REF BC-01-0099

Instructions for Use

BioCore 2019-nCoV Real Time PCR Kit

IVD

For under Emergency Use Authorization only

For prescription use only

For in vitro diagnostic use only

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Invites Ecosystem Company

Contents

BioCore 2019-nCoV Real Time PCR Kit

1. Intended use

BioCore 2019-nCoV Real Time PCR Kit is a reverse-transcription Real time PCR test intended for qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as anterior nasal, mid-turbinate nasal, oropharyngeal, and nasopharyngeal swabs) and lower respiratory specimens (such as sputum, bronchoalveolar lavage (BAL), and tracheal aspirates) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The BioCore 2019-nCoV Real Time PCR Kit is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The BioCore 2019-nCoV Real Time PCR Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Principle of the examination method

The BioCore 2019-nCoV Real Time PCR Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe sets are designed to detect RNA from the *N* and the *RdRp* genes from the 2019-nCoV genome in upper and lower respiratory specimens from patients with signs and symptoms of infection who are suspected of COVID-19. The sequences of *N* gene and the *RdRp* gene are specific for SARS-CoV-2.

BioCore 2019-nCoV Real Time PCR Kit uses rRT-PCR and Taqman chemistry which is specifically applied to the reaction site. During PCR, a reaction product is formed by a specific primer at the target gene region of the 2019-nCoV, and at the same time, fluorescence is formed as the specific Taqman probe is decomposed in each gene. This kit is used together with the CFX96 Dx System (Bio-Rad Inc.), Applied Biosystems 7500 Real-Time PCR Instrument System (Thermo Fisher Scientific Inc.), or SLAN-96P (Shanghai Hongshi Medical Technology Co. Ltd). The RNA sample is extracted manually by using the RNA extraction kit, QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904).

3. Materials provided

3.1. BioCore 2019-nCoV Real Time PCR Kit (Cat No. BC-01-0099, 100T)

Kit Component	Volume in the Kit (for 100 Tests)	Description
2019-nCoV RT PCR Reaction Mixture	1100 µl/tube (10 µl/PCR reaction)	Reverse transcriptase DNA polymerase RNase Inhibitor DW
2019-nCoV RT PCR Primer/Probe Mixture	550 µl/tube (5 µl/PCR reaction)	Primer Probe DW
2019-nCoV RT PCR Positive Control	50 µl/tube (5 µl/PCR reaction)	*N* gene & *RdRp* gene Clone (plasmid) Human β globin Clone (plasmid)
2019-nCoV RT PCR Negative Control	500 µl/tube (in extraction step, use 200 µl)	DEPC-treated Demineralized Water

3.2 BioCore 2019-nCoV Real Time PCR Kit (Cat No. BC-01-0099 x4, 400T)

Kit Component	Volume in the Kit (for 400 Tests)	Description
2019-nCoV RT PCR Reaction Mixture	1100 µl/tube x4 (10 µl/PCR reaction)	Reverse transcriptase DNA polymerase RNase Inhibitor DW
2019-nCoV RT PCR Primer/Probe Mixture	550 µl/ tube x4 (5 µl/PCR reaction)	Primer Probe DW
2019-nCoV RT PCR Positive Control	50 µl/ tube x4 (5 µl/PCR reaction)	*N* gene & *RdRp* gene Clone (plasmid) Human β globin Clone (plasmid)
2019-nCoV RT PCR Negative Control	500 µl/ tube x4 (in extraction step, use 200 µl)	DEPC-treated Demineralized Water

4. Instruments and materials required but not provided

	Product name	Manufacturer	Cat. No.
Instruments	CFX96 Dx System	Bio-Rad	BR185-5484
	Applied Biosystems 7500	Thermo Fisher Scientific Inc	4351104 4351105
	SLAN-96P	Shanghai Hongshi Medical Technology Co.,Ltd	RM98000
	Bench top centrifuge	-	-
	Vortex mixer	-	-
Nuclease free water	To be used as negative control	-	-
4% NaOH	4% NaOH in nuclease free water; used for sputum pretreatments	User Prepared	-
Extraction Kit (Manual)	QIAamp DSP Viral RNA Mini Kit	Qiagen	61904
	Pipet (adjustable)	-	-
Consumables	Sterile pipet tips with filters	-	-
	Sterile 1.5 ml microtube	-	-
	MicroAmp Optical 8-Tube Strip	Applied Biosystems	4316567
	MicroAmp Optical 8-Cap Strip	Applied Biosystems	4323032
	MicroAmp Optical Adhesive Film	Applied Biosystems	4311971
	MicroAmp optical 96-well Reaction Plate	Applied Biosystems	N8010560
	Low-Profile PCR tubes 8-tube Strip, White	Bio-rad	TCS0851
	Optical Flat 8-Cap Strips for 0.2ml tube strips/plate	Bio-rad	TCS0803
	Microseal 'B' PCR Plate Sealing Film, adhesive, optical	Bio-rad	MSB-1001
	Multiplate 96-Well PCR Plates, low profile, unskirted, white	Bio-rad	MLL9651
	Biofact™ 0.2 ml PCR tube	BioFact	PW211-120

⚠ ***Every instrument requires qualification as well as periodic maintenance and calibration. Always read and understand the manufacturer's manual before using them.***

5. Reagent preparation

Frozen reagent should be thawed on ice before being used and should be returned to frozen storage immediately after use. Do not freeze-thawing test reagents more than 4 times.

Mix every reagent well with a vortex mixer and spin down before using it.

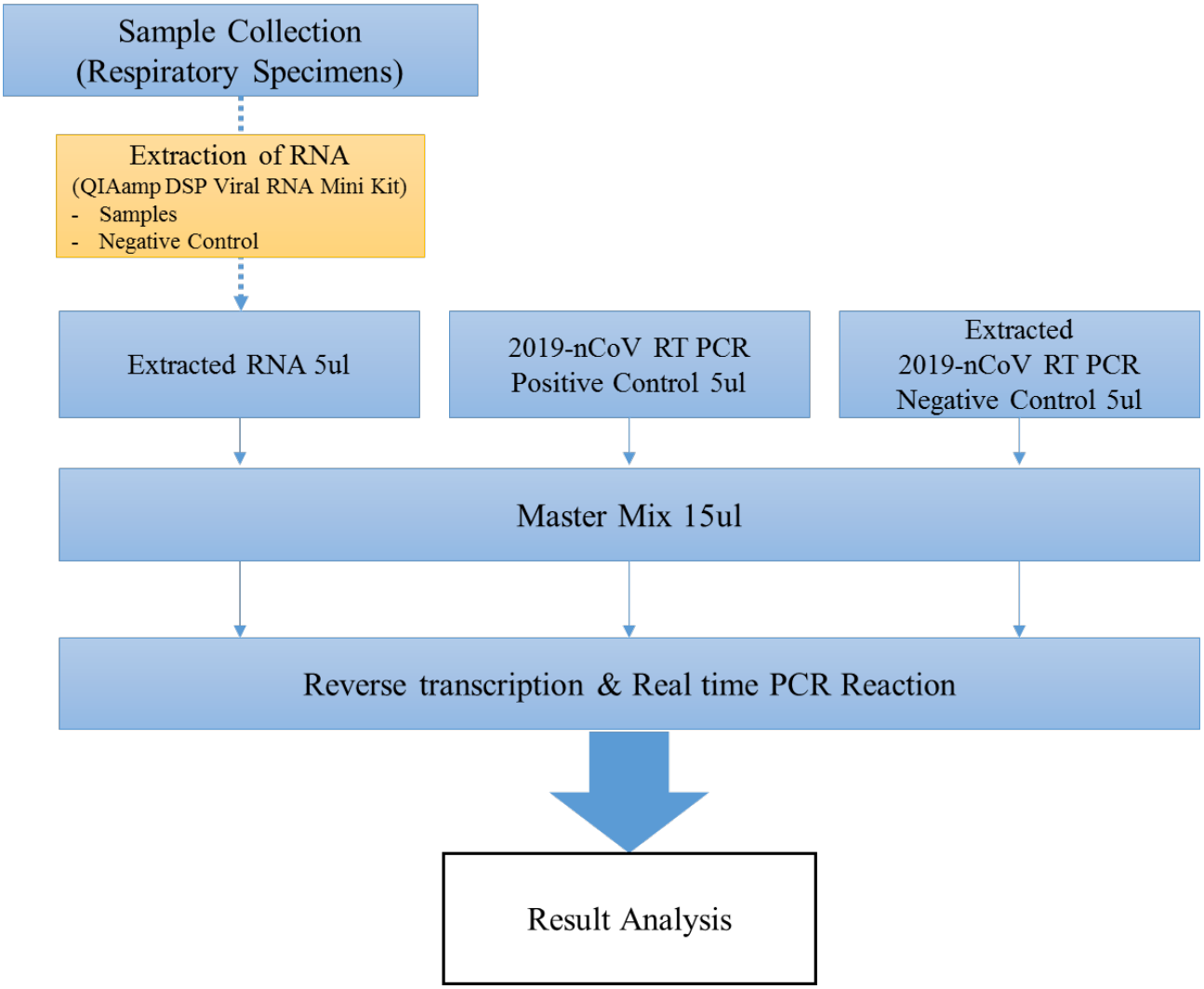
6. Storage and shelf life

Product name	Storage condition	Shelf life
BioCore 2019-nCoV Real Time PCR Kit	≤ -20 ºC	12 months

7. Warnings and precautions

1) Special Conditions for Use Statements:
    - **For use under Emergency Use Authorization only**
    - For *in vitro* diagnostic use only
    - For prescription use only
2) This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
3) This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
4) The emergency use of this product 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. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
5) Prevent contamination of reagents by following standard contamination prevention procedures including but not limited to the use of personal protective equipment and separation of sample handling and extraction locations from the PCR set up locations.
6) Reagents should be stored under the indicated storage conditions (-20 ℃) before and after use.
7) Waste should be disposed of in accordance with infectious waste disposal regulations.
8) Do not mix different lots of reagents.
9) The Positive control may cause contamination if not handled with care and separate from all other PCR reagents and samples.
10) We recommend using sterile disposable pipettes and DNase & RNase-free pipette tips. Never reuse the consumables (for example, tips, test gloves, and test tubes).
11) Always wear protective gloves, experiment clothing, protective goggles, etc. when handling the samples and test reagents. Take care to prevent the reagents from contacting your skin, eyes, mucous, etc. If you contact them, immediately wash them out with a lot of water. Thoroughly wash your hands after handling samples and test reagents.
12) Do not use the test reagent if the package is damaged or reagent bottle is leaking (there is a possibility of contamination or performance degradation, leading to misjudgment).
13) Do not use test reagents after their expiration date.
14) Safely discard unused reagents, wastes, samples, etc. in accordance with the regulations, as those are potentially infectious.
15) Decontaminate surfaces with 0.5% sodium hypochlorite (bleach) diluted with deionized or purified water.

