COVID-19 Nucleic Acid RT-PCR Test Kit

Instruction for Use

20 Tests/Kit (SC-COVID19-20)

100 Tests/Kit (SC-COVID19-100)

For use under the Emergency Use Authorization (EUA) Only

For *in vitro* diagnostic use

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Version 1

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Product Name

COVID-19 Nucleic Acid RT-PCR Test Kit

Specification

SC-COVID19-20: 20 Tests/Kit
SC-COVID19-100: 100 Tests/Kit

Intended Use

The COVID-19 Nucleic Acid RT-PCR Test Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in throat swabs, nasal swabs, nasopharyngeal, and oropharyngeal swabs specimens from individuals with signs and symptoms of infection who are suspected of COVID-19. 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, or by similarly qualified non-U.S. laboratories.

The COVID-19 Nucleic Acid RT-PCR Test Kit is intended for use by trained 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 (EUA).

Negative results do not preclude 2019-nCoV 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. Therapeutic action should not be taken based solely on these results. The final interpretation of results for clinical management of the patient is the responsibility of the managing physician.

US laboratories are required to report test results to the appropriate public health authorities.

Principles of the Procedure

The COVID-19 Nucleic Acid RT-PCR Test Kit is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The COVID-19 primer and probe set(s) is designed to detect RNA from the COVID-19 in throat swabs, nasal swabs, nasopharyngeal, and oropharyngeal swabs specimens from patients with signs and symptoms of infection who are suspected of COVID-19.

This kit uses specific primers and TaqMan probes (5' end-labeled FAM, VIC or ROX fluorescent probes) to simultaneously amplify the COVID-19 ORF1ab gene, N gene fragments, and actin gene in one tube. In the case where the COVID-19 RNA template is contained in the reaction system, a fluorescent signal is released during the PCR amplification process. The instrument is used to monitor the signal intensity of the corresponding channel in real time during the PCR process to achieve qualitative detection results.

This kit uses dUTP and uracil-DNA N-glycosylase (UNG) to minimize PCR cross-contamination from previously amplified PCR products. To avoid possible false positive results, the reaction mixture undergoes a 2 min incubation at 37 °C prior to PCR amplification, allowing UNG to fully degrade potential PCR product contamination.

The kit uses the human β-actin gene as an internal reference gene. This internal control is detected using specific primers and probes (5'end-labeled ROX fluorescent probes) in order to exclude false negative results via confirming the success of the reaction in real time.

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. The PCR reaction solution includes COVID-19 ORF1ab primers and probes, N gene primers and probes, internal control (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
PCR reaction solution (COVID-19 RT-PCR Assay Multiplex: ORF1ab, N gene, and Actin)	500 µL	1 Tube
Enzyme mix	20 µL	1 Tube
Positive control (Synthetic ORF1ab and N gene RNA)*	100 µL	1 Tube
Negative control (TE Buffer)*	100 µL	1 Tube

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

Kit Components (SC-COVID19-100)	Volume	Quantity
PCR reaction solution (COVID-19 RT-PCR Assay Multiplex: ORF1ab, N gene, and Actin)	1000 µL	2 Tubes
Enzyme mix	100 µL	1 Tube
Positive control (Synthetic ORF1ab and N gene RNA)*	200 µL	1 Tube
Negative control (TE Buffer)*	200 µL	1 Tube

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

Materials Required but Not Provided

Reagents

- QIAamp® Viral RNA Mini Kit (QIAGEN, Cat. No. 52904 or 52906)

Required Equipment and consumables

- ABI 7500 Real-Time PCR system (Software version: v2.3)
- Class II biosafety cabinet(s)
- 0.2 ml PCR strip tubes with optical caps
- Aerosol barrier pipette tips
- Disposable powder-free gloves and surgical gowns
- Ice buckets or cold blocks for 96 well PCR plates
- MicroAmp clear adhesive film
- MicroAmp optical 96-well reaction plate
- Microcentrifuge
- Micropipettes (2 µl, 10 µl, 200 µl and 1000 µl)
- Multichannel micropipettes (5-50 µl)
- Nuclease-free water
- PCR workstation
- Plate centrifuge
- Racks for 1.5 ml microcentrifuge tubes
- Vortex mixer

Optional Equipment and Consumables

- QIAcube instrument with protocols and consumables

Reagent Storage and Handling

The kit should be stored below -15 °C away from light and are stable until the expiration date stated on the label (12 months from the manufacture date). The kit should be used within one month after opening; the reagent components should not be frozen and thawed more than 3 times. Dry ice or ice packs should be used during transportation. The transportation time should not exceed 7 days. 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: handling specimens and control samples as if they are capable of transmitting infectious agents.

Specimen type:

- Upper respiratory tract specimens: These specimens include throat swabs, nasal swabs, nasopharyngeal and oropharyngeal swabs.

Specimen collection:

- Patient specimens must be collected according to appropriate laboratory guidelines.
- Follow manufacturer's instruction for collection.
- 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

FDA –

https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/coronavirus-disease-2019-covid-19-frequently-asked-questions

Specimen packaging:

- Store specimen at 2-8 ℃ for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimen at -70 ℃ 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., serum) and the date the sample was collected.
- Specimen should be packed according to International Air Transport Association (IATA) regulations. COVID-19 specimens should be packed in compliance with regulations for UN3373 Biological Substance, Category B. Personnel must be trained to pack and ship according to the regulations and in a manner that corresponds to their function-specific responsibilities.

Specimen storage:

- Specimens for virus isolation and nucleic acid detection should be tested as soon as possible.
- Specimens that can be tested within 24 hours can be stored at 4 ℃.
- Specimens that cannot be tested within 24 hours should be stored at -70 ℃ or below; if -70 ℃ storage is not available; samples can be temporarily stored at -20 ℃.
- Serum can be stored at 4 ℃ for 3 days or below -20 ℃ indefinitely.
- A designated storage space should be set up to separate potential coronavirus specimens from other specimens. Repeated freeze-thaw cycles should be avoided during specimen transport.

Specimen transport:

- Specimens should be sent to the laboratory as soon as possible after collection.
- If the specimens need to be transported over long distances, it is recommended to use dry ice for preservation.

