Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2

For Emergency Use Only

Instructions for Use

(50 reactions/kit)

For *in vitro* Diagnostic (IVD) Use

Rx Only

Labeling Revised: April, 2022

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Intended Use

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* is an *in vitro* diagnostic real-time reverse transcription-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acids in throat (oropharyngeal) swabs, nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates 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, that meet requirements to perform high complexity tests.

Test results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable 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 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-CoV-2* is intended for use by qualified 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-CoV-2* is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

COVID-19 has spread throughout the world. A novel coronavirus (SARS-CoV-2) was identified as the pathogen. Cases of asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 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-CoV-2 RNA in respiratory specimens.

Principles and Procedure

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* 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 (oropharyngeal) swabs and Broncho Alveolar Lavage Fluid (BALF), as well as in nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, and nasal aspirates, from patients who are suspected of COVID-19 by their healthcare provider.

To develop the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*, 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, human 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-CoV-2 **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.
  - Alternatively, ABI 7500 Fast Real-Time PCR System with software v2.0.6, Roche LightCycler® 480 System with software v1.5.0, or QuantStudio 5 Real-Time PCR System with software v1.5.1.
- QIAamp Virus RNA Mini Kit (cat. #52904 or 52906).
  - Alternatively, MGIEasy Nucleic Acid Extraction Kit (cat. #1000020261or 1000020471).
  - Optionally, High-throughput Automated Sample Preparation System: MGISP-960RS software v1.2.
- 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).
- DNAZap™ (Ambion, cat. #AM9890) or equivalent.
- RNAseAWAY™ (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

Caution: 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).

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use in authorized laboratories; 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 *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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 declaration is terminated or authorization is revoked sooner.

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 SARS-CoV-2 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-CoV-2* 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.

Deviations from the recommended setup as described in the Instructions for Use need additional validation before use, as they could cause false results. These changes include using a 384-well format for the assay, changing volumes for reagent preparation, changing PCR cycle parameters, changing the PCR instrument running mode to a fast mode, and using different PCR instruments with different versions of software other than those specified.

The assay signal threshold should be set above the maximum noise level of the no template control curve. The threshold should be either set by auto threshold or manually adjusted after every PCR run based on no template control results following the data analysis procedures for each instrument and software version described in the Instructions for Use for the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*. Manual threshold adjustment and subsequent interpretation of results should take place only when signal introduced from contamination can be ruled out. Deviations in the assay threshold setup could lead to false results. Please contact BGI if you require additional assistance.

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 Area—Process the specimen and controls: c) 3rd: Amplification Area—Carry out PCR.

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 by directly pipetting into the reaction mix in PCR tubes. Do not deposit samples by pipetting to the inside of the 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.

To monitor laboratory contamination, each PCR run should include a no template control reaction. Contamination in the PCR reaction by aerosols, amplicon, or operators can lead to amplification being observed in the no template control samples and run failure. Follow laboratory best practices such as those described in CLSI MM19-A, *Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline, 2011* and the procedures laid out in this Instructions for Use, including those under "Equipment Preparation," to prevent or to eliminate contamination.

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 containing 10% bleach and discarded after decontamination.

Reagent Storage, Handling, and Stability

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* should be stored at temperature lower than -18°C in dark. The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* is stable with shelf 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-CoV-2* 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 (oropharyngeal) 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 processed 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) or MGIEasy Nucleic Acid Extraction kit manually or using MGISP-960RS according to the manufacturer's instructions. 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 except the Enzyme mix 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. Do not change the volumes for reagent preparation specified in Table 1 or the volume of the sample addition in the process below. Such changes could cause false results.

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)

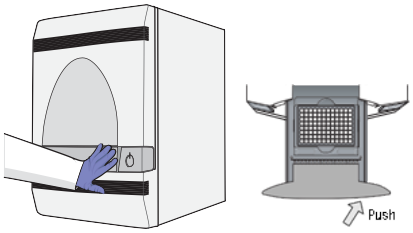
Sample Addition: Add 10µL of the extracted sample RNA to the well pre-filled with PCR-Mix in the following order: no template (negative) control, patient specimen(s), and positive control. Seal the plate and centrifuge at 2000 rpm for 10 seconds. Place the plate into real-time RT-PCR system and record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

Running a Test / Data Analysis

1-1. Running a test in Applied Biosystems™ Real Time PCR System 7500 and ABI 7500 Fast Real Time PCR System (software v2.0.5 or v2.0.6):

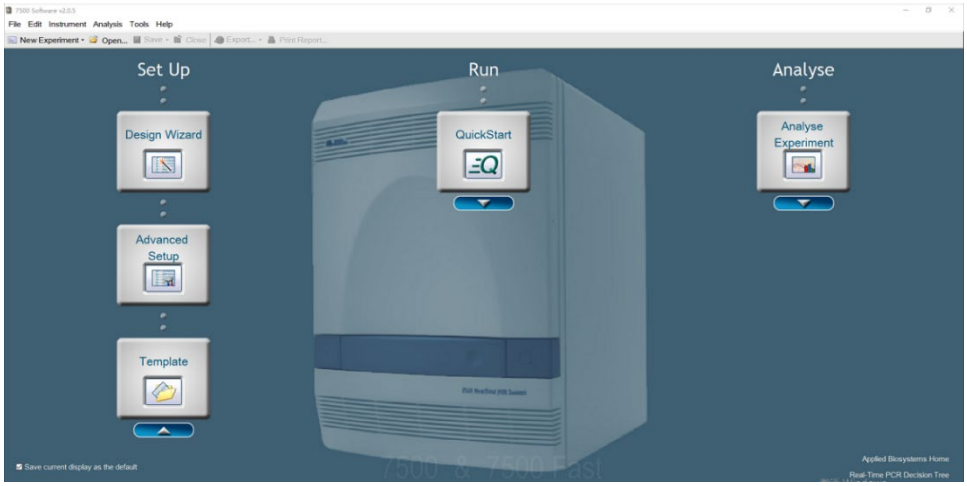
(1) Start Applied Biosystems™ Real Time PCR System 7500: Turn on the computer connected to the system first, then turn on Applied Biosystems™ Real Time PCR System 7500.

(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.

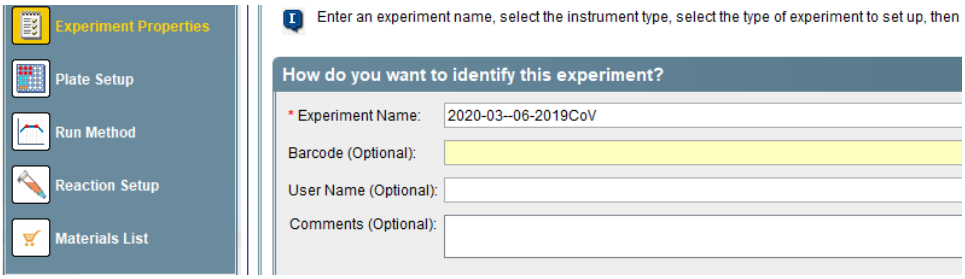


(3) Set up the experiment run:

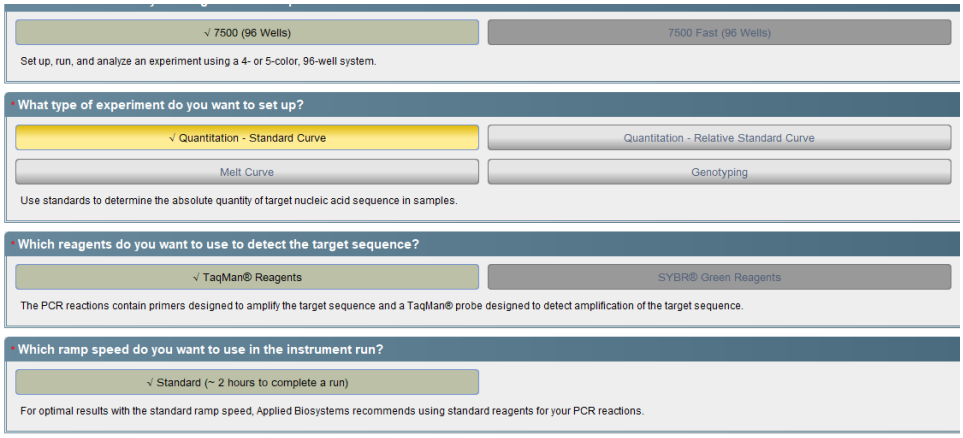
(3.1) Double-click (7500 software v2.0.5 or v2.0.6) or select Start>>All Programs>>Applied Biosystems>>7500 Software v2.0.5 (or v2.0.6).



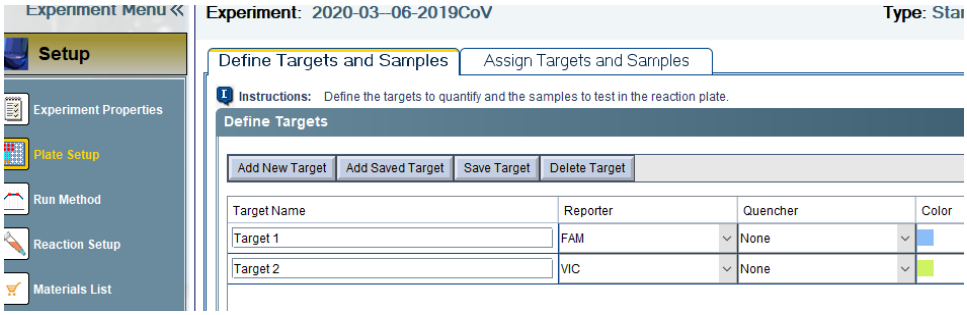
(3.2) **Click** New Experiment to enter Experiment menu. In the Experiment Properties screen, **enter** identifying information for the experiment; you can leave other fields empty.



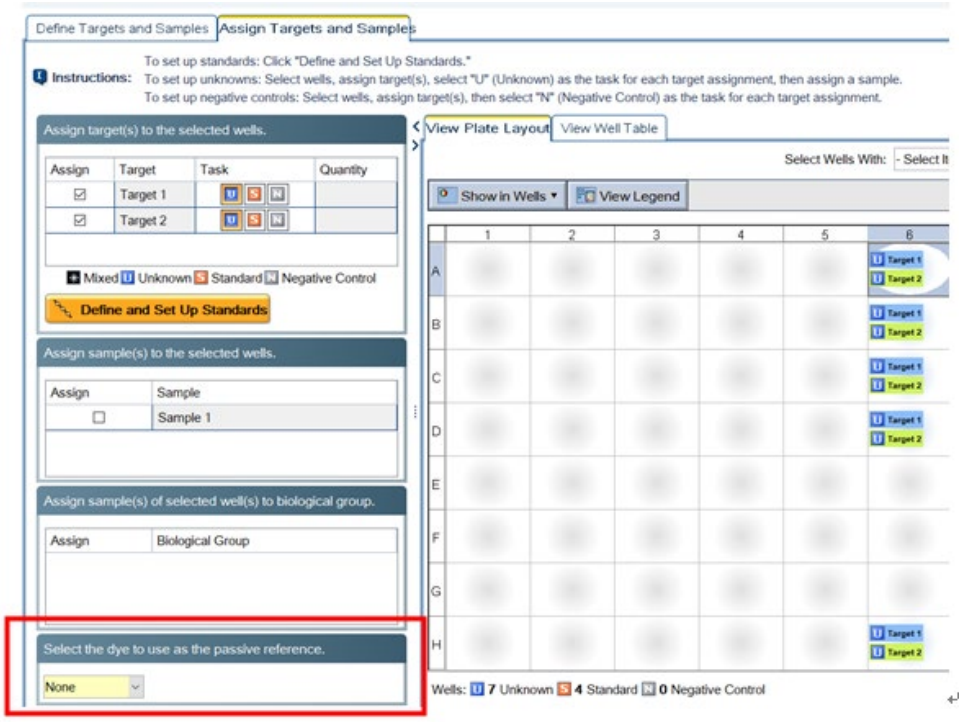
Select **7500 (96 Wells)**; **Quantitation-Standard Curve** (for the experiment type); **TaqMan Reagents** (for reagent); and **Standard** (for ramp speed).



(3.3) Click Plate Setup, in the Targets screen, under the tab Define Targets and Samples, **set** Target 1 with FAM reporter and Target 2 with VIC/HEX reporter as showed in the figure.



