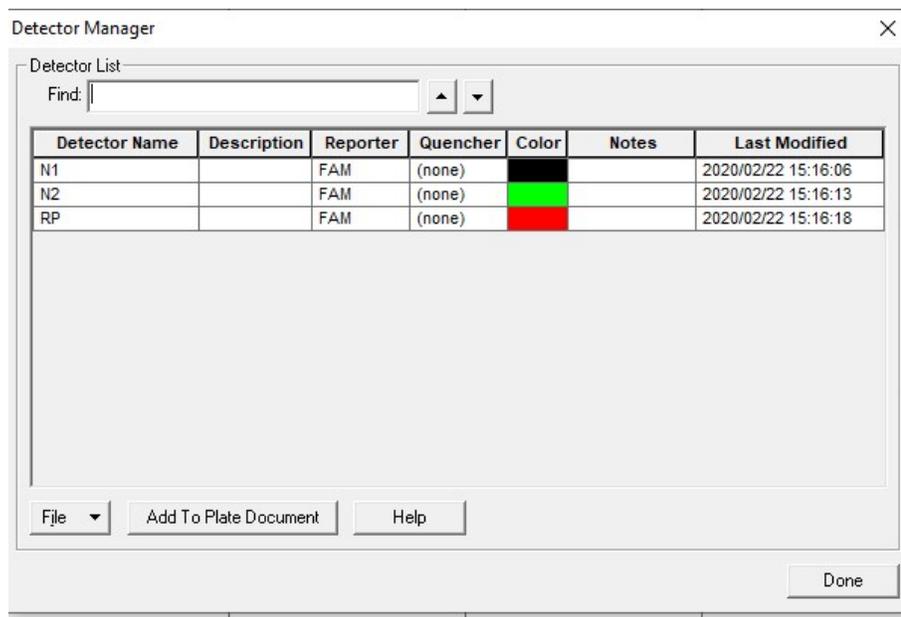
- 18) After clicking “Finish”, there will be a brief pause allowing the Applied Biosystems 7500 Fast Dx to initialize. This initialization is followed by a clicking noise. **Note: The machine must be turned on for initialization.**
- 19) After initialization, the **Plate** tab of the Setup (**Figure 10**) will appear.
- 20) Each well of the plate should contain colored U icons that correspond with the detector labels that were previously chosen. To confirm detector assignments, select **Tools** from the file menu, then select **Detector Manager**.

**Figure 10. Plate Set-up Window**



- 21) The Detector Manager window will appear (**Figure 11**).

**Figure 11. Detector Manager Window**



- 22) Confirm all detectors are included and that each target has a **Reporter** set to **FAM** and the **Quencher** is set to **(none)**.
- 23) If all detectors are present, select **Done**. The detector information has been created and assigned to wells on the plate.

### Defining the Instrument Settings

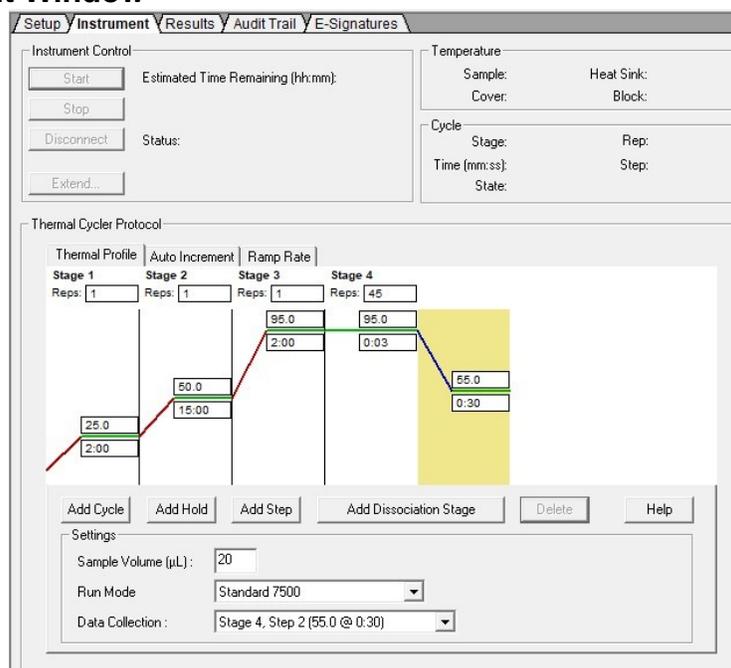
- 1) After detectors have been created and assigned, proceed to instrument set up.
- 2) Select the **Instrument** tab to define thermal cycling conditions. 3) Modify the thermal cycling conditions as follows (**Figure 12**):

**Table 2 Cycling for One-step RTdPCR**

Stage	Temperature (°C)	Time	No. Cycles
Stage 1	25	2 min	1X
Stage 2	50	15 min	
Stage 3	95	2 min	
Stage 4	95	3 sec	45X
	55	30 sec	