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.

- This product should be only used for *in vitro* diagnosis under the EUA. Please read this manual carefully before use.
- 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-sources 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 (P2) 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 pollution.
- Only trained personnel proficient in handling infectious materials and the use of ABI 7500 Real-Time PCR system 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 Level II biological safety hood to protect operator safety and prevent environmental contamination.
- 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.

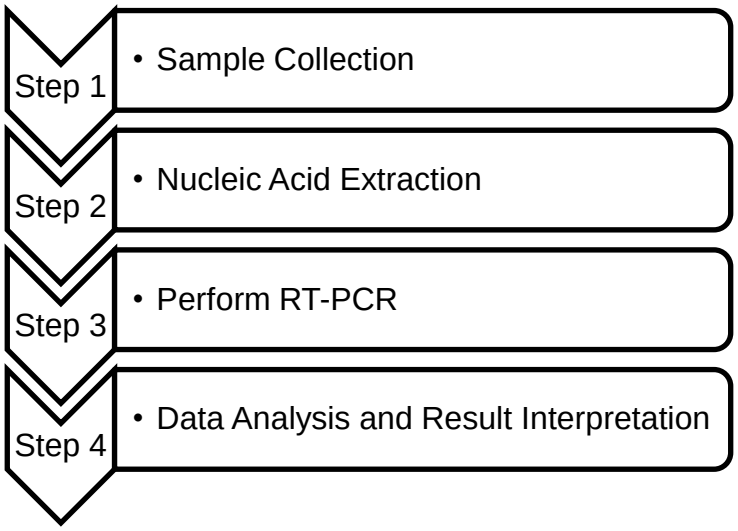
- Try to avoid the generation of air bubbles when the reaction solution is dispensed, and check whether the reaction tubes are tightly closed before going on the machine to avoid leaking and contaminating the instrument.
- After the amplification, the reaction plate is taken out, sealed in a special 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.

Procedure

Overview

The procedure consists of 4 consecutive steps:

1. Sample collection: Specimens are collected according to the manufacturer's instructions and "Specimen Handling and Storage" section on page 7.
2. Nucleic acid extraction: Extract viral RNA.
3. Perform RT-PCR: Prepare the PCR reaction for the extracted RNA and positive and negative control material. Perform the RT- PCR using the COVID-19 Nucleic Acid RT-PCR Test Kit in combination with the ABI 7500 Real-Time PCR system (Software version: v2.3).
4. Data analysis and result interpretation: The results of positive and negative controls should be evaluated to determine if the run is valid. If the run is valid, the internal control and target-specific results of each specimen are evaluated.



Nucleic Acid Extraction

If the sample collection tube does not contain a virus sample storage solution with virus inactivation function, it is optional to inactivate the samples by putting the collection tubes in a 56 °C water bath for 30 minutes prior to testing.

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 20 µl of each control to 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)
QIAamp® Viral RNA Mini Kit	Qiagen	52904 or 52906	Manual or Automated on QiaCube	140	60

Perform RT-PCR

Reagent and Sample Preparation

1. Thaw the direct-use PCR reaction solution at room temperature (~25 ° C). Mix the solution for 30s and centrifuge at 2000 rpm 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 1.5 ml microcentrifuge tube.
3. Dispense the master mix into n 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.
   n = number of samples + negative control (1 serving) + positive control (1 serving) + 1 extra (overage for pipette error)
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 Sinochips Template File

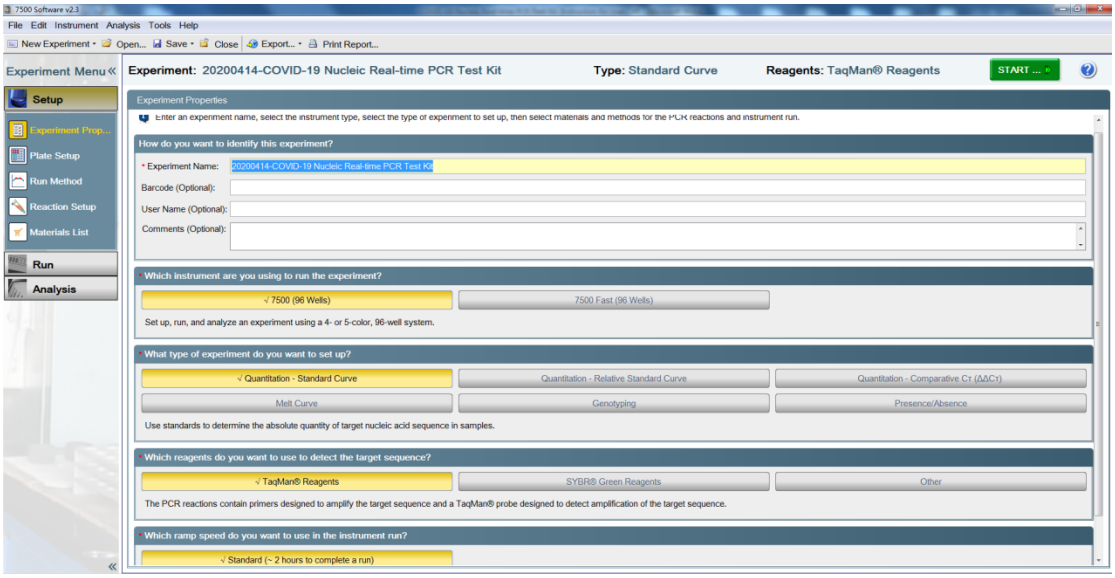
1. Turn on the computer connected to the on ABI 7500 real time PCR instrument.
2. Turn on the ABI 7500 real time PCR instrument.
3. Double click the ABI 7500 real time PCR instrument software (v2.3).
4. Click New experiments › Setup Experiment Properties.
5. Check following run settings and choose the correct settings:

   Instrument: 7500 (96 wells)

