Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV

For Emergency Use Only

Instructions for Use

(50 reactions/kit)

For *in vitro* Diagnostic (IVD) Use

Rx Only

Labeling Revised: March 26, 2020

BGI Genomics Co. Ltd. (“BGI”)
Building No. 7, BGI Park
No. 21 Hongan 3rd Street
Yantian District, Shenzhen, 518083
China, (+86) 400-706-6615

Table of Contents

Intended Use

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* is an *in vitro* diagnostic real-time reverse transcription-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acids in throat swabs and bronchoalveolar lavage fluid (BALF) 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 USC §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in throat swabs and BALF 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 *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* 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 *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-2019-nCoV), which has resulted in thousands of confirmed human infections in many countries including the United States. Cases of asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers, labeled oligonucleotide probes, and control material used in real-time RT-PCR for the in vitro qualitative detection of SARS-2019-nCoV RNA in respiratory specimens.

Principles and Procedure

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in throat swabs and Broncho alveolar Lavage Fluid (BALF) from patients who meet CDC SARS-CoV-2 clinical criteria (e.g., signs and symptoms associated SARS-CoV-2 infection) in conjunction with CDC SARS-CoV-2 epidemiological criteria (e.g., history of residence in or travel to a geographic region with active SARS-CoV-2 transmission at the time of travel, or other epidemiologic criteria for which SARS-CoV-2 testing may be indicated).

To develop the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV*, the whole genome of SARS-CoV-2 was sequenced and compared to other known Coronavirus genes to deliberately select a specific target region in the ORF1ab region of SARS-CoV-2 genome. Further, Haman housekeeping gene β-Actin was developed as the target gene for the internal control.

Materials Provided

***Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* contents.**

Item (50 tests/kit)	Specifications	Quantity
SARS-CoV-2 Reaction Mix	1 mL/vial	1 vial
SARS-CoV-2 Enzyme Mix	80 μL/vial	1 vial
SARS-CoV-2 Positive Control	750 μL/vial	1 vial
SARS-CoV-2 No Template Control	750 μL/vial	1 vial

Materials and Equipment Required But Not Provided

- Applied Biosystems™ Real time PCR system 7500 with software v2.0.5.
- QIAamp Virus RNA Mini Kit (cat. #52904 or 52906).
- Vortex mixer.
- Microcentrifuge.
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL).
- Multichannel micropipettes (5-50 µL).
- Racks for 1.5 mL microcentrifuge tubes.
- -20°C cold blocks.
- Molecular grade water, nuclease-free.
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach).
- DNAZapTM (Ambion, cat. #AM9890) or equivalent.
- RNAse AwayTM (Fisher Scientific; cat. #21-236-21) or equivalent.
- Disposable powder-free gloves and surgical gowns.
- Aerosol barrier pipette tips.
- 1.5 mL microcentrifuge tubes (DNase/RNase free).
- 96-well 0.2 mL PCR reaction plates (Applied Biosystems).

Warnings and Precautions

Federal Law restricts this device to sale by or on the order of a licensed practitioner.

For emergency use only.

For *in vitro* diagnostic use only (IVD).

Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Please read the package insert carefully prior to operation. The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* is only for emergency use with a prescription, as an in vitro diagnostic test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.

False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. a) 1st Area: Preparation Area—Prepare testing reagent: b) 2nd Area: Sample processing—Process the specimen and controls: c) 3rd: Amplification Area—PCR conducted.

All materials used in one area should remain in that area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.

All contents in this package are prepared and validated for the intended testing purpose. Replacement of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

Prior to beginning each assay, each component must be thoroughly thawed and briefly centrifuged. Avoid repeated freeze-thaw cycles.

Immediately after the addition of the Nucleic Acid reaction Mix, the 96 well plate for real-time PCR should be covered and transferred to specimen processing area.

To prevent contamination from exogenous RNA, samples should be prepared in the following sequence: 1) no template (negative) control, 2) specimen RNA, and 3) positive control. In addition, filtered pipette tips are required and should be replaced after the addition of each reagent or sample.

Be sure to deposit samples with the pipette directly into the reaction mix in PCR tubes. Do not deposit samples with the pipette to the inside plate well wall. The plates should be sealed immediately after the addition of sample.

Following the amplification protocol, PCR plates should be placed into a sealable plastic bag for autoclaving and decontamination.

Be sure not to introduce any foam or bubbles into the tubes when aliquoting Nucleic Acid reaction Mix. All PCR plates should be sealed prior to being loaded into the thermocycler to avoid any possible leakage and contamination.

All lab workbench and supplies should be cleaned and disinfected regularly using 75% Ethanol or UV light.

All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in waste bin with bleach and discarded after decontamination.

Reagent Storage, Handling, and Stability

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* should be stored at temperature lower than -18°C in dark. The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* is stable with self-life at 2-8°C for 5 days and at -18°C for 12 months. Unpacked kits should avoid repeated freeze-thaw cycles (4X). The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* can be transported at -18°C in the dark and will remain stable for 5 days.

Specimen Collection, Storage, and Transfer

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

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

Sample collection: Collect fresh specimen of throat swabs or BALF from individuals suspected of having COVID-19. Specimen collection should avoid possible contamination in collection, storage, and transportation. The specimen should be presumed contagious and be operated according to related regulations. Throat swabs: Carefully take out the swab from package and quickly rotate it around two sides of the fauces, throat, and tonsil a few times applying pressure to collect as much secretion as possible. Avoid touching tongue. Break the swab stick and put the head into sampling solution in specimen tube. Screw the tube cap tightly to ensure that there is no leakage. BALF**:** Collect 3ml of unprocessed BALF in sterile, dry and clean DNase/RNase free Cryotubes. Screw the tube cap tightly to ensure no leakage and seal the tube with film.

Sample Storage: The specimen may be tested immediately after collection, or it may be stored at 2-8°C for up to 72 hours before testing. If a delay in testing or shipping is expected, the specimen may be stored at -18°C for no longer than 1 week or at -70°C for no longer than 6 months. Avoid repeated freeze-thaw cycles.

Sample Transportation: The specimen should be shipped in low temperature conditions using dry ice or an ice bag.

Laboratory Procedures

Sample processing: RNA should be collected from fresh a specimen to ensure suitable RNA quality and quantity. The positive control and no template (negative) control should be processed simultaneously alongside the specimen. RNA should be extracted using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. Extracted RNA should be eluted in a final volume of 60 µL. Following extraction, the RNA should be used immediately or stored at -70°C for use later. When handling the positive control, please take precautions to avoid contamination of the specimen sample. Failure to take proper precautions when handling the positive control could result in a false positive result.

Reagent preparation: Prepare all reagent mix in preparation area. To begin, take out the kit contents and thaw thoroughly at ambient temperature. Vortex and centrifuge briefly. The Enzyme Mix should be kept on ice at all times. Next, calculate the number of reactions (N) that will be included in the test. Be sure to include the no template (negative) control (1 tube), the positive control (1 tube), and each specimen. Prepare 96-well plates for real-time RT-PCR based on the estimated number of reactions (N) and prepare the PCR-Mix ingredients as described in Table 1. Pipette 20µL of PCR-Mix into each well. Cover and transfer the plate into sample processing area. The remaining Reaction Mix and Enzyme Mix must be stored at -18°C immediately.

Table 1: Sample reagent preparation calculation

	SARS-CoV-2 Reaction Mix (µL)	**SARS-CoV-2 Enzyme Mix (µL)**
PCR-Mix (µL)	18.5 µL × number of specimens and controls (N)	1.5 µL × number of specimens and controls (N)

Sample Addition: Add 10µL of the extracted sample RNA to the well pre-filled with reagent mix in the following order: no template (negative) control, patient specimen(s), and positive control. Seal the plate and centrifuge at 2000 rpm for 10 seconds. Place the plate into real-time RT-PCR system and record the exact location of controls and each specimen.

