COVID-19 Nucleic Acid RT-PCR Test Kit

Instruction for Use

20 Tests/Kit (SC-COVID19-20)

100 Tests/Kit (SC-COVID19-100)

For Emergency Use Authorization (EUA) Only

For Prescription Use Only

For *In Vitro* Diagnostic Use Only

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Version 1

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Intended Use

COVID-19 Nucleic Acid RT-PCR Test Kit is a real-time RT-PCR test intended for the qualitative detection of RNA from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) 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, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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. 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 COVID-19 Nucleic Acid RT-PCR Test Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The COVID-19 Nucleic Acid RT-PCR Test Kit is only for use under the Food and Drug Administration’s Emergency Use Authorization.

Product Description

The COVID-19 Nucleic Acid RT-PCR Test Kit is a real-time RT-PCR test that contains two primer and probe sets to detect regions in the SARS-CoV-2 ORF1ab and nucleocapsid (N) genes and one primer and probe set to detect human *β-actin* in a clinical sample. RNA isolated from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems™ ABI 7500 Real-Time PCR system or Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument. During the amplification 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 bound probe, causing the reporter dye (FAM, VIC, and ROX) to separate from the quencher dye (BQ1-3), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the thermocycler.

The COVID-19 Nucleic Acid RT-PCR Test Kit also contains a positive RNA control that is specific to the SARS-CoV-2 genomic regions targeted by the assay and a negative control. Both controls are intended to be taken through the entire sample processing procedure, including the extraction.

Materials Provided

The contents of the COVID-19 Nucleic Acid RT-PCR Test Kit are sufficient for 20 or 100 reactions.

The kit consists of a PCR reaction solution, an enzyme reaction mix, a positive control, and a negative control (**Table 1**). The PCR reaction solution includes COVID-19 ORF1ab primers and probes, N gene primers and probes, internal control (*β-actin*) primers and probes, and deoxyribonucleoside triphosphates, etc.

Table 1. COVID-19 Nucleic Acid RT-PCR Test Kit Content

Kit Components (SC-COVID19-20)	Volume	Quantity	Storage
PCR reaction solution (COVID-19 RT-PCR Assay Multiplex: ORF1ab, N gene, and *β-actin*)	500 µL	1 Tube	≤ -15 °C
Enzyme mix	20 µL	1 Tube	≤ -15 °C
Positive control (Pseudovirus - RNA capsuled by protein contains the ORF1a/b, E, and N gene target sequences)*	100 µL	1 Tube	≤ -15 °C
Negative control (TE buffer)*	100 µL	1 Tube	≤ -15 °C

*Positive and negative controls are taken through the entire sample processing procedure.

Kit Components (SC-COVID19-100)	Volume	Quantity	Storage
PCR reaction solution (COVID-19 RT-PCR Assay Multiplex: ORF1ab, N gene, and *β-actin*)	1000 µL	2 Tubes	≤ -15 °C
Enzyme mix	100 µL	1 Tube	≤ -15 °C
Positive control (Pseudovirus - RNA capsuled by protein contains the ORF1a/b, E, and N gene target sequences)*	200 µL	1 Tube	≤ -15 °C
Negative control (TE buffer)*	200 µL	1 Tube	≤ -15 °C

*Positive and negative controls are taken through the entire sample processing procedure.

General Consumables Required but Not Provided

- Micropipettes (2 µl, 10 µl, 200 µl and 1000 µl)
- Multichannel micropipettes (5-50 µl)
- Sterile aerosol barrier (filtered) pipette tips
- Disposable powder-free gloves and surgical gowns
- Ice buckets or cold blocks for 96 well PCR plates
- Plate centrifuge
- Vortex mixer
- Laboratory freezers
    - o -30 °C to -10 °C
    - o ≤-70 °C
- Biological Safety Cabinet, Class II, Type A2, or equivalent
- Microcentrifuge tubes
- Microcentrifuge

Manual RNA Extraction Reagents and Consumables Required but Not Provided

Reagents	Manufacturer	Cat. #
QIAamp® Viral RNA Mini Kit	Qiagen	52904 or 52906
UltraPureTM Distilled Water, DNAse, RNAse, Free, or equivalent	Invitrogen	10977-015
Absolute Ethanol (200 proof), molecular biology grade, or equivalent	Fisher Scientific	BP2818-500

Automated RNA Extraction Instruments, Reagents and Consumables Required but Not Provided

Instruments and Equipment	Manufacturer	Cat. #
KingFisher™ Flex Magnetic Particle Processor with 96 Deep- Well Head	Thermo Scientific	5400630
KingFisher™ Flex 96 Deep-Well Heating Block	Thermo Scientific	24075430
Reagents	**Manufacturer**	**Cat. #**
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Applied Biosystems	A42352
Absolute Ethanol (200 proof), molecular biology grade, or equivalent	Fisher Scientific	BP2818-500
UltraPureTM Distilled Water, DNAse, RNAse, Free, or equivalent	Invitrogen	10977-015
Consumables	**Manufacturer**	**Cat. #**
KingFisher™ Deepwell 96 Plate	Thermo Scientific	95040450, A48305, A48424, 95040455
96-well plate for the tip comb, one of the following: • KingFisher™ 96 KF microplate • ABgene™ 96–Well Polypropylene Storage Microplate • ABgene™ 96–Well 1.2–mL Polypropylene Deepwell Storage Plate • Nunc™ F96 MicroWell™ Black and White Polystyrene Plate	Thermo Scientific or equivalent	•97002540 •AB0796 •AB1127 •136101
KingFisher™ 96 tip comb for DW magnets	Thermo Scientific	97002534, A48438, A48414
KingFisher™ Plastics for 96 standard and PCR formats	Thermo Scientific	97002540

Real-Time PCR Instruments and Equipment Required but Not Provided

Instruments and Equipment	Manufacturer	Cat. #
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)	Applied Biosystems	4406984 (with laptop computer) 4406985 (with tower computer)
Applied Biosystems™ 7500 Real-Time PCR Instrument (used with 7500 Software v2.3)	Applied Biosystems	4351104 (with laptop computer) 4351105 (with desktop computer)
Consumables	**Manufacturer**	**Cat. #**
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode (0.1 ml) – For ABI 7500 Fast Dx Instrument	Applied Biosystems	4346906
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL – For ABI 7500 Instrument	Applied Biosystems	N8010560, 4316813
MicroAmp™ Optical Adhesive Film	Applied Biosystems	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	Applied Biosystems	4333183
Calibration Plates	**Manufacturer**	**Cat. #**
Applied Biosystems™ 7500 Fast Real-Time PCR Systems Spectral Calibration Kit I – For ABI 7500 Fast Dx Instrument	Applied Biosystems	4360788
Applied Biosystems™ 7500 Real Time PCR Systems Spectral Calibration Kit I – For ABI 7500 Instrument	Applied Biosystems	4349180

Warnings and Precautions

As with any PCR test procedure, good laboratory practice is essential to the proper performance of this assay. Care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use (IVD) only.
- For Emergency Use Authorization only.
- For Prescription Use only.
- Please read this manual carefully before use.
- The COVID-19 Nucleic Acid RT-PCR Test Kit has not been FDA cleared or approved.
- The COVID-19 Nucleic Acid RT-PCR Test Kit has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- The COVID-19 Nucleic Acid RT-PCR Test Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The COVID-19 Nucleic Acid RT-PCR Test Kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

- ALWAYS use aerosol barrier pipet tips for RNA extraction and pre-and post-PCR work. Never leave the lid off the pipet tip box while working; replace lid after each pipet tip is removed.
- All patient specimens should be considered potentially infectious and should be handled with universal precautions. All human-source materials should be considered as infectious substances. Wear PPE and frequently replace gloves during the experiment to avoid cross-contamination between samples; sample handling and waste disposal must meet relevant regulatory requirements.
- In order to prevent the virus from spreading, COVID-19 testing laboratories must meet bio-safety level 2 (BSL2) and above. Laboratory management should strictly follow the management specifications of PCR gene amplification laboratories, and experimental operations must be strictly partitioned. The instruments, equipment, consumables, and PPE used in the area must be dedicated and must not be used crosswise to avoid contamination.
- Only trained personnel proficient in handling infectious materials and the use of ABI 7500 Real-Time PCR system or Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument should perform this procedure.
- Before testing, please familiarize yourself with the operation methods and precautions of various instruments to be used and perform quality control for each experiment.
- This product should be fully thawed at room temperature, and mixed and centrifuged at low speed immediately before use.
- Sample processing should be performed in a certified Class II biological safety cabinet (BSC) to protect operator safety and prevent environmental contamination, and should be performed in accordance with national biological safety regulations.
- A negative control and a positive control should be included for each experiment. Do not mix reagents of different batches. Use the kit within the validity period.
- The samples to be tested should be as fresh as possible, and the extraction process should be strictly protected against RNA degradation caused by RNase.
- Samples stored at -70 °C should be thawed, mixed and centrifuged at room temperature for a short time before use.
- When adding the sample, the sample should be completely added to the reaction solution, and no sample should adhere to the tube wall. The tube cap should be closed as soon as possible after the sample is added.
- Try to avoid the generation of air bubbles when the reaction solution is dispensed, and check whether the reaction tubes are tightly closed before going on the machine to avoid leaking and contaminating the instrument.
- After the amplification, the reaction plate is taken out, sealed in a special plastic bag, and discarded at the designated place.
- The workbench and various experimental equipment are often disinfected with 10% sodium hypochlorite, 75% alcohol, and UV lamps when applicable.
- The real-time PCR instrument requires frequent calibration and proper maintenance should be performed to ensure the accuracy of the assay.