   Run type: Quantitation – Standard Curve

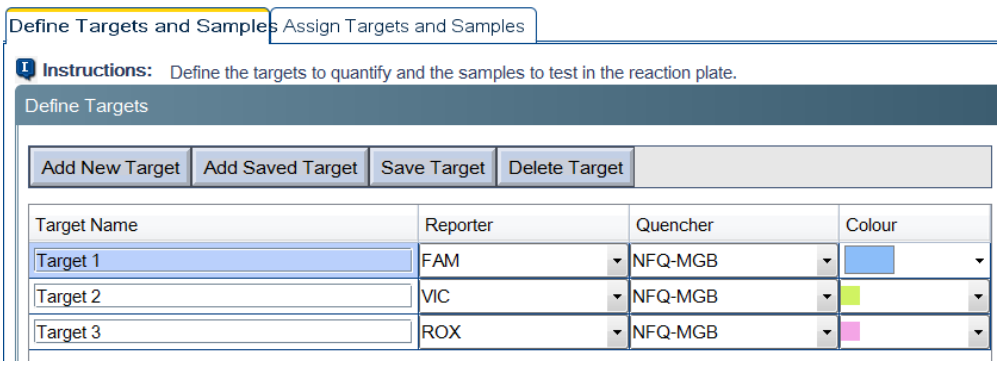
   Run reagent: TaqMan reagents

   Run mode: Standard



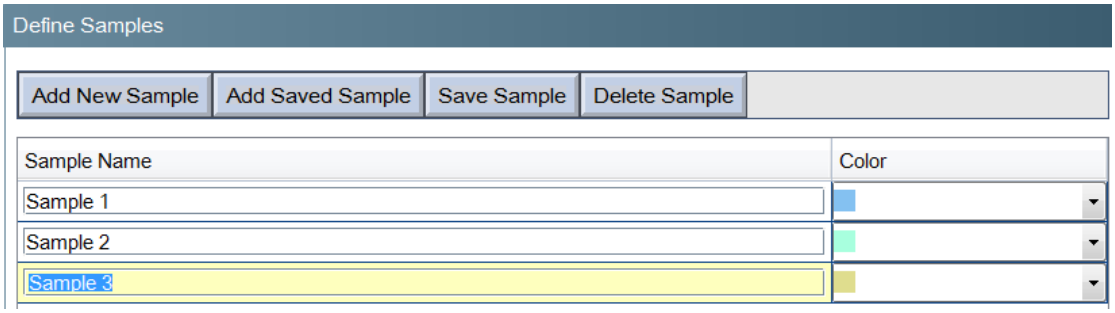
6. Define Targets and Samples.

Select › Setup the Targets and Samples in Plate Setup.



7. To add a sample, click "Add New Sample". If you need to change the name, click the name

of the sample to modify it.