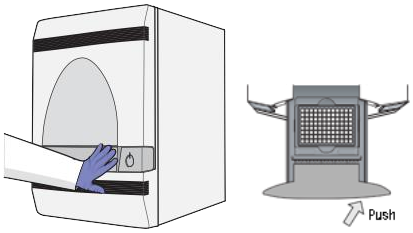
Running a Test

Real-time RT-PCR:

See below for step-by-step operation of ABI 7500 using 7500 software v2.0.5:

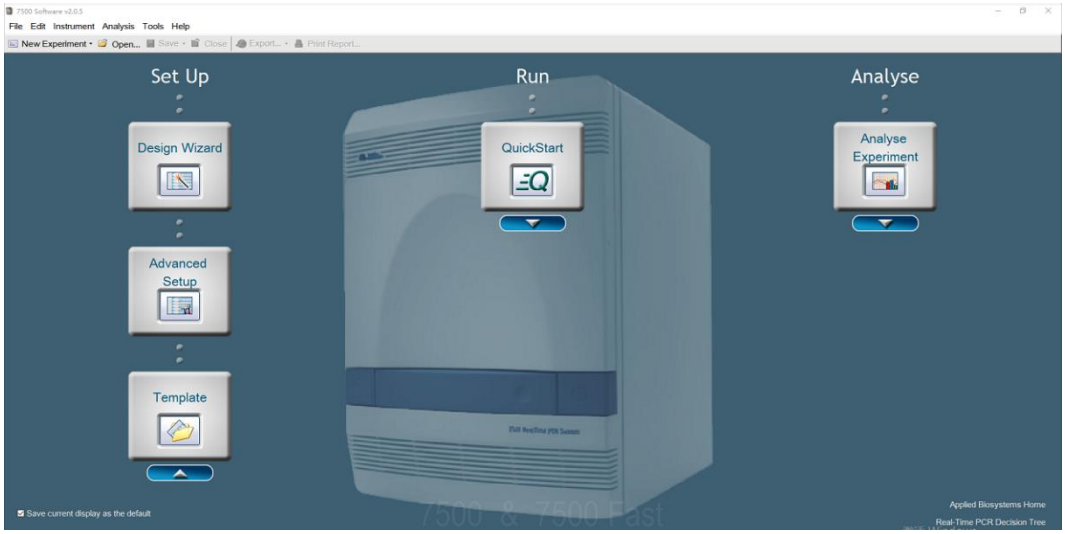
1. Start ABI 7500 real time PCR system: Turn on the computer connected to the system first, then turn on ABI 7500 real time PCR system.

2. Load the instrument: 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.

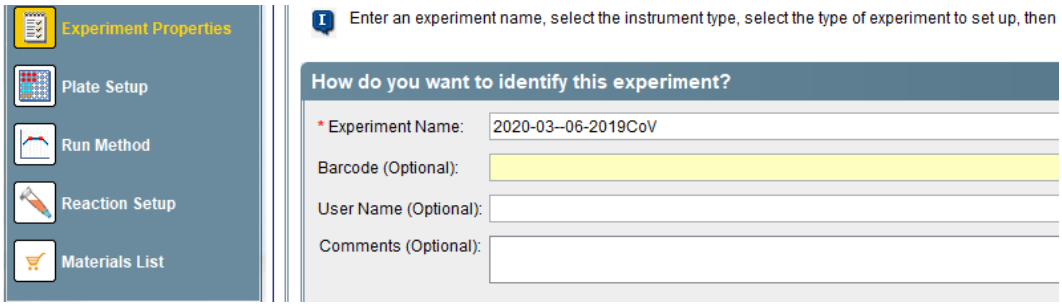


3. Set up the experiment run:

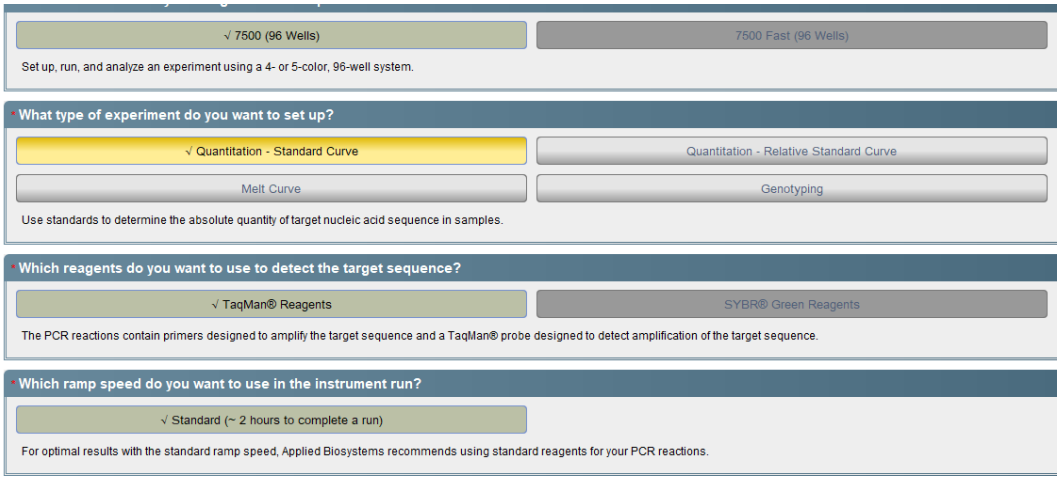
3.1. Double-click (7500 software v2.0.5) or select Start>>All Programs>>Applied Biosystems>>7500 Software v2.0.5.



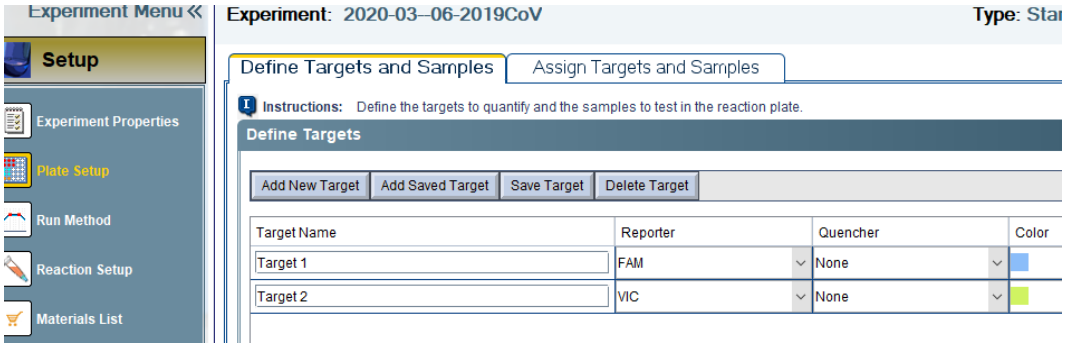
3.2. **Click** New Experiment to enter Experiment menu. In the Experiment Properties screen, **enter** identifying information for the experiment; you can leave other fields empty.