Reagent Storage and Handling

The kit should be stored below -15 °C away from light and is stable until the expiration date stated on the label. The kit should be used within one month after opening; the reagent components should not undergo excessive freeze/thaw cycles. Note the production date and expiration date listed on the label. Reagents from different lot numbers should not be mixed.

Specimen Handling and Storage

Note: Handle specimens and control samples as if they are capable of transmitting infectious agents.

Specimen type:

Upper respiratory specimens such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens.

Specimen collection:

- Patient specimens must be collected according to appropriate laboratory guidelines.
- Follow manufacturer's instruction for collection.
- 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 should not be used and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2–3 mL of viral transport media.
- Nasopharyngeal wash/aspirate or nasal aspirate specimens should be collected in sterile, DNase/RNase free containers without preservative.
- Follow the "Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)" by Centers for Disease Control and Prevention. For more information, visit the CDC's and FDA's websites in the following addresses:
  CDC – https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Specimen storage and transport:

- Specimens should be sent to the laboratory as soon as possible after collection.
- Store specimens at 2-8 °C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70 °C or below.
- Label each specimen container with the patient's ID number (e.g., medical record number), specimen ID (e.g., laboratory requisition number), specimen type (e.g., nasopharyngeal swab specimen) and the date the sample was collected.
- Specimens should be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Personnel must be trained to pack and ship according to the regulations and in a manner that corresponds to their function-specific responsibilities.

Procedure

Overview

Nucleic acids are isolated and purified from upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) using one of the kits in the table below. The QIAamp Viral RNA Mini Kit is intended to be used upstream of the Applied Biosystems ABI 7500 Real-Time PCR system and the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit is intended to be used upstream of the Applied Biosystems ABI 7500 Fast Dx Real-Time PCR system.

Kits	Manufacturer	Cat. No.	Method	Input volume (µl)	Elution volume (µl)
QIAamp® Viral RNA Mini Kit	Qiagen	52904 or 52906	Manual	140	60
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Applied Biosystems	A42352	Automated*	200	50

* KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head

The purified nucleic acid (5 µl) is reverse transcribed using COVID-19 Nucleic Acid RT- PCR Test Kit (19.2 µl PCR reaction solution and 0.8 µl enzyme mix) into cDNA which is then subsequently amplified in Applied Biosystems™ 7500 Real-Time PCR system or Applied Biosystems™ 7500 Fast Dx Real-Time PCR System. 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 intensity. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems™ 7500 Real-Time PCR system or Applied Biosystems™ 7500 Fast Dx Real-Time PCR System.

Steps		Temperature	Time	Cycles
1	UNG incubation	37 °C	2 min	1
2	Reverse transcription amplification	50 °C	15 min	1
3	TaqMan enzyme activation	95 °C	5 min	1
4	DNA denaturation	95 °C	15 sec	45
	Annealing, extension, and fluorescence acquisition	62 °C*	30 sec	

*Collect fluorescence signal during the final 62 °C step.

Procedure using QIAamp Viral RNA Mini Kit and Applied Biosystems™ ABI 7500 Real-Time PCR system

Nucleic Acid Extraction

For RNA extraction, refer to the instructions of commercial virus RNA extraction kits. Appropriate kits include the QIAamp® Viral RNA Mini Kit (Cat No. 52904 or 52906).

Important: Positive and negative controls must be taken through the entire extraction procedure by spiking 28 µl of each control to 112 µl Nuclease-free water in separate containers per batch. The total volume should add up to the recommended input volume listed below.

Recommended methods, input and elution volumes are listed below:

Kits	Manufacturer	Cat. No.	Method	Input volume (µl)	Elution volume (µl)
QIAamp® Viral RNA Mini Kit	Qiagen	52904 or 52906	Manual	140	60

Perform RT-PCR

Reagent and Sample Preparation

1. Thaw the PCR reaction solution at room temperature. Mix the solution for 30s and centrifuge briefly for 10s.
2. Calculate the number of reactions (n) required for the current experiment. Given that one reaction requires 19.2 µL PCR reaction solution and 0.8 µL enzyme reaction mixture, pipet the appropriate volumes into a master mixture tube.
   n = number of samples + negative control (1 serving) + positive control (1 serving) + 1 extra (overage for pipette error)
3. Dispense the master mix into reaction tubes or a 96-well PCR plate at a volume of 20 µL/well. Transfer the PCR reaction tubes to the sample preparation area and return the remaining reagents to storage at -15 °C, away from light.
4. For each well containing master mix, add 5 µL of the negative control, the positive control, or the RNA to be tested.

Example reaction plate set up (up to 94 samples):

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	PC	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
B	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22
C	Sample 23	Sample 24	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30	Sample 31	Sample 32	Sample 33	Sample 34
D	Sample 35	Sample 36	Sample 37	Sample 38	Sample 39	Sample 40	Sample 41	Sample 42	Sample 43	Sample 44	Sample 45	Sample 46
E	Sample 47	Sample 48	Sample 49	Sample 50	Sample 51	Sample 52	Sample 53	Sample 54	Sample 55	Sample 56	Sample 57	Sample 58
F	Sample 59	Sample 60	Sample 61	Sample 62	Sample 63	Sample 64	Sample 65	Sample 66	Sample 67	Sample 68	Sample 69	Sample 70
G	Sample 71	Sample 72	Sample 73	Sample 74	Sample 75	Sample 76	Sample 77	Sample 78	Sample 79	Sample 80	Sample 81	Sample 82
H	Sample 83	Sample 84	Sample 85	Sample 86	Sample 87	Sample 88	Sample 89	Sample 90	Sample 91	Sample 92	Sample 93	Sample 94

5. Cap the PCR reaction tubes or seal the 96-well PCR plate with optical adhesive film.
6. Centrifuge the PCR reaction tubes or the 96-well PCR plate briefly to collect the content at the bottom.
7. Load the PCR tubes or 96-well PCR plate to ABI 7500 Real-Time PCR system when the instrument is ready. If the reaction tubes or plate cannot be immediately loaded into the instrument, temporarily store the tubes at 2-8 °C. The tubes can be stored for up to 2 hours but should be used as soon as possible within that timeframe.

Set Up the ABI 7500 Real-Time PCR system

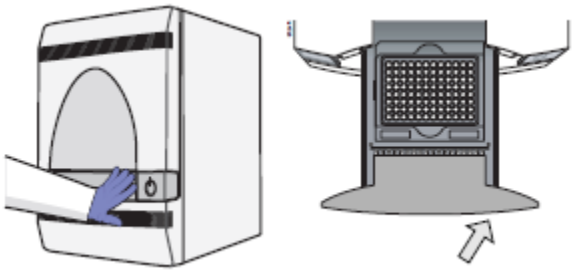
For more information about maintenance and calibration of the ABI 7500 Real-Time PCR system, see the instrument user's manual.