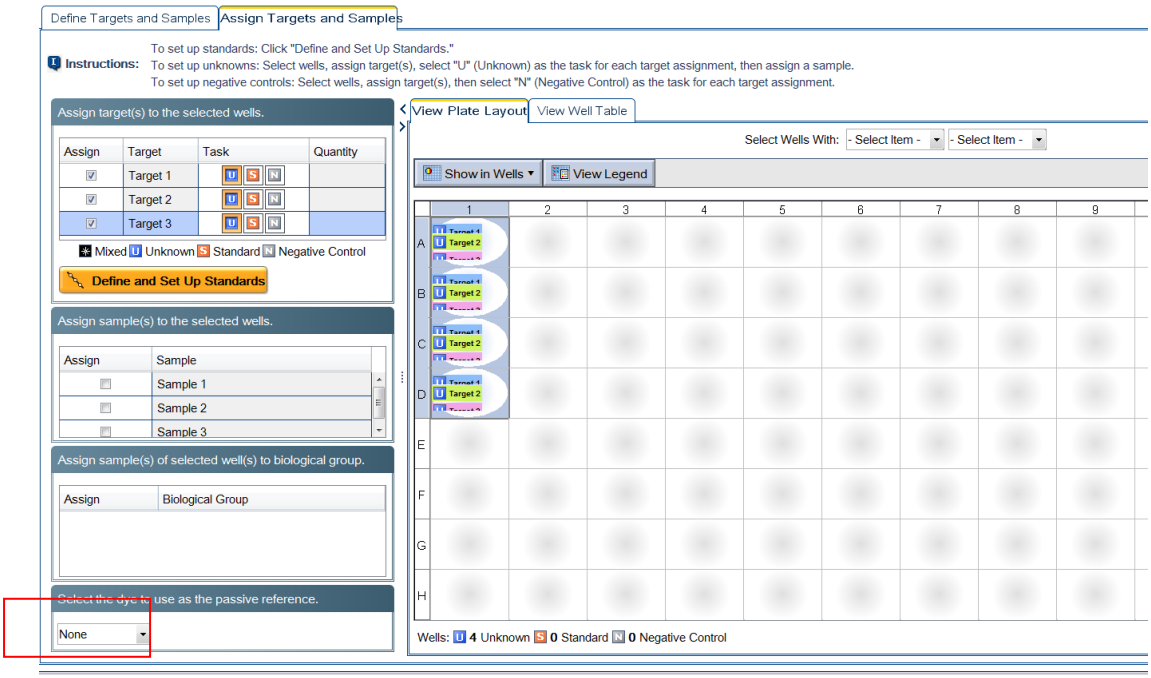


8. Set up the plate layout by assigning a unique sample name to each well.
9. Assign a task to each well:

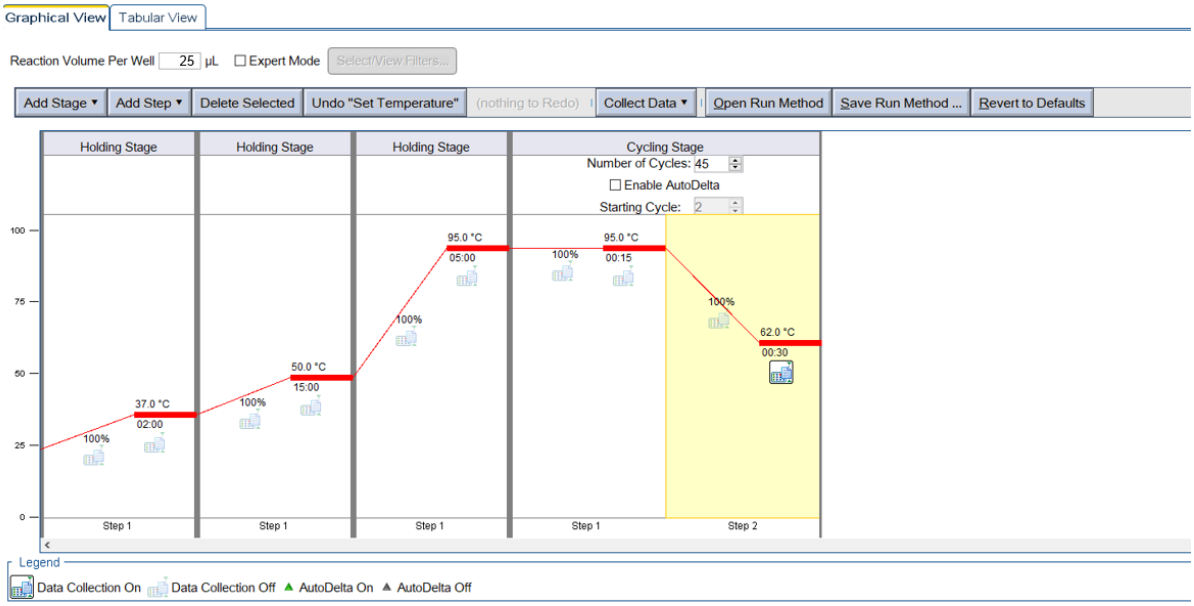
   U-Unknown: for patient samples

   S-Standard: for positive control

   N-Negative control: for negative control



10. The passive reference must be set as None.
11. Under the "Run Method" example table, click the "Graphical View" tab to set up the amplification program. Pull the bar to set the temperature.



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.

12. Set the reaction volume to 25 µL and start the reaction program.

13. Save the file as a template as “COVID-19 Sinochips”.

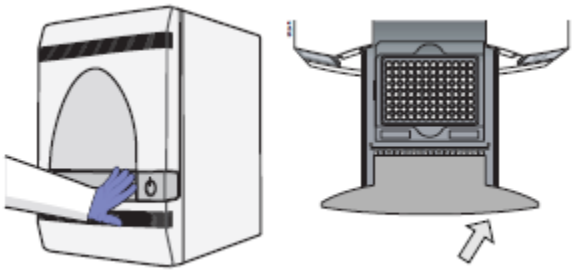
Running a Test

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).

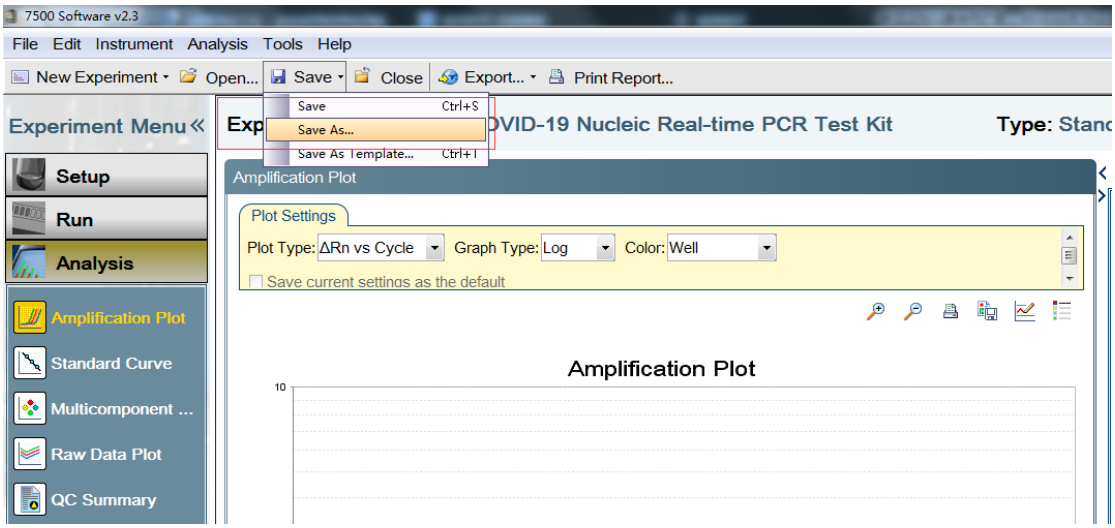
4. Push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door. Apply pressure to the right side of the tray and at an angle.