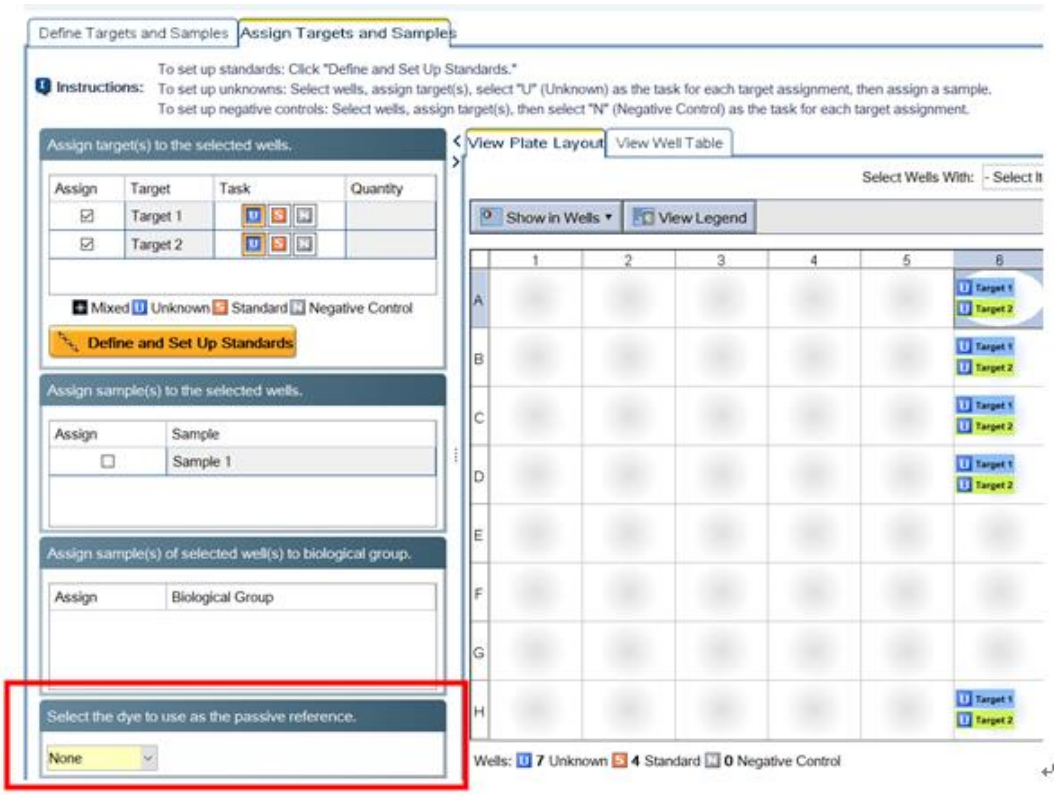
Select **7500 (96 Wells)**; **Quantitation-Standard Curve** (for the experiment type); **TaqMan Reagents** (for reagent); and **standard** (for ramp speed).



3.3. Click Plate Setup, in the Targets screen, under the tab Define Targets and Samples, enter targets as showed in the figure.



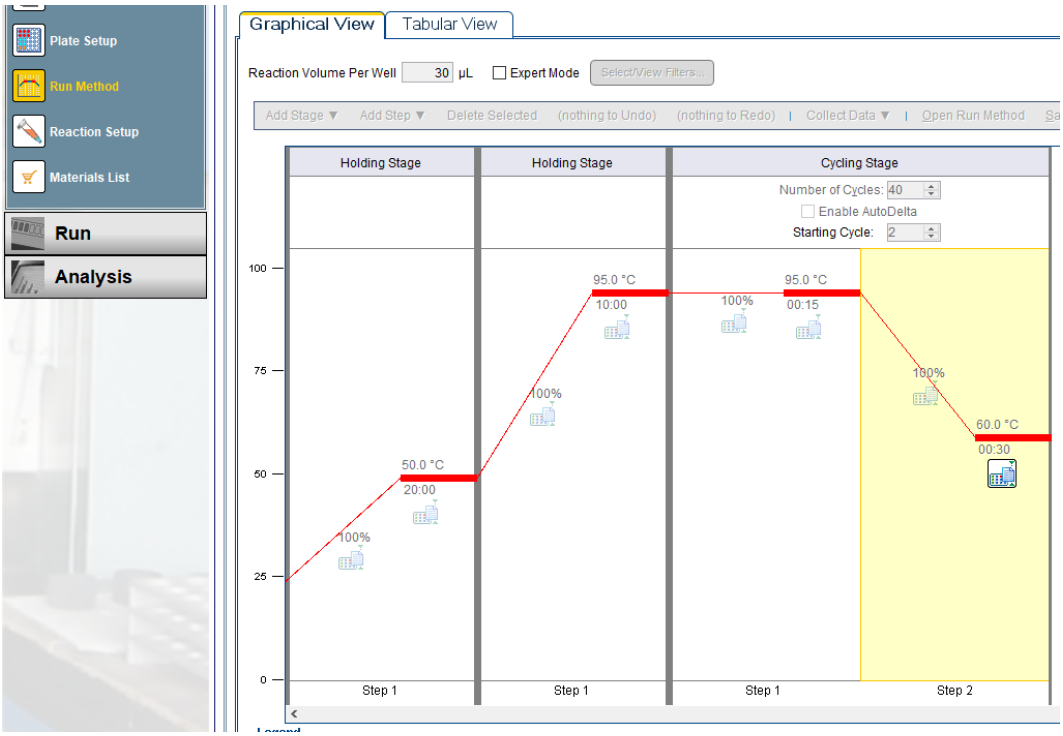
3.4. **Click** Assign Targets and Sample tab, in the Samples screen, **enter** the name of samples and controls to include in the reaction plate in corresponding well, and **select** the sample/target reactions to set up. **Select** None for passive reference.



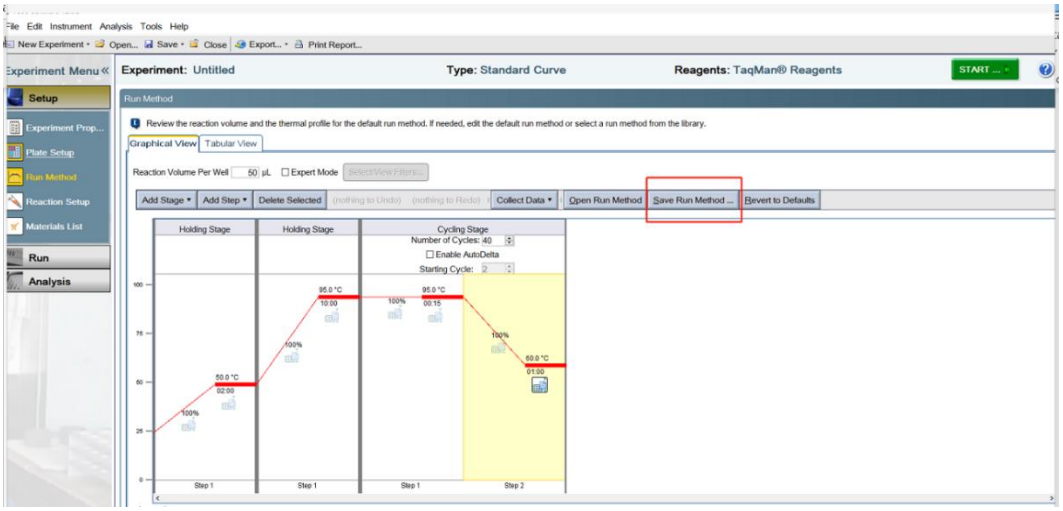
3.5. **Click** Run Method. On the Run Method screen, **select** either the Graphical View tab (default) or the Tabular View to edit the run method. Make sure the thermal profile displays the holding and cycling stages shown below. Enter **30 µL** in the Reaction Volume Per Well field. The FAM channel (Reporter: FAM, Quencher: None) will be set up for detection of SARS-CoV-2 RNA and the VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) will be set up for the detection of the internal reference (β-actin); Reference Dye: None. Configure PCR protocol as shown in Table 2.