Set Up the COVID-19 RT-PCR Reaction on ABI 7500 System

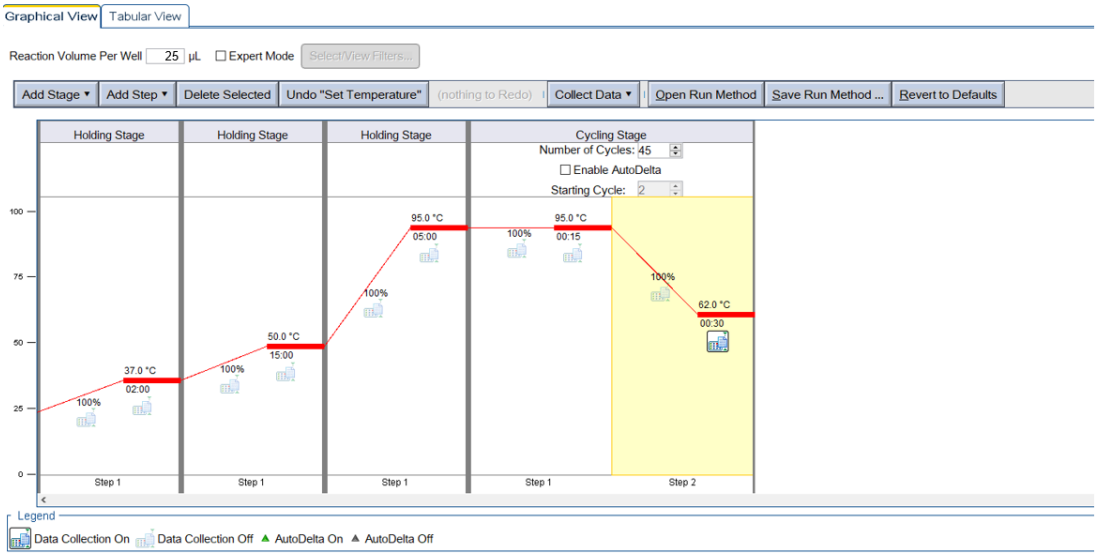
Obtain the template (SCD-COVID-19-ABI 7500.edt) from SCDChina@outlook.com and save the template to the computer connected to the ABI 7500 real time PCR instrument.

Running a Test on ABI 7500 System

1. Turn on the computer connected to the on ABI 7500 real time PCR instrument.
2. Turn on the ABI 7500 real time PCR instrument.
3. Double click the ABI 7500 real time PCR instrument software (v2.3).
4. Push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door. Apply pressure to the right side of the tray and at an angle.



5. Use the “SCD-COVID-19-ABI 7500.edt” template file to setup the run parameters.
6. Under the "Run Method" example table, click the "Graphical View" tab to verify the amplification program.

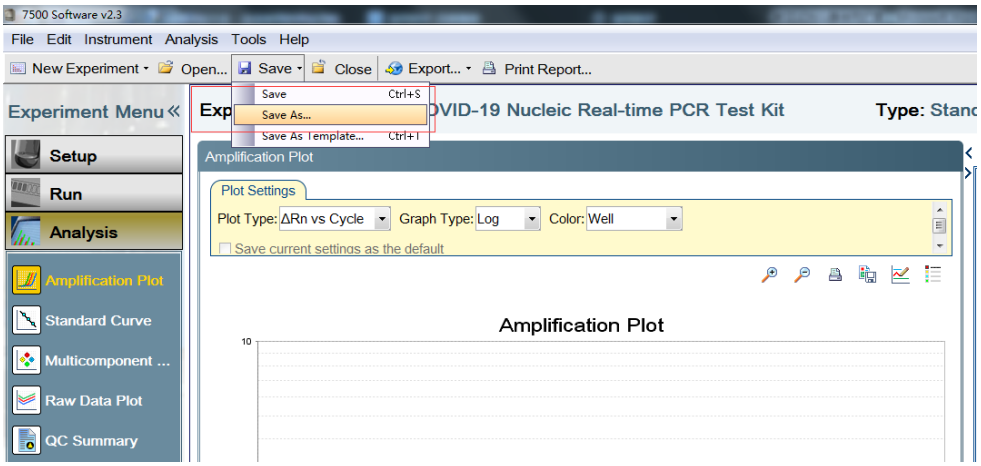


Steps		Temperature	Time	Cycles
1	UNG incubation	37 °C	2 min	1
2	Reverse transcription amplification	50 °C	15 min	1
3	TaqMan enzyme activation	95 °C	5 min	1
4	DNA denaturation	95 °C	15 sec	45
	Annealing, extension, and fluorescence acquisition	62 °C*	30 sec	

*Collect fluorescence signal during the final 62 °C step.

7. Double check all settings then click Run and Start to initialize amplification.

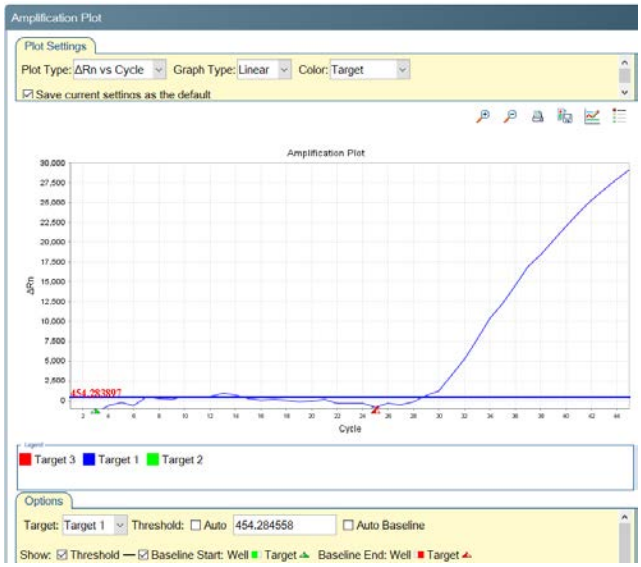
8. After the run completes, saved the data and proceed to data analysis.

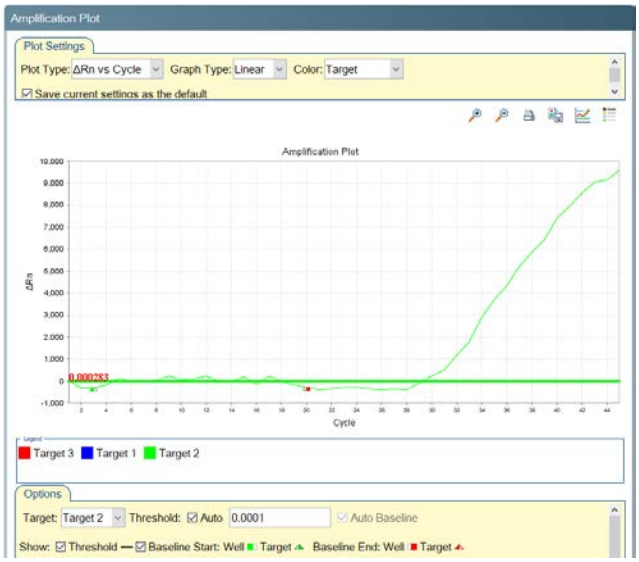


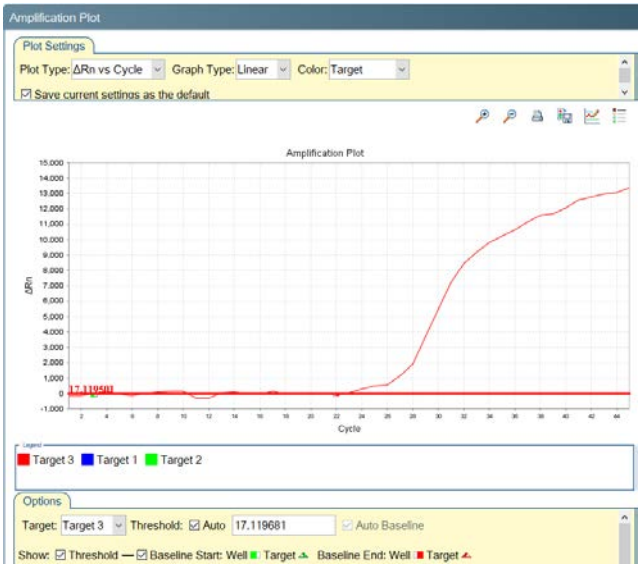
Data Analysis and Result Interpretation

1. Click Analysis. In the Amplification Plot screen under Plot Settings tab:
   a. In the Plot Type drop-down list, select ΔRn vs Cycle (default).
   b. In the Graph Type drop-down list, select Linear.
   c. In the Plot Color drop-down list, select Target as showed in the figure below.

2. Set the baseline starting point at cycle 2-15 and ending at cycle 10-25.
3. Threshold setting principle: The threshold line just exceeds the highest point of the negative control.
4. Click Analyze.
5. To review a Ct value of a sample, click the well containing the sample as shown in the figure below. In the Target drop down, select the target for review. Example for a positive sample.