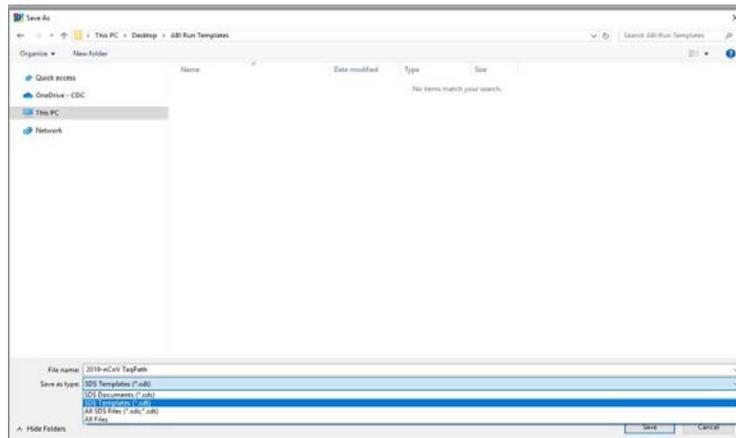
- a. Under **Settings** (**Figure 12**), bottom left-hand box, change volume to 20 µL.
- b. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
- c. Step 2 of Stage 4 should be highlighted in yellow to indicate data collection (see **Figure 12**).

**Figure 12. Instrument Window**



- 4) After making changes to the **Instrument** tab, the template file is ready to be saved. To save the template, select **File** from the top menu, then select **Save As**.
- 5) Save the template as **2019-nCoV TaqPath** as appropriate in the desktop folder labeled “**ABI Run Templates**” (you must create this folder). Save as type should be SDS Templates (\*.sdt) (Figure 13).