Table 2: Thermocycler protocol

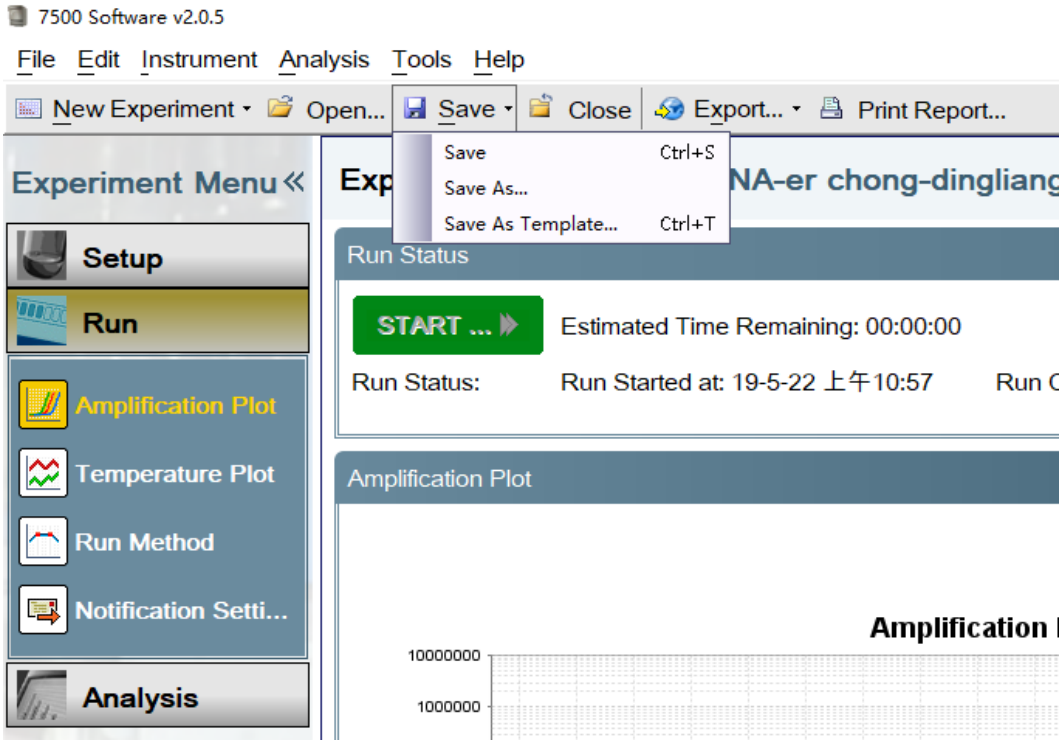
Step	Cycle	Temperature	Duration	Fluorescence measured (Y/N?)
1	1 cycle	50 °C	20 minutes	N
2	1 cycle	95 °C	10 minutes	N
3	40 cycles	95 °C	15 seconds	N
		60 °C	30 seconds	Y



You may save a run method as shown in the figure below and use the method for future experiments.



3.6. **Click** Run, in the Run screen, save the experiment. **Click** START



3.7. After the run completes, unload the instrument and proceed to data analysis

*Procedure and Images Adapted from ABI 7500 User Manual

Data Analysis

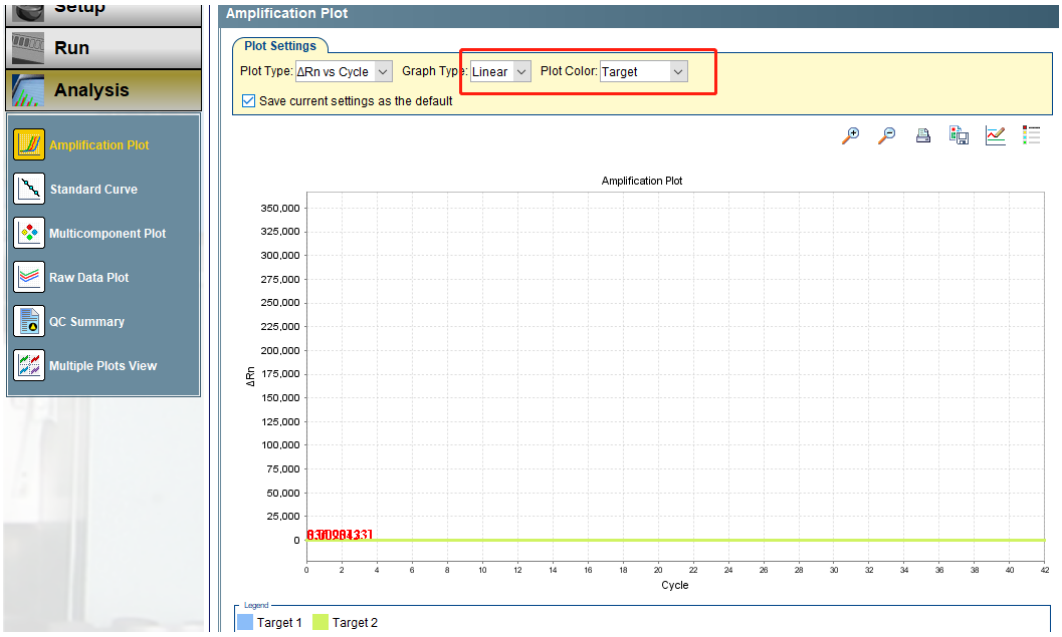
See below for step-by-step operation of ABI 7500 using 7500 software v2.0.5 for Data analysis:

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 3 and ending at cycle 15.

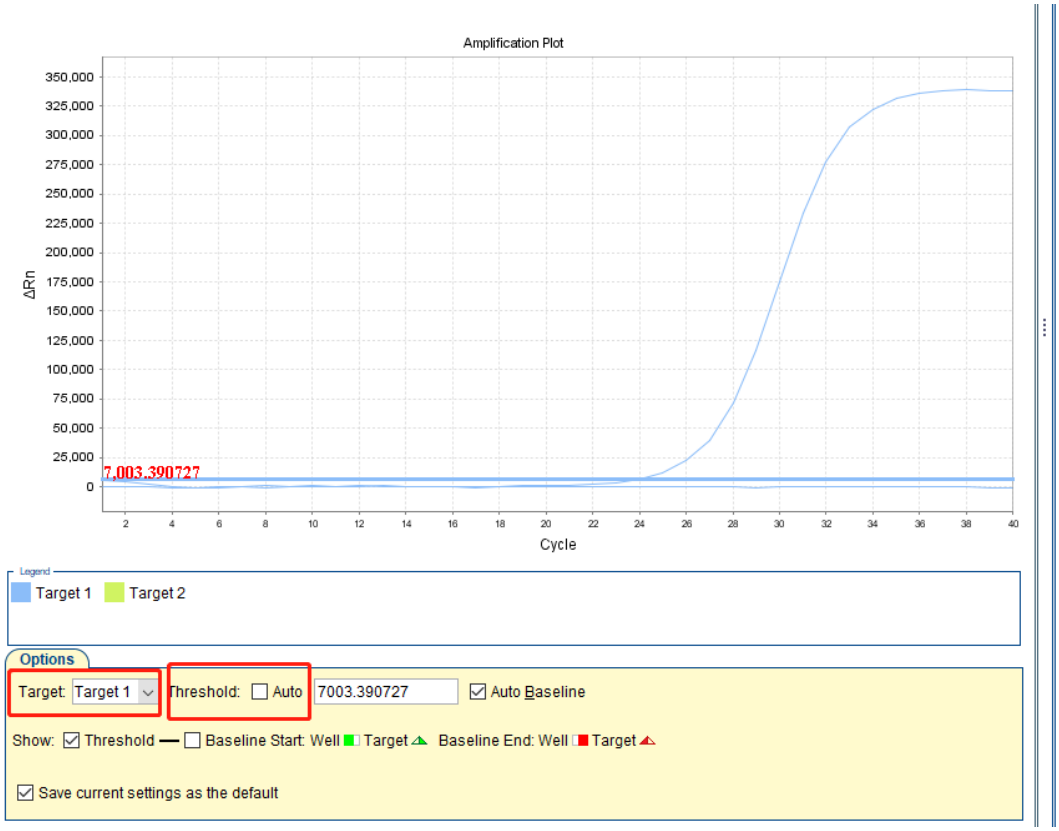
3. Manually set thresholds:

    a. In the Target drop-down list, select Target 1.