Amplification Plot
Plot Settings
Plot Type: ΔRn vs Cycle
Graph Type: Linear
Color: Target
Save current settings as the default
Amplification Plot
ΔRn
Cycle
Target 3
Target 1
Target 2
Options
Target: Target 1
Threshold: Auto 454.284558
Auto Baseline
Show: Threshold — Baseline Start: Well Target Baseline End: Well Target


Amplification Plot
Plot Settings
Plot Type: ΔRn vs Cycle
Graph Type: Linear
Color: Target
Save current settings as the default
Amplification Plot
ΔRn
Cycle
Target 3
Target 1
Target 2
Options
Target: Target 2
Threshold: Auto 0.0001
Auto Baseline
Show: Threshold — Baseline Start: Well Target Baseline End: Well Target


Amplification Plot
Plot Settings
Plot Type: ΔRn vs Cycle
Graph Type: Linear
Color: Target
Save current settings as the default
Amplification Plot
ΔRn
Cycle
Target 3
Target 1
Target 2
Options
Target: Target 3
Threshold: Auto 17.119681
Auto Baseline
Show: Threshold — Baseline Start: Well Target Baseline End: Well Target

Interpretation of Control Results

The COVID-19 Nucleic Acid RT-PCR Test Kit provides positive and negative controls to monitor the reliability of the results. All controls should be examined prior to interpreting patient results. Control materials should meet the requirements listed in the table below. If the controls are not valid, the run should be rejected.

- Negative Control (NTC): TE buffer that checks for possible contamination with target nucleic acid during extraction and PCR setup. It is used in each batch/run starting at extraction.
- Positive Control: Pseudovirus (RNA capsuled by protein) that contains the ORF1ab, E, and N gene target sequences to monitor the reliability of the results from sample extraction to PCR amplification. It is used in each batch/run.
- Internal control: Primers/probe that detects β-actin from each clinical specimen. It monitors possible PCR inhibition and/or failure of RNA extraction. It is co-detected in each clinical specimen.

Control Type	**Ct Value**		
	FAM (*ORF1ab* Gene)	**VIC (*N* Gene)**	**ROX (*β-actin* Gene)**
Positive	≤ 36	≤ 36	Undet
Negative (NTC)	Undet or >40	Undet or >40	Undet
Internal control	**N/A**	**N/A**	≤ 45

N/A: Not applicable
Undet: Undetected

Interpretation of Sample Results

After assessing the positive and negative controls, the patient sample result can be assessed. The assay reports Ct values for each individual target from which the user will need to interpret independently according to the table below.

Ct Value			**RESULT**	**ACTION**
FAM (*ORF1ab* Gene)	**VIC (*N* Gene)**	**ROX (*β-actin* Gene)**		
Undet or >40	Undet or >40	≤ 45	Negative/Not Detected	Report results to sender.
Ct ≤ 40	Ct ≤ 40	≤ 45*	Positive/Detected	Report results to sender and appropriate public health authorities.
Ct ≤ 40	Undet or >40	≤ 45*	Inconclusive	Repeat extraction and rRT-PCR once. If the repeated result remains the same, it is reported as Inconclusive and recommend recollection if clinically indicated.
Undet or >40	≤ 40	≤ 45*	Inconclusive	Repeat extraction and rRT-PCR once. If the repeated result remains the same, it is reported as Inconclusive and recommend recollection if clinically indicated.
Undet	Undet	Undet	Invalid	Repeat extraction and rRT-PCR once. If the repeated result remains invalid, consider collecting a new specimen from the patients.

* If the result for a specimen is positive, the Ct value of the internal control (ROX) is not required to be considered valid.
Undet: Undetected

Procedure using MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit and Applied Biosystems™ ABI 7500 Fast Dx Real-Time PCR system

Nucleic Acid Extraction

For RNA extraction, refer to the instructions of commercial virus RNA extraction kits. Appropriate kits include the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A42352).

Important: Positive and negative controls must be taken through the entire extraction procedure by spiking 50 µl of each control to 150 µl nucleus free water in separate containers per batch. The total volume should add up to the recommended input volume listed below.

Recommended methods, input and elution volumes are listed below:

Kits	Manufacturer	Cat. No.	Method	Input volume (µl)	Elution volume (µl)
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Applied Biosystems	A42352	Automated*	200	50

* KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head

Perform RT-PCR

Reagent and Sample Preparation

1. Thaw the PCR reaction solution at room temperature. Mix the solution for 30s and centrifuge briefly for 10s.
2. Calculate the number of reactions (n) required for the current experiment. Given that one reaction requires 19.2 µL PCR reaction solution and 0.8 µL enzyme reaction mixture, pipet the appropriate volumes into a master mixture tube.
   n = number of samples + negative control (1 serving) + positive control (1 serving) + 1 extra (overage for pipette error)
3. Dispense the master mix into reaction tubes or a 96-well PCR plates at a volume of 20 µL/well. Transfer the PCR reaction tubes to the sample preparation area and return the remaining reagents to storage at -15 °C, away from light.
4. For each well containing master mix, add 5 µL of the negative control, the positive control, or the RNA to be tested.

Example reaction plate set up (up to 94 samples):

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	PC	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
B	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22
C	Sample 23	Sample 24	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30	Sample 31	Sample 32	Sample 33	Sample 34
D	Sample 35	Sample 36	Sample 37	Sample 38	Sample 39	Sample 40	Sample 41	Sample 42	Sample 43	Sample 44	Sample 45	Sample 46
E	Sample 47	Sample 48	Sample 49	Sample 50	Sample 51	Sample 52	Sample 53	Sample 54	Sample 55	Sample 56	Sample 57	Sample 58
F	Sample 59	Sample 60	Sample 61	Sample 62	Sample 63	Sample 64	Sample 65	Sample 66	Sample 67	Sample 68	Sample 69	Sample 70
G	Sample 71	Sample 72	Sample 73	Sample 74	Sample 75	Sample 76	Sample 77	Sample 78	Sample 79	Sample 80	Sample 81	Sample 82
H	Sample 83	Sample 84	Sample 85	Sample 86	Sample 87	Sample 88	Sample 89	Sample 90	Sample 91	Sample 92	Sample 93	Sample 94

5. Cap the PCR reaction tubes or seal the 96-well PCR plate with optical adhesive film.
6. Centrifuge the PCR reaction tubes or the 96-well PCR plate briefly to collect the content at the bottom.
7. Load the PCR tubes or 96-well PCR plate to ABI 7500 Real-Time PCR system when the instrument is ready. If the reaction tubes or plate cannot be immediately loaded into the instrument, temporarily store the tubes at 2-8 °C. The tubes can be stored for up to 2 hours but should be used as soon as possible within that timeframe.

Set Up the ABI 7500 Fast Dx Real-Time PCR system

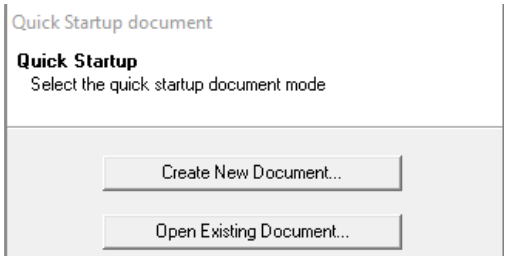
For more information about maintenance and calibration of the ABI 7500 Fast Dx Real-Time PCR system, see the instrument user's manual.

Set Up the COVID-19 RT-PCR Reaction on ABI 7500 Fast Dx System

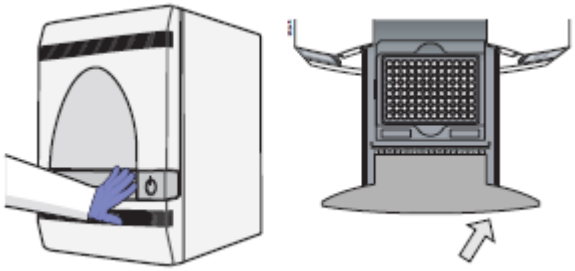
Obtain the template (SCD-COVID-19-ABI 7500 Fast Dx.sdt) from SCDChina@outlook.com and save the template to the computer connected to the ABI 7500 Fast Dx real time PCR instrument.

Running a Test on ABI 7500 Fast Dx System

1. Turn on the computer connected to the on ABI 7500 Fast Dx real time PCR instrument.
2. Turn on the ABI 7500 Dx Fast real time PCR instrument.
3. Double click the ABI 7500 Dx Fast real time PCR instrument software (v1.4.1).
4. Click "Create New Document".