5. Use the “COVID-19 Sinochips” template file to setup the run parameters.

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

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



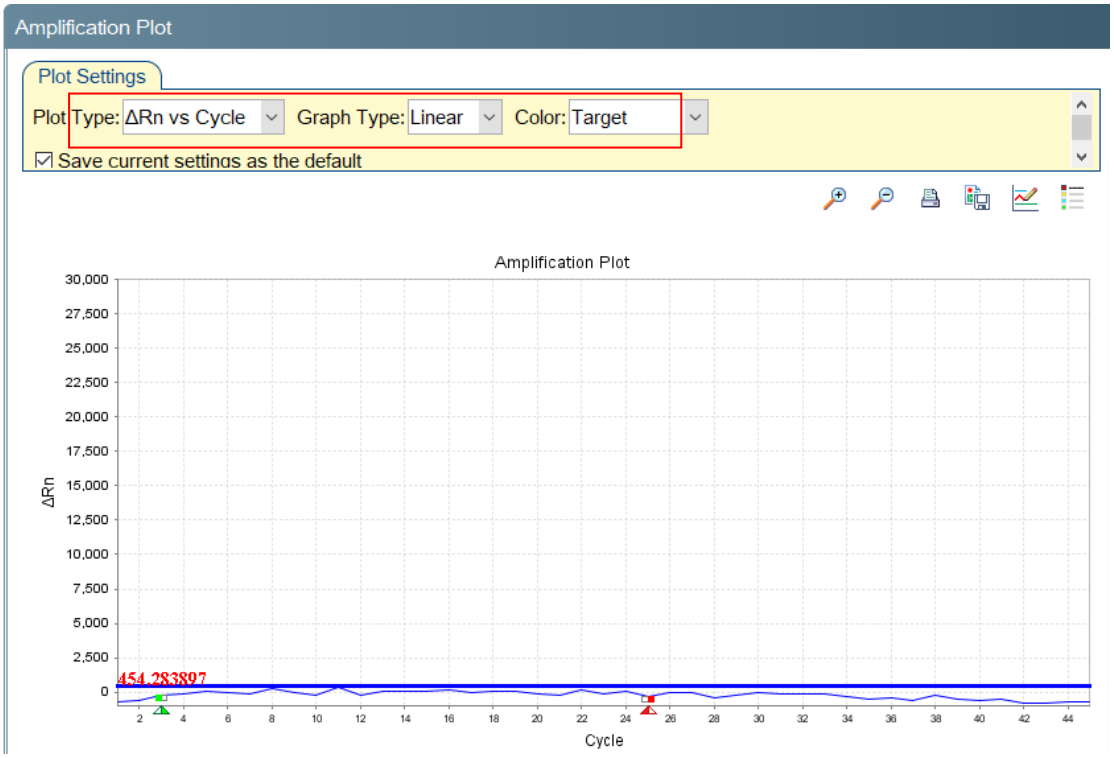
Data analysis and result interpretation

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.

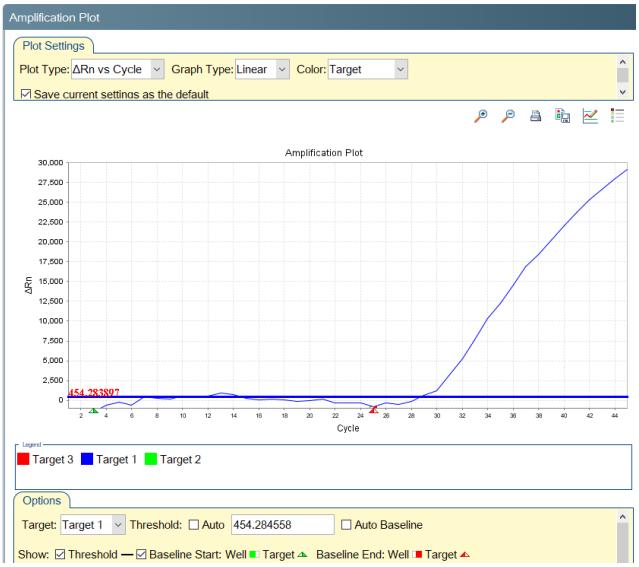


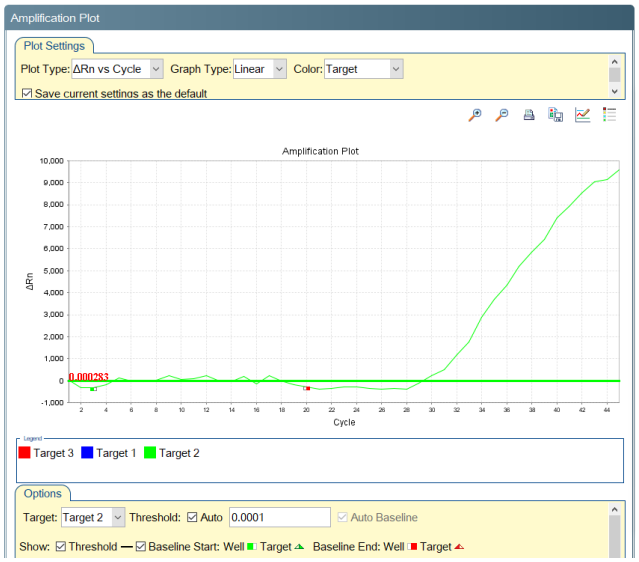
2. Set the baseline starting point at cycle 2-15 and ending at cycle 10-25.

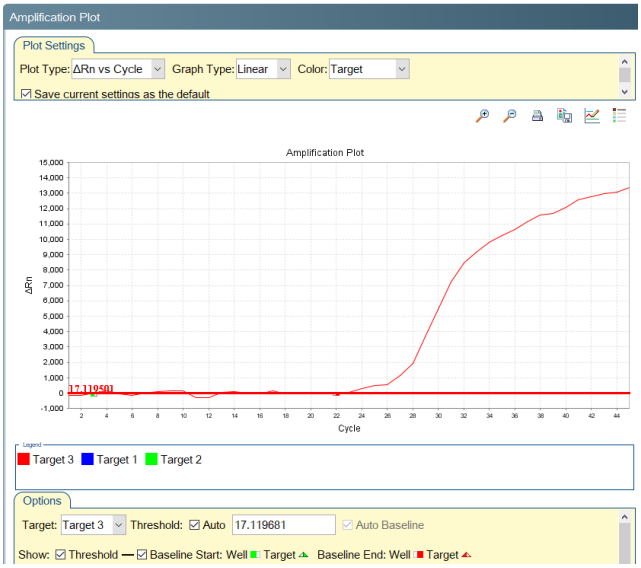
3. Threshold setting principle: The threshold line just exceeds the highest point of the negative control.

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 positive and negative 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 interpret patient results. Positive and negative control should meet the requirements listed in Table 2. If the controls are not valid, the run should be rejected.

Table 2. Expected control results.

Control Type	**Ct Value**		
	FAM (*Orf1ab* Gene)	VIC （*N* Gene)）	ROX （*β-actin* Gene）
Positive	30 ≤ Ct ≤ 36	30 ≤ Ct ≤ 36	-*
Negative	Undet	Undet	-*

* No requirements on the Ct value
Undet: Undetermined

Interpretation of sample results

After assessing the positive and negative controls, the patient sample result can be assessed. Table 3 lists the expected results for the kit with valid positive and negative controls:

Table 3. Expected results for the COVID-19 Nucleic Acid RT-PCR Test Kit.

Ct Value			**RESULT**
FAM (*Orf1ab* Gene)	VIC （*N* Gene)）	ROX （*β-actin* Gene）	
Undet	Undet	Ct ≤ 45	Negative
Ct ≤ 40	Ct ≤ 40	Ct ≤ 45*	Positive
Undet	Undet	Undet	Invalid, specimen needs to be retested.
Ct ≤ 40	40< Ct ≤45	Ct ≤ 45	Indeterminate, recommend to retest specimen, if second test gives the same result, then report as indeterminate.
40< Ct ≤45	Ct ≤ 40	Ct ≤ 45	Indeterminate, recommend to retest specimen, if second test gives the same result, then report as indeterminate.
40< Ct ≤45	40< Ct ≤45	Ct ≤ 45	Indeterminate, recommend to retest specimen, if second test gives the same result, then report as indeterminate.