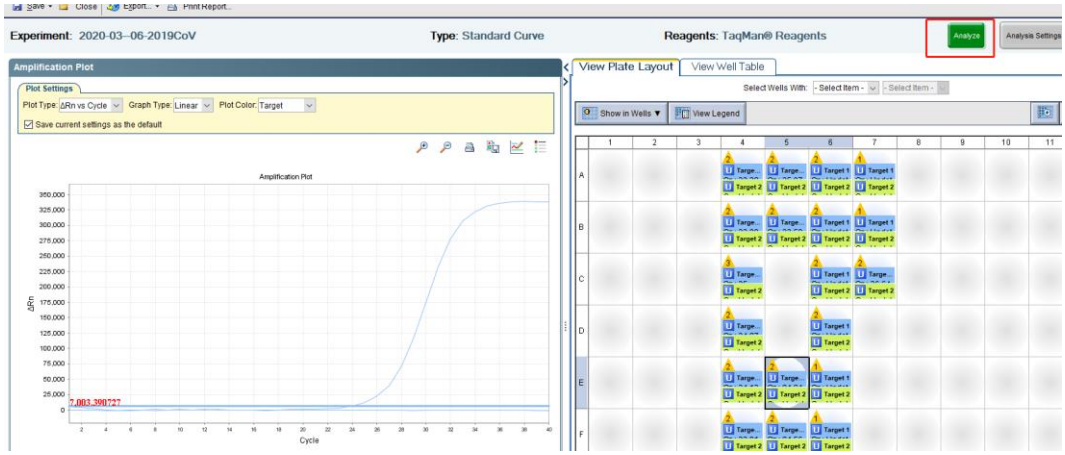
    b. Uncheck ☑Auto to □Auto as shown in the figure below.

    c. Adjust the threshold just above the curve from NTC (noise).

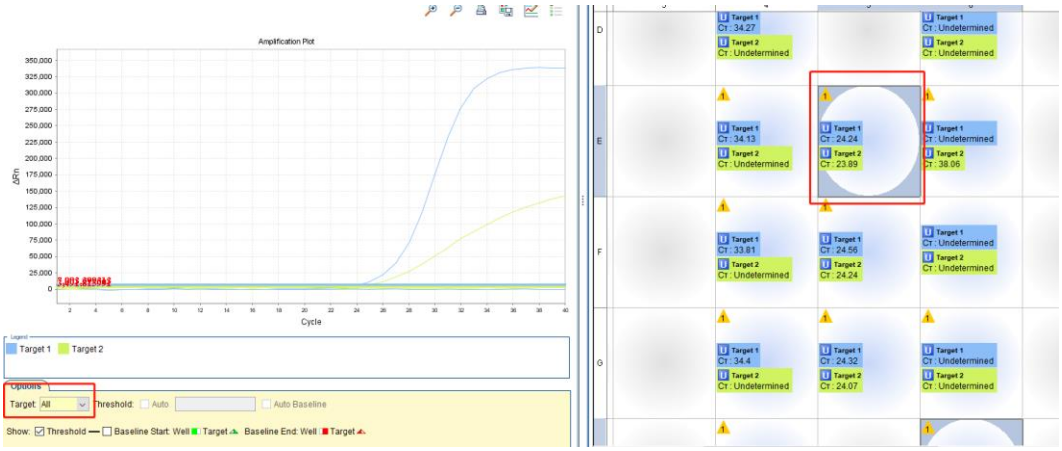
    d. Repeat the steps for Target 2.



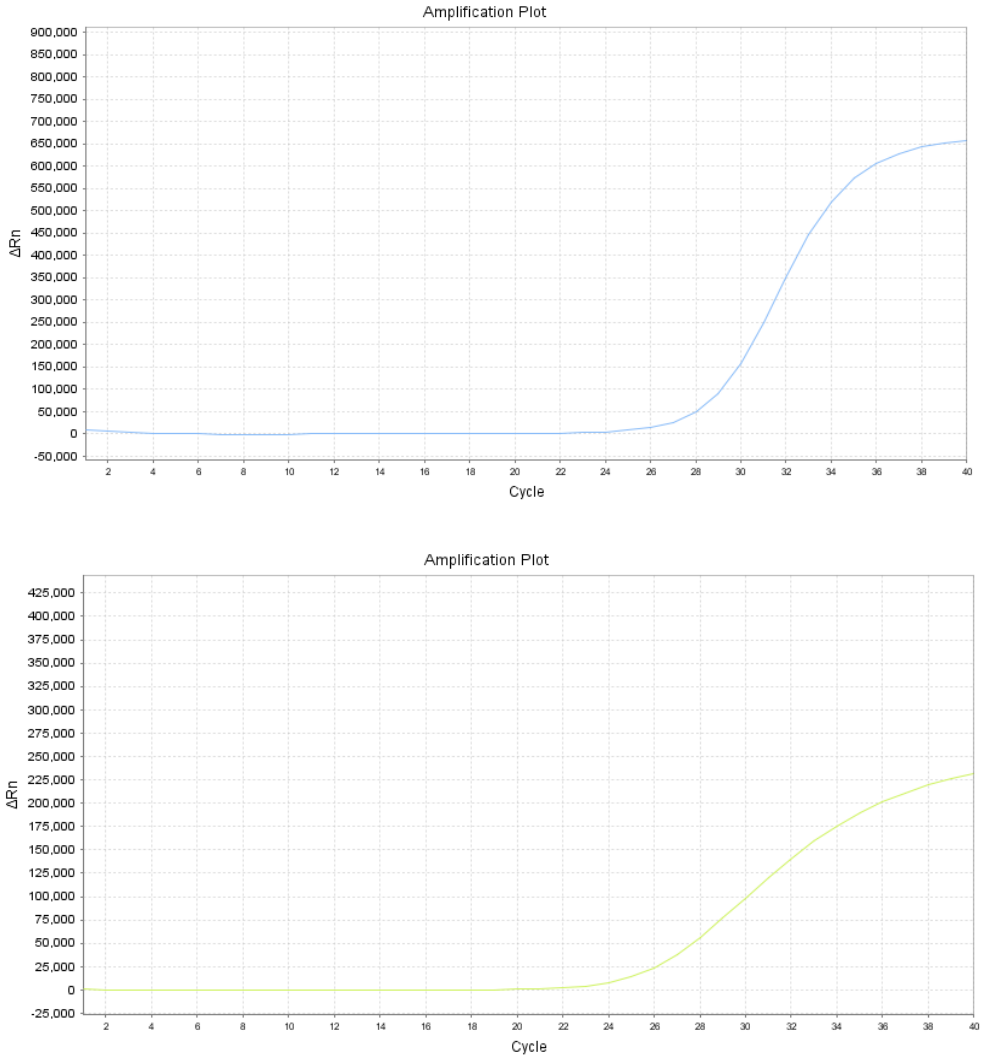
4.3. Click Analyze. The software analyzes the data with the settings.



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



4.4. Example of a positive sample amplification curve (2019-nCoV FAM in blue and internal reference VIC in green).



*Procedure and Images Adapted from ABI 7500 User Manual.

Quality Control and Interpretation of Results

Quality control:

Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. Quality control procedures are intended to monitor reagent and assay performance. Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly. A positive extraction control should be included in each nucleic acid isolation batch. Always include a no template (negative) control and positive control in each amplification and detection run.

The no template (negative) control should provide no amplification curve and Ct values in the FAM and VIC/HEX channels of "0" or "no data available". The positive control should provide an amplification curve in both the FAM and VIC/HEX channels that appear to be in a sigmoidal shape. Further, the Ct values in the FAM and VIC/HEX channel should not higher than 37 and 35 respectively. The amplification curve for the test specimen should appear to be in a sigmoidal shape with a Ct value not higher than 35 in the VIC/HEX channel. Notably, each of the above requirements for the no template (negative) control, positive control, and internal standard for the test specimen, should be met in a single test. If each requirement is not met in an individual test, the test is invalid. Table 3 provides further details for interpretation of results for quality control.

Table 3. Interpretation of results for quality control.

Quality control metrics	VIC (observation)	FAM (observation)	Interpretation
No template control	No amplification	No amplification	Pass; proceed to sample analysis
Positive control	Sigmoidal amplification curve and Ct value is <35.	Sigmoidal amplification curve and Ct value is <37.	
No template control	Sigmoidal amplification curve and Ct value is <35.	Sigmoidal amplification curve and Ct value is <37.	Fail; repeat run before proceeding to sample analysis.
Positive control	No amplification or Ct value is >35.	No amplification or Ct value is >37.	Fail; repeat run before proceeding to sample analysis.