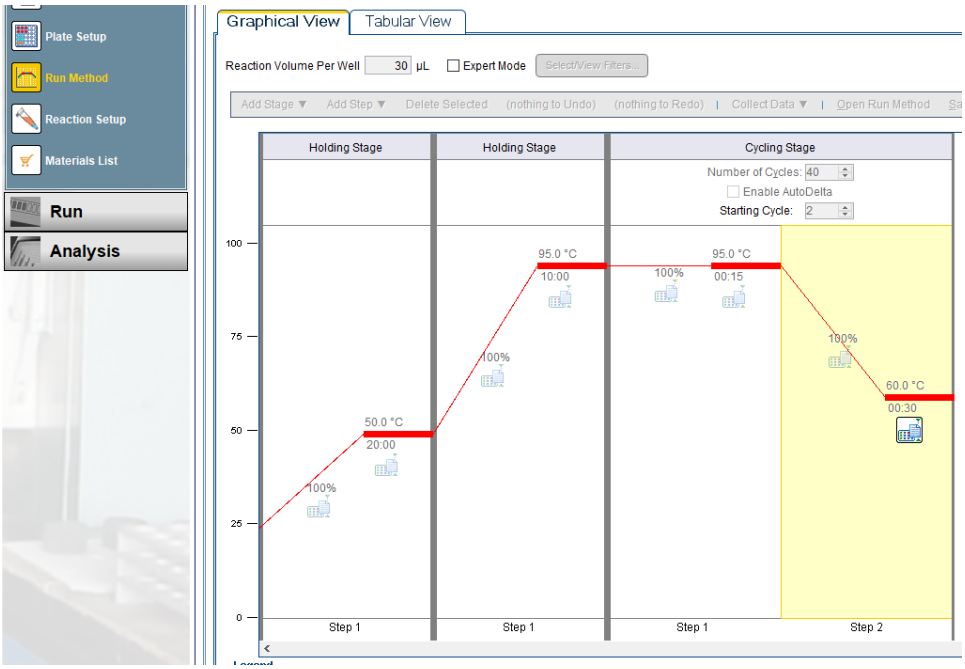
(3.4) **Click** Assign Targets and Sample tab, in the Samples screen, **enter** the name of samples and controls to include in the reaction plate in corresponding well, and **select** the sample/target reactions to set up. **Select** None for passive reference.



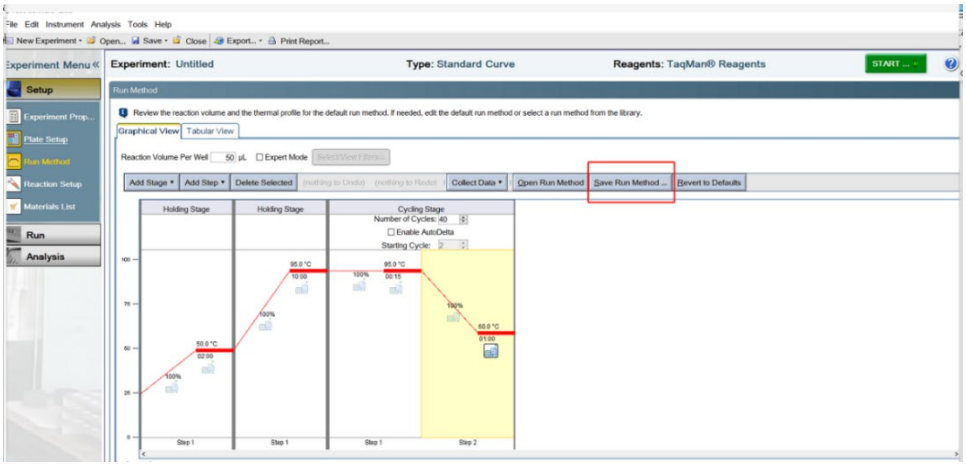
(3.5) **Click** Run Method. On the Run Method screen, **select** either the Graphical View tab (default) or the Tabular View to edit the run method. Make sure the thermal profile displays the holding and cycling stages shown below. **Enter 30 µL** in the Reaction Volume Per Well field. The FAM channel (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: PCR protocol

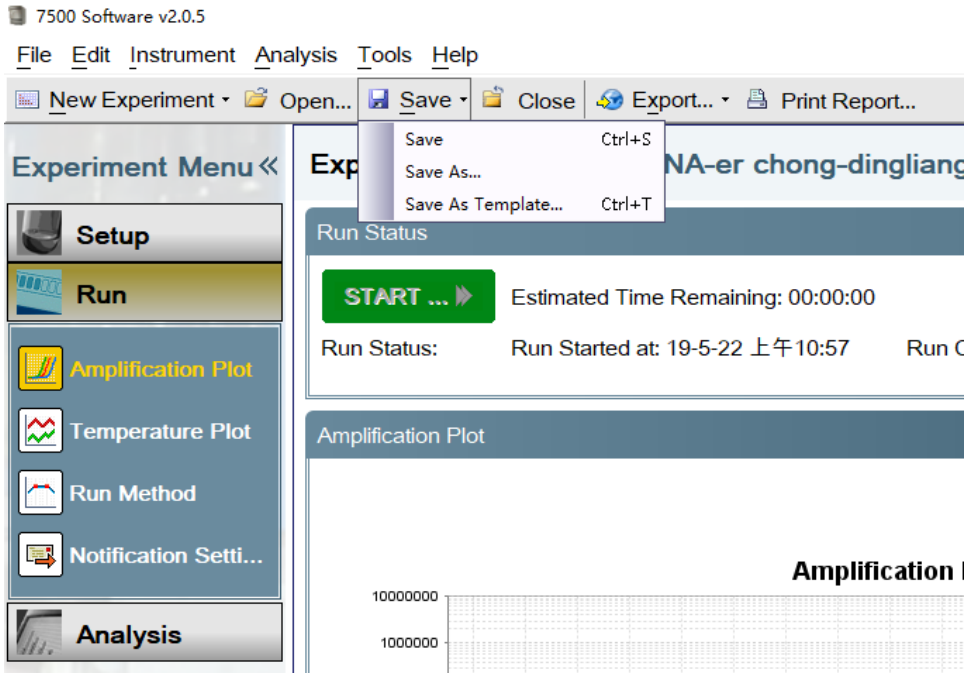
Step	Cycle	Temperature	Duration	Fluorescence measured (Y/N?)
1	1 cycle	50 °C	20 minutes	N
2	1 cycle	95 °C	10 minutes	N
3	40 cycles	95 °C	15 seconds	N
		60 °C	30 seconds	Y



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 Applied Biosystems™ Real Time PCR System 7500 User Manual

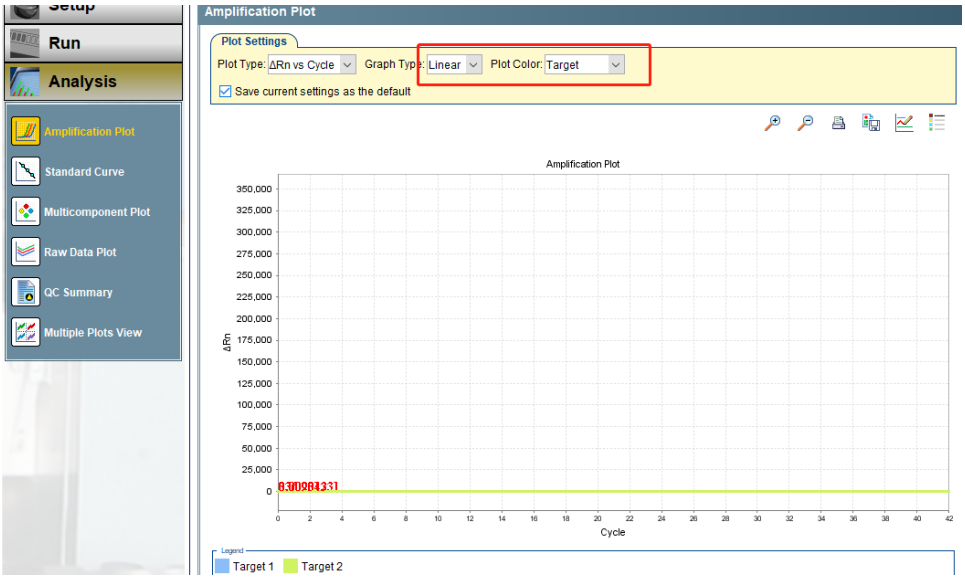
1-2. Analyzing data in Applied Biosystems™ Real Time PCR System 7500 and ABI 7500 Fast Real Time PCR System (software v2.0.5 or v2.0.6):

(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) Threshold Setup:

(3.1) Use auto threshold to check the result of the no template control (NTC). If the NTC shows no Ct values for both the FAM and the VIC/HEX channels, then go to step (4) directly, and use auto threshold for sample analysis.

(3.2) Manually set threshold if the NTC shows a Ct value > 37 for the FAM channel or a Ct value > 35 for the VIC/HEX channel and does not exhibit sigmoidal amplification curves:

a. In the Target drop-down list, select Target 1 or Target 2, depending on the channel that requires manual threshold setting.

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

c. **Adjust the threshold to be equal to the maximum level of the no-template control curve (i.e., equal to the maximum value of the random noise curve)**.

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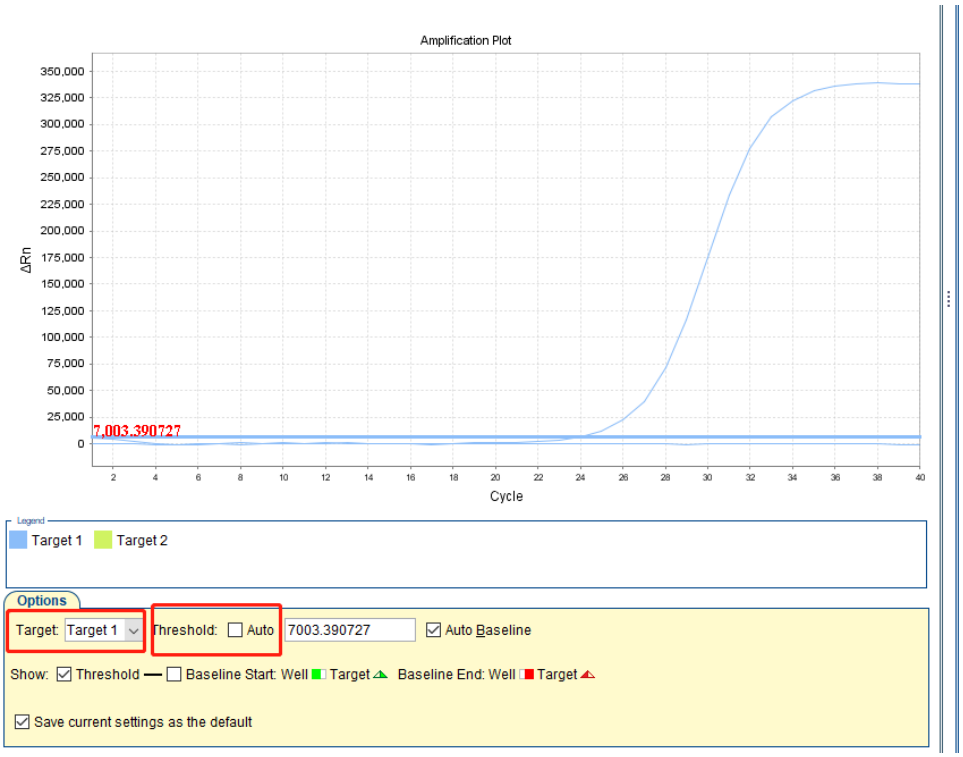
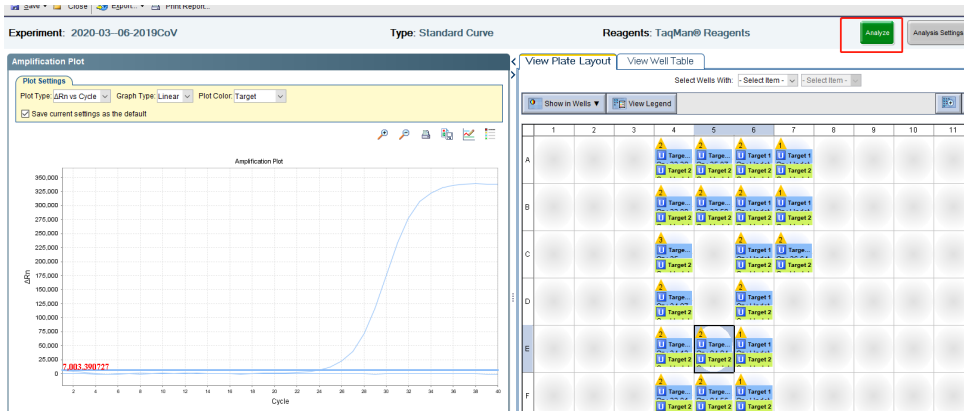
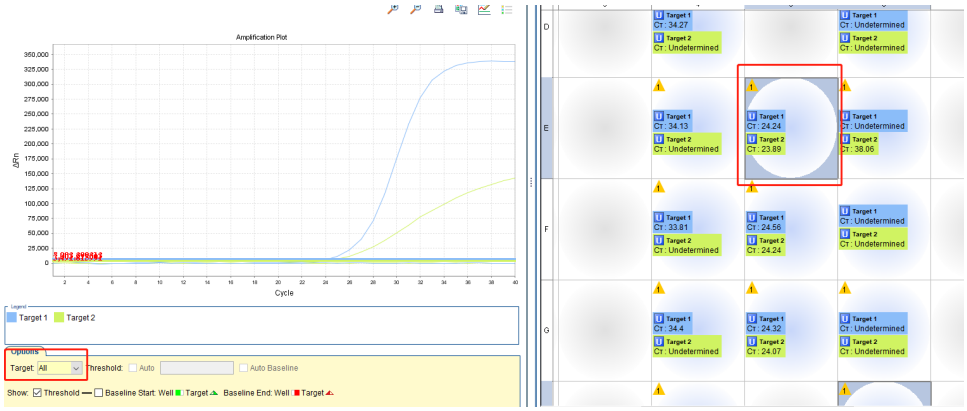