8. Overview of procedure



9. Pre-laboratory sample preparation

9.1. Specimen Collection

All specimens should be by a medical professional at a designated place following collection instructions of the collection device manufacturer. The following additional instructions are provided for sputum and for combined nasopharyngeal and oropharyngeal specimens:

a) Sputum Collection:

- Sputum is always contaminated by oral and upper respiratory bacteria, so rinse your mouth well with sterile saline before collection and spit sputum into the container to prevent contamination
- Collect sputum in sterile collection containers conventionally used for sputum collection.

b) Combined nasopharyngeal and an oropharyngeal swab collection

- Collect a nasopharyngeal and an oropharyngeal specimen from each patient using a cotton swab and combine then into the same transport tube with VTM
- The nasopharyngeal swab is collected by scraping the secretions from the lower and middle nasopharyngeal (oropharyngeal) by inserting a cotton swab deep into the nasal cavity.
- The oropharyngeal swab is collected by pressing the tongue and scraping the discharge from the pharyngeal wall.

9.2. Pretreatment of Sputum

1) Mix sputum with an equal volume 4% of NaOH solution (40mg/ml nuclease free water).
2) Vortex for 30 seconds, then leave it for 1 minute at 15 ~ 30 °C.
3) Please repeat the step 2) 4 times.
4) Centrifuge for 10 minutes at 13000 rpm and remove the supernatant.
5) Resuspend the remaining pellet using 1XPBS and use it for RNA extraction.

9.3. Sample Storage

Keep the sample refrigerated at 2~8 ºC when storing it for a short period (not longer than one day) and at -70 ºC or lower when storing it for a long time.

10. Examination procedure

10.1. RNA extraction from samples

RNA extraction is performed using the QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904) according to the package insert using 200 µl Sample input volume and elution in 50µl Elution buffer

The extraction must include one negative control per batch of extracted samples consisting of 200 µl of nuclease free water or TE buffer and taken through the entire sample processing procedure.

10.2. Setting up the 2019-nCoV RT PCR

1) Add 5 µl of 2019-nCoV RT PCR Primer/Probe Mixture into 10 µl of 2019-nCoV RT PCR Reaction Mixture for one sample (Table 1).

Table 1. 2019-nCoV RT PCR Master Mixture

Number of Samples / Solution	1	3
2019-nCoV RT PCR Reaction Mixture	10	30
2019-nCoV RT PCR Primer/Probe Mixture	5	15
Total (µl)	15	45

2) Add 5µl of extracted RNA into 15µl of the Master Mixture (made from step 1)) and mix well by pipetting.
3) Also, prepare the 5µl of 2019-nCoV RT PCR Positive Control (PC) and an extracted 2019-nCoV RT PCR Negative Control (extracted NC). Then, put each of them into 15µl of Master Mixture (made from step 1)). Mix well by pipetting.
4) Place the tube in a Real Time PCR machine and start the machine using following condition (Table 2).

Be sure to mix and spin down the sample because it affects the baseline formation unless it is mixed.

10.3. Setting the 2019-nCoV Real Time PCR condition in each instruments.

Set the program in each instruments under the following conditions (Table 2).

Table 2. 2019-nCoV RT PCR condition

Temperature	Time	Cycles
50 ºC	30 min	1
95 ºC	15 min	1
95 ºC	15 sec	45
60 ºC	**30 sec**	
→ Fluorescence acquiring		
N gene → FAM *RdRp* gene → CalRed610 TexasRed IC → Cy5		

Note: Check the fluorescence selectable by each device and the fluorescence of the product. The CalRed 610 is interchangeable with TexasRed.

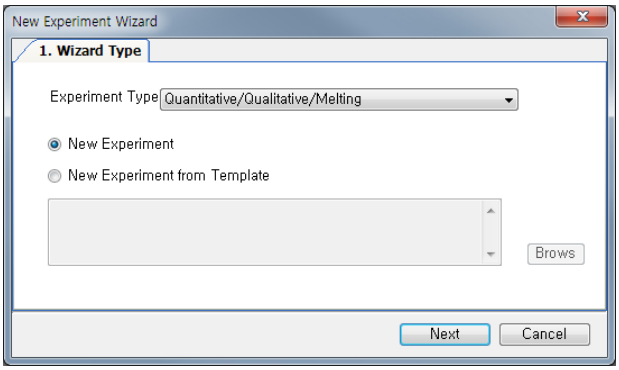
A. SLAN-96P

1) Double click 

(SLAN 96P software 8.2)

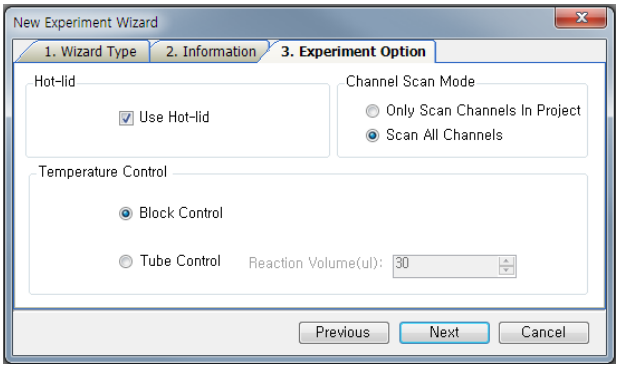
2) Click  to make a new experiment.

3) Check the default value and click **Next**.

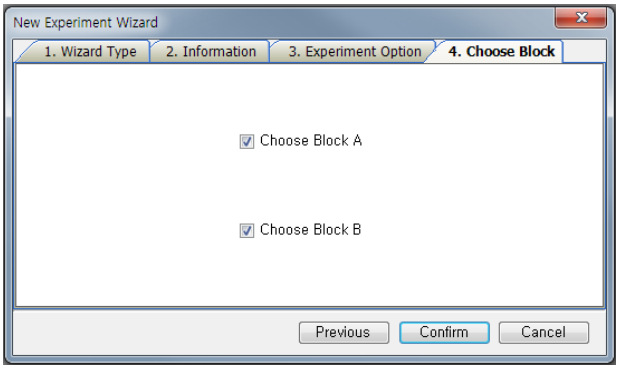


4) Enter the **Experiment Name** field and click **Next**.

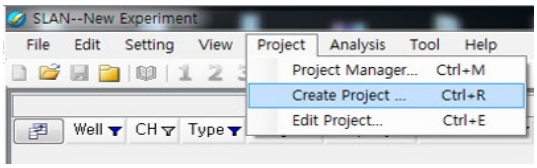
5) Check the default value. If it is same with below, click **Next**.



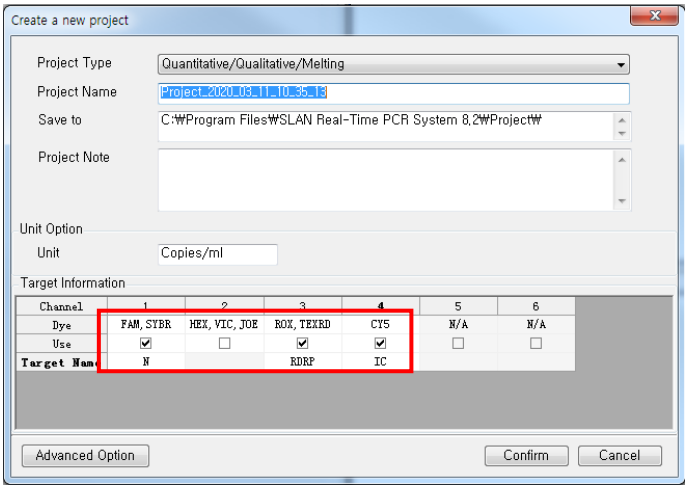
6) User can choose the block. If the block is chosen, click **Confirm**.



7) Click **Create Project** to make a protocol.

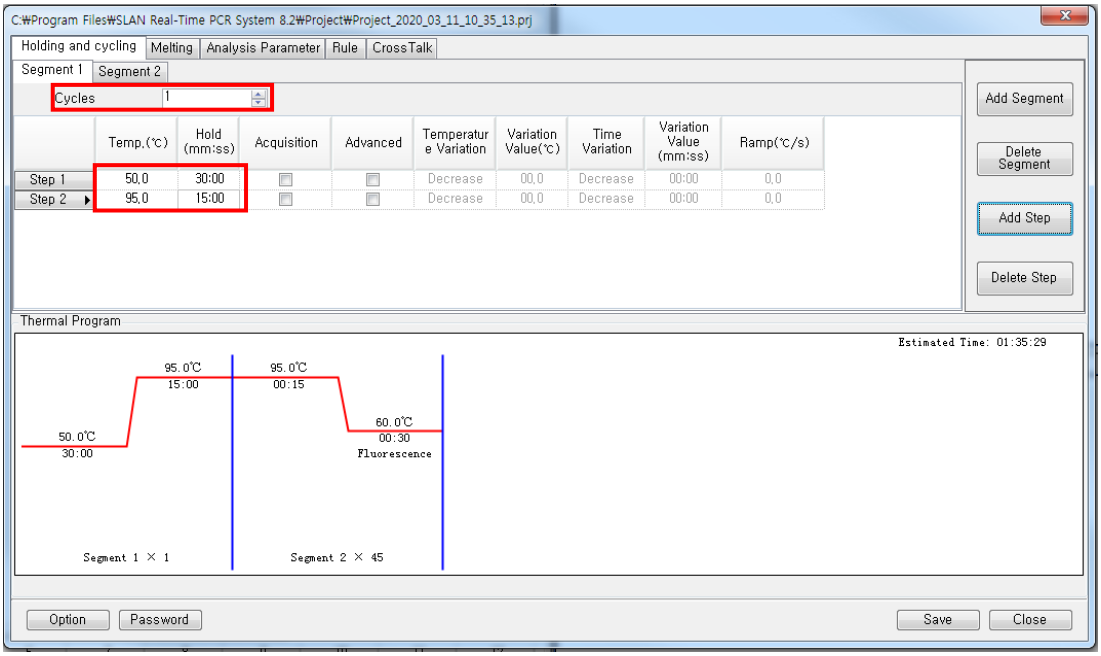


8) Enter the **Project Name** in the cell. Check the **Target Information** (channel 1, 3, 4) and write the **Target Name**. Then, click **Confirm** to go next step.