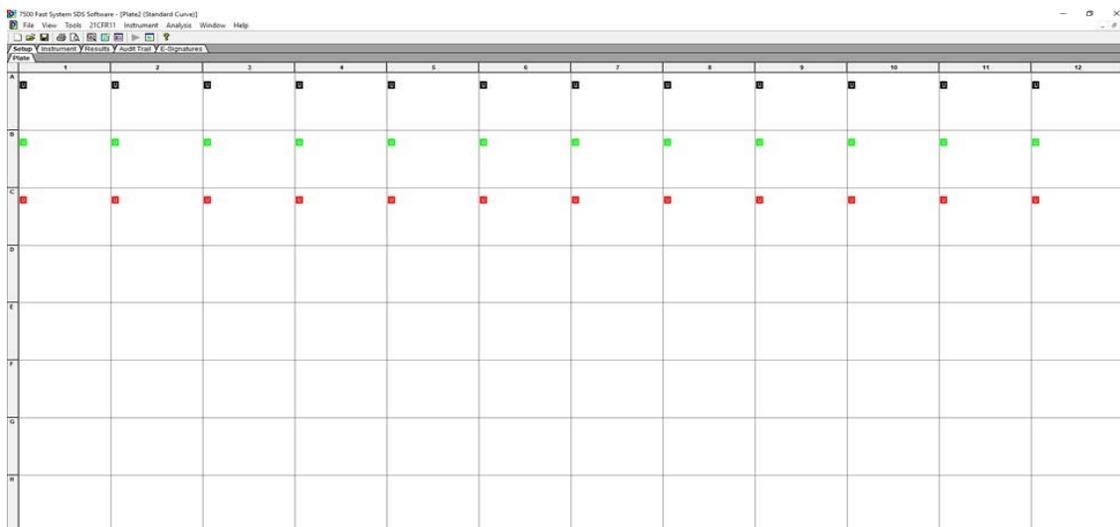
**Figure 13. Saving Template**



### Running a Test

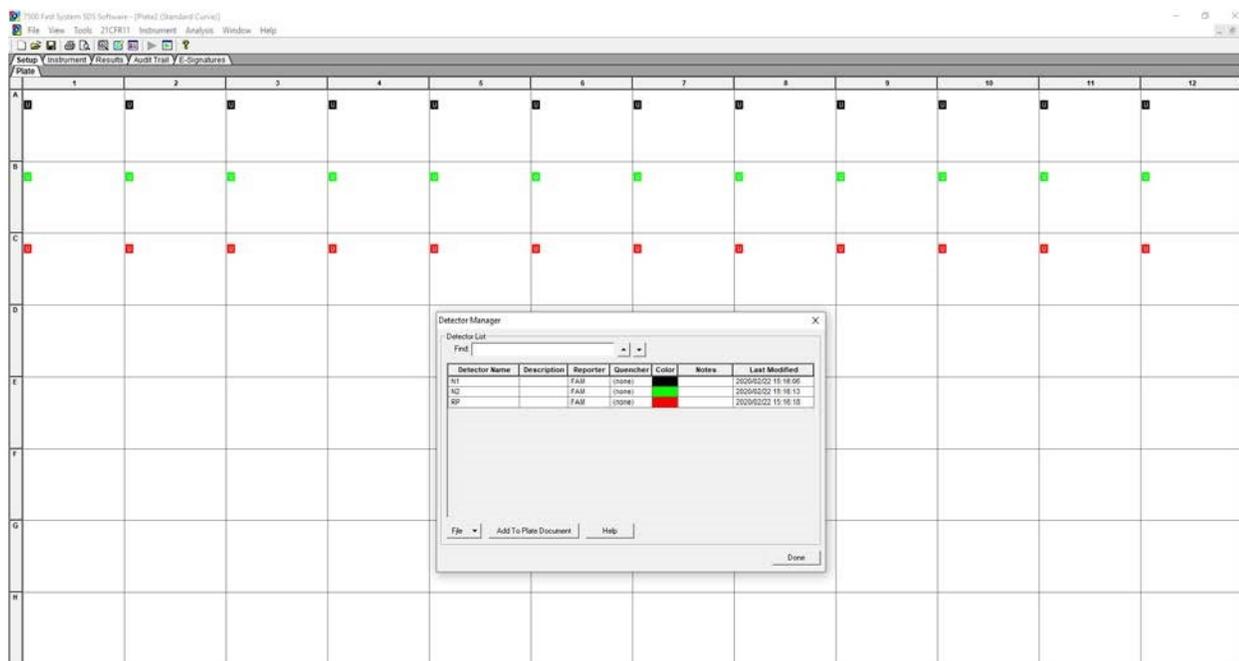
- 1) Turn on the ABI 7500 Fast Dx Real-Time PCR Instrument.
- 2) Launch the Applied Biosystems 7500 Fast Dx Real-time PCR System by double clicking on the 7500 Fast Dx System icon on the desktop.
- 3) A new window should appear, select **Open Existing Document** from the menu.
- 4) Navigate to select your ABI Run Template folder from the desktop.
- 5) Double click on the appropriate template file (**2019-nCoV TaqPath**)
- 6) There will be a brief pause allowing the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument to initialize. This initialization is followed by a clicking noise. **Note: The machine must be turned on for initialization.**

**Figure 14. Plate Set-up Window**



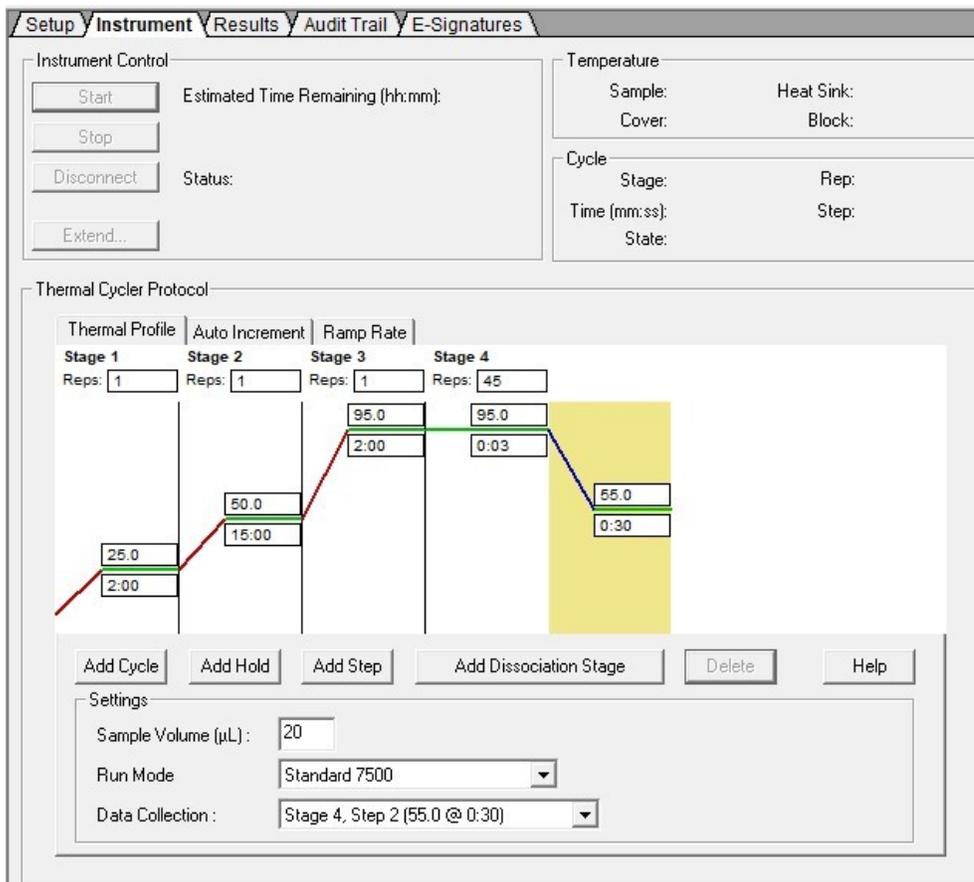
- 7) After the instrument initializes, a plate map will appear (**Figure 14**). The detectors and controls should already be labeled as they were assigned in the original template.
- 8) Click the **Well Inspector** icon  from the top menu.
- 9) Highlight specimen wells of interest on the plate map.
- 10) Type sample identifiers to **Sample Name** box in the **Well Inspector** window (**Figure 15**).