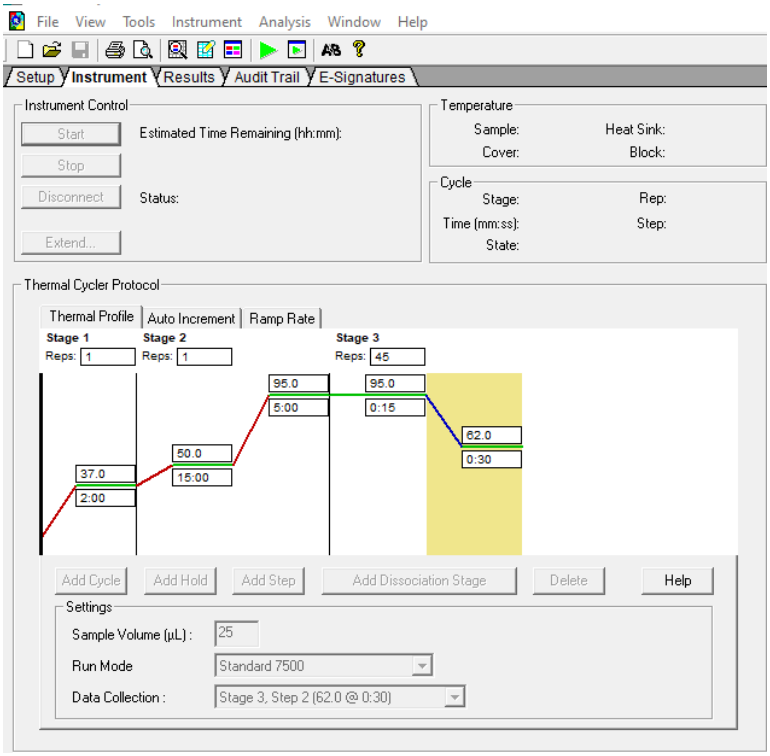
5. Navigate to select your ABI 7500 Fast Dx Run template file (SCD-COVID-19-ABI 7500 Fast Dx.sdt).
6. There will be a brief pause allowing the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument to initialize.
7. After the instrument initializes, a plate map will appear. The detectors and controls should already be labeled as they were assigned in the original template.
8. Push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door. Apply pressure to the right side of the tray and at an angle.



9. Click the Instrument tab at the upper left corner and check the thermal cycler protocol.

Steps		Temperature	Time	Cycles
1	UNG incubation	37 °C	2 min	1
2	Reverse transcription amplification	50 °C	15 min	1
3	TaqMan enzyme activation	95 °C	5 min	1
4	DNA denaturation	95 °C	15 sec	45
	Annealing, extension, and fluorescence acquisition	62 °C*	30 sec	

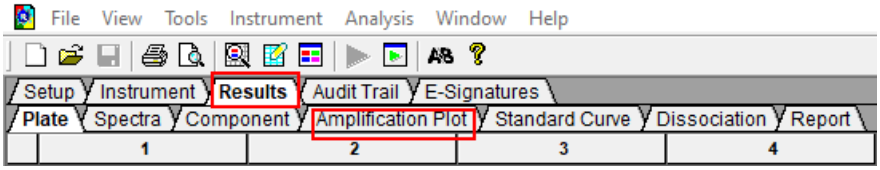
*Collect fluorescence signal during the final 62 °C step.
Sample volume (µL): 25
Run mode: Standard 7500
Data Collection: Stage 3, Step 2 (62.0 @0:30)



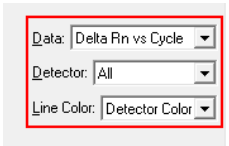
10. Click “Start” tab to initiate the RT-PCR run.
11. The run file must be saved; from the main menu, select File, then Save As. Save in appropriate run folder designation.

Data Analysis and Result Interpretation

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.

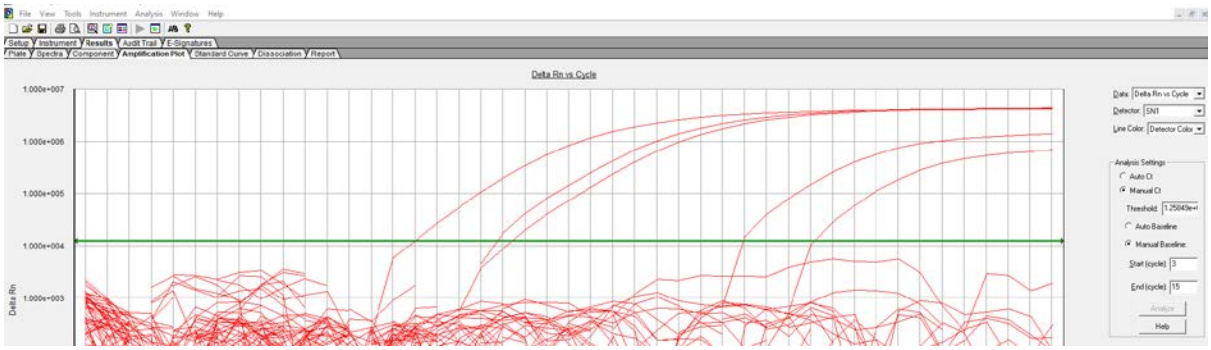


3. Highlight all samples.
4. On the right-hand side of the window, the data drop down selection should be set to **Delta Rn vs. Cycle.**
5. Chose “**Detector Color**” as the line color.

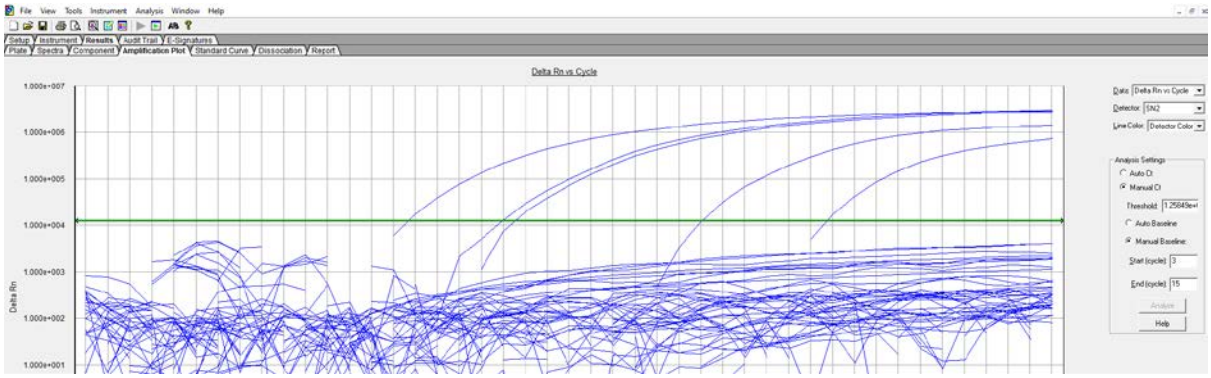


6. Using the mouse, click and drag the red threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal.
   Note: Set up the threshold above the straight line.
7. Click **Analyze** button in the lower right corner of the window. The red threshold line will turn to green, indicating the data has been analyzed.

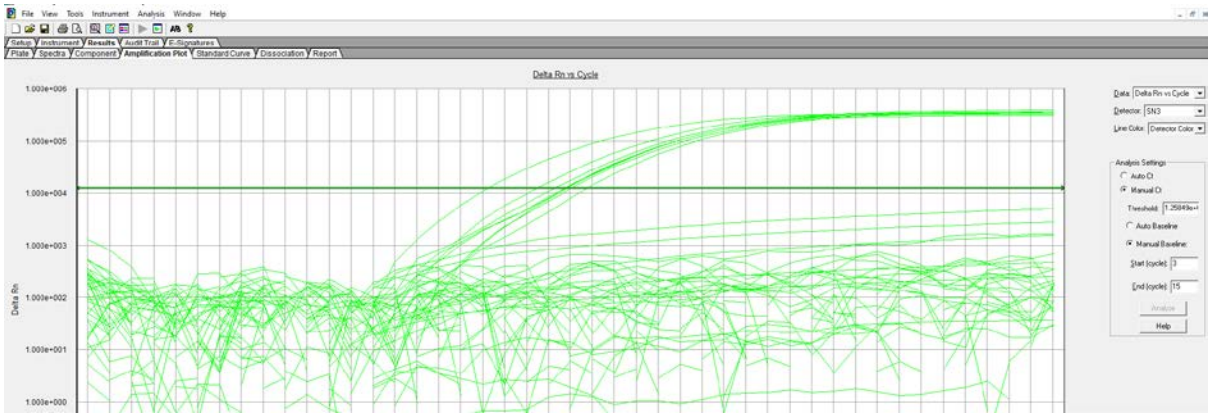
8. Select **SN1** detector.
   Visualize the threshold and adjust if needed.
   Note: if adjusted, click the **Analyze** button again.