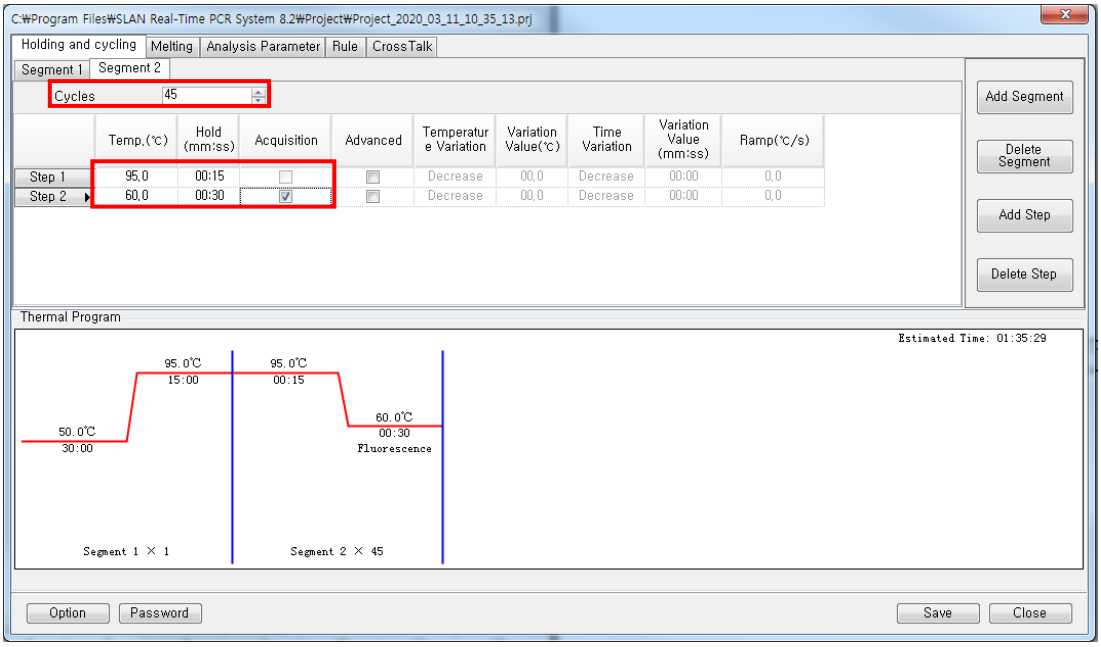


9) **Add Segment** to make a 2019-nCoV RT PCR condition.

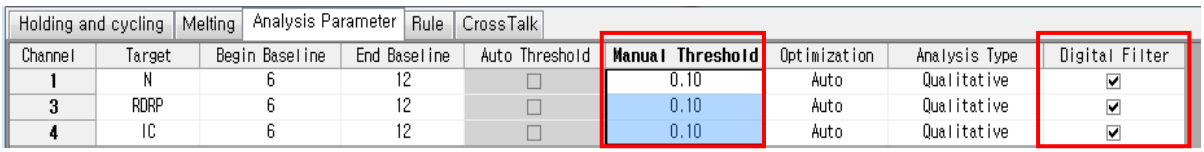
10) In **Segment 1**, **Add Step** to make two steps (see below). Enter the correct temperature and holding time.



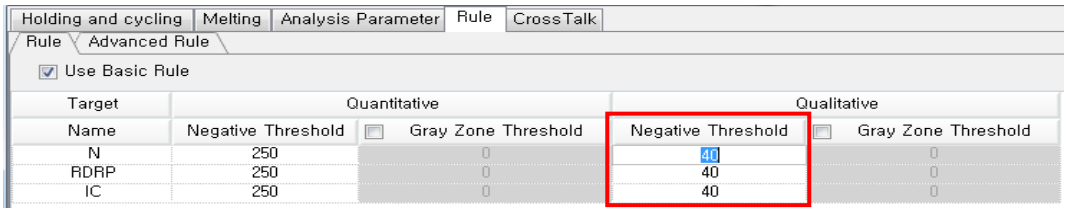
11) In **Segment 2**, **Add Step** to make two steps and enter the cycle number (see below). Enter the correct temperature and holding time. Please check the **Acquisition** in second step.



12) In the **Analysis Parameter**, edit the **Manual Threshold** to "**0.10**", and check the **Digital Filter**.



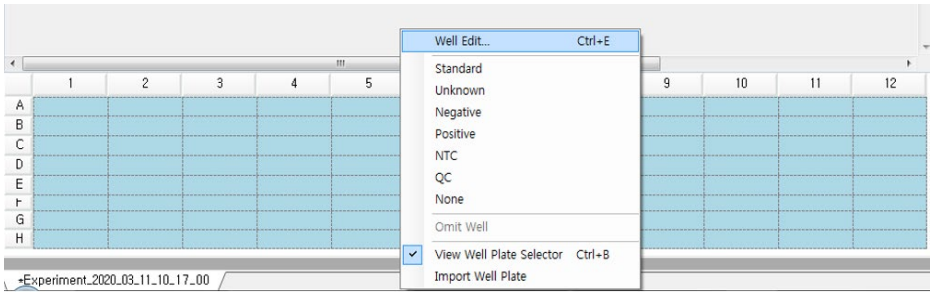
13) In the **Rule**, edit the **Negative Threshold** to "**40**".



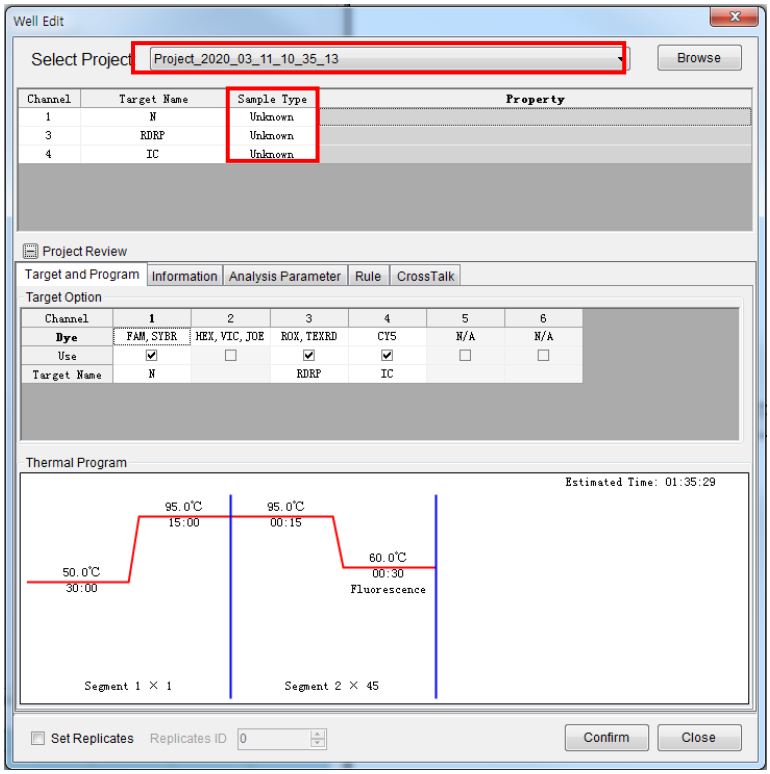
14) Click **Save** to save the project and **Close**.

> ***Please be sure to set the thresholds of each targets manually before running the RT PCR process. Refer to Section 11. in page 25.***

15) Drag the wells and right-click the selected wells. Then, go to the **Well Edit.**



16) In the **Select Project** drop-down list, click the project which were made at 14) step. Then, change all of the **Sample Type** to "Unknown". Click **Confirm**.
(User can review the project and check again the whole PCR condition)



17) At last, click the 

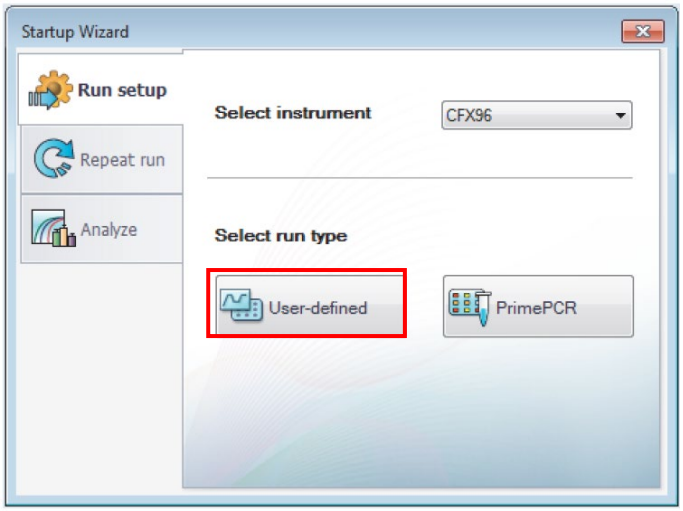
to run the real time PCR.

This procedure is based on the "version 8.2" of SLAN software. If you want to use the latest SLAN software, please contact to the website of Sansure Biotech Inc.

B. CFX96 Dx System

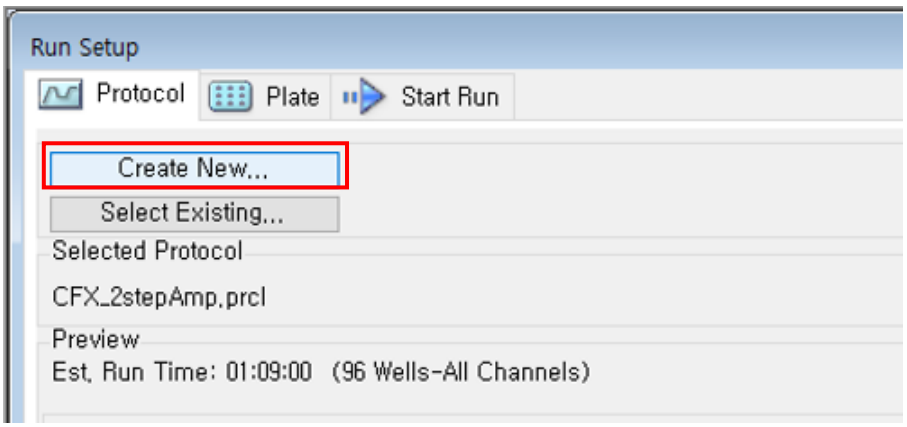
1) Double click the icon  to open the Home window of CFX96.