**Figure 15. Labeling Wells**



- 11) Repeat steps 9-10 until all sample identifiers are added to the plate setup.
- 12) Once all specimen and control identifiers are added click the **Close** button on the **Well Inspector** window to return to the **Plate** set up tab.
- 13) Click the **Instrument** tab at the upper left corner. 14) The reaction conditions, volumes, and type of 7500 reaction should already be loaded. (**Figure 16**).

**Figure 16. Instrument Settings**

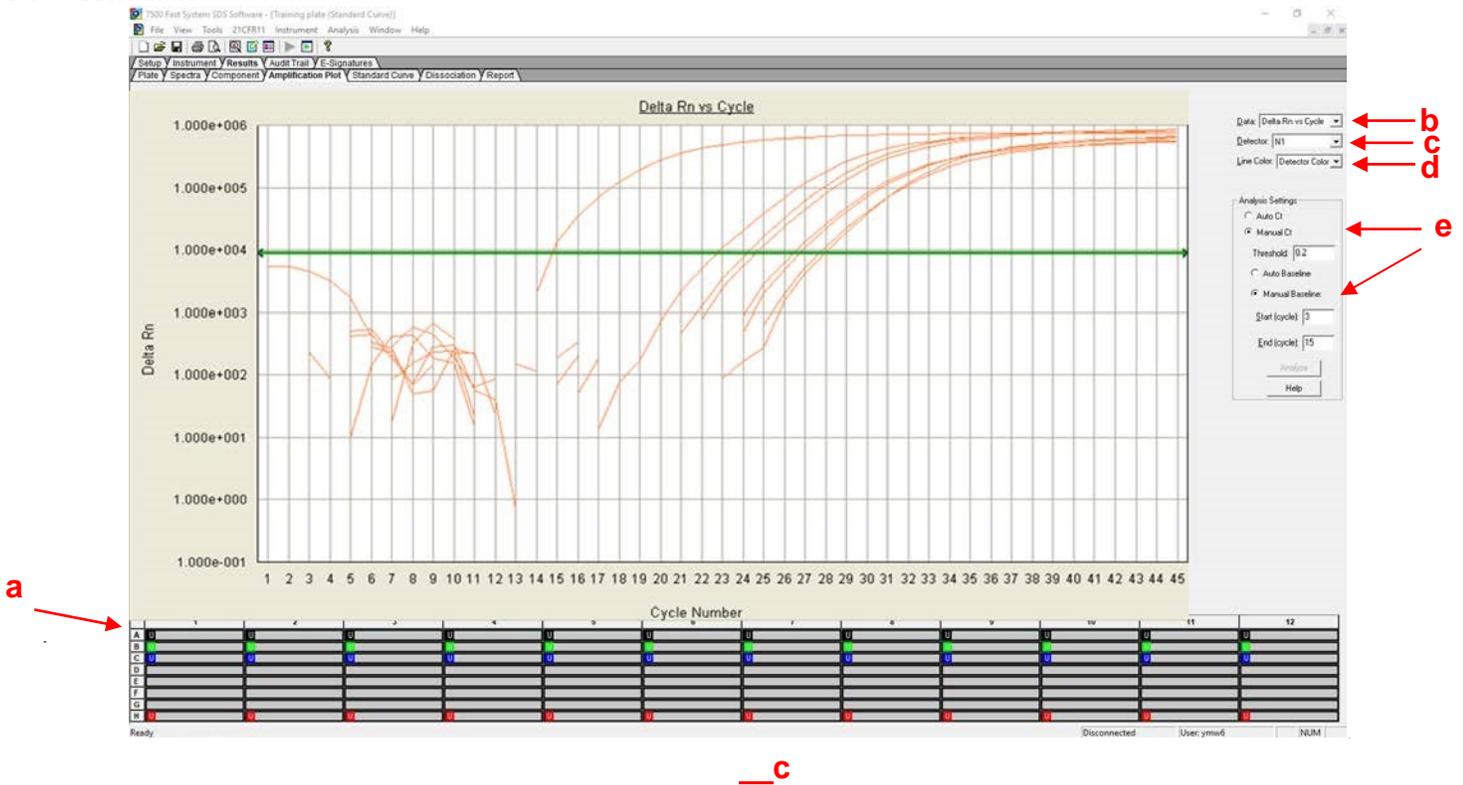


- 15) Ensure settings are correct (refer to the *Defining Instrument Settings*).
- 16) Before proceeding, the run file must be saved; from the main menu, select **File**, then **Save As**. Save in appropriate run folder designation.
- 17) Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.
- 18) Once the run file is saved, click the **Start** button. *Note: The run should take approximately 1hr and 20 minutes to complete.*