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

Limitations

- The use of this assay as an *In vitro* diagnostic under the FDA EUA is limited to laboratories that are certified under the CLIA, 42 U.S.C. § 263a, to perform high complexity tests.
- 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.
- Amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure.
- False-positive results may arise from:
  a) Cross contamination during specimen handling or preparation
  b) Cross contain nation between patient samples
  c) Specimen mix-up
  d) RNA contamination during product handling
  e) Failure to follow instructions for use
- False-negative results may arise from:
  a) Degradation of COVID-19 RNA during transportation
  b) Insufficient RNA input
  c) Failed RNA extraction
  d) Specimen mix-up
  e) The presence of RT-PCR inhibitors
  f) Mutation in COVID-19 virus
  g) Failure to follow instructions for use
- Positive result cannot directly reflect the viral load in the original specimens.
- Negative results do not preclude infection with COVID-19 virus and should not be the sole basis of patient management decision.
- The limit of detection (LoD) is determined based on a 95% confidence of detection. When COVID-19 RNA presents at or above the LoD concentration in any specimen, there will be a low probability that COVID-19 testing result is negative. When COVID-19 RNA presents below the LoD concentration in any specimen, it is possible that the COVID-19 testing result is positive.

Performance characteristics

Limit of detection (Analytical Sensitivity)

Limit of Detection (LoD) studies were used to determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates tested positive. A two-phase approach was used to determine the preliminary LoD. In phase I, a recombinant virus containing COVID-19 RNA (2019-nCoV_N_Positive Control, Integrated DNA Technologies, US, Reference number: 26624238, Lot NO. 0000500326) was serially diluted into the concentration at 2E+05, 2E+04, 2E+03, 2E+02 and 2E+01 Copies/mL. The preliminary LoD was confirmed by testing 20 replicates per concentration on a PCR system (Applied Biosystems™ Real-Time PCR System 7500). The results of phase I are summarized in Table 4.

Table 4. Phase I LoD Study.

Concentration / Replicate	C_t Value									
	2E+01copies/mL		2E+02 copies/mL		2E+03 copies/mL		2E+04 copies/mL		2E+05 copies/mL	
	ORF1ab	N	ORF1ab	N	ORF1ab	N	ORF1ab	N	ORF1ab	N
1	36.94	Undet	32.89	32.59	29.48	28.87	25.18	25.65	23.42	22.79
2	Undet	Undet	32.28	32.81	29.68	29.70	25.23	24.97	23.60	22.89
3	35.92	36.24	31.88	33.64	28.79	28.28	25.14	24.74	23.40	23.29
4	34.25	Undet	31.85	33.89	30.08	28.04	25.13	25.02	22.63	21.72
5	36.15	Undet	30.93	32.62	26.89	27.77	24.54	24.24	22.66	22.24
6	Undet	38.23	29.64	33.46	28.23	27.76	23.64	24.32	22.64	21.89
7	Undet	Undet	30.99	32.56	27.60	27.59	24.71	23.99	21.97	21.55
8	32.53	Undet	31.16	31.47	27.30	27.38	23.63	24.00	22.67	21.06
9	33.07	Undet	27.07	28.76	26.57	26.47	21.68	22.42	23.61	21.55
10	35.13	Undet	31.15	32.07	27.80	26.79	22.98	23.84	22.21	20.77
11	36.26	39.59	32.31	33.38	28.97	28.48	25.41	25.76	20.85	21.19
12	35.69	38.59	32.71	32.73	29.34	28.95	25.56	25.63	22.49	21.26
13	37.83	Undet	31.68	32.67	28.15	28.78	25.62	25.06	21.85	21.38
14	34.45	34.76	31.27	31.76	29.33	28.61	24.82	24.15	21.49	20.97
15	34.84	35.02	31.12	31.58	28.22	27.17	25.85	24.99	22.41	22.01
16	34.24	30.88	31.12	31.03	26.27	25.68	25.33	25.02	21.80	19.77
17	Undet	34.32	29.85	31.19	26.63	26.96	20.98	24.45	21.53	20.89
18	Undet	33.91	29.92	31.55	26.19	27.30	23.46	23.48	21.99	21.07
19	35.76	36.44	28.89	30.97	26.66	27.65	23.31	24.40	20.79	20.30
20	32.65	35.57	30.73	30.91	26.84	27.39	23.22	24.08	20.27	19.36
% Positive	75%	55%	100%	100%	100%	100%	100%	100%	100%	100%

Undet=Undetermined

As shown in Table 4 the lowest concentration level with observed positive rates ≥ 95% was 200 virus copies/mL. In phase II, a lower concentration based on 200 copies/mL (3-fold dilution, 200/3 copies/mL and 200/6 copies/mL) were tested to perform a refined tentative LoD

study. The results of phase II are summarized in Table 2.

Table 5. LoD Determination Using Recombinant Virus Containing COVID-19

Concentration / Replicate	C_t Value					
	22.2 copies/mL		66.7 copies/mL		200 copies/mL	
	ORF1ab	N	ORF1ab	N	ORF1ab	N
1	Undet	Undet	33.79	33.26	36.44	32.89
2	37.42	Undet	33.91	33.59	35.92	32.57
3	35.15	Undet	33.41	32.51	35.66	32.54
4	Undet	36.33	33.00	33.34	35.53	32.18
5	33.98	Undet	34.16	33.20	34.36	32.30
6	34.68	33.81	33.00	33.80	33.99	32.02
7	29.88	33.12	33.89	33.51	34.03	32.12
8	35.42	33.37	33.75	31.86	33.75	31.89
9	35.42	34.07	32.31	31.93	33.26	31.36
10	35.69	35.76	32.55	32.22	32.44	30.73
11	33.05	Undet	31.76	32.23	34.64	31.95
12	33.27	Undet	31.32	Undet	35.09	32.40
13	34.72	35.54	32.21	30.32	36.15	33.04
14	33.07	33.36	32.02	Undet	34.94	32.25
15	33.72	Undet	32.15	32.11	34.12	32.33
16	33.55	Undet	33.52	39.68	33.69	31.81
17	38.73	32.16	29.70	31.00	33.20	31.41
18	35.77	35.08	29.70	33.16	33.73	31.81
19	36.68	Undet	31.04	33.41	32.54	30.75
20	Undet	33.15	27.88	31.70	32.16	30.34
% Positive	85%	55%	100%	90%	100%	100%

Undet=Undetermined

As shown in Table 5，The lowest concentration level with observed positive rates ≥ 95% was 200 virus copies/mL. Considering the complexity of clinical specimens, the preliminary LoD of the kit was set at 200 copies/mL.