2) Use the Startup Wizard to quickly set up and click the **User-defined** experiments.
The Protocol Editor will be shown to create a protocol.



3) Then, the Protocol Editor will be shown to create a protocol.

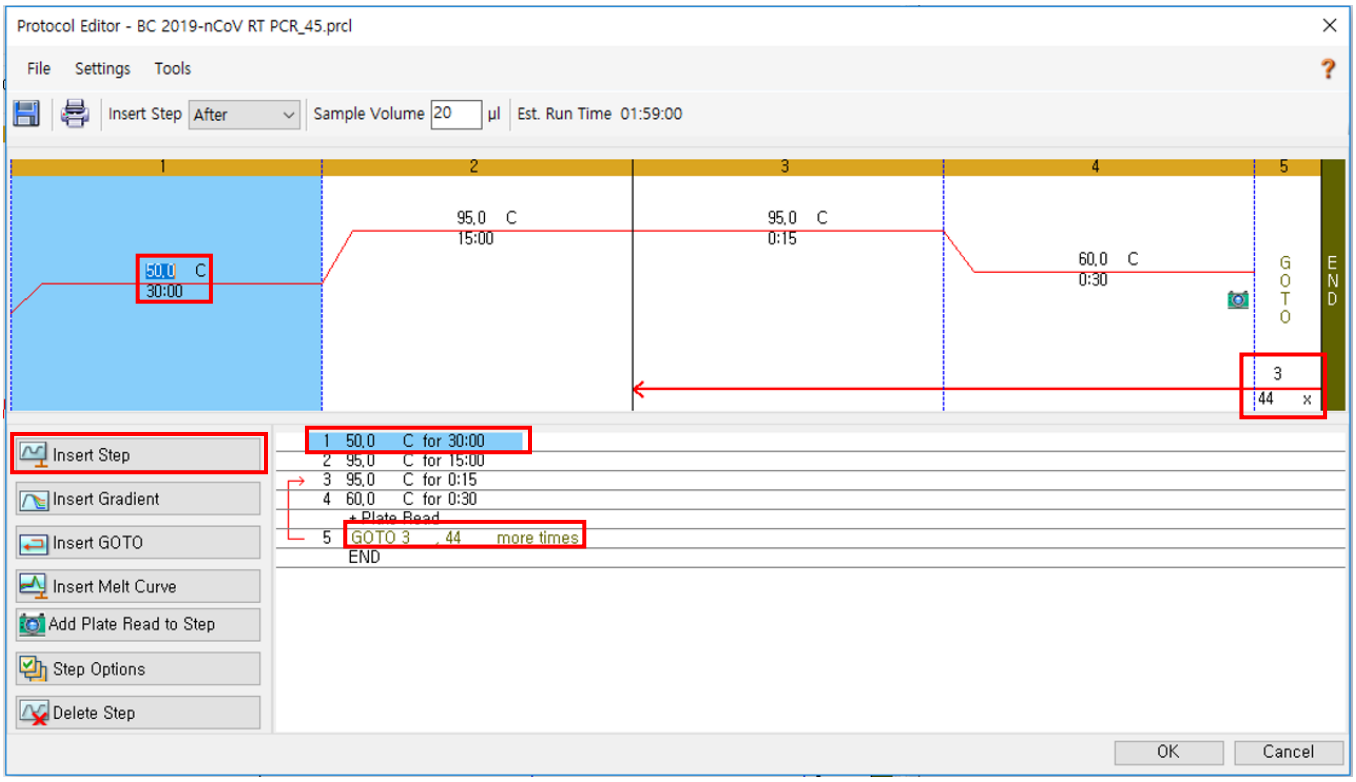
4) Click the **Create New** for set the PCR condition.



5) First, set the **Sample Volume** of the reaction to "**20 µl**".

6) Next, add the steps by selecting **Insert Step** and set the correct temperature and time.

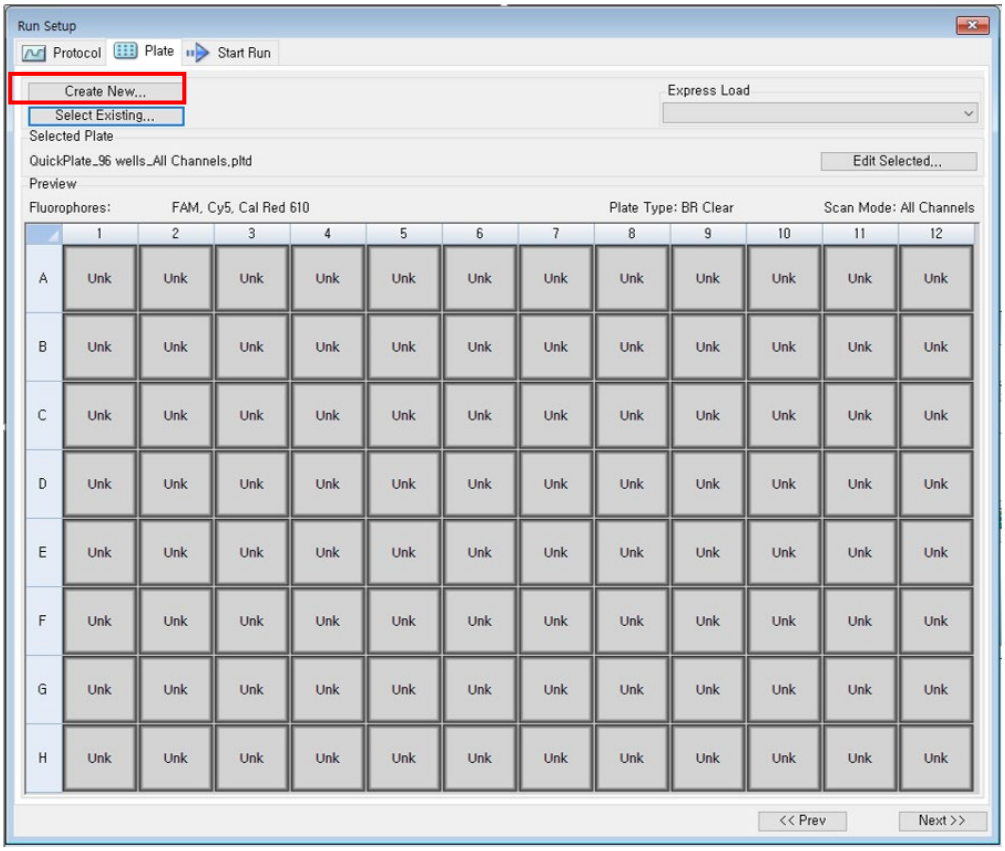
7) If you want to repeat the cycle, click the GOTO stage for setting the repeat stages and repeat times.



8) If you finish the setting PCR condition, please click "**OK**" for saving the experiment protocol.

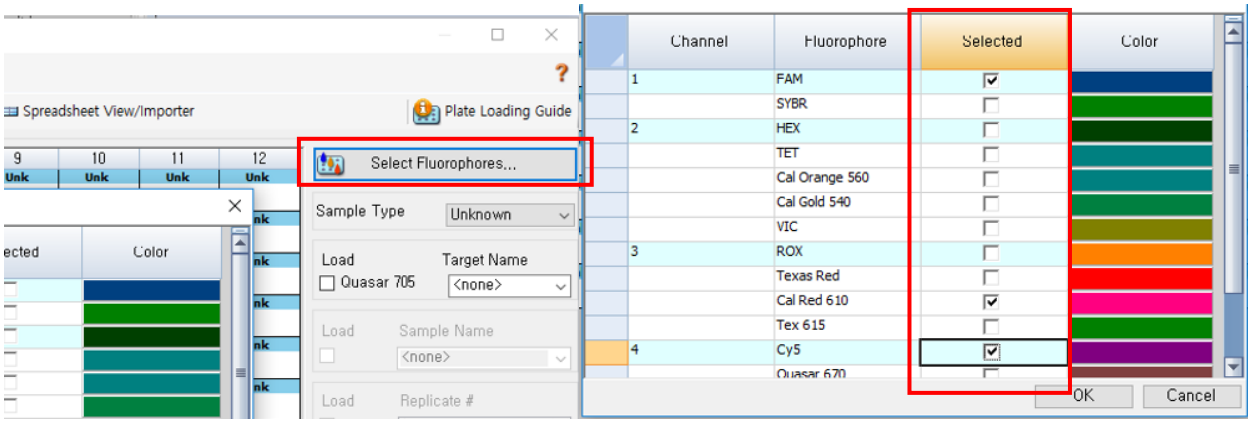
9) Next, click the **Plate** tap for set up the Fluorophores and naming the wells.

10) Click the **Create New** button for make plate template.