### Data Analysis

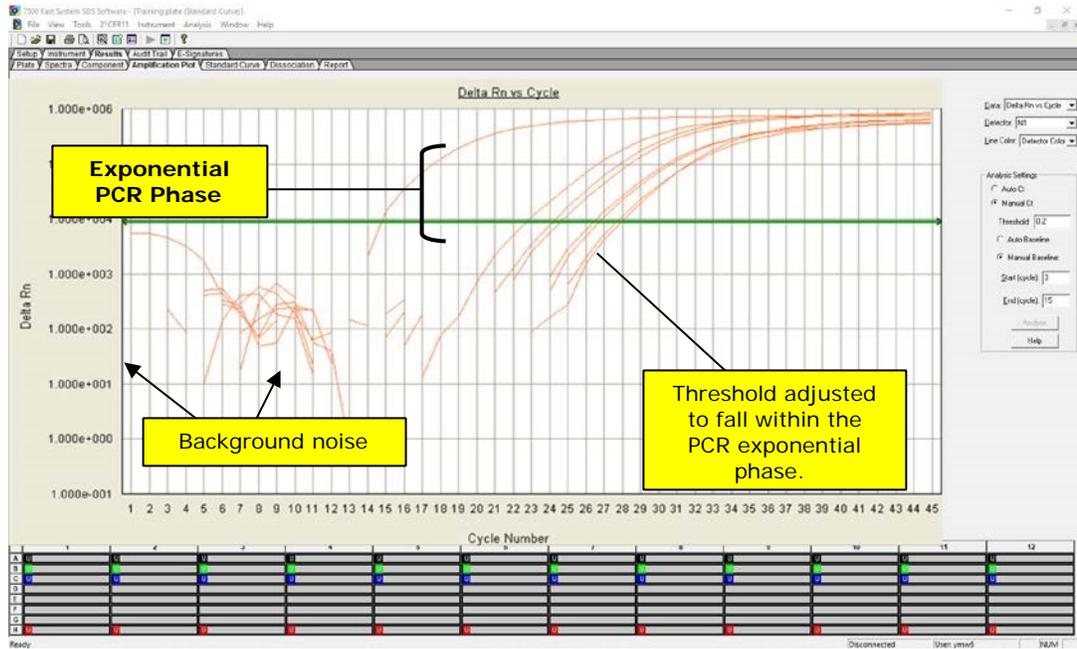
- 1) After the run has completed, select the **Results** tab at the upper left corner of the software.
- 2) Select the **Amplification Plot** tab to view the raw data (**Figure 17**).

**Figure 17. Amplification Plot Window**



- 3) Start by highlighting all the samples from the run; to do this, click on the upper left-hand box (a) of the sample wells (Figure 17). All the growth curves should appear on the graph.
- 4) On the right-hand side of the window (b), the **Data** drop down selection should be set to **Delta Rn vs. Cycle**.
- 5) Select **N1** from (c), the **Detector** drop down menu, using the downward arrow.
  - a. Please note that each detector is analyzed individually to reflect different performance profiles of each primer and probe set.
- 6) In the **Line Color** drop down (d), **Detector Color** should be selected.
- 7) Under **Analysis Settings** select **Manual Ct** (e).
  - b. Do not change the **Manual Baseline** default numbers.
- 8) Using the mouse, click and drag the red threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal (Figure 18).

**Figure 18. Amplification Plot**



- 9) Click the **Analyze** button in the lower right corner of the window. The red threshold line will turn to green, indicating the data has been analyzed.
- 10) Repeat steps 5-9 to analyze results generated for each set of markers (N1, N2, RP).
- 11) Save analysis file by selecting **File** then **Save As** from the main menu.
- 12) After completing analysis for each of the markers, select the **Report** tab above the graph to display the Ct values (**Figure 19**). To filter report by sample name in ascending or descending order, simply click on **Sample Name** in the table.

**Figure 19. Report**

Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tim
A1	NTC	N1	Unknown	Undet						
A2	hCoVPC 1	N1	Unknown	20.2502						
A3	hCoVPC 2	N1	Unknown	20.8418						
A4	hCoVPC 3	N1	Unknown	20.4583						
A5	APC	N1	Unknown	25.4229						
B1	NTC	N2	Unknown	Undet						
B2	hCoVPC 1	N2	Unknown	20.8181						
B3	hCoVPC 2	N2	Unknown	21.6657						
B4	hCoVPC 3	N2	Unknown	21.2075						
B5	APC	N2	Unknown	28.445						
C1	NTC	RP	Unknown	Undet						
C2	hCoVPC 1	RP	Unknown	20.9186						
C3	hCoVPC 2	RP	Unknown	21.3587						
C4	hCoVPC 3	RP	Unknown	20.7028						
C5	APC	RP	Unknown	25.9686						

## Interpretation of Results and Reporting

### **Extraction and Positive Control Results and Interpretation No Template Control (NTC)**

The NTC consists of using nuclease-free water in the Real-Time RT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

### **COVID-19 Positive Control**

The COVID-19 Positive Control A combination of cDNA plasmids encoding the SARS-CoV-2 N gene and RP target. The COVID-19 Positive Control will yield a positive result with the following primer and probe sets: N1, N2 and RP.