Interpretation of Results:

Examination and Interpretation of Controls – Positive, Negative and Internal:

The controls for the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* are evaluated using the nucleic acid amplification curve and Ct values generated by the RT-PCR system software. The Ct cut-off values were determined using the receiver operator characteristic curves of the tested clinical samples. The Ct value in the FAM channel for a valid no template (negative) control should be "0" and there should be no sigmoidal amplification curve. Experimental analysis found that the Ct values for positive SARS-CoV-2 samples should be no higher than 37. Thus, the Ct value in the FAM channel for a valid positive control should be no higher than 37 and there should be a sigmoidal amplification curve. Experimental analysis found that the Ct values for the internal positive control samples should be no higher than 35. Thus, the Ct value in the VIC/HEX channel for a valid internal positive control should be no higher than 35 and there should be a sigmoidal amplification curve.

Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and no template (negative) controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. For instance, the no template (negative) control should provide Ct values at FAM and VIC/HEX channels of "0" or "no data available". The positive control should provide an amplification curve in both the FAM and VIC/HEX channels that appear to be in a sigmoidal shape. Further, the Ct values in the FAM and VIC/HEX channel should not higher than 37 and 35 respectively. To be deemed valid, a test must satisfy both the no template (negative) control and positive control requirements noted above. If neither requirement is satisfied, or if only one requirement is satisfied, the test is invalid.

A specimen is positive for SARS-CoV-2 if there is a sigmoidal amplification curve in the FAM channel, the Ct value is not higher than 37, there is a sigmoidal amplification curve in the VIC/HEX channel, and the Ct value is not higher than 35 (Table 4, Sample 1). The specimen is negative for SARS-CoV-2 if there is no sigmoidal amplification curve in the FAM channel, there is a Ct value of "0" or "no data available", there is a sigmoidal amplification curve in the VIC/HEX channel, and the Ct value is not higher than 35 (Table 4, Sample 2). The specimen should be retested if the amplification curve in the VIC/HEX channel has a Ct higher than 35, even if there is a sigmoidal amplification curve in the FAM channel (Table 4, Sample 3). The specimen should be retested if the amplification curve in the FAM and VIC/HEX channels have a Ct higher than 37 and 35 respectively (Table 4, Sample 4).

Upon retesting, the specimen can be reported as positive for SARS-CoV-2 if there is a sigmoidal amplification curve in the FAM channel, the Ct value is not higher than 37, there is a sigmoidal amplification curve in the VIC/HEX channel, and the Ct value is not higher than 35 (Table 4, Sample 1). Further, upon retesting, the specimen can be reported as negative for SARS-CoV-2 if there is no sigmoidal amplification curve in the FAM channel or the Ct value is higher than 37, and there is a sigmoidal amplification curve in the VIC/HEX channel and the Ct value is not higher than 35 (Table 4, Sample 2).

An exemplary interpretation of the test results using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* are provided in Table 4.

Table 4. Exemplary interpretation of test results for *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV*

	VIC/HEX Observation	**FAM Observation**	**Interpretation**
Sample 1	Sigmoidal amplification curve and Ct value is <35.	Sigmoidal amplification curve and Ct value is <37.	**Positive for SARS-COV-2 RNA**; amplification detected in both channels and Ct is below threshold.
Sample 2	Sigmoidal amplification curve and Ct value is <35.	Sigmoidal amplification curve and Ct value is >37.	**Negative for SARS-COV-2 RNA**; amplification detected in both channels but Ct is above threshold for FAM channel.
Sample 3	Sigmoidal amplification curve and Ct value is >35.	Sigmoidal amplification curve and Ct value is <37.	**Invalid test, please repeat***; amplification detected in FAM channel, but Ct for VIC channel above the threshold.
Sample 4	Sigmoidal amplification curve and Ct value is >35.	Sigmoidal amplification curve and Ct value is >37.	**Invalid test, please repeat***; Ct for VIC and FAM channel above the threshold.

*First, conduct the repeat test by re-extracting RNA from the same specimen. If the test fails again, collect a new specimen from the patient and repeat the test.

Limitations

The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* was established using throat swab and bronchoalveolar lavage samples. Nasal swabs, mid-turbinate nasal swabs and nasopharyngeal swabs are also considered acceptable specimen types for use with the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

Samples 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 the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:

- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- Using unauthorized extraction or assay reagents
- The presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus
- Failure to follow instructions for use

False-positive results may arise from:

- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

A positive result indicates the detection of nucleic acid from the relevant virus.

Nucleic acid may persist even after the virus is no longer viable.

Laboratories are required to report all positive results to the appropriate public health authorities.

Conditions of Authorization for the Laboratory

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/ EmergencySituations/ucm161496.htm.

However, to assist clinical laboratories using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

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

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and BGI Americas Corp. (info@bgiamericas.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

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

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

Performance Characteristics

Limit of Detection (LoD):

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

Preparation of the manufacturer's standards:

First, RNA was extracted from the pseudo-virus described above, using the QIAamp Virus RNA Mini Kit manufactured by QIAGEN. Then, the concentration of the extracted pseudo-virus RNA was calculated from the ng/μL concentration (determined by optical density of the extracted RNA solution) and the molecular weight of the pseudo-virus RNA. This concentration was also confirmed with ddPCR, as summarized in Table 7 below. Finally, the pseudo-virus RNA was diluted into 10^4, 10^3, and 10^2 Copies/mL to be used as the manufacturer's standards. Note, the concentration of the pseudo-virus was not determined using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV*.

LoD with Pseudo-virus
The LoD of the Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV was estimated by testing the standardized dilutions of pseudo-virus described above (n = 3 each). The lowest target level at which all three replicates produced positive results was 100 copies/mL. This value was then confirmed by testing 20 replicates at five different concentrations above and below the estimated LoD (**Table 5**).

Table 5. LoD confirmation with pseudo-virus

Concentration Estimated by Digital PCR (copies/mL)	**Number Positive/ Number Tested**	**Proportion Positive**
500	20/20	100%
300	20/20	100%
150	20/20	100%
100	***20/20***	***100%***
75	15/20	75%

LoD with Clinical Specimens
The quantity of SARS-CoV-2 in three clinical specimens that were known to be positive was estimated by quantitative digital PCR. The remainder of each specimen was then diluted in SARS-CoV-2 negative clinical matrix to achieve the approximate concentrations shown in **Table 6**.

Table 6. Dilution of clinical specimens for LoD determination

Concentration estimated by Digital PCR [1] (copies/mL)	Dilution Factor		
	Throat swab (1.33×10^4 copies/mL)	**BALF1 (1.25×10^4 copies/mL)**	**BALF2 (1.55×10^4 copies/mL)**
500	26.5	25.1	31
300	44.2	41.8	51.7
150	88.3	83.5	103.4
100	132.5	125.3	155.2
75	176.7	167.1	206.9

[1] Note: this concentration may not accurately reflect the number of genomic equivalents present