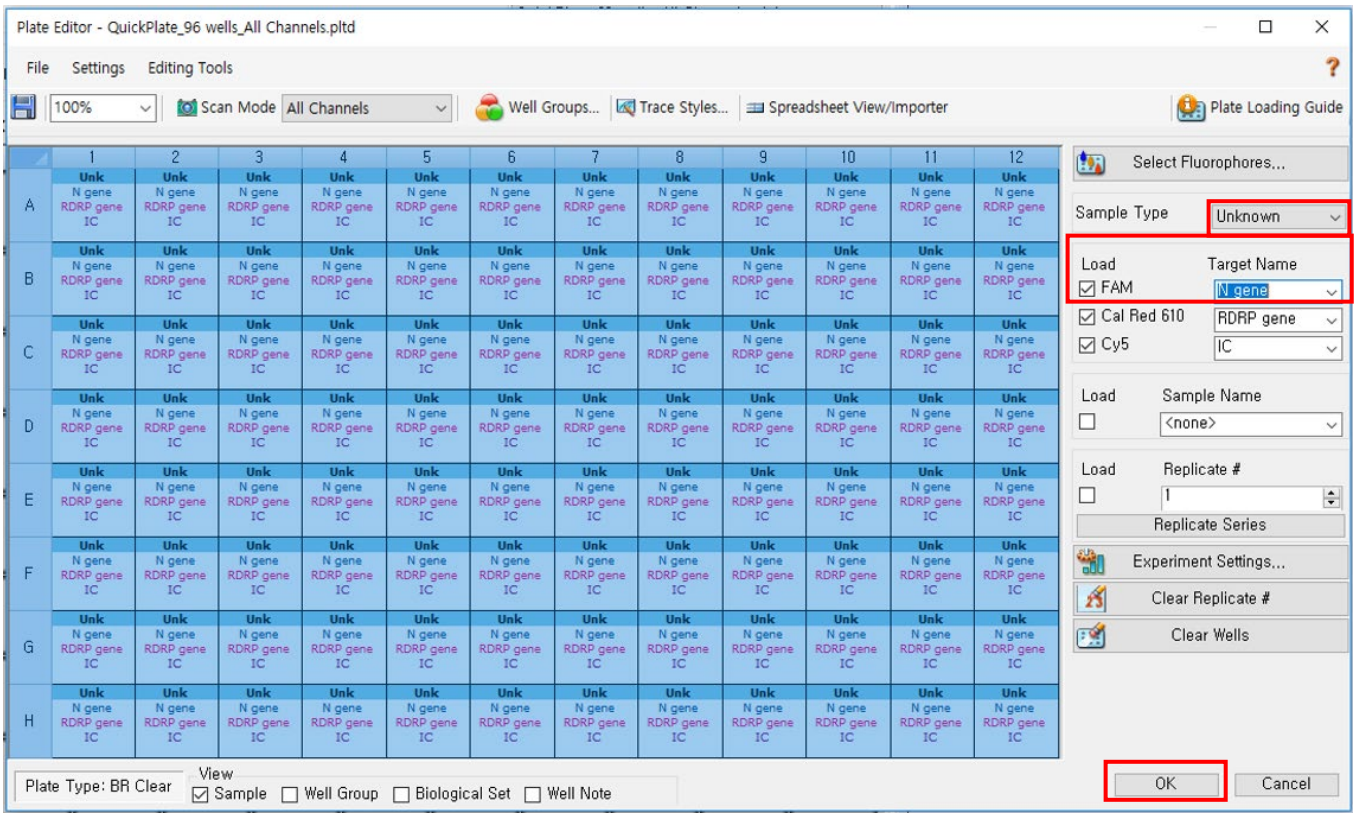


11) Then, click the **Select Fluorophores** and check the box to select "**FAM**", "**Cal Red 610**", and "**Cy5**".

12) Click "**OK**" and save the information.



13) Drag the whole wells and check the targets. Then, edit the "**Target Name**". When you wrote the target name, **please enter it** for changing the well type.



14) If you finish the setting Plate, please click "**OK**" for saving the Plate template.

15) Finally, go to the **Start Run** tab and load the tubes in the instrument.

16) Click 

to perform the real time PCR.

> ***After end of the test, please set the thresholds of each targets manually and analyze the result. Refer to Section 11. in page 25.***

C. Applied Biosystems 7500 Real-Time PCR Instrument System

1) Double click  (7500 software)

2) In the Home screen, click  **Advanced Setup** to open the Advanced Setup.

3) Click the **Experiment Name** field, then enter the name.

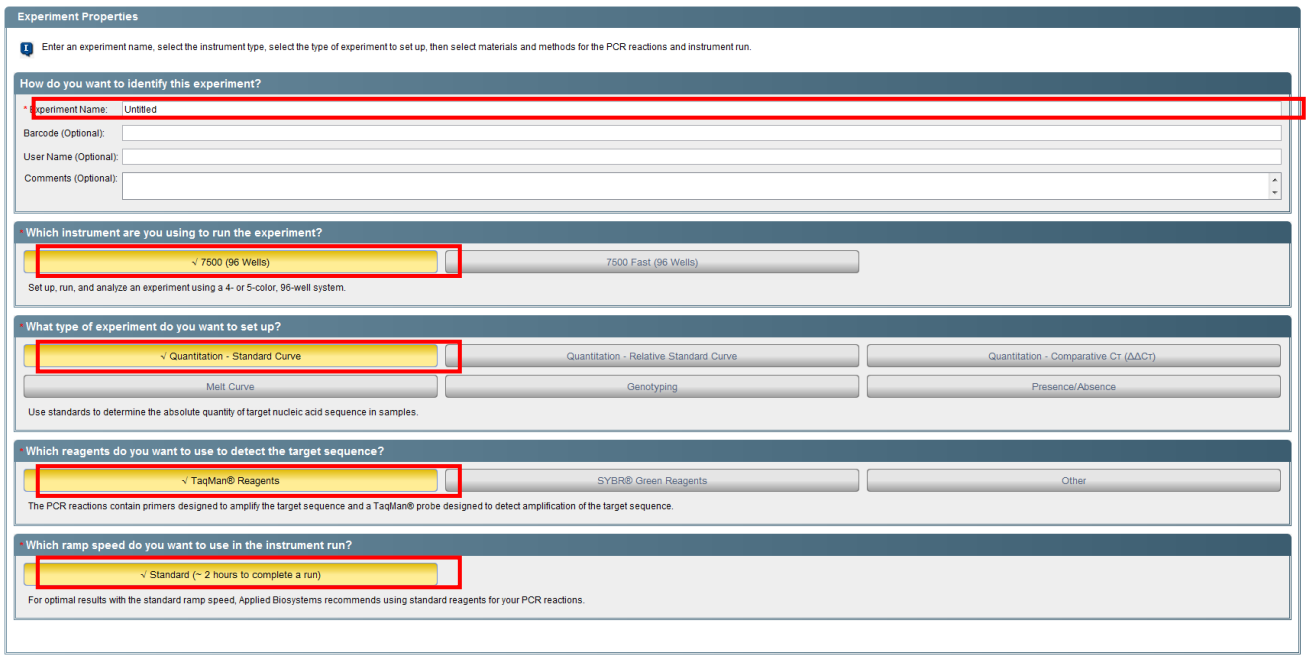
(Enter an experiment name that is descriptive and easy to remember. You can enter up to 100 characters in the Experiment Name field. You cannot use the following characters in the Experiment Name field: / \ > < * ? " | : ;)

4) Select **7500 (96 Wells)**.

5) Select **Quantitation** for the experiment type.

6) Select **TaqMan® Reagents** for the reagents.

7) Select **Standard (~ 2 hours to complete a run)** for the ramp speed.



8) Click the **Plate Setup** in the side tap, for set up the targets.

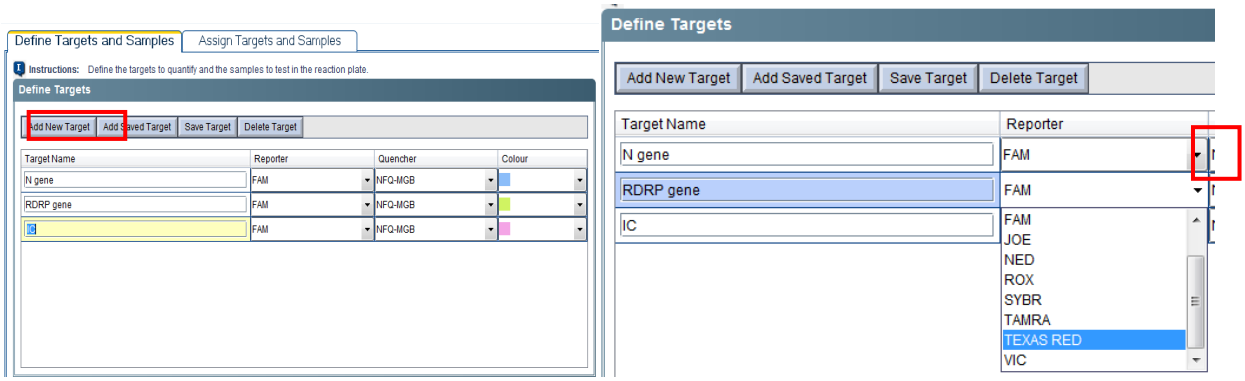
9) In the Targets Screens, click the **Add New Target** and enter the target names.

Click the **Target Name cell**, then enter the target names.

a. In the Reporter drop-down list, select FAM (default).

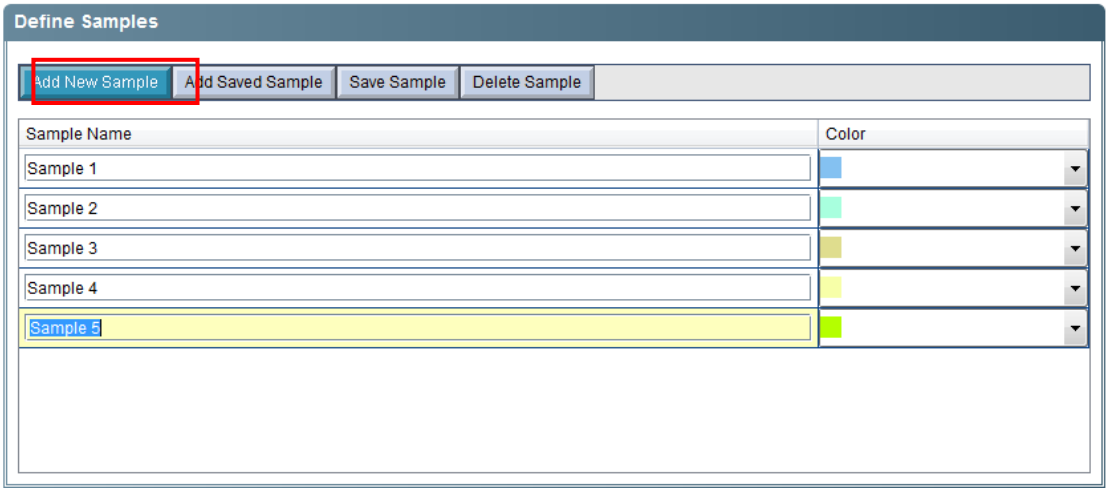
b. In the Quencher drop-down list, select NFQ-MGB (default).

c. In the Color field, leave the default.