### **Human Specimen Control (HSC) (Extraction Control)**

The HSC is used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified nucleic acid from the HSC should yield a positive result with the RP primer and probe set and negative results with all COVID-19 markers, N1 and N2.

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the assay may have been set up and/or executed improperly, or reagents or equipment malfunction may have occurred. Repeat the test run using residual extraction material.

**Table 3 Expected Results for Test Controls**

<b>Control Type</b>	<b>External Control Name</b>	<b>Used to Monitor</b>	<b>N1</b>	<b>N2</b>	<b>RP</b>	<b>Expected Ct Values</b>
Positive	COVID-19 Positive Control	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40.00 Ct

## **RNase P (Extraction Control)**

- All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P 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 RP assay does not produce a positive result for human clinical specimens, interpret as follows:
  - If the COVID-19 N1 and N2 are positive even in the absence of a positive RP, the result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.
  - If all COVID-19 markers (N1 and N2) AND RNase P 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.

## **COVID-19 Markers (N1 and N2)**

- When all controls exhibit the expected performance, a specimen is considered negative if all COVID-19 marker (N1, N2) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the RNase P 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 positive for SARS-CoV-2 if all COVID-19 markers (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct). The RNase P may or may not be positive as described above, but the 2019-nCoV result is still valid.
- When all controls exhibit the expected performance and the growth curves for the COVID-19 markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is invalid. The extracted RNA from the specimen should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the retested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.
- When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test.
- If HSC is positive for N1 or N2, then contamination may have occurred during extraction or sample processing. Invalidate all results for specimens extracted alongside the HSC. Re-extract specimens and HSC and re-test.

### Gnomegen COVID-19 RT-qPCR Detection Kit Results Interpretation Guide

The table below lists the expected results for the Gnomegen COVID-19 RT-qPCR Detection Kit. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact Gnomegen for consultation and possible specimen referral.

**Table 4 Gnomegen COVID-19 RT-qPCR Detection kit Results Interpretation**

<b>N1 Assay</b>	<b>N2 Assay</b>	<b>RP Assay</b>	<b>Final Interpretation of the Results</b>	<b>Report</b>	<b>Actions</b>
+	+	±	Positive Result for the <b>2019-nCoV</b>	Positive for the <b>2019-nCoV</b>	Report positive results to healthcare provider and appropriate public health authorities
Only 1 of the two targets are positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR.
-	-	+	Negative Result for the <b>2019-nCoV</b>	Not Detected	Report negative results to healthcare provider. Consider testing for other pathogens
-	-	-	Invalid Result	Invalid result	Repeat extraction and RT-qPCR.

### Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- Always include a NTC, negative control, and positive control in each amplification and detection run. Per CLIA regulations, HSC must be tested at least once per day.
- All clinical samples should use VIC positive numbers to control for specimen quality and extraction.

### Limitations

- The use of this assay as an in vitro diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical

Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Performance of the Gnomegen COVID-19 RT-qPCR Detection Kit has only been established in nasopharyngeal and oropharyngeal swab specimens.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and PCR systems have not been evaluated.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the virus mutates in the rRT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and, during the course of infection, when these specimens are most likely to contain levels of viral RNA that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

### **Conditions of Authorization for the Laboratory**

The Gnomegen COVID-19 RT-qPCR Detection 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-useauthorizations#coronavirus2019>

However, to assist clinical laboratories using the Gnomegen COVID-19 RT-qPCR Detection Kit, the relevant Conditions of Authorization are listed below:

- Authorized laboratories<sup>1</sup> using the Gnomegen COVID-19 RT-qPCR Detection 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.
- Authorized laboratories using the Gnomegen COVID-19 RT-qPCR Detection Kit will use your 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.
- Authorized laboratories that receive the Gnomegen COVID-19 RT-qPCR Detection Kit will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using the Gnomegen COVID-19 RT-qPCR Detection Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the Gnomegen COVID-19 RT-qPCR Detection 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 Gnomegen (via email: [info@gnomegendx.com](mailto:info@gnomegendx.com)) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using the Gnomegen COVID-19 RT-qPCR Detection 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.
- Gnomegen LLC, authorized distributors, and authorized laboratories using Gnomegen COVID-19 RT-qPCR Detection Kit 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.”

## Performance Characteristics

### Analytical Performance:

#### *Limit of Detection (LoD):*

The LoD studies determined the lowest detectable concentration of the SARS-CoV-2 at which approximately 95% of all (true positives) replicates tested positive using the Gnomegen COVID-19 RT-qPCR Detection Kit. A two-phase approach was used to determine the LoD for the upper respiratory specimen type. In phase I, the preliminary LoD

was established and confirmed in phase II by testing 20 replicates. Using this two-phase approach, the LoD was determined to be **10 copies** of viral RNA per reaction.