To confirm the LoD, COVID-19 virus samples were spiked into negative patient virus transport media at the final concentration of 22.2, 66.7, and 200 copies/mL. RNA isolated using the Qiagen QIAamp® Viral RNA Mini Kit is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems 7500 Real-Time PCR instrument with software version 2.3.

Table 6. LoD Determination.

Concentration / Replicate	C_t Value								
	22.2 copies/mL			66.7 copies/mL			200 copies/mL		
	ORF1ab (FAM)	N (VIC)	Actin (ROX)	ORF1ab (FAM)	N (VIC)	Actin (ROX)	ORF1ab (FAM)	N (VIC)	Actin (ROX)
1	36.35	34.36	33.02	35.95	36.23	33.25	33.71	34.11	32.21
2	44.28	Undet	33.23	37.19	34.64	33.21	34.60	36.85	32.74
3	37.46	36.89	33.81	35.38	34.09	33.07	34.76	36.19	33.15
4	43.05	Undet	34.22	35.52	Undet	34.30	32.98	33.23	32.99
5	35.02	Undet	34.15	37.21	Undet	33.85	34.36	34.20	33.23
6	37.75	Undet	34.19	37.90	Undet	33.50	33.38	33.81	33.03
7	41.00	Undet	3.72	Undet	34.13	33.41	34.80	33.38	32.76
8	36.68	Undet	33.00	36.90	33.49	32.72	33.07	32.93	32.54
9	35.64	Undet	32.44	Undet	36.15	31.95	32.26	32.23	32.09
10	35.86	Undet	32.46	34.33	35.29	32.17	35.52	31.52	31.39
11	36.10	Undet	32.51	34.57	38.79	32.47	34.34	33.61	32.24
12	38.02	37.82	33.2	36.84	36.27	32.89	34.39	33.45	32.42
13	37.68	35.93	33.85	Undet	34.40	33.21	34.70	35.90	33.10
14	Undet	17.87	34.5	34.72	34.91	33.35	33.38	32.68	32.81
15	Undet	Undet	34.75	35.52	36.62	33.33	34.19	35.10	33.40
16	37.64	36.01	3418	36.33	33.77	33.25	35.40	33.82	33.03
17	Undet	Undet	33.73	35.56	35.61	33.29	31.16	32.31	32.51
18	35.85	Undet	3.99	35.17	35.16	32.85	33.37	32.01	32.36
19	35.32	Undet	32.17	36.37	Undet	31.91	34.39	33.20	32.09
20	36.38	36.38	31.58	33.50	Undet	31.91	33.00	32.15	31.45
% Positive	85%	35%	100%	85%	75%	100%	100%	100%	100%

As shown in Table 6， The lowest concentration level with observed positive rates ≥ 95% was 200 virus copies/mL. The LoD of the kit was set at 200 copies/mL.

Reactivity/Inclusivity

The 2019 novel coronavirus (COVID-19) belongs to the beta gene of the Corona virus family, which is a single-stranded positive-strand RNA virus. Primers and probes were designed against an open reading frame (ORF1ab) and nucleoprotein (N). The primers and probes were designed using the NCBI COVID-19 virus sequence (MN908947.1) as reference. BLASTn analysis queries alignments were performed with the COVID-19 ORF1ab and N oligonucleotide primer and probe sequences with all publicly available nucleic acid sequences for COVID-19 in GenBank demonstrate the predicted reactivity/inclusivity of the COVID-19 Nucleic Acid RT-PCR Test Kit.

All the alignments show 100% identity to the available COVID-19 sequences.

Cross-reactivity

Nineteen types of organisms (see Table 7 and 8) were used for cross-reactivity analysis and evaluation of the COVID-19 Nucleic Acid RT-PCR Test Kit. Among them, the SA, *E.* coli, *Chlamydia pneumoniae* , *Mycobacterium tuberculosis,* IAV, IBV, and Parainfluenza virus were tested with the kit for cross-reaction detection. The results showed that these pathogens were negative, indicating that there was no cross-reaction between the twenty-two pathogens and the COVID-19 nucleic kit.

Table 7. In silico cross-reactivity analysis (ORFab).

#	Pathogen	Accession	ORF Result			
			ORFab Forward	ORFab Reverse	ORFab Probe 1	ORFab Probe 2
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 (e.g. C1 Ad. 71)	J01917.1	81.82%	46.15%	50.00%	60.00%
8	*Human Metapneumo-virus* (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%

#	Pathogen	Accession	ORF Result			
			ORFab Forward	ORFab Reverse	ORFab Probe 1	ORFab Probe 2
15	*Influenza* C	NC_006310.2	55.00%	46.15%	46.15%	60.00%
16	Enterovirus (e.g. EV68)	JF896312.1	59.09%	53.85%	61.54%	64.00%
17	Respiratorysyncytial 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%

Table 8. In silico cross-reactivity analysis (N).