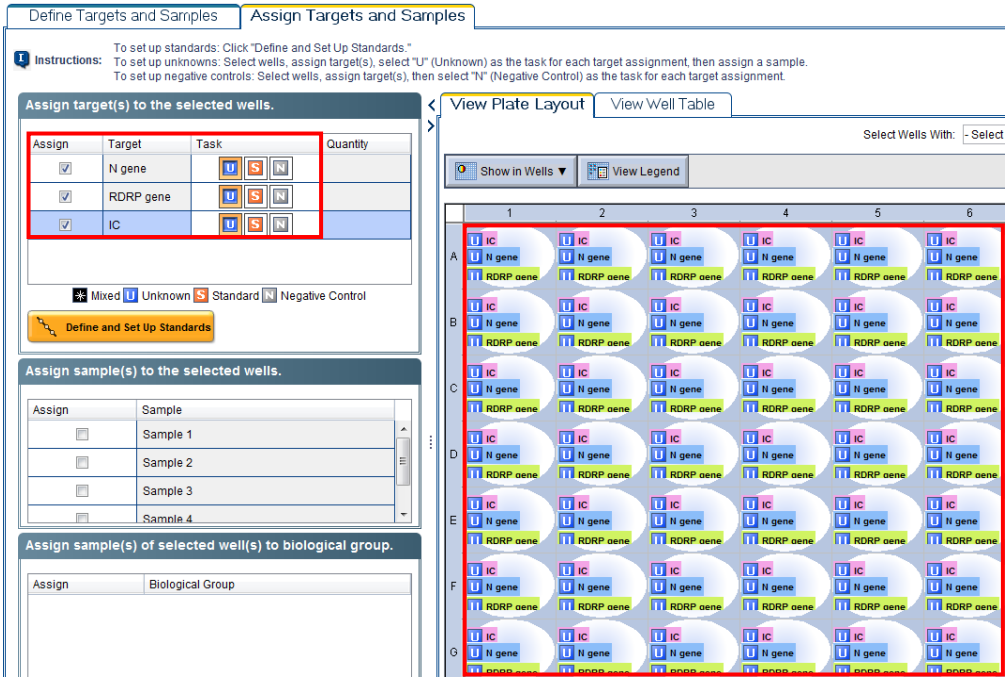


10) Next to the Targets Screens, click the **Add New Sample** and enter the sample names.

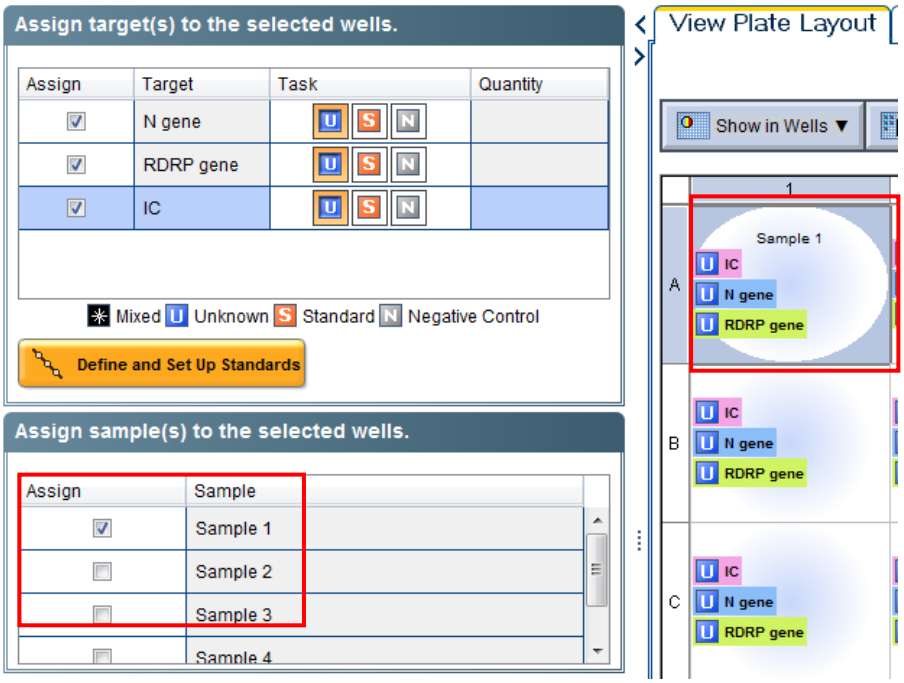
Click the **Sample Name cell**, then enter the sample names. In the Color field, leave the default.



11) In the **Assign Targets and Samples** screen, drag the whole well and check the targets to assign targets to the selected wells.



12) Click the well and check the **Sample** name to assign samples to the selected wells.

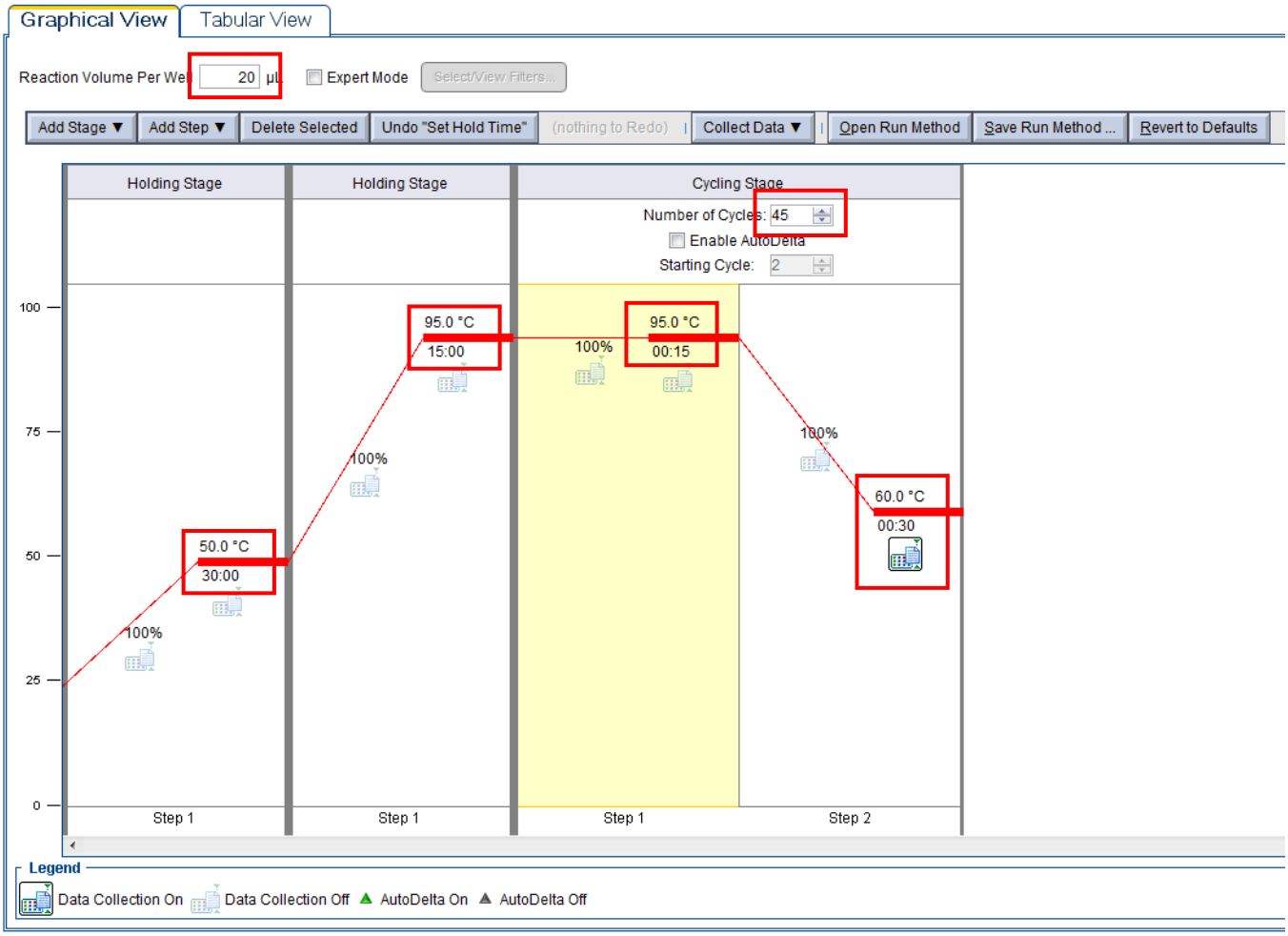


13) In the **Select the dye to use as the passive reference** drop-down list, select **None** (default is “Rox”).

14) Go to the **Run Method** (in the side tap). In the Run Method screen, select the **Graphical View** tab (default).

15) Make sure the Reaction Volume per Well field displays "**20 µl**".

16) Make sure the thermal profile displays the holding and cycling stages shown below. If necessary, click a temperature or time setting, then change it.



17) Click **START RUN** 

> ***1) This procedure is based on the "v2.0.6" of 7500 software. If you want to use the latest 7500 software, please contact to the website of Thermo Fisher Scientific.***
> ***2) After end of the test, please set the thresholds of each targets manually and analyze the result. Refer to Section 11. in page 25.***

11. Threshold manual set up for data analysis

1) SLAN-96P

Channel	Item	Threshold	Negative Threshold	Analysis Type
1	*N* gene	0.1	40	Qualitative
3	*RdRp* gene	0.1	40	Qualitative
4	IC	0.1	40	Qualitative

2) CFX96 Dx System

Channel	Item	Manual Threshold
1	*N* gene	200
3	*RdRp* gene	200
4	IC	200

3) Applied Biosystems 7500 Real-Time PCR Instrument System

Channel	Item	Manual Threshold
1	*N* gene	10,000
3	*RdRp* gene	10,000
4	IC	10,000

Note 3-1) When setting the experimental program named "Setup", set the "Select the dye to use as the passive reference" to "None", in "Assign Targets and Samples" of "Plate Setup"

Note 3-2) Deselect "Auto Baseline" of "Amplification Plot" when analyzing results in "Analysis" section

12. Quality Control

12.1. Control Materials for BioCore 2019-nCoV Real Time PCR Kit

a) 2019-nCoV RT PCR Negative Control (NC)

The negative control is needed to monitor contamination of extraction and amplification reagents and is comprised of nuclease free water. The negative control should be tested one time per every batch of extracted specimens and yield a negative result for each target (*N*, *RdRp* and b-globin) in the BioCore 2019-nCoV Real Time PCR Kit.

b) 2019-nCoV RT PCR Positive Control (PC)

The positive control is needed to confirm functionality of the PCR reagents and is comprised with the *N* gene, *RdRp* gene of SARS-CoV-2, and Human β globin gene. The positive control should be tested one time per every test run and yield a positive result for each target (*N*, *RdRp* and β-globin) in the BioCore 2019-nCoV Real Time PCR Kit.

c) Internal control (IC) targeting Human β globin is needed to verify that nucleic acid is present in every sample and is used for every sample processed. Because the positive control is a DNA template (circular plasmid), this serves as a control to ensure that the reverse transcription step is proceeding as intended. This also serves as the extraction positive control to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

12.2. Verification of Control Materials

Each test must include the 2019-nCoV RT PCR Positive control and 2019-nCoV RT PCR Negative control result.