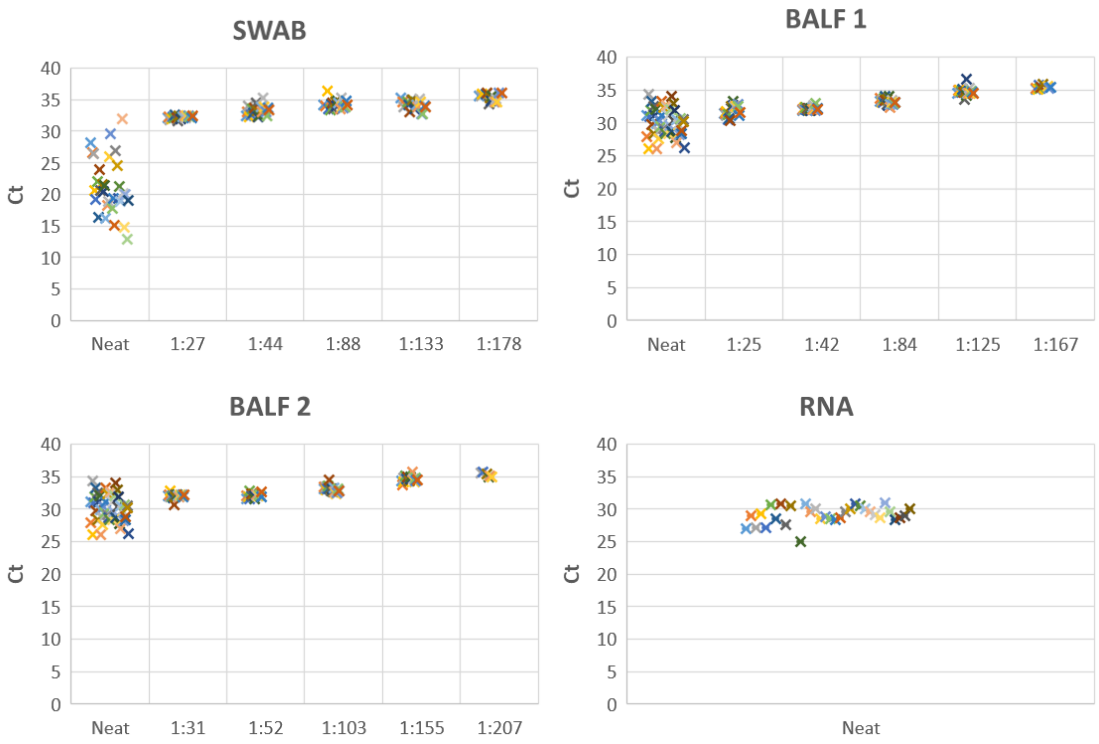
The LoD of the Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV was evaluated by testing the dilutions of each clinical specimen described above (n = 20 each). The LoD was determined to be the highest dilution at which ≥19/20 results were positive (i.e., ≥95% proportion positive) (**Table 7**).

Table 7. LoD confirmation.

Specimen	Concentration of SARS-CoV-2 estimated by Digital PCR (copies/mL) [1]	Number Positive/ Number Tested	Proportion Positive
Throat swab	500	20/20	100%
	300	20/20	100%
	150	***19/20***	***95%***
	100	18/20	90%
	75	15/20	75%
BALF1	500	20/20	100%
	300	20/20	100%
	150	20/20	100%
	100	***20/20***	***100%***
	75	10/20	50%
BALF2	500	20/20	100%
	300	20/20	100%
	150	20/20	100%
	100	***19/20***	***95%***
	75	6/20	30%

[1] Note: this concentration may not accurately reflect the number of genomic equivalents present

Scatter plots of Ct values obtained from the dilutions of SARS-CoV-2 positive specimens in the LoD Study are shown in below, together with the Ct values from testing of undiluted specimens in the Clinical Evaluation.



Further validation:

The LoD (150 Copies/mL) for each clinical matrix was further validated for 3 lots of kits on a PCR system (Applied Biosystems™ Real Time PCR System 7500) in 20 replicates, where at least 19 tests confirmed positive for every matrix/kit.

a) *Reactivity/Inclusivity:*

Currently, different SARS-CoV-2 isolates are not available for the validation of reactivity/inclusivity of the kit. Primer/probe inclusivity was therefore evaluated by BLASTn analysis against 284 publicly available SARS-CoV-2 sequences on March 10, 2020. The Primer NPC1-YF22 and probe NPC1-P2 exhibited 100% homology with all the available sequences. Primer NPC1-YR21 exhibited a single mismatch with one published sequence (homology of 96%).

In addition to *in silico* analysis, 10 specimens from different regions of China confirmed as SARS-CoV-2 positive based on clinical criteria were used to validate the lower detection limit. The concentration of SARS-CoV-2 in each specimen was estimated with ddPCR. Further, each specimen was diluted to estimated concentrations of 5×10^3 Copies/mL and 100 Copies/mL (LoD concentration) and tested in replicates of 10 to evaluate the reproducibility of the test. The coefficient of Variation (CV) of Ct values at 5 x 10^3 copies/mL was lower than 5%. Table 8 below summarizes the results.

Table 8. Reactivity and Inclusivity testing

	Concentration (Copies/mL)	Testing results				
		Reproducibility			LoD	
		Diluted concentration (Copies/mL)	Detection rate	CV	Diluted concentration (Copies/mL)	Detection rate
BALF3	1.15×10^5	5×10^3	100%	0.32%	100	100%
BALF4	7.13×10^4	5×10^3	100%	0.48%	100	100%
BALF5	9.49×10^4	5×10^3	100%	0.52%	100	100%
BALF6	4.45×10^3	5×10^3	100%	0.66%	100	100%
BALF1	1.25×10^4	5×10^3	100%	0.74%	100	100%
BALF7	5.25×10^4	5×10^3	100%	0.99%	100	100%
Throat swab 1	1.33×10^4	5×10^3	100%	0.51%	100	90%
Throat swab 2	6.88×10^3	5×10^3	100%	0.46%	100	100%
BALF2	1.55×10^4	5×10^3	100%	1.12%	100	100%
BALF8	8.89×10^4	5×10^3	100%	0.87%	100	100%

*Note, this concentration may not accurately reflect the genomic equivalent copies GEC/mL of the viral RNA from specimens.

Specificity/Cross-reactivity:

The fifty-four pathogens listed in Table 9 below were wet tested with the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* for cross-reactivity. No false positive results were observed.

Table 9. Pathogens tested in cross-reactivity evaluation

No	**Pathogen**	**Provider**	**Tested concentration**
1	New type A H1N1 influenza virus (2009)	National institutes for food and drug control (People's Republic of China)	8×10^7 Copies/mL
2	Seasonal H1N1 influenza virus		1.8×10^7 Copies/mL
3	Influenza A virus (H3N2)		1.2×10^7 Copies/mL
4	Influenza A virus (H5N1)		4.3×10^5 Copies/mL
5	Influenza A virus (H7N9)		6.2×10^5 Copies/mL

6	Influenza B virus (Yamagata)		2.1×10^5 Copies/mL
7	Influenza B virus (Victoria)		2.0×10^7 Copies/ mL
8	Respiratory syncytial virus A	National institutes for food and drug control (People's Republic of China)	5.3×10^5 Copies/mL
9	Respiratory syncytial virus type B		1.2×10^6 Copies/mL
10	Parainfluenza virus 1		7.1×10^5 Copies/mL
11	Parainfluenza virus 2		3.9×10^5 Copies/mL
12	Parainfluenza virus 3		1.8×10^6 Copies/mL
13	Rhinovirus A	BGI Biotechnology (Wuhan) Co., Ltd	$> 10^5$ Copies/mL
14	Rhinovirus B		$> 10^5$ Copies/mL
15	Rhinovirus C		$> 10^5$ Copies/mL
16	Adenovirus type 1		$> 10^5$ Copies/mL
17	Adenovirus type 2		$> 10^5$ Copies/mL
18	Adenovirus type 3		$> 10^5$ Copies/mL
19	Adenovirus type 4		$> 10^5$ Copies/mL
20	Adenovirus type 5		$> 10^5$ Copies/mL
21	Adenovirus type 7		$> 10^5$ Copies/mL
22	Adenovirus type 55		$> 10^5$ Copies/mL
23	Enterovirus A	National institutes for food and drug control (People's Republic of China)	2.2×10^5 Copies/mL
24	Enterovirus B		6.2×10^5 Copies/mL
25	Enterovirus C		4.2×10^5 Copies/mL
26	Enterovirus D		3.7×10^5 Copies/mL
27	Human interstitial pneumovirus	BGI Biotechnology (Wuhan) Co., Ltd	$> 10^5$ Copies/mL
28	Epstein-Barr virus	National institutes for food and drug control (People's Republic of China)	1.6×10^6 Copies/mL
29	Measles virus		4.8×10^5 Copies/mL
30	Cytomegalovirus		5.1×10^5 Copies/mL
31	Rotavirus	BGI Biotechnology (Wuhan) Co., Ltd	$> 10^5$ Copies/mL
32	Norovirus		$> 10^5$ Copies/mL