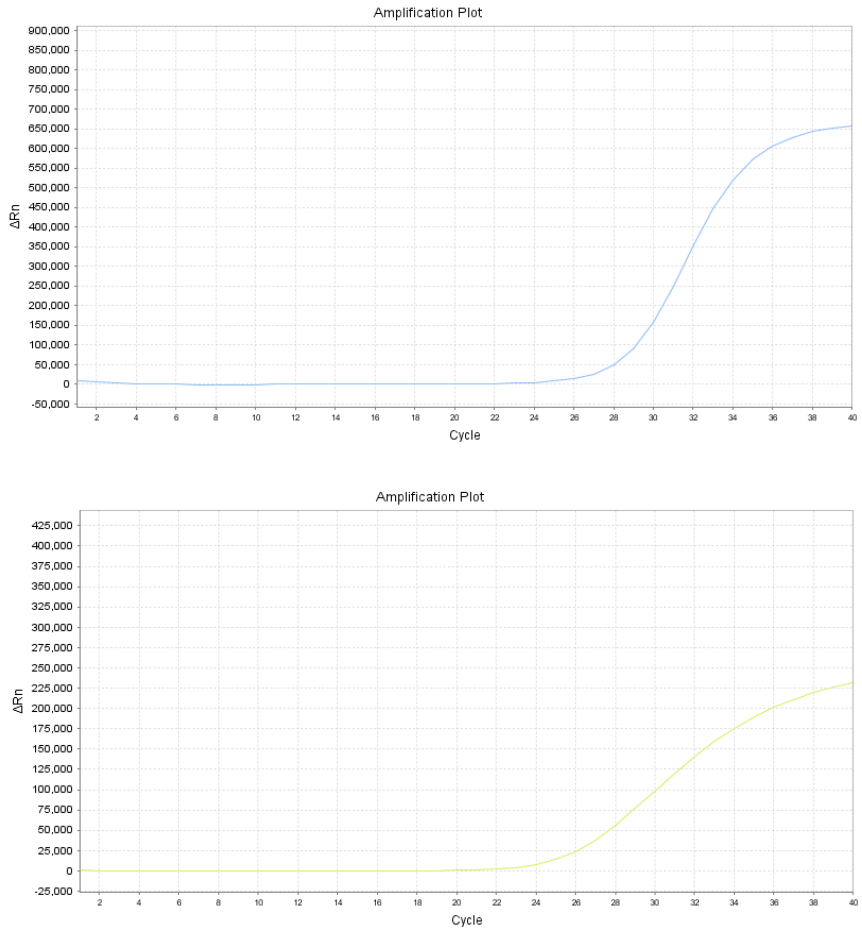
(4) **Click** Analyze. The software analyzes the data with the settings.



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



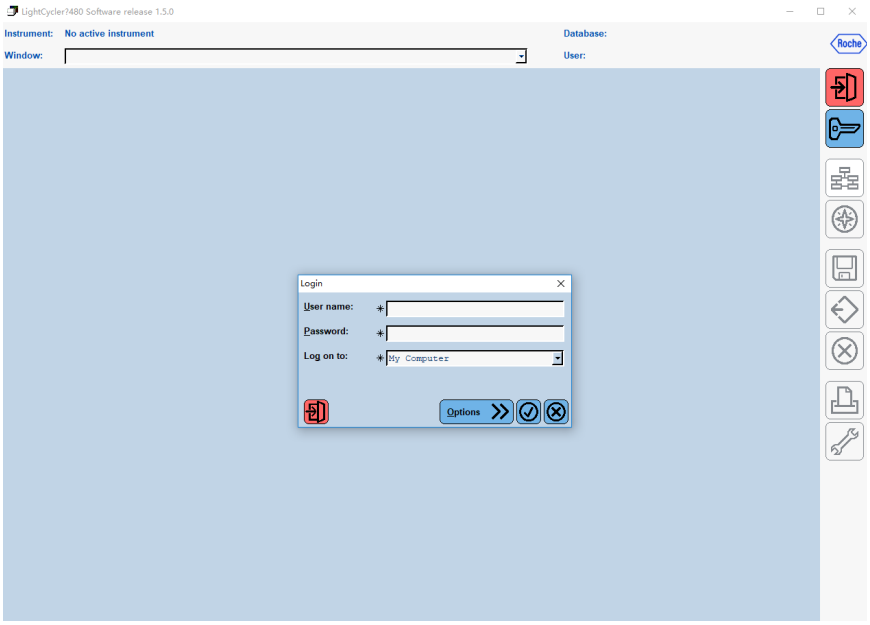
(4.1) Example of a positive sample amplification curve (SARS-CoV-2 FAM in blue and internal reference VIC/HEX in green).



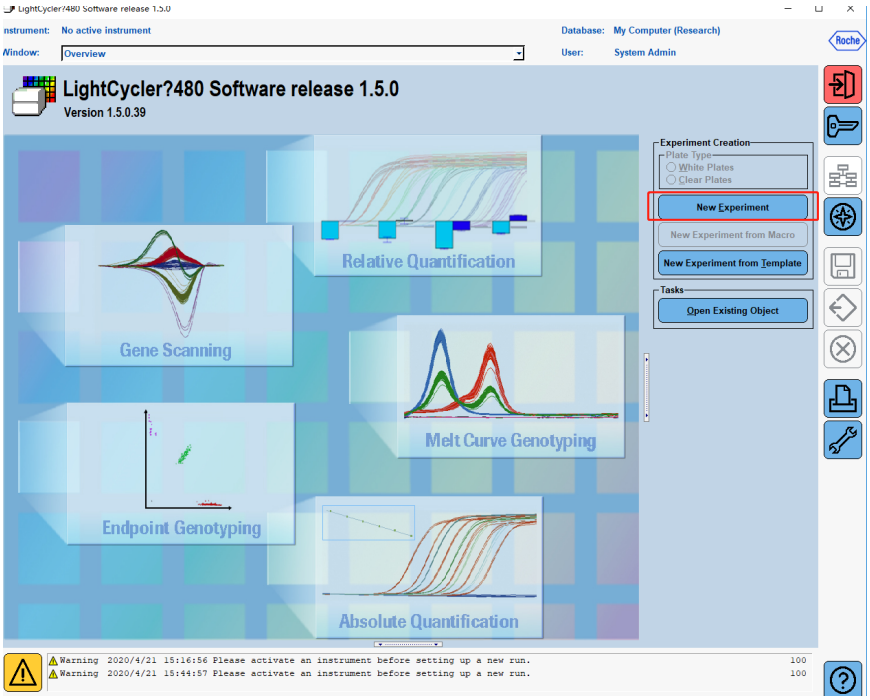
*Procedure and Images Adapted from Applied Biosystems™ Real Time PCR System 7500 User Manual.

2. Running a test and analyzing data in Roche LightCycler® 480 System (software v1.5.0):

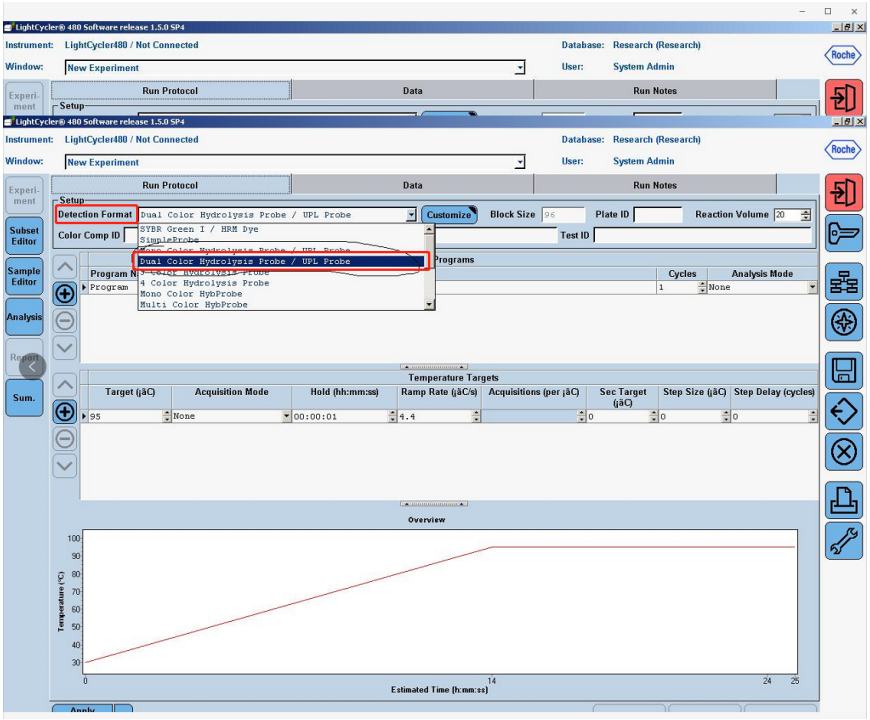
(1) Double click LightCycler480 software icon on the desktop, and when prompted, enter user name and password to log into the software interface (see screenshot below).



(2) Click **New Experiment** (see screenshot below).



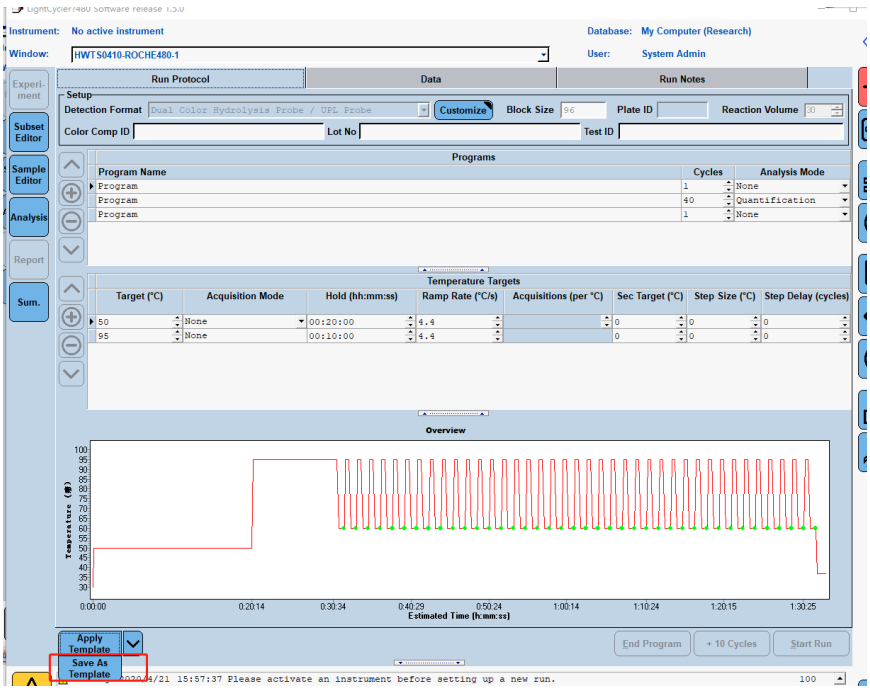
(3) In the drop-down menu next to **Detection Format**, select **DualColor Hydrolysis Probe/UPL Probe** (see screenshot below).



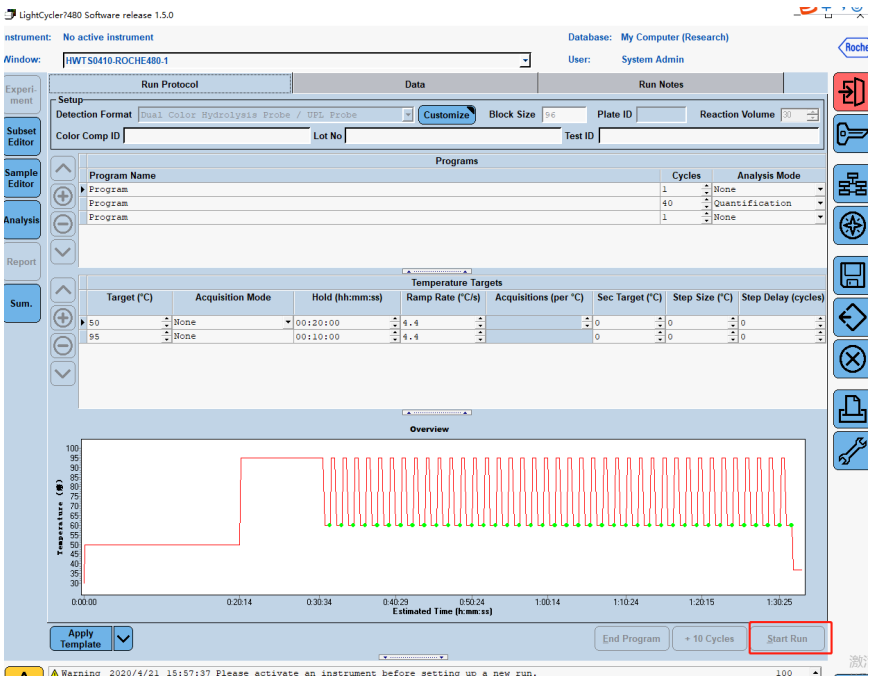
(4) Designate individual program under **Program Name**, and set temperature and time parameters for each program in the **Program Temperature Targets** panel below, referring to the steps, number of cycles, temperature, and duration in the table below. Use (+) and (–) buttons to add or delete steps in the interface.