<Acceptance Criteria of Controls>

Control	Ct value			Result
	***N* gene (FAM)**	***RdRp* gene (CalRed610 / TexasRed)**	**IC (Cy5)**	
2019-nCoV RT PCR Positive Control	≤ 40	≤ 40	≤ 40	Positive control (valid)
2019-nCoV RT PCR Negative Control (NC)	No Ct or > 40	No Ct or > 40	No Ct or > 40	Negative control (valid)

If the negative and/or positive control included in the run is invalid, the entire run is invalid and patient results cannot be interpreted. In this case a root cause analysis needs to be performed, and all patient specimens need to be retested after the root cause has been identified and eliminated.

13. Interpretation of results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

No.	Ct value			Result	Action
	***N* gene (FAM)**	***RdRp* gene (CalRed610 / TexasRed)**	**IC (Cy5)**		
1	No Ct or > 40	No Ct or > 40	≤ 40	2019-nCoV Negative	Report Result
2	≤ 40	≤ 40	Any [a]	2019-nCoV Positive	Report Result
3	No Ct or > 40	≤ 40			
4	≤ 40	No Ct or > 40			
5	No Ct or > 40	No Ct or > 40	No Ct or > 40	Invalid	Invalidate sample. Do NOT report. Re-test

a) If the concentration of SARS CoV-2 is high, the amplification of the IC may not occur.

14. Trouble Shooting

Perform a retest in any of the following cases. Check the following situations if there is no amplification reaction or the reaction is weak.

1) Abnormal QC results

1. Trouble shooting of 2019-nCoV RT PCR Positive Control result
   - Check whether the consumables used in the experiment have been sterilized.
   - Check whether tubes and tips have been not reused and make sure that they are not contaminated.
   - Check the storage condition of 2019-nCoV RT PCR Positive Control.
   - Check the Freeze-thaw repeat count of 2019-nCoV RT PCR Positive Control.

2. Trouble shooting of 2019-nCoV RT PCR Negative control result
   - Check whether the consumables used in the experiment have been sterilized.
   - When the experiment repeated with a lot of sample, the amplification product is present in the laboratory so that the amplification reaction may occur in 2019-nCoV RT PCR Negative control. Keep clean the laboratory and experimental equipment before the experiment.
   - Check whether kit components were contaminated with the amplification product.

2) Result analysis

1. Not detecting Ct value of the *N* gene, *RdRp* gene, and/or the IC gene
   - Check whether test tube was in the Real time PCR machine and the machine was run
   - Check the amount of solution in the test tube
   - Measure the nucleic acid content in the extracted sample/s. If measurement indicates that no RNA or too little RNA was extracted, re-extract the nucleic acid from sample. If RNA is present in sufficient amount, repeat the PCR reaction.
   - If the repeat testing in the PCR still yields no amplification products, consider inhibition of the sample/s. For trouble shooting purposes only you may test those samples diluted. If the diluted result is negative, you must not report the result and instead you must obtain a new sample for testing.

2. Not detecting Ct value of internal control
   - Check the *N* gene and *RdRp* gene Ct value:
     - If the *N* gene and *RdRp* gene were detected, missing the IC signal is acceptable as the amplification of the IC may not occur when high concentrations of SARS CoV-2 are present.
     - If the *N* gene and/or the *RdRp* gene were not detected, consider inhibition of the sample/s. For trouble shooting purposes only you may test those samples diluted. If the diluted result is negative, you must not report the result and instead you must obtain a new sample for testing.

3. Check the storage condition of kit components.

4. Missing amplification of individual targets in positive result may be due to a sample at concentrations near or below the limit of detection of the test.

15. Limitation of the examination procedure

Specimens must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

Extraction and amplification of nucleic acid from clinical specimens must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.

The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.

False-negative results may arise from:

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

False-positive results may arise from:

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

As with any molecular test, mutations within the target regions of BioCore 2019-nCoV Real Time PCR Kit could affect primer and/or probe binding resulting in failure to detect the presence of virus. The clinical performance of this test has not been established in 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.

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. Follow up testing should be performed according to the current CDC recommendations.

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.

16. Conditions of Authorization for the Laboratories

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

To assist clinical laboratories using the BioCore 2019-nCoV Real Time PCR Kit, the relevant Conditions of Authorization are listed below.

1) Authorized laboratories[1] using the BioCore 2019-nCoV Real Time PCR Kit must include results and reports of the BioCore 2019-nCoV Real Time PCR Kit. Under exigent circumstances, other appropriate methods for disseminating may be used, which may include mass media.

2) Authorized laboratories using the BioCore 2019-nCoV Real Time PCR Kit must perform the BioCore 2019-nCoV Real Time PCR Kit as outlined in the authorized labeling. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the BioCore 2019-nCoV Real Time PCR Kit are not permitted.

3) Authorized laboratories that receive the BioCore 2019-nCoV Real Time PCR Kit must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

4) Authorized laboratories using the BioCore 2019-nCoV Real Time PCR Kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities.

5) Authorized laboratories must collect information on the performance of the BioCore 2019-nCoV Real Time PCR Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and INVITES BIOCORE CO., LTD. (hjchoi@bio-core.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

6) All laboratory personnel using the BioCore 2019-nCoV Real Time PCR Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal

[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.”

protective equipment when handling this kit, and use the BioCore 2019-nCoV Real Time PCR Kit in accordance with the authorized labeling.

7) INVITES BIOCORE CO., LTD., its authorized distributor(s) and authorized laboratories using the BioCore 2019-nCoV Real Time PCR Kit 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.

17. Performance characteristics

17.1. Analytical Sensitivity: Limit of Detection (LoD)

This study was conducted to evaluate the LoD of the BioCore 2019-nCoV Real Time PCR Kit using a SARS CoV-2 (COVID-19) RNA distributed by National Culture Collection for Pathogens, Korea (NCCP No. 43326). All sample replicates were prepared by spiking the SARS CoV-2 (COVID-19) RNA into negative clinical sputum matrix pretreated with 4% NaOH.

A preliminary LoD study was performed, testing eight (8) replicates at each of four (4) different concentrations: SARS CoV-2 RNA - 2000, 1000, 500, 250 copies/mL. Testing was performed according to the Instructions for Use provided in this document, using the QIAamp DSP Viral RNA Mini Kit (Qiagen; catalog #61904) for extraction and all three claimed instruments, SLAN-96P, CFX96 Dx System, and Applied Biosystems 7500 Real-time PCR Instrument System. The tentative LoD was estimated as 500 copies/mL.

The preliminary LoD was confirmed by testing twenty replicates of different concentrations of SARS CoV-2 (COVID-19) RNA on the Applied Biosystem 7500 Real-time PCR, the SLAN 96P, and the CFX96 Dx System. The final study results are summarized in the following Tables 3 and 4. While the LoD of the two SARS target varies between 500 and 1000 depending on the target and the instrument, the final LoD based on the result interpretation of the test, established the LoD for the BioCore 2019-nCoV Real Time PCR Kit measured on the SLAN 96P, CFX96 Dx System and Applied Biosystem 7500 Real-time PCR as 500 copies/mL.

Table 3. LoD Summary of each target gene

PCR Instrument	Target	Positive Rate	Limit of Detection (copies/mL)	Mean Ct	Detection Rate
SLAN-96P	*N* gene	19/20	1000	38.24	95.0
	RdRp gene	20/20	500	36.20	100.0
CFX96 Dx System	*N* gene	19/20	500	34.56	100.0
	RdRp gene	20/20	1000	35.23	100.0
Applied Biosystem 7500 Real-time PCR	*N* gene	19/20	500	35.61	95.0
	RdRp gene	20/20	500	35.90	100.0

Table 4. LoD Summary of each Real time PCR instruments

PCR Instrument	Limit of Detection* (copies/mL)
SLAN-96P	500
CFX96 Dx System	500
Applied Biosystem 7500 Real-time PCR	500

* LoD per result interpretation considering results of both SARSCoV-2 targets

17.2. Analytical Sensitivity: Inclusivity

The inclusivity of the BioCore 2019-nCoV Real Time PCR Kit was evaluated using *in silico* analysis of the assay primers and probes in relation to 3007 of SARS-CoV-2 sequences available in the NCBI. In "Severe acute respiratory syndrome coronavirus 2 data hub" of NCBI, 3,007 of Nucleotide complete (complete DNA) was analyzed which are registered from November 30, 2019 to April 30, 2020. Mismatched complete DNAs have never been detected in both N gene and RdRp gene. Through these results, it is expected that 3,007 of the SARS-COV-2 complete DNAs (reported until 2020.04.30) can be detected with a positive result.

Table 5. *in silico* Analysis of Mismatched DNAs

No.	Accession	Description	Target 1 (*N* gene)			Target 2 (*RdRp* gene)		
			FWD P	REV P	PROBE	FWD P	REV P	PROBE
1	MT365028	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/HKG/HKU-905a/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
2	MT114414	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/HKG/HKU-903a/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
3	MT114415	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/HKG/HKU-903b/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
4	MT370516	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-03/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
5	MT370518	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-05/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
6	MT370904	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NY1-PV08001/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
7	MT374102	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-06/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
8	MT374103	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/TWN/CGMH-CGU-07/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
9	MT459922	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-	100%	**95% (19/20)**	100%	100%	100%	100%

		2/human/GRC/264_32497/2020, complete genome						
10	MT450973	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC58/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
11	MT450980	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC66/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
12	MT451007	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC97/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
13	MT451158	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC262/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
14	MT451168	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC272/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
15	MT451176	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC280/2020, complete genome	100%	100%	100%	100%	100%	**97% (30/31)**
16	MT451186	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC294/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
17	MT451194	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC302/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
18	MT451197	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/AUS/VIC305/2020, complete genome	100%	100%	100%	100%	100%	**97% (30/31)**
19	MT459979	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/SRB/Novi Pazar-363/2020, complete genome	100%	100%	**96% (24/25)**	100%	100%	100%
20	MT461626	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-4270/2020, complete genome	**95% (21/22)**	100%	100%	100%	100%	100%
21	MT450872	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/SRB/KV26/2020, complete genome	100%	100%	**96% (24/25)**	100%	100%	100%

17.3. Analytical Specificity: Cross Reactivity

The cross-reactivity of the BioCore 2019-nCoV Real Time PCR Kit was evaluated using both wet testing and *in silico* analysis. For wet testing, the cross-reactivity test was conducted with the viruses that are likely to infect the respiratory tract. The pooled human nasal wash and twenty-seven (27) viruses which are spiked into SARS-CoV2 negative transport medium at the concentrations indicated in Table 6 were extracted by QIAamp DSP Viral RNA Mini Kit. Then, the samples tested according to the Instructions for Use provided in this document. Testing was performed on the SLAN-96P instrument. *In silico* cross-reactivity was defined as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. The results from the cross-reactivity, both wet testing and *in silico* analysis, are shown in Table 6 and 7.