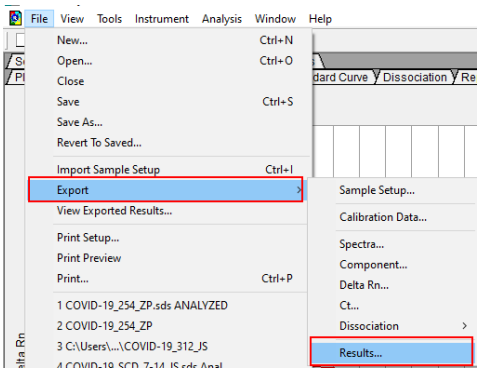
9. Select **SN2** detector.
   Visualize the threshold and adjust if needed.
   Note: if adjusted, click the **Analyze** button again.



10. Select **SN3** detector.
    Visualize the threshold and adjust if needed.
    Note: if adjusted, click the **Analyze** button again.



11. Save the analyzed file.
12. After completing analysis for each of the markers, select the **File** tab.
13. Click **Export** button the top panel.
14. Choose **Results**.



15. Save the results to the computer or other storage unit and proceed to interpretation results.

Interpretation of Control Results

The COVID-19 Nucleic Acid RT-PCR Test Kit provides control materials to monitor the reliability of the results. All controls should be examined prior to interpreting patient results. Controls should meet the requirements listed in the table below. If the controls are not valid, the run should be rejected.

Control Type	Ct Value		
	FAM (*ORF1ab* Gene)	**VIC (*N* Gene)**	**ROX (*β-actin* Gene)**
Positive	≤ 36	≤ 38	Undet or >40
Negative (NTC)	Undet or >36	Undet or >38	Undet or >40
Internal control	**N/A**	**N/A**	≤ 40

N/A: Not applicable
Undet: Undetected

Interpretation of Sample Results

After assessing the positive and negative controls, the patient sample result can be assessed. The assay reports Ct values for each individual target from which the user will need to interpret independently according to the table below.

- Negative Control (NTC): TE buffer that checks for possible contamination with target nucleic acid during extraction and PCR setup. It is used in each batch/run starting at extraction.
- Positive Control: Pseudovirus (RNA capsuled by protein) that contains the ORF1ab, E, and N gene target sequences to monitor the reliability of the results from sample extraction to PCR amplification. It is used in each batch/run.
- Internal control: Primers/probe that detects β-actin from each clinical specimen. It monitors possible PCR inhibition and/or failure of RNA extraction. It is co-detected in each clinical specimen.

Ct Value			RESULT	ACTION
FAM (*ORF1ab* Gene)	**VIC (*N* Gene)**	**ROX (*β-actin* Gene)**		
Undet or >36	Undet or >38	≤ 40	Negative/Not Detected	Report results to sender.
≤ 36	≤ 38	≤ 40*	Positive/Detected	Report results to sender and appropriate public health authorities.
≤ 36	Undet or >38	≤ 40*	Inconclusive	Repeat extraction and rRT-PCR once. If the repeated result remains the same, it is reported as Inconclusive and recommend recollection if clinically indicated.
Undet or > 36	≤ 38	≤ 40*	Inconclusive	Repeat extraction and rRT-PCR once. If the repeated result remains the same, it is reported as Inconclusive and recommend recollection if clinically indicated.
Undet	Undet	Undet	Invalid	Repeat extraction and rRT-PCR once. If the repeated result remains invalid, consider collecting a new specimen from the patients.

* If the result for a specimen is positive, the Ct value of the internal control (ROX) is not required to be considered valid.
Undet: Undetected

Limitations

- This assay is for *in vitro* diagnostic use under the FDA Emergency Use Authorization (EUA). Testing is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
- The performance of the COVID-19 Nucleic Acid RT-PCR Test Kit was established using nasopharyngeal swab samples. Mid-turbinate, nasopharyngeal and oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens are also considered acceptable specimen types for use with the COVID-19 Nucleic Acid RT-PCR Test Kit but performance has not been established.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic, immunosuppressant drugs or cold medications have not been evaluated.
- Specimens must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- 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 processing systems have not been validated.
- False-positive results may arise from:
  a) Cross contamination during specimen handling, preparation, nucleic acid extraction, PCR assay set-up or product handling
  b) Cross contamination between patient samples

c) Specimen mix-up
d) Failure to follow instructions for use

- False-negative results may arise from:
  a) Improper collection, transport or handling of specimens
  b) Insufficient RNA input
  c) Failed RNA extraction
  d) Specimen mix-up
  e) The presence of amplification inhibitors in specimen
  f) Mutation of SARS-CoV-2 in the test target region
  g) Failure to follow instructions for use

Conditions of Authorization

The COVID-19 Nucleic Acid RT-PCR Test 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 FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the COVID-19 Nucleic Acid RT-PCR Test Kit, the relevant Conditions of Authorization are listed below.

- Authorized laboratories[b] using the COVID-19 Nucleic Acid RT-PCR Test Kit will include with test result reports, 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 COVID-19 Nucleic Acid RT-PCR Test Kit will use the test as outlined in the authorized labeling. 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 the COVID-19 Nucleic Acid RT-PCR Test Kit are not permitted.
- Authorized laboratories that receive the COVID-19 Nucleic Acid RT-PCR Test Kit will notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the COVID-19 Nucleic Acid RT-PCR Test 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 COVID-19 Nucleic Acid RT-PCR Test Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and ZhuHai Sinochips Bioscience Co., Ltd (Website: http://www.sinochips.cn; Customer/Technical Support: scdchina@outlook.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the COVID-19 Nucleic Acid RT-PCR Test Kit of which they become aware.

- All laboratory personnel using the COVID-19 Nucleic Acid RT-PCR Test Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your test in accordance with the authorized labeling.
- ZhuHai Sinochips Bioscience Co., Ltd, its authorized distributor(s) and authorized laboratories using the COVID-19 Nucleic Acid RT-PCR Test 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.

[b] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Performance Characteristics

Limit of Detection (Analytical Sensitivity)

LoD Evaluation using the QIAamp Viral RNA Mini Kit and Applied Biosystems™ ABI 7500 Real-Time PCR system

The LoD is defined from this study as the lowest concentration of SARS-CoV-2 that can be reliably detected at least 95% of the time. The preliminary LoD of the COVID-19 Nucleic Acid RT-PCR Test Kit was determined using the pseudovirus (RNA capsuled by protein, contains the ORF1ab, E, and N gene target sequences) obtained from Fubio (Suzhou) Biotechnology Co., Ltd, (Cat. #: FNV-2019-nCOV-abII-EMN). The pseudovirus was spiked into negative nasopharyngeal swab specimens and diluted 10-fold across the range of 20 to 0.02 copies/µl in twenty replicates. The spiked specimens were extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Cat #52906 or 52904). For all extractions, 140 µL of spiked specimen input was eluted into 60 µL. The eluate (5 µl) was evaluated with the COVID-19 Nucleic Acid RT-PCR Test on Applied Biosystems™ ABI 7500 Real-Time PCR system with 7500 Software v2.3. The lowest copy to yield ORF1ab and N detections was 2 copies/µl (**Table 2)** which was set as the preliminary LoD.

Table 2. Preliminary LOD Summary Results using the QIAamp Viral RNA Mini Kit and the Applied Biosystems™ ABI 7500 Real-Time PCR system

Target Level	Valid tested replicates	SARS-CoV-2 ORF1ab Positive			SARS-CoV-2 N Positive			Internal Control β-actin Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
20 cp/µL	20	20	28.30	100%	20	29.81	100%	20	29.97	100%
2 cp/µL	20	20	32.10	100%	19	32.72	95%	20	30.25	100%
0.2 cp/µL	20	18	35.78	90%	7	36.34	35%	20	30.22	100%
0.02 cp/µL	20	10	36.98	50%	3	38.54	15%	20	29.34	100%

To confirm the preliminary LoD, pseudovirus (RNA capsuled by protein) obtained from Fubio (Suzhou) Biotechnology Co., Ltd, (Cat. #: FNV-2019-nCOV-abII-EMN) was spiked into negative nasopharyngeal swab specimens in 2-fold dilutions across the range of 2 to 0.5 copies/µl in twenty replicates. The spiked specimens were extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Cat #52906 or 52904). For all extractions, 140 µL of spiked specimen

input was eluted into 60 µL. The eluate (5 µl) was evaluated with the COVID-19 Nucleic Acid RT-PCR Test on Applied Biosystems™ ABI 7500 Real-Time PCR system with 7500 Software v2.3. The LoD for this testing method was confirmed at 2 copies/µl (19/20 replicates detected the N gene, 20/20 replicates detected the ORF1ab gene) for the Applied Biosystems™ ABI 7500 Real-Time PCR system with 7500 Software v2.3 (**Table 3**).