33	Mumps virus		> 10^5 Copies/mL
34	Varicella zoster virus	Beijing Union Medical College Hospital	2.7×10^5 Copies/mL
35	Endemic human coronavirus (HKU1)	BGI Biotechnology (Wuhan) Co., Ltd	1.5×10^5 Copies/mL
36	Endemic human coronavirus (OC43)		1.1×10^5 Copies/mL
37	Endemic human coronavirus (NL63)		1.0×10^6 Copies/mL
38	Endemic human coronavirus (229E)		3.8×10^5 Copies/mL
39	SARS coronavirus		1.7×10^5 Copies/mL
40	MERS coronavirus		2.1×10^5 Copies/mL
41	Mycoplasma pneumoniae		> 10^6 CFU/mL
42	Chlamydia pneumoniae		> 10^6 CFU/mL
43	Legionella	National institutes for food and drug control (People's Republic of China)	5.4×10^8 CFU/mL
44	Pertussis	BGI Biotechnology (Wuhan) Co., Ltd	> 10^6 CFU/mL
45	Haemophilus influenzae	National institutes for food and drug control (People's Republic of China)	5.0×10^8 CFU/mL
46	Staphylococcus aureus		2.3×10^9 CFU/mL
47	Streptococcus pneumoniae		1×10^7 CFU/mL
48	Streptococcus pyogenes		2.2×10^8 CFU/mL
49	Klebsiella pneumoniae		1.8×10^8 CFU/mL
50	Mycobacterium tuberculosis attenuated strains		3.1×10^6 CFU/mL
51	Aspergillus fumigatus	Beijing Union Medical College Hospital	1.9×10^6 CFU/mL
52	Candida albicans	National institutes for food and drug control (People's Republic of China)	4×10^6 CFU/mL
53	Candida glabrata		9.6×10^6 CFU/mL
54	Cryptococcus neoformans	Beijing Union Medical College Hospital	2.3×10^7 CFU/mL
55	Human genome	BGI Biotechnology (Wuhan) Co., Ltd	/

The *in silico* analysis of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* primers and probes against the sequences of 48 pathogens showed the kit would be specific to the target SARS-CoV-2 gene and not cross-react with these pathogens. Although sequence homology greater than or equal to 80% for one of the primers could be found against some pathogens such as Bacillus spp., Bacteroidetes, and Influenza A, the potential for exponential amplification was determined to be low.

Five microorganisms (SARS coronavirus, Adenoviridae, Influenza A, Bacillus, and Bacteroidetes) out of the 48 tested showed ≥80% homology with respect to one of the primers. Among these five, wet testing confirmed no cross-reactivity with SARS coronavirus, Adenoviridae, and Influenza A.

For Bacillus and Bacteroidetes, sequences were found that exhibit ≥80% homology with one of the SARS-CoV-2 primers, but not with any other primers included in the assay. Cross-reaction and/or interference with the assay due to the presence of these organisms is therefore unlikely to occur.

A study was performed to evaluate the potential for interference with the Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV by the presence of high concentrations of human β-actin mRNA. No interference was observed in the presence of up to 1.76E+09 copies of β-actin internal control transcript, the highest level tested (Table 10). The average level of β-actin RNA in throat swab specimens was estimated to be ~4.65E+05 copies/mL by digital PCR.

Table 10. Effect of high concentration of human beta actin on detection of 2019-nCov

Human beta-actin (Copies/mL)	Pseudo-virus (Copies/mL)	FAM (virus) Ct value	Average FAM (virus) Ct value	VIC (beta actin) Ct value	Average VIC (beta actin) Ct value
1.76E+09	200	35.44	35.35	11.34	11.33
		35.06		11.31	
		35.5		11.32	
		35.47		11.3	
		35.29		11.37	
1.76E+08	200	33.77	33.49	14.57	14.51
		33.46		14.44	
		33.3		14.48	
		33.24		14.48	
		33.68		14.57	
1.76E+07	200	33.34	33.15	17.91	17.89
		33.17		17.92	
		33.55		17.92	
		32.67		17.88	
		33.04		17.84	

none	200	32.92 33.07 33.23 33.14 33.19	33.11	/	/
negative samples without spiked in virus		Negative	/	22.5	/

Clinical performance:

A retrospective study was conducted with 384 clinical specimens collected by National Institute for Viral Disease Control and Prevention under China CDC, and Wuhan CDC, and BGI's clinical laboratories in Wuhan, Tianjin and Shenzhen. The 384 specimens included BALF and throat swabs (Table 11).

Table 11. Brief summary of specimens by types in the clinical evaluation

	Cases		
	Positive	**Negative**	**Total**
BALF	58	165	223
Throat swab	34	67	101
RNA-BALF	34	26	60
Total	126	258	384

Clinical diagnostic criteria (patient status determination):

Criterion 1. Fourteen days prior to the onset of illness, the patient (i) traveled to or resided in Wuhan, (ii) had contact with a patient with a fever and respiratory symptoms, or (iii) was exposed to a cluster of COVID-19 patients.

Criterion 2. Clinical presentation indicates that (i) the patient has a fever, (ii) the patient's chest images shows multiple mottling, consolidation, or ground glass opacities, or (iii) the patient shows leukopenia or lymphopenia.

Criterion 3. Laboratory test of sputum, oropharyngeal swabs, or lower respiratory specimens for SARS-Cov-2 returns positive. Laboratory detection of SARS-CoV-2 virus includes RT-PCR detection and viral sequencing showing high homology with known SARS-CoV-2 sequence.

*Clinical status of a patient is determined as positive if all three criteria above are met.

Summary of the result:

A total 384 specimens were enrolled and tested in the study to evaluate the performance of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV* in detecting SARS-CoV-2 from of throat swab specimens, BALF, and extracted RNA obtained from National Institute for Viral Disease Control and Prevention under Chinese Center for Disease Control. Compared to the clinical diagnosis of COVID-19, RT-PCR of SARS-CoV-2 showed overall positive and negative percent agreement across all specimens of 88.1% (95% CI: 81.2% to 92.7%) and 99.6% (95% CI: 97.8% to 99.9%). See Table 12 below for summary of clinical results.

Table 12. Summary of clinical results.

BALF	Diagnosis positive	Diagnosis negative	Total
Test positive	47	0	47
Test negative	11	165	176
Total	58	165	223
PPA =	81.0%	69.1-89.1%	
NPA =	100%	97.7-100%	

Throat swab	Diagnosis positive	Diagnosis negative	Total
Test positive	31	0	31
Test negative	3	67	70
Total	34	67	101
PPA =	91.2%	77.0-97.0%	
NPA =	100%	94.6-100%	

RNA	Diagnosis positive	Diagnosis negative	Total
Test positive	33	1	34
Test negative	1	25	26
Total	34	26	60
PPA =	97.1%	85.1-99.5%	
NPA =	96.2%	81.1-99.3%	

Combined	Diagnosis positive	Diagnosis negative	Total
Test positive	111	1	112
Test negative	15	257	272
Total	126	258	384
PPA =	88.1%	81.2-92.7%	
NPA =	99.6%	97.8-99.9%	

References

1. LU Rou-jian, ZHANG Ling-lin, TAN Wen-jie, ZHOU Wei-min, WANG Zhong, PENG Kun, RUAN Li. Development and Comparison of Real-Time and Conventional RT-PCR Assay for Detection of Human Coronavirus NL63 and HKU1[J]. CHINESE JOURNAL OF VIROLOGY, 2008(4).

2. NIU P, LU R, LAN J, LIU G, WANG W, TAN W. Development of Novel Multiplex Real-time RT-PCR Assays for Detection of MERS-CoV Infection[J]. CHINESE JOURNAL OF VIROLOGY, 2016(3).

3. CHEN Yu-jing. Development of two-panel reactions of real-time PCR for detection of 18 types/subtypes of respiratory viruses [D]. 2015

Contact Information and Product Support

For technical and product support, contact BGI Genomics directly:

Service hotline: (+86) 400-706-6615

Product support website: https://www.bgi.com/global/molecular-genetics/2019-ncov-detection-kit/.