Table 6. Cross-reactivity analysis by wet testing (2019-nCoV negative)

No	Strain	Origin	Concentration		Result		
			ng/ul	cp/ul	Test 1	Test 2	Test 3
1	Influenza virus A H1N1	ZeptoMetrix panel	1	6.8×10^7	-	-	-
2	Influenza virus A H3N2	ZeptoMetrix panel	1	6.8×10^7	-	-	-
3	Influenza virus A Pdm2009	ZeptoMetrix panel	1	6.8×10^7	-	-	-
4	Influenza virus B	ZeptoMetrix panel	1	6.4×10^7	-	-	-
5	RSV A2	ZeptoMetrix panel	1	6.2×10^7	-	-	-
6	Rhinovirus 1A	ZeptoMetrix panel	1	1.3×10^8	-	-	-
7	Corona Virus_229E	ZeptoMetrix panel	1	3.4×10^7	-	-	-
8	Corona Virus_OC43	ZeptoMetrix panel	1	3.0×10^7	-	-	-
9	Corona Virus_NL63	ZeptoMetrix panel	1	3.4×10^7	-	-	-
10	Corona Virus_HKU-1	ZeptoMetrix panel	1	3.1×10^7	-	-	-
11	Metapneumovirus	ZeptoMetrix panel	1	7.0×10^7	-	-	-
12	Rhinovirus A	ATCC VR-1131	1	1.3×10^8	-	-	-
13	Rhinovirus B	ATCC VR-284	1	1.3×10^8	-	-	-
14	Parainfluenza virus 1	ZeptoMetrix panel	1	6.2×10^7	-	-	-
15	Parainfluenza virus 2	ZeptoMetrix panel	1	6.0×10^7	-	-	-
16	Parainfluenza virus 3	ZeptoMetrix panel	1	6.2×10^7	-	-	-
17	Parainfluenza virus 4	ZeptoMetrix panel	1	6.0×10^7	-	-	-

18	Adenovirus type 1	ZeptoMetrix panel	1	2.6×10^7	-	-	-
19	Adenovirus type 3	ZeptoMetrix panel	1	2.6×10^7	-	-	-
20	Adenovirus type 31	ZeptoMetrix panel	1	2.7×10^7	-	-	-
21	Coxsackievirus A3	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
22	Coxsackievirus B3	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
23	Echovirus 6	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
24	Echovirus 7	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
25	Enterovirus 68	ATCC VR-1076	1	1.3×10^8	-	-	-
26	Enterovirus 71	Pathogenic Virus Bank	1	1.3×10^8	-	-	-
27	SARS-coronavirus	In vitro transcribed RNA	1	5.3×10^8	-	-	-
28	Pooled human nasal wash	Clinical Specimen			-	-	-

Table 7. Cross-reactivity analysis by *in silico* analysis

Microorganism	Target	
	N	*RdRp*
MERS-coronavirus	None	None
Chlamydia pneumoniae	None	None
Haemophilus influenzae	None	None
Legionella pneumophila	None	None
Mycobacterium tuberculosis	None	None
Streptococcus pneumoniae	None	None
Streptococcus pyogenes	None	None
Bordetella pertussis	None	None
Mycoplasma pneumoniae	None	None
Pneumocystis jirovecii (PJP)	None	None
Candida albicans	None	None
Pseudomonas aeruginosa	None	None
Staphylococcus epidermis	None	None
Streptococcus salivarius	None	None

17.4. Interference Study

The interference study was conducted for confirming the analytical specificity of BioCore 2019-nCoV Real Time PCR Kit. In this study, 2 endogenous potentially interfering substances and 3 exogenous potentially interfering substances were tested in 6 SARS-CoV-2 negative specimens (2 of sputum, 2 oropharyngeal swabs, and 2 nasopharyngeal swabs) and 6 SARS-CoV-2 positive specimens (2 of sputum, 2 oropharyngeal swabs, and 2 nasopharyngeal swabs). None of the tested substances caused interference with SARS-CoV-2 positive or negative samples.

Table 8. Summary of interference substance test

Interferent	C[2]	Matrix	No SARS-CoV2	SARS-CoV-2 [5000 copies/mL = 10xLoD]				
			Negativity Rate	Positivity Rate	Target	Mean Ct	SD [Ct]	Mean Difference in Ct
Control	-	Sputum	8/8 100%	8/8 100%	N	32.2	0.2	32.2 ± 0.5
					RdRp	33.2	0.2	33.2 ± 2.8
		NP	8/8 100%	8/8 100%	N	32.0	0.2	32.0 ± 1.2
					RdRp	33.4	0.4	33.4 ± 3.8
		OP	8/8 100%	8/8 100%	N	31.8	0.3	31.8 ± 1.8
					RdRp	33.8	0.7	33.8 ± 1.9
Mucin (60ug/ml)	-	Sputum	4/4 100%	4/4 100%	N	32.2	0.3	32.2 ± 0.3
					RdRp	33.3	0.4	33.3 ± 0.6
		NP	4/4 100%	4/4 100%	N	31.8	0.1	31.8 ± 0.1
					RdRp	33.2	0.4	33.2 ± 0.5
		OP	4/4 100%	4/4 100%	N	31.6	0.6	31.6 ± 0.8
					RdRp	34.2	0.5	34.2 ± 0.6
Blood (5% v/v)	60 g/L	Sputum	4/4 100%	4/4 100%	N	32.1	0.5	32.1 ± 0.7
					RdRp	33.1	0.1	33.1 ± 0.2
		NP	4/4 100%	4/4 100%	N	31.7	0.2	31.7 ± 0.3
					RdRp	33.2	0.3	33.2 ± 0.4
		OP	4/4 100%	4/4 100%	N	32.1	0.3	32.1 ± 0.4
					RdRp	34.0	0.7	34.0 ± 0.6
Oxymeta-zoline 0.05% (v/v)	0.2 g/L	Sputum	4/4 100%	4/4 100%	N	32.5	1.0	32.5 ± 0.9
					RdRp	34.6	1.6	34.6 ± 1.7
		NP	4/4 100%	4/4 100%	N	32.1	0.3	32.1 ± 0.4
					RdRp	33.6	0.4	33.6 ± 0.3
		OP	4/4 100%	4/4 100%	N	33.0	0.2	33.0 ± 0.2
					RdRp	34.7	1.0	34.7 ± 1.5
Zanamivir 5mg/ml	0.2 g/L	Sputum	4/4 100%	4/4 100%	N	32.5	1.4	32.5 ± 1.4
					RdRp	34.0	1.3	34.0 ± 1.7
		NP	4/4 100%	4/4 100%	N	32.7	1.4	32.7 ± 1.7
					RdRp	35.3	1.5	35.3 ± 1.4
		OP	4/4 100%	4/4 100%	N	33.1	1.1	33.1 ± 1.4
					RdRp	34.9	1.1	34.9 ± 1.4

[2] Concentration

Oseltamivir 75mg/ml	2 mg/L	Sputum	4/4 100%	4/4 100%	N	32.5	1.0	32.5 ± 1.4
					RdRp	34.4	1.3	34.4 ± 1.5
		NP	4/4 100%	4/4 100%	N	32.4	0.7	32.4 ± 0.8
					RdRp	33.8	1.0	33.8 ± 1.5
		OP	4/4 100%	4/4 100%	N	33.2	0.2	33.2 ± 0.3
					RdRp	34.6	0.3	34.6 ± 0.4

17.5. Clinical Evaluation

For the clinical validation one hundred twenty (120) retrospective, deidentified clinical samples were tested, of which 60 (20 positive and 40 negative) were lower respiratory samples (sputum pretreated with 4% NaOH as described in the instructions for use) and 60 (20 positive and 40 negative) were upper respiratory samples (combined oropharyngeal and nasopharyngeal swabs). Samples were extracted by QIAamp DSP Viral RNA Mini Kit and tested with the BioCore 2019-nCoV Real Time PCR Kit and a comparator device EUA authorized by FDA (US) and the Korea Authority (MFDS) using the CFX96 Dx System (Bio-rad). Testing is summarized in Table 8.

Table 9. Summary of Clinical Evaluation Result

Sputum		**Comparator assay**	
		POSITIVE	NEGATIVE
BioCore 2019-nCoV RT PCR Kit	POSITIVE	20	0
	NEGATIVE	0	40
Positive Percent Agreement: 20/20 = 100% (95% CI: 83.89%-100.00%) **Negative Percent Agreement: 40/40 = 100%** (95% CI: 91.24%-100.00%)			

Combined Oropharyngeal swabs & Nasopharyngeal swabs		**Comparator assay**	
		POSITIVE	NEGATIVE
BioCore 2019-nCoV RT PCR Kit	POSITIVE	20	0
	NEGATIVE	0	40
Positive Percent Agreement: 20/20 = 100% (95% CI: 83.89%-100.00%) **Negative Percent Agreement: 40/40 = 100%** (95% CI: 91.24%-100.00%)			

Performance was estimated as 100% PPA and 100% NPA for upper and lower respiratory specimen types. No false positive and false negative samples were observed with the BioCore 2019-nCoV Real Time PCR Kit.

18. 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 'QIAamp DSP Viral RNA Mini Kit' (Qiagen, cat no. 61904). The results are summarized in Table 10.

Table 10. 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	NP Swab	0.6 x 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

19. Reference

[1] Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Interim guidance. 17 January 2020

[2] Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR-Protocol and preliminary evaluation as of Jan 13, 2020

[3] CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

20. Technical support

Please contact us using the following contact information for any product inquiries, complaints and adverse event reporting:

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21. Glossary of Symbols

	Expiry Date
	Batch Code
	Caution
	Manufacturer
	Contains sufficient for <n> tests
	Do not reuse
	In vitro diagnostic Medical device
	Limit of Temperature
	Catalog Number
	Consult included HandBook For use