Table 3: Confirmatory LOD Summary Results using the QIAamp® Viral RNA Mini Kit and the Applied Biosystems™ ABI 7500 Real-Time PCR system

Target Level	Valid tested replicates	SARS-CoV-2 ORF1ab Positive			SARS-CoV-2 N Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
0.5 cp/µl	20	18	35.04	90%	18	34.71	90%
1 cp/µl	20	20	32.95	100%	18	34.72	90%
2 cp/µl	20	20	33.94	100%	19	34.45	95%

LoD Evaluation using the MagMax Viral/Pathogen Nucleic Acid Isolation kit and the KingFisher Flex system with the Applied Biosystems™ ABI 7500 Fast Dx Real-Time PCR system

The LoD is defined from this study as the lowest concentration of SARS-CoV-2 that can be reliably detected at least 95% of the time. The preliminary LoD of the COVID-19 Nucleic Acid RT-PCR Test Kit was determined using the pseudovirus (RNA capsuled by protein, contains the ORF1ab, E, and N gene target sequences) obtained from Fubio (Suzhou) Biotechnology Co., Ltd, (Cat. #: FNV-2019-nCOV-abII-EMN). The pseudovirus was spiked into negative nasopharyngeal swab specimens with 5 replicates at 1.25 copies/µl, 2.5 copies/µl, and 5 copies/µl. The specimens were extracted using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems, Cat A42352) and the KingFisher Flex system (Thermo Scientific). For all extractions, 200 µL of specimen input was eluted into 50 µL using the MVP_2Wash_200_Flex protocol. The eluate (5 µl) was evaluated with the COVID-19 Nucleic Acid RT-PCR Test on Applied Biosystems™ ABI 7500 Fast Dx Real-Time PCR system with SDS Software v1.4.1. The preliminary LoD for this testing method was confirmed at 2.5 copies/µl (5/5 replicates were positive, **Table 4**).

Table 4. Preliminary LOD Summary Results using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and the KingFisher Flex system with the Applied Biosystems™ ABI 7500 Fast Dx Real-Time PCR system

Target Level	Valid tested replicates	SARS-CoV-2 ORF1ab Positive			SARS-CoV-2 N Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
5 cp/µL	5	5	32.53	100%	5	35.77	100%
2.5 cp/µL	5	5	34.72	100%	5	37.24	100%
1.25 cp/µL	5	0	37.56	0%	0	N/A	0%

To confirm the preliminary LoD, pseudovirus (RNA capsuled by protein) obtained from Fubio (Suzhou) Biotechnology Co., Ltd, (Cat. #: FNV-2019-nCOV-abII-EMN) was spiked into negative nasopharyngeal swab specimens at 2.5 copies/ µl in twenty replicates using MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (KingFisher Flex) on Applied

Biosystems™ ABI 7500 Fast Dx Real-Time PCR system. Result is listed in **Table 5**.

Table 5. Confirmatory LOD Summary Results using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and the KingFisher Flex system with the Applied Biosystems™ ABI 7500 Fast Dx Real-Time PCR system

Target Level	Valid tested replicates	SARS-CoV-2 ORF1ab Positive			SARS-CoV-2 N Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
2.5 cp/µl	20	20	34.84	100%	19	36.75	95%

The entire LoD study is summarized in **Table 6**.

Table 6. Limit of detection confirmation testing summary

Instrument	Extraction Kit	Extraction Method/Instrument	Concentration (copies/µl) *	Detected rate ORF1ab gene	Detected rate N gene
ABI 7500	QIAamp® Viral RNA Mini Kit	Manual	2	20/20	19/20
ABI 7500 Fast DX	MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	KingFisher Flex/Automated	2.5	20/20	19/20

Inclusivity (Analytical Sensitivity)

Inclusivity was demonstrated by analyzing the sequence of each of the primers and probes for homology with the Betacoronavirus GenBank available in NCBI (last updated on 8/1/2020, number of sequences: 15,530), by *in silico* analysis using NCBI Nucleotide BLAST (BLASTn) alignment tool.

For all primers and probes, there was 100% homology with the vast majority of COVID-19 strains (100% for N-gene and 99% for ORF1ab, **Table 7**). A single mismatch (99% homology) with the ORF1ab reverse primer for the ORF1ab region is not located at the critical position of the primer and is not expected to affect test performance.

Table 7. *In silico* Inclusivity Analysis

Characteristic	ORF1ab region	N-gene
Total Primer Length (nt)	99	64
Total # of Strains Evaluated	15,178	13,553
100% Match	14,986	13,553
1 Mismatch	192	0
2 Mismatch	0	0
>2 Mismatch	0	0

Cross-Reactivity

The primer and probe sequences of the kit were used in a BLASTn analysis with the nucleic acid sequences of 31 normal and pathogenic organisms associated with the respiratory tract using the NCBI database. The cross-reactivity study results are listed in **Table 8**.

Table 8. *In silico* cross-reactivity analysis

#	Pathogen	Accession	ORF1ab Result			
			ORF1ab-F	ORF1ab-R	ORF1ab-P1	ORF1ab-P2
1	Human coronavirus 229E	NC_002645.1	59.09%	69.23%	76.92%	72.00%
2	Human coronavirus OC43	NC_006213.1	81.82%	76.92%	76.92%	72.00%
3	SARS-coronavirus	NC_004718.3	63.64%	50.00%	50.00%	64.00%
4	Human coronavirus HKU1	NC_006577.2	86.36%	76.92%	53.85%	56.00%
5	MERS-coronavirus	NC_019843.3	59.09%	84.62%	53.85%	68.00%
6	Human coronavirus NL63	NC_005831.2	45.45%	65.38%	46.15%	52.00%
7	Adenovirus	J01917.1	81.82%	46.15%	50.00%	60.00%
8	Human Metapneumovirus (hMPV)	NC_039199.1	63.64%	57.69%	50.00%	52.00%
9	Parainfluenza virus 1	D01070.1	68.18%	61.54%	46.15%	56.00%
10	Parainfluenza virus 2	DQ072589.1	54.55%	50.00%	50.00%	72.00%
11	Parainfluenza virus 3	D10025.1	45.45%	46.15%	50.00%	60.00%
12	Parainfluenza virus 4	JN651406.1	50.00%	50.00%	46.15%	52.00%
13	Influenza A (IAV)	KT388711.1	40.91%	50.00%	53.85%	48.00%
14	Influenza B (IBV)	MK459627.1	54.55%	69.23%	42.31%	64.00%
15	Influenza C	NC_006310.2	55.00%	46.15%	46.15%	60.00%
16	Enterovirus	JF896312.1	59.09%	53.85%	61.54%	64.00%
17	Respiratory syncytial virus	BD081932.1	54.55%	53.85%	61.54%	60.00%
18	*Chlamydia pneumoniae*	FR747827.1	63.64%	61.54%	57.69%	60.00%
19	Rhinovirus	FJ869955.1	40.91%	53.85%	53.85%	56.00%
20	*Haemophilus influenzae*	JUZZ01000002.1	68.18%	80.77%	65.38%	60.00%
21	*Legionella pneumophila*	RBGB01000022.1	68.18%	65.38%	61.54%	60.00%
22	*Mycobacterium tuberculosis*	CAACAK010000030.1	63.64%	61.54%	73.08%	60.00%
23	*Streptococcus pneumoniae*	FR671403.1	68.18%	61.54%	65.38%	60.00%
24	*Streptococcus pyogenes*	NZ_JULO01000040.1	72.73%	53.85%	57.69%	60.00%
25	*Bordetella pertussis*	CP011448.1	68.18%	46.15%	73.08%	68.00%
26	*Mycoplasma pneumoniae*	KQ758408.1	59.09%	73.08%	65.38%	52.00%
27	*Pneumocystis jirovecii (PJP)*	HAAA01000003.1	59.09%	73.08%	69.23%	52.00%
28	*Candida albicans*	BD267550.1	50.00%	53.85%	50.00%	64.00%
29	*Pseudomonas aeruginosa*	AF440524.1	63.64%	69.23%	73.08%	68.00%
30	*Staphylococcus epidermis*	MT125873.1	72.73%	57.69%	57.69%	64.00%
31	*Streptococcus salivarius*	NZ_JWGR01000139.1	72.73%	53.85%	61.54%	60.00%