To determine the LoD, 5 upper respiratory samples (oropharyngeal swabs) from healthy donors were pooled and individually spiked with serially diluted quantified whole viral genomic RNA extracted from cells infected with SARS-CoV-2 obtained from a COVID-19 positive specimen in Hefei, China (COV20851-2) to prepare a simulated, contrived clinical matrix. Contrived clinical matrix with viral spike-ins were individually purified and tested. Samples were extracted manually using the QIAGEN DSP Viral RNA Mini kit (cat # 61904). Purified RNA was tested using the Gnomegen COVID-19 RT-qPCR Detection Kit on the Applied Biosystems 7500 Fast Dx Real-Time PCR System. The approximate LoD was identified by testing 10, 5 and 2-fold serial dilutions of the characterized stock of purified whole viral RNA. For each dilution, a total of 3 replicates were tested. Data are shown in Table 5.

**Table 5: Tentative LoD in upper respiratory specimen**

2019-nCoV Strain Tested	Stock Concentration	Concentration in Dilution Tested [copies of viral RNA /reaction ]	N1 C <sub>t</sub>	N2 C <sub>t</sub>	RP 3 C <sub>t</sub>	# of Positive N1 Target Samples	# of Positive N2 Target Samples	Lowest Concentration with Uniform Positivity per Analyte
COVID-19 Virus	4X10 <sup>4</sup> copies/ul	2000	26.62	27.67	32.61	3/3 samples	3/3 samples	10 copies/ Reaction (20ul)
		2000	26.90	28.03	33.15			
		2000	26.90	28.02	32.63			
		200	29.87	30.87	33.24	3/3 samples	3/3 samples	
		200	29.91	31.24	33.02			
		200	30.23	31.39	33.29			
		20	33.36	32.66	30.73	3/3 samples	3/3 samples	
		20	33.97	34.86	32.18			
		20	33.34	33.61	30.93			
		10	33.46	34.96	32.20	3/3 samples	3/3 samples	
		10	35.40	36.00	30.68			
		10	33.61	36.33	32.20			
		2	Undetermined	Undetermined	30.39	1/3 samples	1/3 samples	
		2	Undetermined	Undetermined	31.18			
		2	34.91	38.93	42.28			
		1	Undetermined	Undetermined	31.15	2/3 samples	0/3 samples	
		1	35.54	Undetermined	32.54			
		1	35.57	Undetermined	32.40			

From there, the approximate LoD was confirmed by testing 20 additional extraction replicates prepared at the tentative LoD (10 RNA copies/reaction). The contrived samples were prepared and extracted following the same procedure above. The LoD was confirmed to be 10 copies of viral RNA per reaction as (20/20) of the replicates were positive. Data are shown in Table 6.

**Table 6: LoD determination in upper respiratory specimen - confirmation**

Effective Concentration	Replicate	N1 Ct Values	N2 Ct Values	RP Ct Values	Interpretation	# of Positive Samples	Percentage
LoD (10 copies/reaction)	1	34.80	35.96	26.90	Positive	20/20	100%
	2	33.79	35.18	27.54	Positive		
	3	35.75	34.50	26.99	Positive		
	4	35.49	34.70	26.74	Positive		
	5	34.48	34.65	26.94	Positive		
	6	34.34	35.15	26.54	Positive		
	7	35.72	35.83	27.17	Positive		
	8	35.06	35.36	26.93	Positive		
	9	33.95	34.75	26.84	Positive		
	10	35.32	38.03	37.15	Positive		
	11	33.84	36.82	31.91	Positive		
	12	35.68	36.41	27.00	Positive		
	13	36.81	36.80	27.30	Positive		
	14	34.68	36.11	26.74	Positive		
	15	35.11	36.66	27.13	Positive		
	16	33.73	37.03	27.29	Positive		
	17	33.99	35.37	26.56	Positive		
	18	33.50	38.01	26.71	Positive		
	19	33.67	37.63	27.50	Positive		
	20	33.84	35.17	26.98	Positive		

***In Silico Analysis of Primer and Probe Sequences:***

The primers and probes were also analyzed in silico with all the known strains/isolates in the NCBI Severe acute respiratory syndrome coronavirus 2 data hub as of April 20<sup>th</sup>, 2020. The sequences were aligned with 458 complete sequences using the nBLAST to the database: Severe acute respiratory syndrome coronavirus 2 (taxid:2697049) which contains 458 complete sequences and 493 total sequences. The search parameters were adjusted to search for a short input sequence.