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

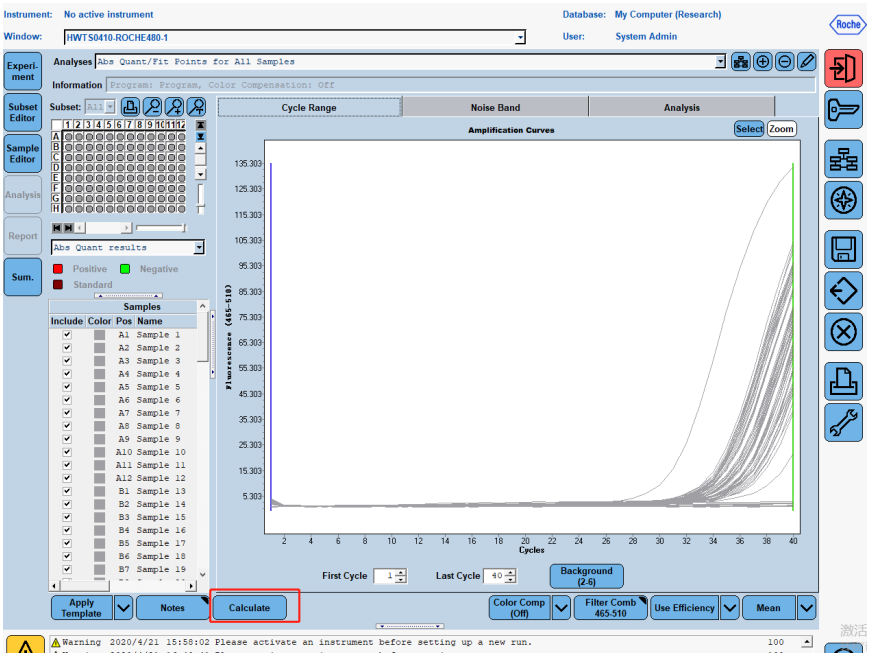
(5) Save the program as a template by clicking **Save As Template**. The template can be used for future experiments by clicking **Apply Template** (see screenshot below).



(6) Select **Start Run** and enter experiment name when prompted.



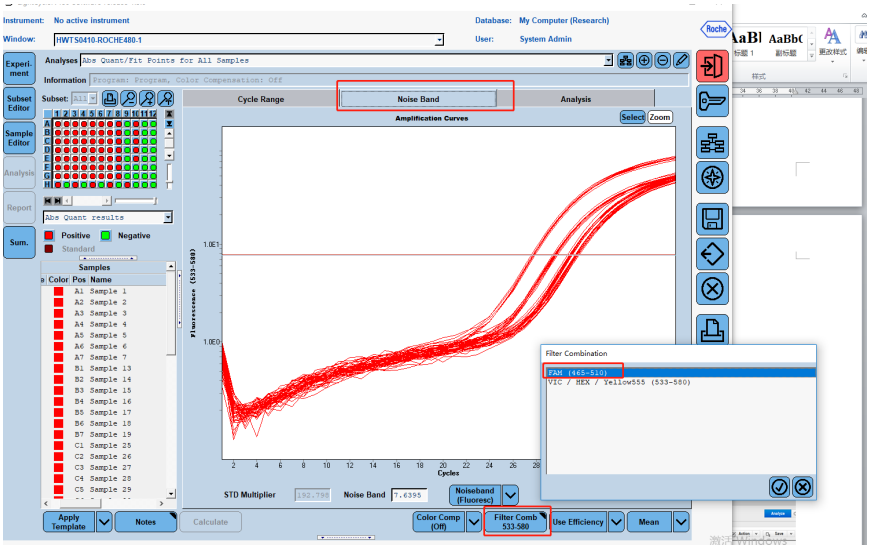
(7) After a run is complete, click **Analysis** on the left panel (see screenshot above) to open the analysis interface, and click **Calculate** (see screenshot below) for auto threshold.



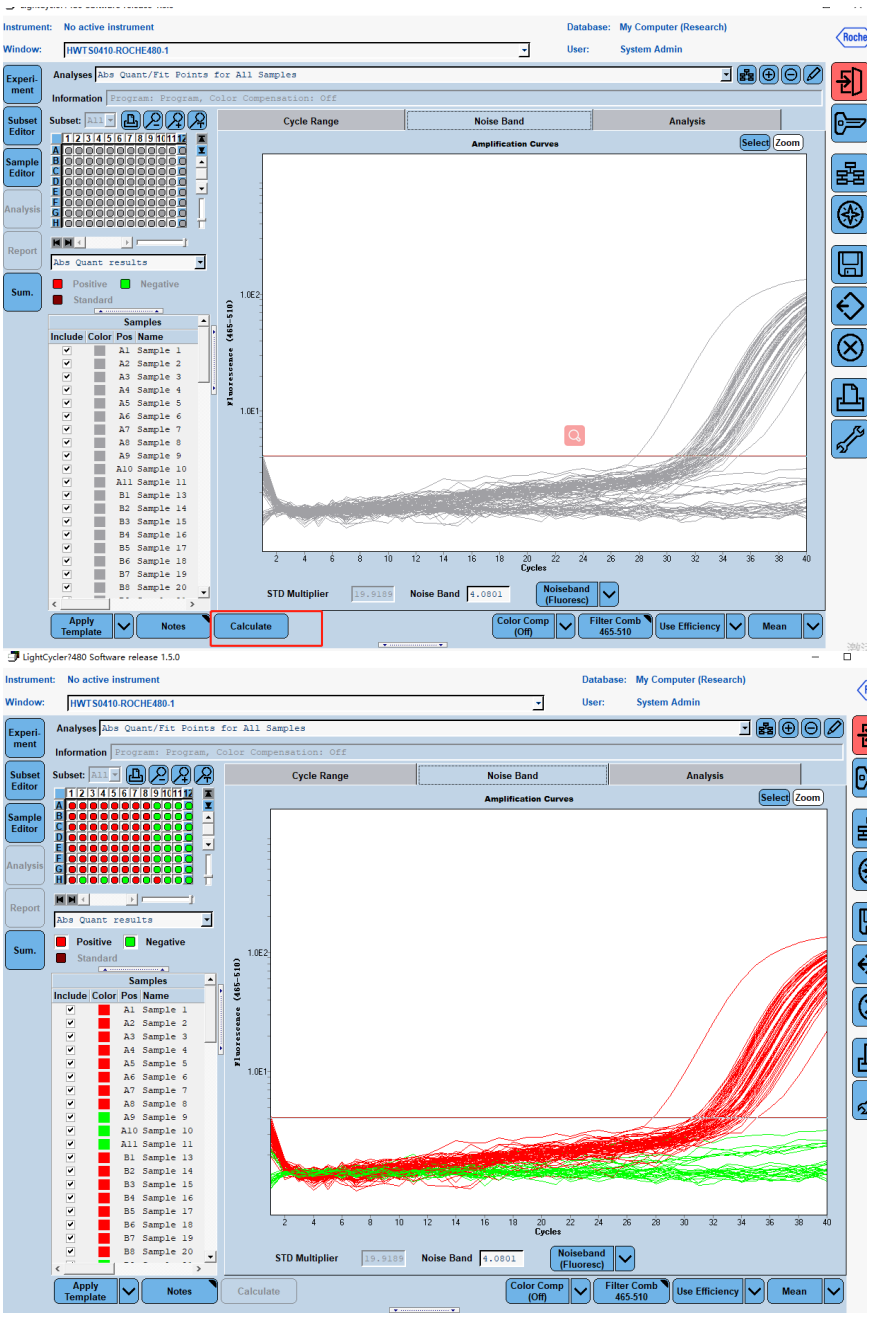
(8) Use auto threshold to check the result of the no template control (NTC). If the NTC shows no Ct values data for both the FAM and the VIC/HEX channels, then go to step (10) directly, and use auto threshold for sample analysis.

(9) Manually set threshold if the NTC shows a Ct value > 37 for the FAM channel or a Ct value > 35 for the VIC/HEX channel and does not exhibit sigmoidal amplification curves:

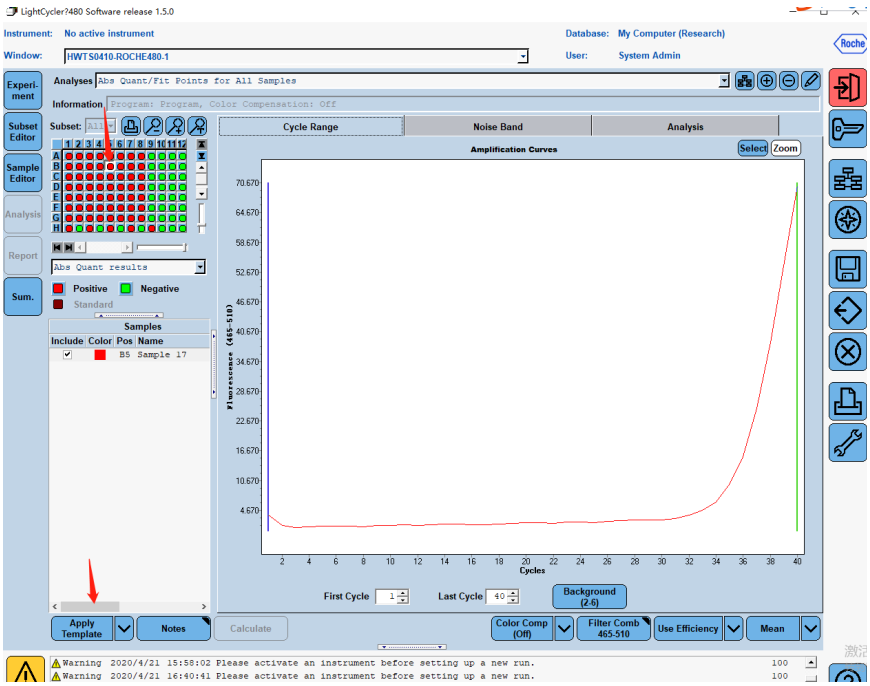
To manually adjust threshold, select the **Noise Band** tab, and select FAM and VIC/HEX channel to set up threshold respectively (see screenshot below).



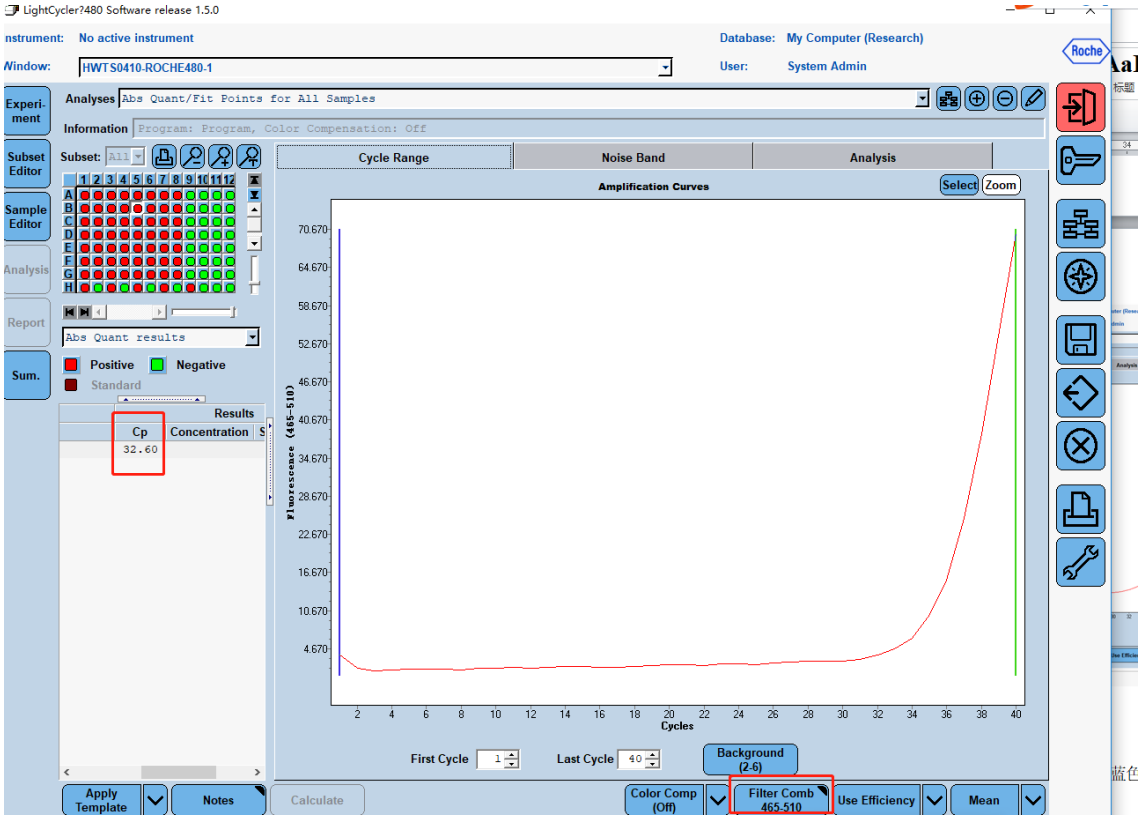
<u>Adjust the threshold to be equal to the maximum level of the no-template control curve (i.e., equal to the maximum value of the random noise curve)</u>, then click **Calculate** to apply the change. The results will be analyzed as shown in the screenshots below.