#	Name of pathogens	Accession	N gene Result		
			N-F	N-R	N-P
1	Human coronavirus 229E	NC_002645.1	63.16%	65.00%	64.00%
2	Human coronavirus OC43	NC_006213.1	63.16%	65.00%	64.00%
3	SARS-coronavirus	NC_004718.3	63.16%	55.00%	72.00%
4	Human coronavirus HKU1	NC_006577.2	63.16%	65.00%	64.00%
5	MERS-coronavirus	NC_019843.3	84.21%	70.00%	72.00%
6	Human coronavirus NL63	NC_005831.2	68.42%	60.00%	56.00%
7	Adenovirus	J01917.1	68.42%	70.00%	56.00%
8	Human Metapneumovirus (hMPV)	NC_039199.1	52.63%	60.00%	52.00%
9	Parainfluenza virus 1	D01070.1	63.16%	50.00%	56.00%
10	Parainfluenza virus 2	DQ072589.1	68.42%	50.00%	80.00%
11	Parainfluenza virus 3	D10025.1	68.42%	60.00%	52.00%
12	Parainfluenza virus 4	JN651406.1	57.89%	65.00%	56.00%
13	Influenza A(IAV)	KT388711.1	57.89%	60.00%	48.00%
14	Influenza B(IBV)	MK459627.1	68.42%	55.00%	68.00%
15	Influenza C	NC_006310.2	52.63%	65.00%	48.00%
16	Enterovirus	JF896312.1	57.89%	55.00%	72.00%
17	Respiratory syncytial virus	BD081932.1	68.42%	65.00%	60.00%
18	*Chlamydia pneumoniae*	FR747827.1	63.16%	65.00%	68.00%
19	Rhinovirus	FJ869955.1	47.37%	65.00%	60.00%
20	*Haemophilus influenzae*	JUZZ01000002.1	73.68%	65.00%	64.00%
21	*Legionella pneumophila*	RBGB01000022.1	73.68%	75.00%	68.00%
22	*Mycobacterium tuberculosis*	CAACAK010000030.1	63.16%	60.00%	64.00%
23	*Streptococcus pneumoniae*	FR671403.1	73.68%	65.00%	64.00%
24	*Streptococcus pyogenes*	NZ_JULO01000040.1	57.89%	60.00%	64.00%
25	*Bordetella pertussis*	CP011448.1	89.47%	65.00%	68.00%
26	*Mycoplasma pneumoniae*	KQ758408.1	63.16%	55.00%	60.00%
27	*Pneumocystis jirovecii (PJP)*	HAAA01000003.1	68.42%	60.00%	72.00%
28	*Candida albicans*	BD267550.1	57.89%	55.00%	56.00%
29	*Pseudomonas aeruginosa*	AF440524.1	73.68%	65.00%	64.00%
30	*Staphylococcus epidermis*	MT125873.1	52.63%	50.00%	44.00%
31	*Streptococcus salivarius*	NZ_JWGR01000139.1	57.89%	60.00%	56.00%

Among the tested organisms, *Human coronavirus HKU1* (NC_006577.2) showed homology (86.36%) for the forward primer for the ORF1ab gene. The reverse primer showed 76.92% homology, while the probes showed 53.85% and 56.00% homology. The risk of the non-specific amplification is low.

Adenovirus (J01917.1) showed an 81.82% homology for the forward primer for the ORF1ab gene. The reverse primer and probes showed low homology (46.15%, 50.00% and 60.00%), therefore the risk of the non-specific amplification is low.

Haemophilus influenzae (JUZZ01000002.1) showed an 80.77% homology for the reverse primer for the ORF1ab gene. The forward primer and probes showed low homology (68.18%, 65.38%, and 60.00%), therefore the risk of the non-specific amplification is low.

Parainfluenza virus 2 (DQ072589.1) showed an 80.00% homology for the N gene probe. The forward and reverse primers showed low homology (68.42% and 50.00%), therefore the risk of the non-specific amplification is low.

Bordetella pertussis (CP011448.1) showed an 89.47% homology for the forward primer for the N gene. The reverse primer and probe showed low homology (65.00% and 68.00%), therefore the risk of the non-specific amplification is low.

MERS-coronavirus (NC_019843.3) showed an 84.62% homology for the reverse primer for the ORF1ab gene. The forward primer and probes showed low homology for the ORF1ab gene (59.09%, 53.85% and 68.00%) therefore the risk of the non-specific amplification is low. MERS-coronavirus (NC_019843.3) showed an 81.21% homology for the forward primer for the N gene. The reverse primer and probe showed low homology (70.00% and 72.00%) therefore the risk of the non-specific amplification is low.

Cross-Reactivity Studies (wet-testing):

Fourteen microbes for microbial cross-reactivity testing were chosen to reflect related and potentially cross-reactive organisms and species, as well as those likely to co-occur in clinical samples.

Reference samples (listed in **Table 9**) were purchased from Beijing Beina Chuanglian Biotechnology Institute, China and diluted to $5X10^6$ copies/mL or 5000 copies/ul (2500X LOD) and spiked into leftover clinical nasopharyngeal swab virus transport media. The spiked specimens were extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Cat #52906 or 52904). For all extractions, 140 µL of spiked specimen input was eluted into 60 µL. The eluate (5 µl) was evaluated with the COVID-19 Nucleic Acid RT-PCR Test on Applied Biosystems™ ABI 7500 Real-Time PCR system with 7500 Software v2.3. Each of the samples was prepared with two lots and tested in triplicates for each lot. All samples tested negative for SARS-CoV-2 using the COVID-19 Nucleic Acid RT-PCR Test Kit.

Table 9. Organisms evaluated for cross-reactivity

Sample ID	Microbial	Sample ID	Microbial
S1	*Staphylococcus aureus*	**S8**	*Rubella virus*
S2	*Escherichia coli*	**S9**	*Respiratory syncytial virus*
S3	*Mycoplasma pneumoniae*	**S10**	*Human cytomegalovirus*
S4	*Chamydiae pneumoniae*	**S11**	*Hepatitis C virus*
S5	*Mycobacterium tuberculosis*	**S12**	*Human adenovirus 5*
S6	*Influenza A virus*	**S13**	*SARS-CoV*
S7	*Influenza B virus*	**S14**	*MERS-CoV*

Clinical Evaluation

A clinical evaluation of the COVID-19 Nucleic Acid RT-PCR Test Kit was performed using clinical nasopharyngeal swab specimens that were extracted using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit and the KingFisher Flex system and that were amplified/detected using the Applied Biosystems™ ABI 7500 Fast Dx Real-Time PCR system. At total of 395 nasopharyngeal specimens (310 negative and 85 positive) were evaluated with comparator results obtained from an FDA EUA test. Clinical evaluation results are summarized in **Table 10**.

Table 10. Clinical performance of the COVID-19 Nucleic Acid RT-PCR Test Kit in nasopharyngeal swab specimens

		FDA EUA Test		
		Positive Patient Specimen	Negative Patient Specimen	**Total**
COVID-19 Nucleic Acid RT-PCR Test Kit	Positive Patient Specimen	**82**	**0**	**82**
	Negative Patient Specimen	**3**	**310**	**313**
	Total (395)	**85**	**310**	

Results:

The positive and negative percent agreements between the COVID-19 Nucleic Acid RT-PCR Test Kit and FDA EUA test is:

PPA = 100% * 82/(82 + 3) = 96.5% (95% C.I. = 90.1% - 98.8%)

NPA = 100% * 310/(310 + 0) = 100% (95% C.I. = 98.8% - 100%)

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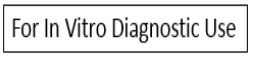
Interpretation of Symbols

	For Prescription Use Only		Caution
	Warning		Manufacturer
	Temperature Limitation		Consult Instructions for Use
	*In Vitro* Diagnostic Medical Device		Lot Number
	Use by Date		For In Vitro Diagnostic Use
	*In Vitro* Test		Keep Dry
	Biological Risks		Sufficient For

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