**Table 7 nBLAST results of Gnomegen COVID-19 RT-qPCR Detection Kit primers and probes**

(Request ID) RID*	Sequence	Sequences producing significant alignment	% Homology
8A58SXD6114	N1 fwd	458	100%
	N1 rev	458	100%
	N1 probe	458	100%
	N2 fwd	458	100%
	N2 rev	458	100%
	N2 probe	458	100%

\*All RIDs and search results were saved and exported into an excel files and kept in a repository. The files can be made available upon request.

For isolate inclusivity, all the alignments for the N1 and N2 targets (primers and probes) show 100% identity of the kit to the 458 available COVID-19 complete sequences from known strains/isolates in the NCBI Severe acute respiratory syndrome coronavirus 2 data hub as of April 20<sup>th</sup>, 2020 (Table 7).

***Exclusivity/Cross-reactivity:***

The Gnomegen COVID-19 RT-qPCR Detection Kit utilizes identical oligonucleotide sequences for the N1 and N2 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel.

- As reported under the CDC EUA, the *in-silico* analysis for the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining the primers and probe results, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive RT-PCR results.
- Analysis of the forward primer of the N2 target showed high homology to Bat SARS-like coronaviruses. However, the reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora was observed. that would predict potential false positive rRT-PCR results. Combining the primers and probe results, there is no prediction of potential false positive RT-PCR results.

**Clinical Performance:**

**Contrived Specimen Testing**

The clinical performance of Gnomegen COVID-19 RT-qPCR Detection Kit on Applied Biosystems 7500 Fast Dx Real-Time PCR System was evaluated using a total of 30 negative and a total of 30 positive contrived clinical samples at various dilutions of characterized purified whole viral RNA (COV20851-2). Prior to testing, the positive and negative contrived samples were blinded and randomized before testing. Clinical samples were collected and handled by qualified personnel according to the package insert of the collection device. Low positive and moderate positive contrived positive clinical samples were prepared by spiking characterized purified whole viral RNA into individual negative clinical samples to approximately 2X LoD (20 contrived positive samples) and the remaining 10 contrived samples were prepared at 4X LoD. Then the samples were extracted manually using the QIAGEN DSP Viral RNA Mini kit (cat # 61904). Purified RNA were tested using the Gnomegen COVID-19 RT-qPCR Detection Kit on the Applied Biosystems 7500 Fast Dx Real-Time PCR System. Results of the clinical validation studies are provided below.

**Table 8: Clinical Performance of the Gnomegen COVID-19 RT-q PCR Detection kit: upper respiratory specimens**

Sample Type	Sample Concentration	Number of Samples	# of Positive Samples	% Positive	Mean N1 Ct Values	Mean N2 Ct Values	Mean RP Ct Values
Upper Respiratory Specimen	Negative	30	0	0%	NA	NA	29.28
	2X LoD	20	20	100%	32.42	34.30	26.65
	4X LoD	10	10	100%	31.96	34.09	27.07

PPA: 100% (88.65-100%)

NPA:100% (88.65-100%)

### Clinical Specimen Testing

The clinical performance of Gnomegen COVID-19 RT-qPCR Detection Kit on Applied Biosystems 7500 Fast Dx Real-Time PCR System was evaluated and validated against a real-time PCR comparator method from among the list of FDA EUA's using a total of 30 COVID-19 negative and 30 COVID-19 positive patient samples. Prior to testing, the positive and negative patient samples were blinded and randomized. Clinical samples were collected and handled by qualified personnel according to the package insert of the collection device. The samples were extracted manually using the QIAGEN DSP Viral RNA Mini kit (cat # 61904). Purified RNA was tested using the Gnomegen COVID-19 RT-qPCR Detection Kit and the comparator method. Results of the clinical validation studies are provided below.

**Table 13: Clinical Performance Gnomegen COVID-19 RT-q PCR Detection Kit Upper Respiratory Specimen with the Comparator Method**

Gnomegen COVID-19 RT-qPCR Detection Kit Result	Comparator Method Result	
	Positive	Negative
Positive	30	0
Inconclusive	0	0
Negative	0	30

PPA:100% (88.65-100%)

NPA:100% (88.65-100%)

As the results show, the COVID-19 negative and positive patient samples tested on Gnomegen COVID-19 RT-qPCR Detection Kit were 100% in agreement to the composite comparator.

## Symbols

	Contains reagents sufficient for <N> number of reactions
	Use by
	In vitro Diagnostic Device
	Catalog Number
	Lot Number
	Serial Number
	Prescription Use Only
	Temperature Limitations
	Manufacturer
	Keep away from sunlight
	Consult Instructions for use



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References

1. Clinical and Laboratory Standards Institute. *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline*. CLSI Document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
2. Centers for Disease Control and Prevention (CDC). *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)*. Available online at: <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>

Revision history: Pub. No. CV03030004

Revision	Date	Description
01	20 April 2020	New Document

TaqPath™, DNAZap™, RNase Away™, Applied Biosystems® is a registered trademark of ThermoFisher Scientific.

QIAamp® is a registered trademark of QIAGEN Group.

Ultrafree® is a registered trademark of Merck KGaA.

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