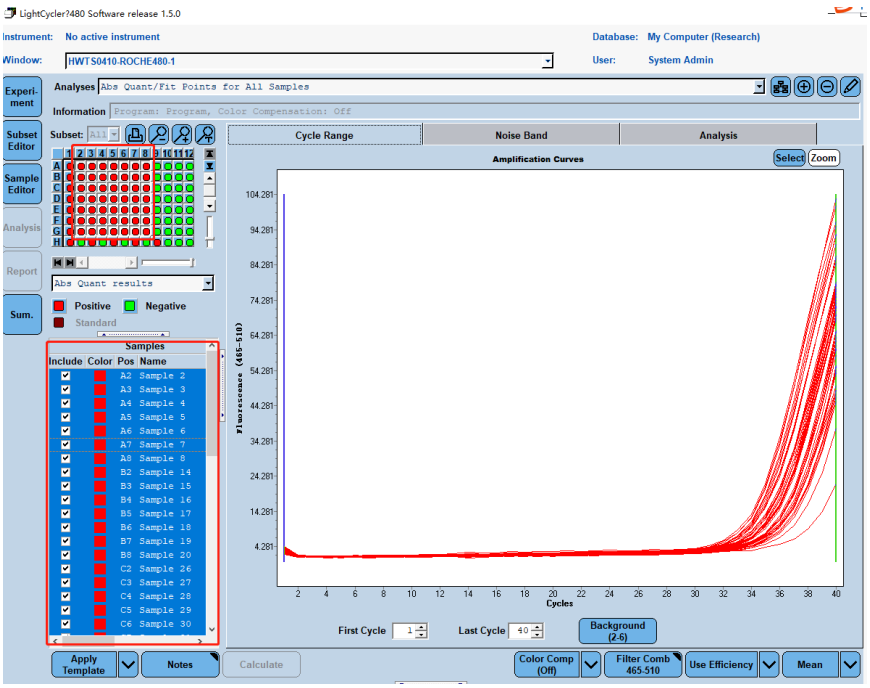
(10) In the upper left corner of the interface, select a well, and corresponding Ct value will show by dragging the bar to the right (see screenshot below and follow red arrows).

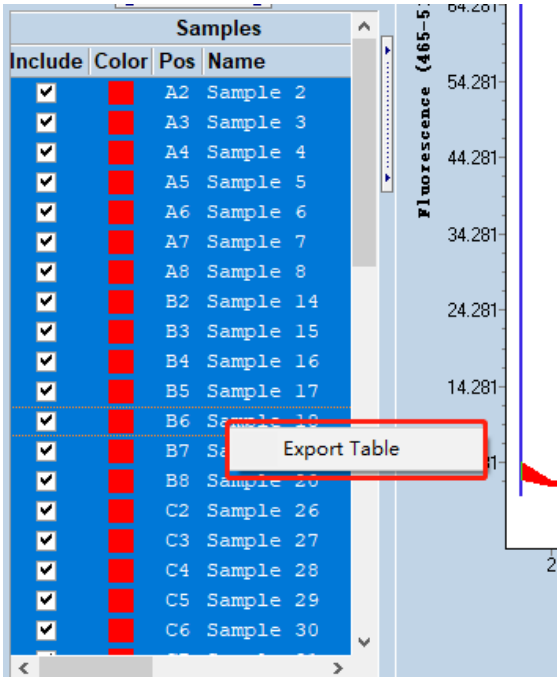


(11) To review Ct values of different channels, select **Filter Comb** (see screenshot below).

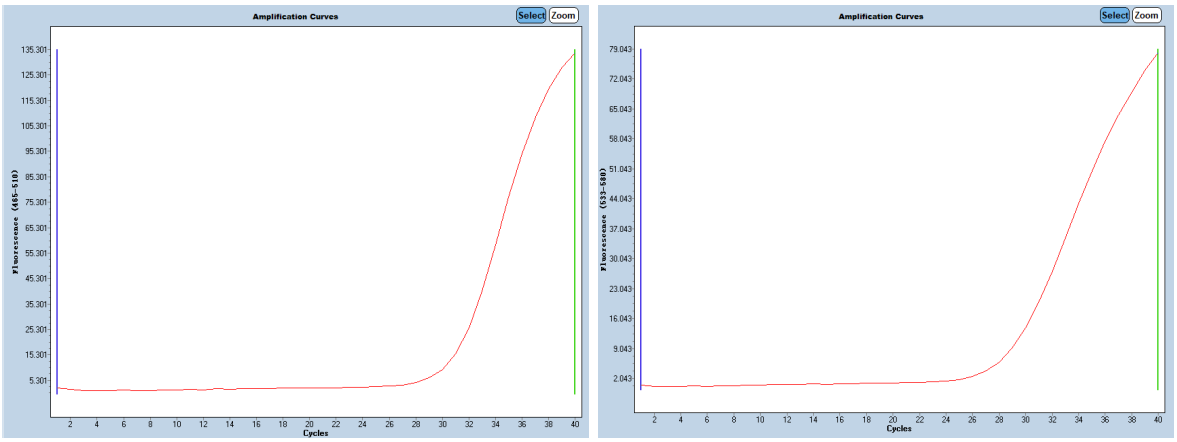


(12) To export results, click **Export Table**.



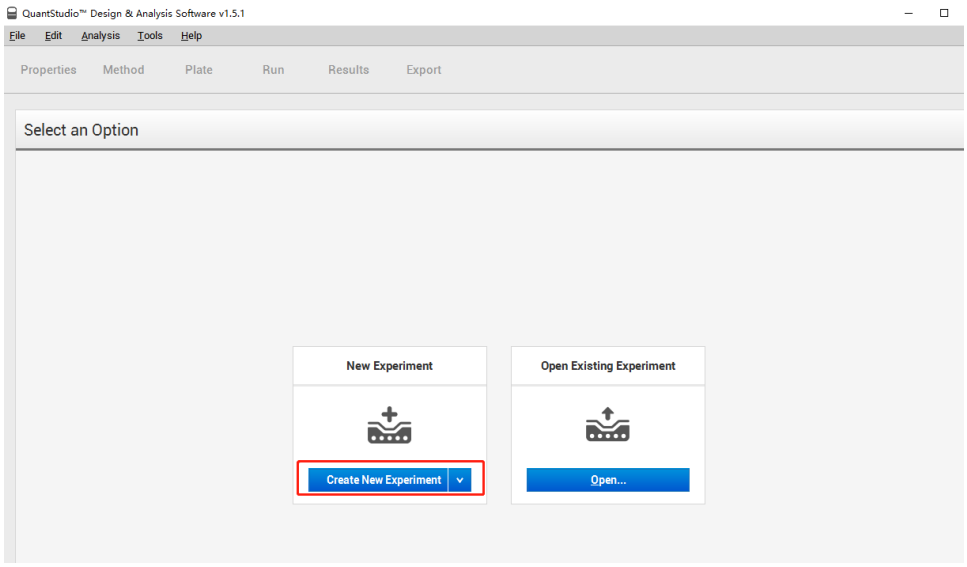


(13) Observe the Ct values of target FAM (left screenshot below) and VIC/HEX (internal reference, right screenshot below) of samples and determine the result of samples referring to instructions for use of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*.



3. Running a test and analyzing data in QuantStudio 5 Real-Time PCR System with (software v1.5.1):

(1) Double click QuantStudio Design & Analysis Software on the desktop, or select Start - All Program - Applied Biosystems - QuantStudio Design & Analysis Software to start the software. Then click **Create New Experiment**.



(2) Enter experiment information as listed below in the **Properties** interface:

a. Enter experiment in the Name field;

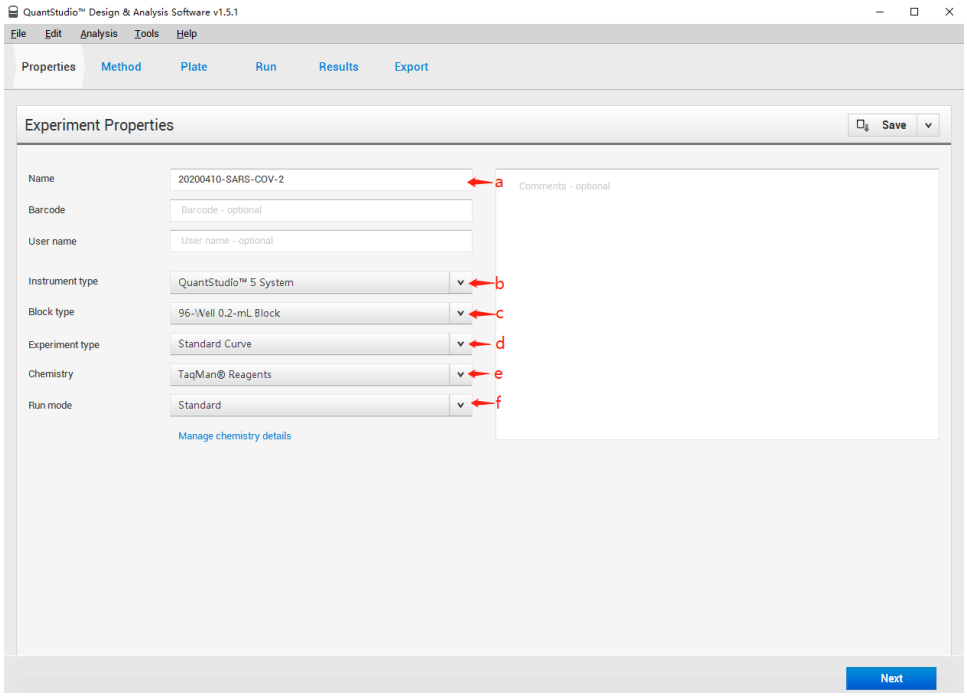
b. Select **QuantStudio™ 5 Systems** for instrument type;

c. Select **96-well 0.2ml Block** for Block type;

d. Select **Standard Curve** for Experiment type;

e. Select **TaqMan Reagents** for Chemistry;

f. Select **Standard** for Run mode.



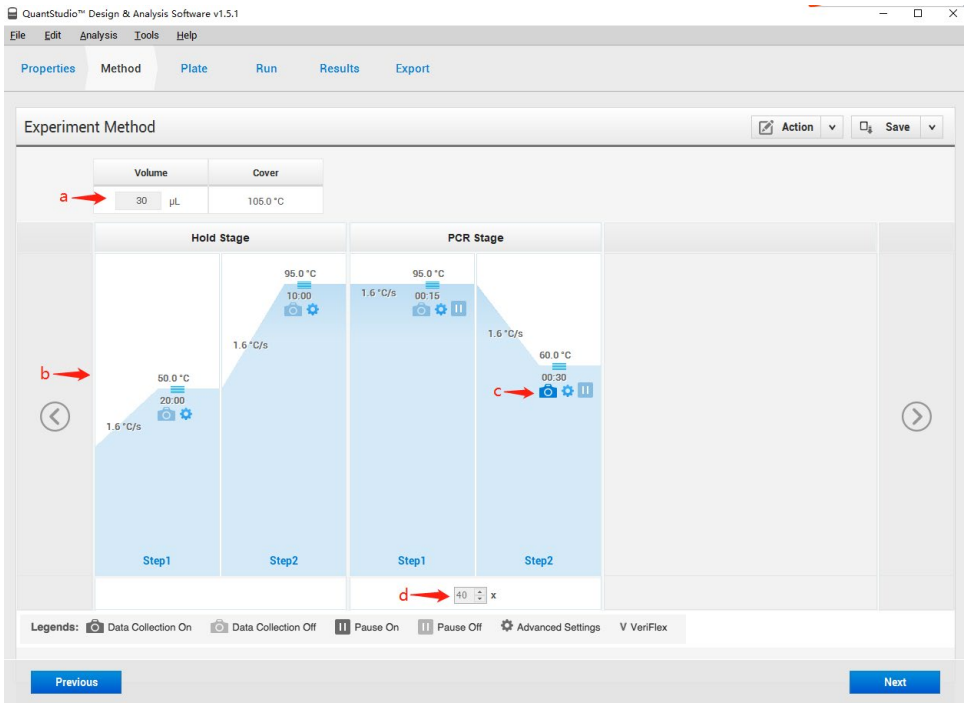
(3) Click **Next** to enter **Method** interface. Set the experiment method as shown below.

a. Set volume at **30** µL;

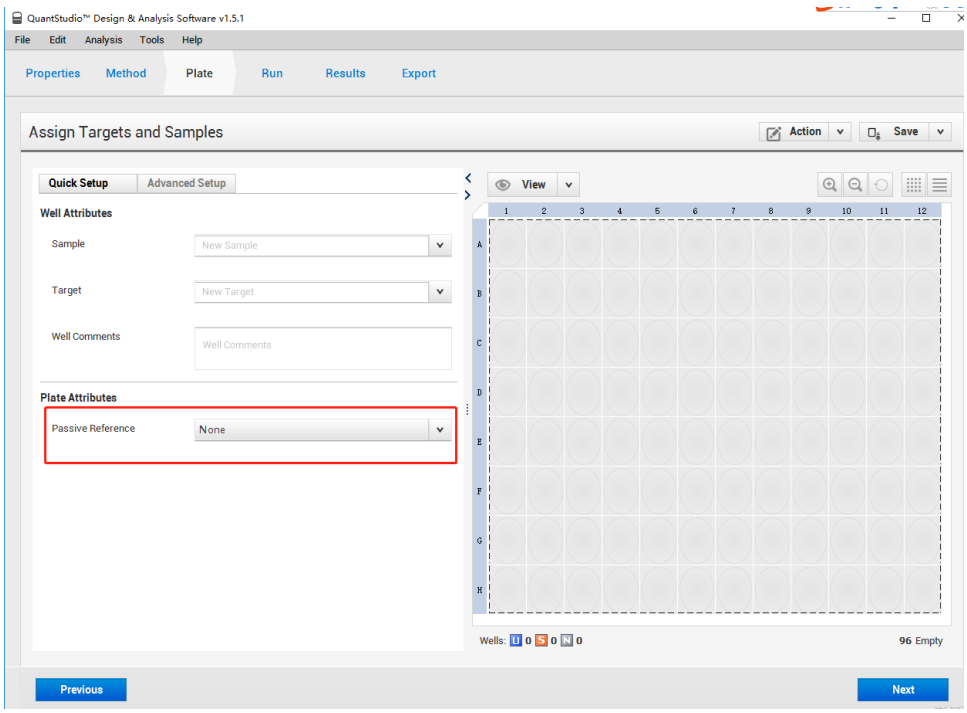
b. Set Holding stage and PCR Stage as shown below;

c. Fluorescence Measured at Step of "60.0°C, 30 s, ensure **Camera** icon is in blue;

d. Enter **40** for cycle number.