#	Name of pathogens	Accession	N gene Result		
			N gene Forward	N gene Reverse	N gene Probe
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 (e.g. C1 Ad. 71)	J01917.1	68.42%	70.00%	56.00%
8	*Human Metapneumo-virus* (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 (e.g. EV68)	JF896312.1	57.89%	55.00%	72.00%
17	Respiratorysyncytial 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%

The primer and probe sequences of the kit were used to blast with the nucleic acid sequences of 19 pathogens. The ORF-R1 reverse primer has sequence homology close to 80% with MERS-coronavirus, but the probe sequence homology are far less than 80%, so there is no nonspecific amplification. The ORF-F1 primer has close to 80% homology with Human coronavirus HKU1, and its reverse primer and probe homology are 66.7% and 64.3%, respectively. Alignment of primer probe sequences (forward primers, reverse primers and

probes) of other pathogens shows that the homology is less than 80%. Therefore, sequence alignment indicates that there is no non-specific amplification when amplified with one or several of the 19 organisms.

Clinical evaluation

The clinical evaluation phase I was performed using 7 positive (P1-P7) and 6 negative (N1-N6) reference specimens. The results are summarized in Table 9.

Table 9 Test results of the company-made reference product.

Result / Specimen ID	LOT：01200204			Result / Specimen ID	LOT:01200205		
	FAM	VIC	Result		FAM	VIC	Result
P1	33.07	33.90	+	P1	32.94	33.95	+
P2	32.52	33.94	+	P2	32.73	33.99	+
P3	29.97	31.18	+	P3	29.53	31.19	+
P4	30.06	31.40	+	P4	29.21	31.04	+
P5	28.06	29.60	+	P5	28.55	29.61	+
P6	28.56	29.94	+	P6	28.39	29.54	+
P7	24.09	25.97	+	P7	24.43	25.83	+
N1	Undet	Undet	-	N1	Undet	Undet	-
N2	Undet	Undet	-	N2	Undet	Undet	-
N3	Undet	Undet	-	N3	Undet	Undet	-
N4	Undet	Undet	-	N4	Undet	Undet	-
N5	Undet	Undet	-	N5	Undet	Undet	-
N6	Undet	Undet	-	N6	Undet	Undet	-
Coincidence rate	100%			Coincidence rate	100%		

Undet=Undetermined

Phase II clinical was performed using 81 patient specimens. The positive and negative clinical specimens were tested as blinded specimens. The results are summarized in Table 10 and demonstrated a Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of 100%.

Table 10 Test results of reagents on 81 clinical samples.

Sample #	FAM	VIC	ROX	Expected Result	Result	Sample #	FAM	VIC	ROX	Expected Result	Result
1	37.19	35.19	37.47	+	+	41	Undet	Undet	33.28	-	-
2	35.6	34.63	38.42	+	+	42	Undet	Undet	33.75	-	-
3	35.64	35.27	35.9	+	+	43	Undet	Undet	38.51	-	-
4	35.09	34.72	37.7	+	+	44	Undet	Undet	34.84	-	-
5	36.4	34.09	39.02	+	+	45	Undet	Undet	34.78	-	-
6	31.59	29.18	34.2	+	+	46	Undet	Undet	33.47	-	-
7	33.28	31.77	36.15	+	+	47	Undet	Undet	33.49	-	-
8	29.83	27.46	32.04	+	+	48	Undet	Undet	33.58	-	-
9	34.44	35.59	31.54	+	+	49	Undet	Undet	35.16	-	-
10	36.33	34.31	32.85	+	+	50	Undet	Undet	35.25	-	-
11	37.75	34.47	29.27	+	+	51	Undet	Undet	34.27	-	-
12	38.2	30.07	26.35	+	+	52	Undet	Undet	33.21	-	-
13	35.16	32.92	25.98	+	+	53	Undet	Undet	34.51	-	-
14	33.18	29.59	25.1	+	+	54	Undet	Undet	34.57	-	-
15	37.38	32.03	26.5	+	+	55	Undet	Undet	35.7	-	-
16	Undet	Undet	30.47	-	-	56	Undet	Undet	35.49	-	-
17	Undet	Undet	30.35	-	-	57	Undet	Undet	34.26	-	-
18	Undet	Undet	30.94	-	-	58	Undet	Undet	34	-	-
19	Undet	Undet	29.02	-	-	59	Undet	Undet	34.54	-	-
20	Undet	Undet	28.6	-	-	60	Undet	Undet	34.31	-	-
21	Undet	Undet	29.57	-	-	61	Undet	Undet	35.71	-	-
22	Undet	Undet	27.98	-	-	62	Undet	Undet	35.21	-	-
23	Undet	Undet	30.9	-	-	63	Undet	Undet	35.29	-	-
24	Undet	Undet	28.77	-	-	64	Undet	Undet	33.9	-	-
25	Undet	Undet	29.37	-	-	65	Undet	Undet	34.53	-	-
26	Undet	Undet	27.57	-	-	66	Undet	Undet	34.39	-	-
27	Undet	Undet	30.04	-	-	67	Undet	Undet	41.42	-	-
28	Undet	Undet	28.17	-	-	68	Undet	Undet	35.46	-	-
29	Undet	Undet	27.84	-	-	69	Undet	Undet	35.16	-	-
30	Undet	Undet	29.38	-	-	70	Undet	Undet	32.98	-	-
31	Undet	Undet	28.06	-	-	71	Undet	Undet	33.71	-	-
32	Undet	Undet	31	-	-	72	Undet	Undet	35.04	-	-
33	Undet	Undet	27.44	-	-	73	Undet	Undet	33.98	-	-
34	Undet	Undet	32.09	-	-	74	Undet	Undet	35.48	-	-
35	Undet	Undet	23.51	-	-	75	Undet	Undet	34.1	-	-
36	Undet	Undet	34.03	-	-	76	Undet	Undet	33.77	-	-
37	Undet	Undet	34.48	-	-	77	Undet	Undet	34.23	-	-
38	Undet	Undet	37.13	-	-	78	Undet	Undet	34.92	-	-

Sample #	FAM	VIC	ROX	Expected Result	Result	Sample #	FAM	VIC	ROX	Expected Result	Result
39	Undet	Undet	36.01	-	-	79	Undet	Undet	33.94	-	-
40	Undet	Undet	34.65	-	-	80	Undet	Undet	36.21	-	-
						81	Undet	Undet	34.1	-	-

Undet=Undetermined

+ Positive

- Negative

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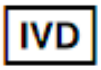
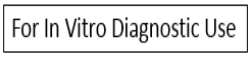
Interpretation of Symbols

	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
	Biologial Risks		Sufficient For

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