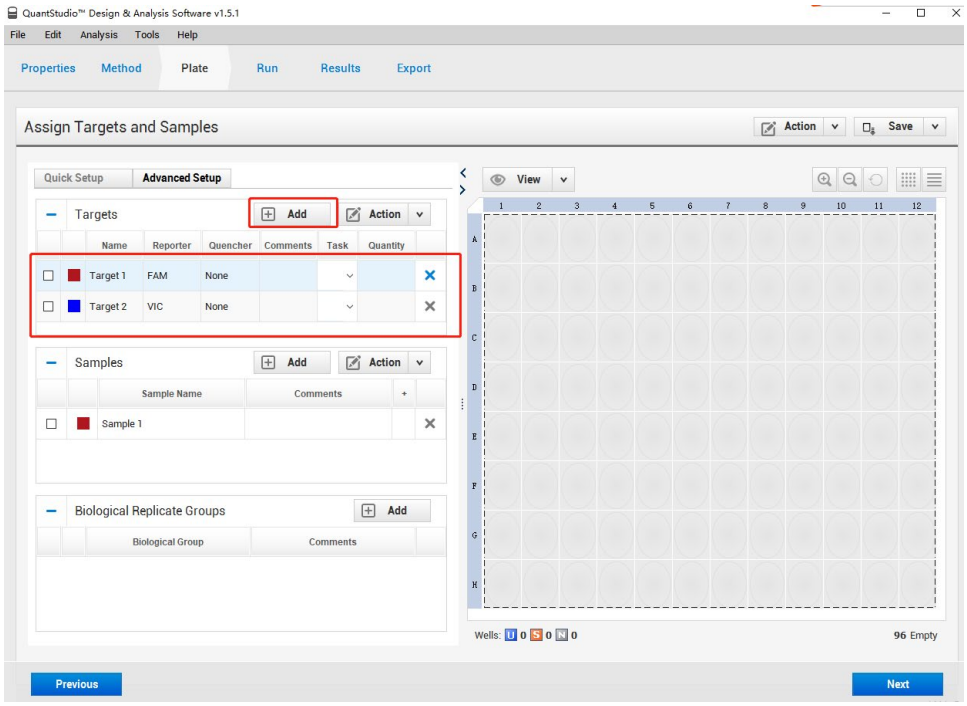


(4) Click on **Next** to enter **Plate** interface. Double click **Quick Setup** and set **None** for passive reference.

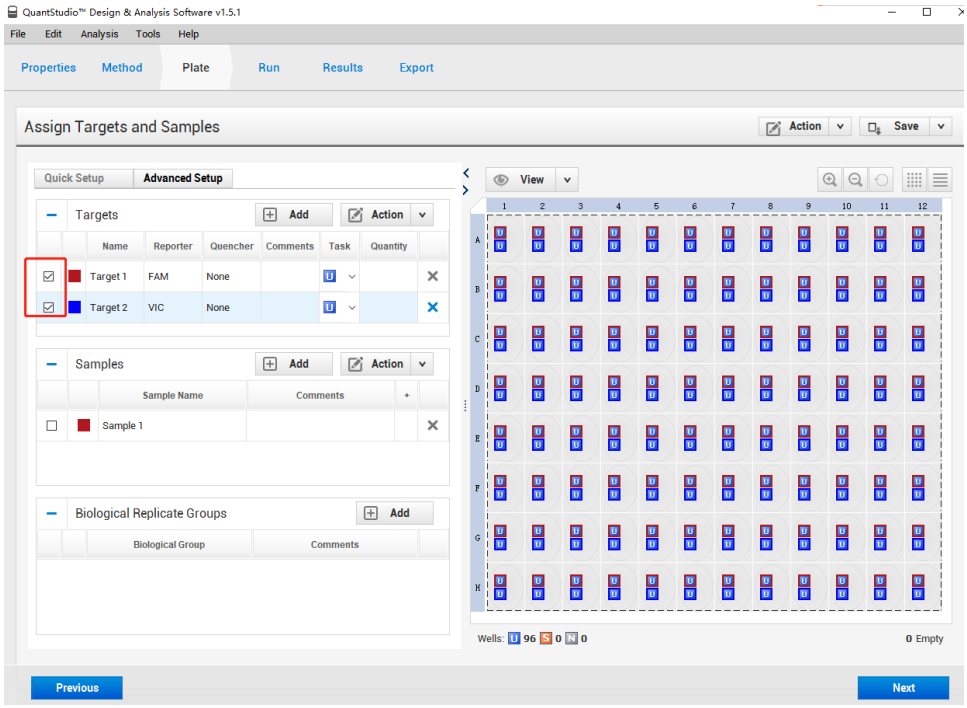


(5) Click **Advanced Setup**, then click **Add** to set target. Target 1 SARS-CoV-2, set Reporter

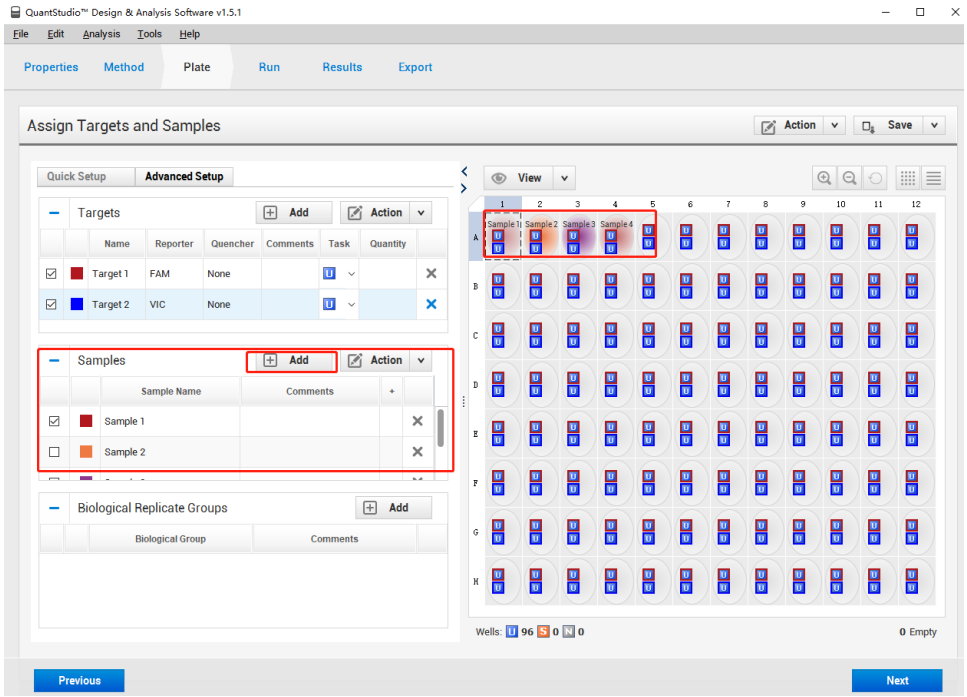
FAM and Quencher None; Add Target 2 internal reference, set Report **VIC/HEX** and Quencher **None**.



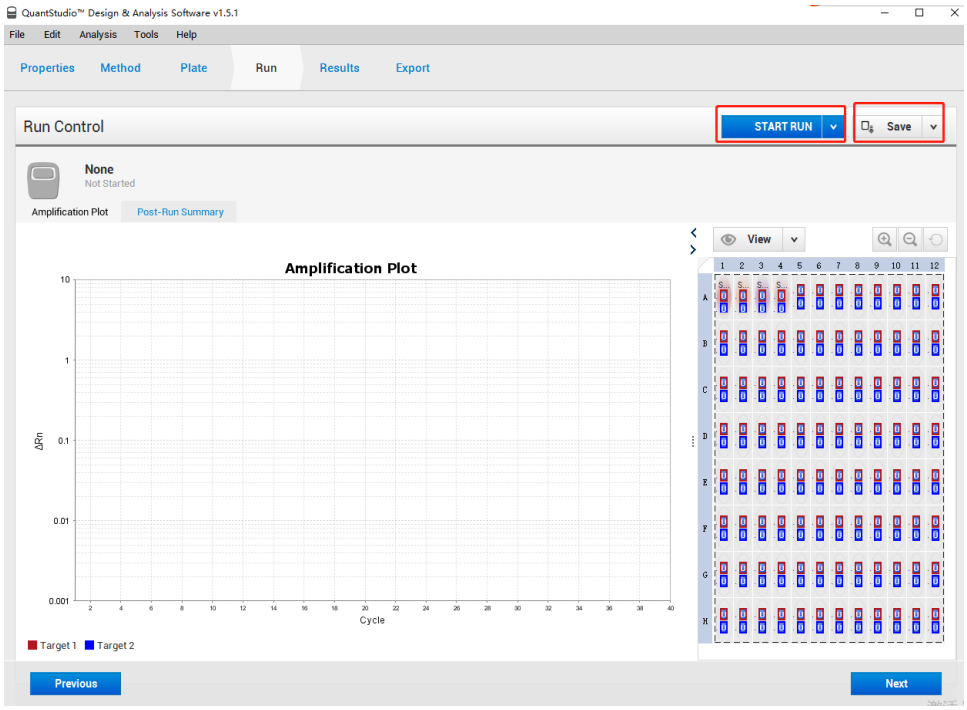
(6) Assign the Targets and Samples in the **Advanced setup** interface. Enter individual sample name in assigned wells and assign all targets.



(7) Click **Add** in the samples field as shown below, and edit individual samples as needed.



(8) Click **Next** to enter **Run** interface, click **Save,** then click **START RUN**.

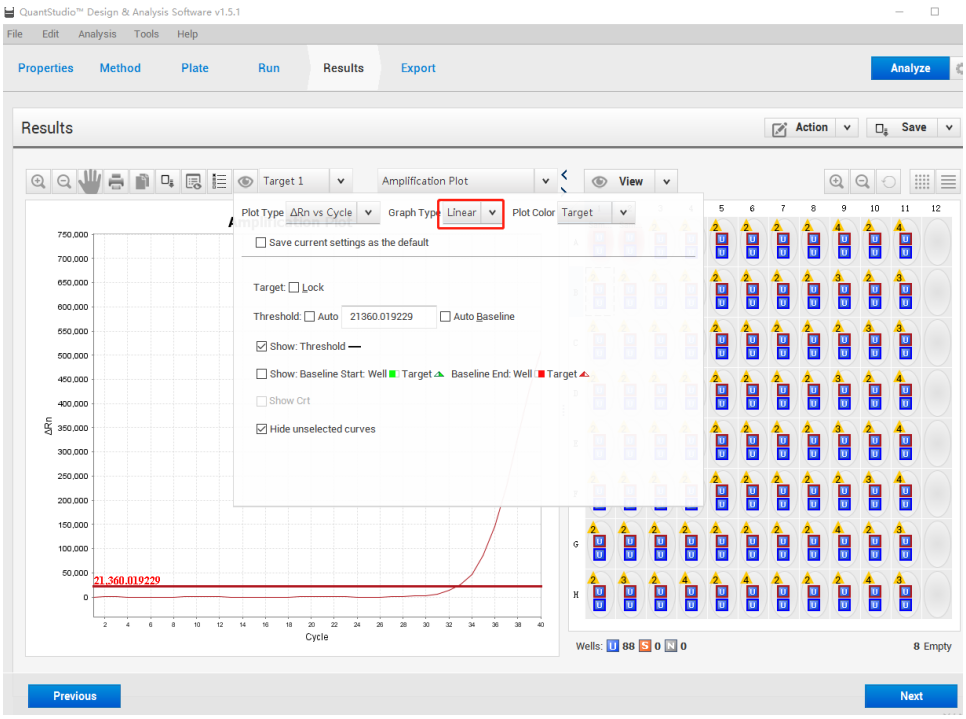


(9.1) After the experiment is done, click **Results** to enter Results interface, click **Analyze** on the top right-hand corner to analyze the data as shown below for auto threshold setting.

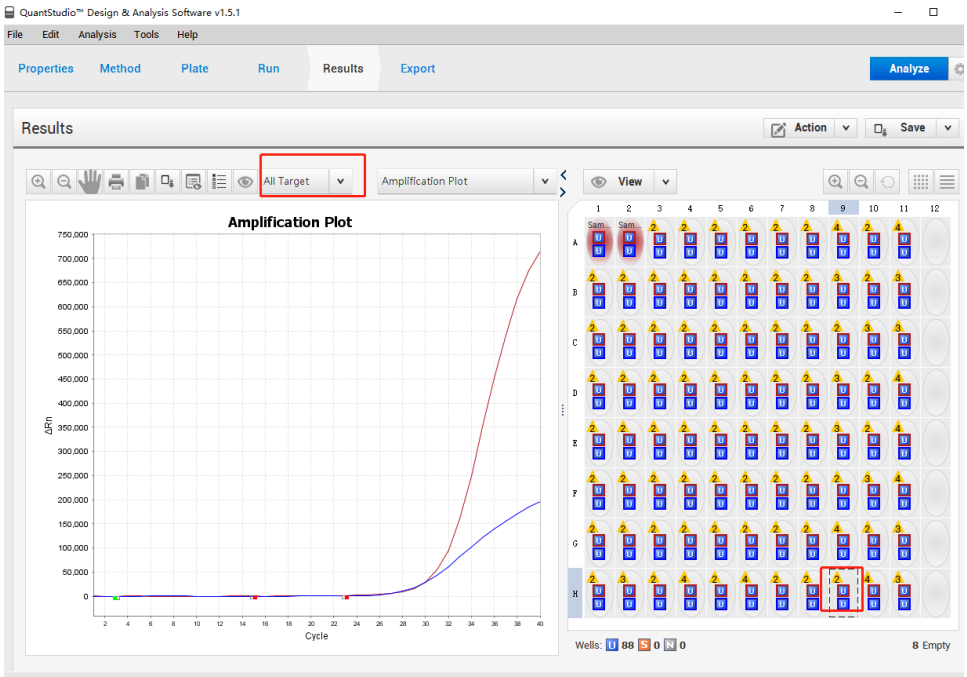
a. Click **Show Plot Setting**, under the **Graph Type**, select **Linear**.
b. Use auto threshold to check the result of the no template control (NTC). If the NTC shows no Ct values for both the FAM and the VIC/HEX channels, then go to step (10) directly, and use auto threshold for sample analysis.

(9.2) Manually set threshold if the NTC shows a Ct value > 37 for the FAM channel or a Ct value > 35 for the VIC/HEX channel and does not exhibit sigmoidal amplification curves:

Manually adjust baseline and threshold for each target. Click **Show Plot Setting**, select a target, tick **Show: Threshold** and **Show: Baseline.** Tick Threshold "☑ Auto" to "□Auto and **adjust the threshold to be equal to the maximum level of no-template control curve (i.e., equal to the maximum value of the random noise curve)**, then click **Analyze**.



(10) Observe the Ct value of target "SARS-CoV-2" and "internal reference" of samples and determine the result of samples referring to instructions for use of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*. See below screenshot depicting amplification curve for SARS-CoV-2 in red and internal reference in blue.



Quality Control and Interpretation of Results

Quality control:

Quality control 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.

Using auto threshold for both the FAM and VIC/HEX channels with baseline setting starting point at cycle 3 and ending at cycle 15, the no template (negative) control should provide (i) Ct value of 0 (i.e., "no data available") or Ct > 37 for the FAM channel (with no sigmoidal amplification curve) and (ii) Ct value of 0 (i.e., "no data available") or Ct > 35 for the VIC/HEX channel (with no sigmoidal amplification curve).

Under either auto threshold or manual threshold, the positive control should provide sigmoidal amplification curves in both the FAM and VIC/HEX channels, and the Ct values in the FAM and VIC/HEX channels should be no higher than 37 and 35, respectively.

The amplification curve for the test specimen should be in a sigmoidal shape with a Ct value no 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 for each test. If any of the requirements is not met in an individual test, the test is invalid. Table 3 provides further details for interpretation of quality control results.

Table 3. Interpretation of results for quality control.

Quality control metrics	VIC/HEX (observation)	FAM (observation)	Interpretation
No template control	No data or No sigmoidal amplification curve and Ct value is > 35	No data or No sigmoidal amplification curve and Ct value is > 37	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.

Quality control metrics	VIC/HEX (observation)	FAM (observation)	Interpretation
Positive control	No data or No sigmoidal amplification curve or Ct value is > 35	No data or No sigmoidal amplification curve 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-CoV-2* 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 operating characteristic curves of the tested clinical samples. The Ct value in the FAM channel for a valid no template (negative) control should be 0 (i.e., "no data available") or Ct > 37 (with 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, using auto threshold for both the FAM and VIC/HEX channels with baseline setting starting point at cycle 3 and ending at cycle 15, the no template (negative) control should provide (i) Ct value of 0 (i.e., "no data available") or Ct > 37 for the FAM channel (with no sigmoidal amplification curve) and (ii) Ct value of 0 (i.e., "no data available") or Ct > 35 for the VIC/HEX channel (with no sigmoidal amplification curve). Based on the NTC result, the positive control can be assessed using auto threshold or manually adjusted threshold. The positive control should provide amplification curves 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 channels 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. Once both requirements are satisfied, assessment of clinical specimen test results can be performed. A workflow diagram is provided at the end of this section.

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 curves in the FAM and VIC/HEX channels have Ct values 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).

Examples illustrating how to interpret test results obtained with the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* are provided in Table 4.

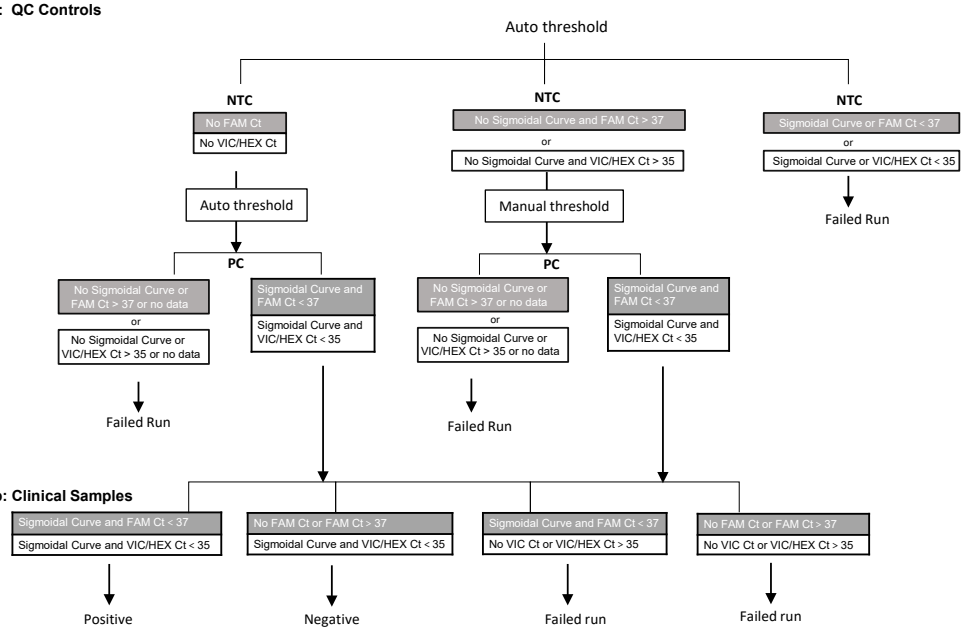
Table 4. Example interpretation of test results for *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*

	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**; Valid Internal Reference
Sample 2	Sigmoidal amplification curve and Ct value is <35.	No Ct data or no sigmoidal amplification curve or Ct value is >37.	**Negative for SARS-COV-2 RNA**; Valid Internal Reference
Sample 3	No Ct data or no sigmoidal amplification curve or Ct value is >35.	Sigmoidal amplification curve and Ct value is <37.	**Invalid test, please retest***
Sample 4	No Ct data or no sigmoidal amplification curve or Ct value is >35.	No Ct or no sigmoidal amplification curve or Ct value is >37.	**Invalid test, please retest***

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

Test result interpretation workflow:

1st Step: QC Controls



2nd Step: Clinical Samples

NTC: No Template Control
PC: Positive Control
Auto threshold: Using auto threshold for both FAM and VIC/HEX channels with baseline setting starting point at cycle 3 and ending at cycle 15.
Manual threshold: Adjusting the threshold to be equal to the maximum level of NTC curve with baseline setting starting point at cycle 3 and ending at cycle 15.

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-CoV-2* was established using throat (oropharyngeal) swabs and bronchoalveolar lavage fluid (BALF) samples. Nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates are also considered acceptable specimen types for use with the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* 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 SARS-CoV-2.

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

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

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

However, to assist clinical laboratories using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*- must 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.

B. Authorized laboratories using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* must perform the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* 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 perform the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* are not permitted.

C. Authorized laboratories that receive the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* 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 the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* 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 the test in accordance with the authorized labeling.

G. BGI Genomics Co. Ltd., its authorized distributor(s), and authorized laboratories using the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* must 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.

[1] 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 (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-CoV-2*.

LoD with Pseudo-virus:
The LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* 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 (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

*Note: this concentration may not accurately reflect the number of genomic equivalents present

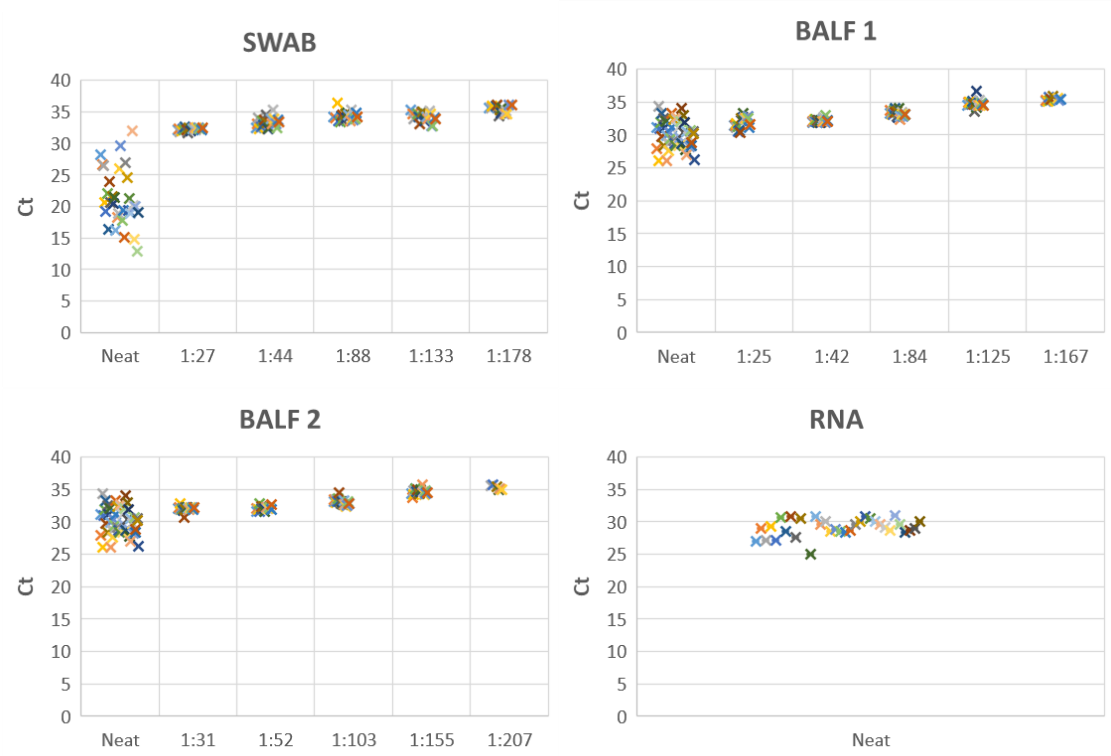
The LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* was evaluated by testing the dilutions of each clinical specimen described above (n = 20 each), using the QIAamp Virus RNA Mini Kit and Applied Biosystems™ Real Time PCR System 7500 for RNA extraction and PCR. The LoD was determined to be the highest dilution at which ≥19/20 results were positive (i.e., ≥95% positive) (Table 7-1).

Table 7-1. LoD confirmation.

Specimen	Concentration of SARS-CoV-2 estimated by Digital PCR (Copies/mL)*	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 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.

Validation of additional RNA extraction kit:
Data shown in Table 7-2 below were obtained by diluting known SARS-CoV-2 positive clinical specimens with SARS-CoV-2 negative throat swab or BALF matrix, as appropriate. The results demonstrate that the LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* used with MGIEasy Nucleic Acid Extraction Kit (manual) is comparable to the LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* used with QIAamp Virus RNA Mini Kit (manual). Applied Biosystems™ Real Time PCR System 7500 was used for this validation.

Table 7-2. Validation data for additional extraction kit used manually.

Sample number and specimen type	Concentration of SARS-CoV-2 (Copies/mL)*	MGIEasy Nucleic Acid Extraction Kit (manual)
1. Throat swab (positive)	300 (2x LoD)**	5/5
	150 (1x LoD)	5/5
	75 (0.5x LoD)	2/5
	37.5 (0.25x LoD)	0/5
2. Throat swab (positive)	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	75 (0.5x LoD)	2/5

	37.5 (0.25x LoD)	1/5
3. Throat swab (positive)	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	75 (0.5x LoD)	2/5
	37.5 (0.25x LoD)	0/5
4. BALF (positive)	300 (3x LoD)	5/5
	150 (1.5x LoD)	5/5
	75 (0.75x LoD)	2/5
	37.5 (0.375x LoD)	0/5
Throat swab (negative)	0 (0x LoD)	0/5
BALF (negative)	0 (0x LoD)	0/5

* Concentrations determined by digital PCR. This concentration may not accurately reflect the number of genomic equivalents present.

** LoD as determined using the QIAamp Virus RNA Mini Kit (Table 7-1).

Validation of additional RNA extraction kit using automation system:
Data shown in Table 7-3 below were obtained by diluting known SARS-CoV-2 positive clinical specimens with SARS-CoV-2 negative throat swab or BALF matrix, as appropriate. The results demonstrate that the LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* used with MGIEasy Nucleic Acid Extraction Kit and liquid handler MGISP-960RS is comparable to the LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* used with QIAamp Virus RNA Mini Kit (manual). For this validation, a single MGISP-960RS instrument was used in accordance with MGISP-960RS manufacturer's instruction, to perform all extraction steps, and Applied Biosystems™ Real Time PCR System 7500 was used in subsequent steps.

Table 7-3. Validation data for additional extraction kit used with liquid handler MGISP-960RS.

Sample number and specimen type	**Concentration of SARS-CoV-2 (Copies/mL)***	**MGIEasy Nucleic Acid Extraction Kit on MGISP-960RS**
1. Throat swab (positive)	450 (3x LoD)**	5/5
	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	100 (0.67x LoD)	5/5
	75 (0.5x LoD)	4/5
	37.5 (0.25x LoD)	1/5
2. Throat swab (positive)	450 (3x LoD)	5/5
	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	100 (0.67x LoD)	5/5
	75 (0.5x LoD)	5/5
	37.5 (0.25x LoD)	2/5
3. Throat swab	450 (3x LoD)	5/5

(positive)	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	100 (0.67x LoD)	5/5
	75 (0.5x LoD)	5/5
	37.5 (0.25x LoD)	2/5
4. Throat swab (positive)	450 (3x LoD)	5/5
	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	100 (0.67x LoD)	5/5
	75 (0.5x LoD)	5/5
	37.5 (0.25x LoD)	2/5
5. Throat swab (positive)	450 (3x LoD)	5/5
	300 (2x LoD)	5/5
	150 (1x LoD)	5/5
	100 (0.67x LoD)	5/5
	75 (0.5x LoD)	4/5
	37.5 (0.25x LoD)	1/5
6. BALF (positive)	450 (4.5x LoD)	5/5
	300 (3x LoD)	5/5
	150 (1.5x LoD)	5/5
	100 (1x LoD)	5/5
	75 (0.75x LoD)	4/5
	37.5 (0.375x LoD)	2/5
7. BALF (positive)	450 (4.5x LoD)	5/5
	300 (3x LoD)	5/5
	150 (1.5x LoD)	5/5
	100 (1x LoD)	5/5
	75 (0.75x LoD)	4/5
	37.5 (0.375x LoD)	2/5
Throat swab (negative)	0 (0x LoD)	0/5
BALF (negative)	0 (0x LoD)	0/5

* Concentrations determined by digital PCR. This concentration may not accurately reflect the number of genomic equivalents present.
** LoD as determined using the QIAamp Virus RNA Mini Kit (Table 7-1).

Validation of additional PCR systems:
Data shown in Table 7-4 below were obtained by diluting known SARS-CoV-2 positive clinical specimens with SARS-CoV-2 negative throat swab or BALF matrix, as appropriate. The result demonstrate that the LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* used with (i) ABI 7500 Fast Real Time PCR System, (ii) Roche LightCycler® 480 System, or (iii) QuantStudio 5 Real-Time PCR System is comparable to the LoD of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* used with Applied Biosystems™ Real Time

PCR System 7500. The QIAamp Virus RNA Mini Kit (manual) was used in the extraction step in this validation.

Table 7-4. Validation data for additional PCR systems.

Sample number and specimen type	Concentration of SARS-CoV-2 (Copies/mL)*	ABI 7500 Fast Real Time PCR System	Roche LightCycler® 480 System	QuantStudio 5 Real-Time PCR System
1. Throat swab (positive)	300 (2x LoD)**	5/5	5/5	5/5
	150 (1x LoD)	5/5	5/5	5/5
	100 (0.67x LoD)	5/5	5/5	5/5
	75 (0.5x LoD)	3/5	5/5	4/5
2. Throat swab (positive)	300 (2x LoD)	5/5	5/5	5/5
	150 (1x LoD)	5/5	5/5	5/5
	100 (0.67x LoD)	5/5	5/5	5/5
	75 (0.5x LoD)	2/5	5/5	3/5
3. Throat swab (positive)	300 (2x LoD)	5/5	5/5	5/5
	150 (1x LoD)	5/5	5/5	5/5
	100 (0.67x LoD)	5/5	5/5	5/5
	75 (0.5x LoD)	5/5	4/5	4/5
4. BALF (positive)	300 (3x LoD)	5/5	5/5	5/5
	150 (1.5x LoD)	5/5	5/5	5/5
	100 (1x LoD)	5/5	5/5	5/5
	75 (0.75x LoD)	1/5	5/5	4/5
5. BALF (positive)	300 (3x LoD)	5/5	5/5	5/5
	150 (1.5x LoD)	5/5	5/5	5/5
	100 (1x LoD)	5/5	5/5	5/5
	75 (0.75x LoD)	5/5	5/5	5/5
Throat swab (negative)	0 (0x LoD)	0/5	0/5	0/5

* Concentrations determined by digital PCR. This concentration may not accurately reflect the number of genomic equivalents present.

** LoD as determined using the QIAamp Virus RNA Mini Kit (Table 7-1)

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-CoV-2* 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	8×10^7 Copies/mL
2	Seasonal H1N1 influenza virus		1.8×10^7 Copies/mL

3	Influenza A virus (H3N2)	(People's Republic of China)	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	1.6×10^6 Copies/mL
29	Measles virus		4.8×10^5 Copies/mL

30	Cytomegalovirus	(People's Republic of China)	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-CoV-2* 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-CoV-2* 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 SARS-CoV-2.

Human beta-actin (Copies/mL)	Pseudo-virus (Copies/mL)	FAM (virus) Ct value	Average FAM (virus) Ct value	VIC/HEX (beta actin) Ct value	Average VIC/HEX (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	33.11	/	/
		33.07			
		33.23			
		33.14			
		33.19			
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-CoV-2* 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%	

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples, and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were MGIEasy nucleic acid extraction kit (Cat. No.:1000020471, for 96 preps/kit) and ABI 7500 Real-Time PCR System, respectively. The results are summarized in Table 13.

Table 13: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal (NP) swab	3.0×10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

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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

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

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

Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2

Instructions for Use Appendix A: Instrument Qualification Method

1. Purpose

This appendix is intended to provide a qualification procedure to prepare a panel of spiked samples for verifying the performance of the Roche LightCycler® 480 System with the BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 by the end user. Qualification with the BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 must be achieved by passing the acceptance criteria listed in this procedure prior to usage for diagnostic testing.

Note: ABI 7500 Real-Time PCR System, ABI 7500 Fast Real-Time PCR System and QuantStudio 5 Real-Time PCR System are not required to perform ‘‘Instrument Qualification Method’’. Please print and place the additional label in Appendix B on the front panel of these instruments directly.

2. Required Materials

Description	Included in the kit
Negative control: SARS-CoV-2 No Template Control (NTC)	Yes
Positive control: SARS-CoV-2 Positive Control (PC)	Yes
Other: Negative patient sample	No

3. Precautions

Please refer to the section of warnings and precautions in the BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 IFU. Ensure instrument calibration and maintenance are current prior to performing qualification. Required materials and kit components must be stored at appropriate temperatures as directed and kept on ice once thawed. The extracted RNA samples should be kept cold during testing.

4. Procedure

4-1. Preparation of two positive spike-in samples. Two positive spike-in samples should be prepared by following the procedure below:

(1) Label two 1.5 mL RNase-free tubes as A and B.

(2) Dilute SARS-CoV-2 Positive Control 80-fold with negative patient sample into approximately 4xLoD concentration (600 copies/ml) of SARS-CoV-2 and label as tube A:

Pipette 987.5 µL of negative patient sample into Tube A and add 12.5 µL of SARS-CoV-2 Positive Control.

(3) Dilute SARS-CoV-2 Positive Control 16-fold with negative patient sample into approximately 20xLoD concentration (3000 copies/ml) of SARS-CoV-2 and label as tube B: Pipette 937.5 µL of negative patient sample into Tube B and add 62.5 µL of SARS-CoV-2 Positive Control.

(4) Vortex well and centrifuge tubes A and B to collect the contents at the bottom of each tube.

4-2. Extraction:

Extract the positive spike-in samples (tubes A and B) and negative control (SARS-CoV-2 No Template Control, NTC) in triplicate, extract only one non-diluted positive control (SARS-CoV-2 Positive Control, PC) for a total of ten extractions.

(1) For manual extraction using the QIAGEN QIAamp Viral RNA Mini Kit (cat. #52904 or 52906), please refer to the QIAGEN user manual for the detailed instructions. The following is a brief procedure:

(1.1) Prepare enough Buffer AVL-carrier RNA mix for ten samples.

(1.2) Label ten 1.5 mL microcentrifuge tubes as tubes 1-10.

(1.3) Add 560 µL of Buffer AVL-carrier RNA mix into each of tubes 1-10.

(1.4) Add 140 µL of NTC sample into each of tubes 1-3.

(1.5) Add 140 µL of tube A into each of tubes 4-6.

(1.6) Add 140 µL of tube B into each of tubes 7-9.

(1.7) Add 140 µL of PC sample into tube 10.

(1.8) Extract all samples using the QIAGEN QIAamp Viral RNA Mini Kit following the manufacturer's instructions.

(1.9) Elute the samples using 60 µL of Buffer AVE.

(1.10) After extraction, the RNA should be used immediately or stored at -70°C for use later.

(2) For automated extraction using the High-throughput Automated Sample Preparation System (MGISP-960RS) and MGIEasy Nucleic Acid Extraction Kit (cat. #1000020261 or 1000020471), please follow MGI user manual for the detailed instructions. The following is a brief procedure:

(2.1) Set up the instrument according to MGISP-960RS user manual.

(2.2) Prepare enough consumables for the extraction of ten samples.

(2.3) Add 180 μL sample into a deep-well plate (MGI, cat. # 1000004644) to make sure that there is 160 μL sample can be transfered. 3 replicates per tube A, B and NTC sample; pipette NTC sample into well A1-A3, tube A sample into well A4-A6, tube B sample into well A7-A9. Pipette PC sample into only one well A10.

(2.4) Prepare reagents and add them into individual deep-well plates according to the manufacturer's instructions.

(2.5) Extract the samples using MGIEasy Nucleic Acid Extraction Kit and MGISP-960RS instrument.

(2.6) After the extraction process is finished, the RNA should be used immediately or stored at -70°C for use later.

(3) For manual extraction using MGIEasy Nucleic Acid Extraction Kit (cat. #1000020261 or 1000020471), please follow MGI user manual for the detailed instructions. The following is a brief procedure:

(3.1) Prepare enough Buffer Mixture for ten samples.

(3.2) Label ten 1.5 mL microcentrifuge tubes as tubes 1-10.

(3.3) Dispense 460 μL buffer mixture into each of tubes 1-10.

(3.4) Add 200 μL of NTC sample into each of tubes 1-3.

(3.5) Add 200 μL of tube A sample into each of tubes 4-6.

(3.6) Add 200 μL of tube B sample into each of tubes 7-9.

(3.7) Add 200 μL of PC sample into tube 10.

(3.8) Extract the samples using MGIEasy Nucleic Acid Extraction Kit by following the manufacturer's instructions.

(3.9) Elute the samples with 50 μL nuclease-free water provided in the extraction kit.

(3.10) After extraction, the RNA should be used immediately or stored at -70°C for use later.

4-3. Follow *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* IFU to set up RT-PCR reaction and perform data analysis.

4-4. Expected Results

(1) NTC samples (extracted sample tubes 1-3 or wells A1-A3): 100% (3/3 wells) negative for both SARS-CoV-2 RNA and internal control.

(2) Tube A: 4xLoD samples (extracted sample tubes 4-6 or wells A4-A6): 100% (3/3 wells) positive for both SARS-CoV-2 RNA and internal control.

(3) Tube B: 20xLoD samples (extracted sample tubes 7-9 or wells A7-A9): 100% (3/3 wells) positive for both SARS-CoV-2 RNA and internal control.

(4) Non-diluted PC sample (extracted sample tube 10 or well A10): positive for both SARS-CoV-2 RNA and internal control.

4-5. Acceptance Criteria:

The results should be 100% in concordance with the expected results.

4-6. When acceptance criteria are met, Roche LightCycler® 480 System is considered qualified for use with the *BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* in clinical testing.

5. Questions

If you have questions or comments about these instructions, please contact BGI Genomics Service Hotline: (+86) 400-706-6615.

Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2

Instructions for Use Appendix B: Additional Label

For ABI 7500 Real-Time PCR System, ABI 7500 Fast Real-Time PCR System, Roche LightCycler® 480 System and QuantStudio 5 Real-Time PCR System

Please print the "Emergency Use Only" label below, and place it on the front panel of ABI 7500 Real-Time PCR System, ABI 7500 Fast Real-Time PCR System, Roche LightCycler® 480 System and QuantStudio 5 Real-Time PCR System. Roche LightCycler® 480 System must successfully complete the qualification process before applying the label and using the instrument. If the instrument is labeled as Research Use Only, please cover it with the "Emergency Use Only" label below. The instruments should retain this label throughout the EUA use of *BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*.

* Refer to Appendix A (Instrument Qualification Method) for instructions.

Emergency Use Only

This instrument is authorized for use with *BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2*

WARNINGS

Caution: 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).

The *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use in 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 *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of the *Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2* is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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 declaration is terminated or authorization is revoked sooner.