- 15° C

- 20° C

IV

COVID-19 DetectionKit

For in vitro diagnostic use only

A diagnostic kit for detection of New Coronavirus (COVID-19)
in clinical samples using Real-time PCR

Instructions for Use | v2.0

SS-9011

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Indications of Medical Devices Act

1. Product Category: IVD Reagent for Infectious Agents
2. Product Name: U-TOP™ COVID-19 Detection Kit
3. Product Catalogue Number: SS-9011
4. Purpose of use: See 1. in this User Guide

| Warnings and Precautions |

Contact us for detailed information for the safe use of the U-TOP™ COVID-19 Detection Kit. Please check storage temperature and attention points for accurate diagnosis of the product. Sample and Assay waste must be disposed of in a legally designated manner.

| Warranty and Responsibility |

All products of SEASUN BIOMATERIALS are tested under rigorous quality management processes. SEASUN BIOMATERIALS Inc. guarantees to ensure the quality of the product during warranty period. If any problems relating to the quality of the product are found, please contact the headquarters immediately.

| Quality Control System |

All aspects of the quality management system, product creation, quality assurance, and supplier qualifications are certified to ISO 13485, KGMP.

| Inquiries and customer service (A/S) |

Send us an e-mail (as@seasunbio.com) to inquire about the product.

C·O·N·T·E·N·T·S

SEASUN BIOMATERIALS

1. Intended Use

U-TOP™ COVID-19 Detection Kit is an in vitro diagnostic product intended for Real-time PCR detection of new Coronavirus (2019-nCoV) infection in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals with suspected COVID-19 who present clinical signs or meet COVID-19 epidemiological criteria.

Result are for the identification of 2019-nCoV RNA in the specimens. Positive results are indicative of active or non-active infection of 2019-nCoV but do not indicate the co-infection of other bacteria and viruses.

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 U-TOP™ COVID-19 Detection Kit kit is produced and optimized for using CFX96 real-time PCR detection system (Bio-Rad) or ABI 7500 (Thermofisher) Real-time PCR system. This kit is intended for use by only qualified and well trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
Please read this user guide carefully and check the components in the product before first use.

2. Product Configuration

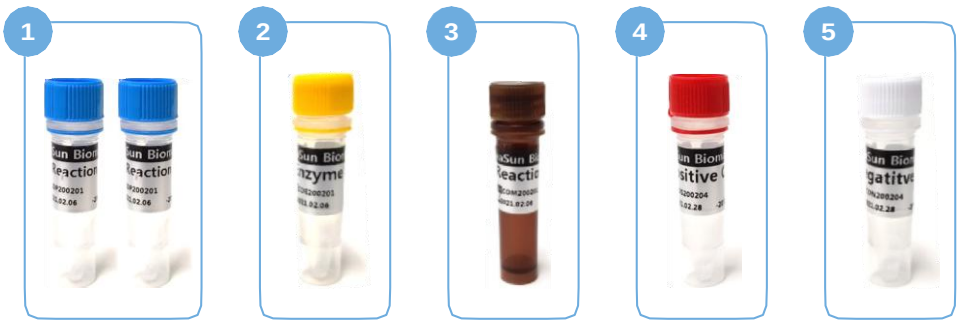
The U-TOP™ COVID-19 Detection Kit uses Peptide Nucleic Acid (PNA) based real-time PCR technique to in vitro reverse transcription of 2019-nCoV RNA followed with DNA amplification and detection. The kit targets 2019-nCoV specific genomic regions of ORF1ab and Nucleocapsid
(N) gene. The PNA probes for the detection of 2019-nCoV are labeled FAM (ORF1ab) and HEX (N gene) fluorescent dyes. PNA is an artificial analog of DNA which has extreme thermo and enzyme stability in addition to high specificity to the target nucleic acid with its neutrally charged

peptide backbone. Since PNA is not cleaved by enzymatically, it does not produce false positive results and the diagnostic grey zone as well.

The kit includes an Internal PCR Control (IPC) to evaluate the detection performance as well as enzyme activity and PCR equipment productivity. IPC probe is labeled with Texas Red fluorescent dye which uses independent fluorescence detection channel from 2019-nCoV targets. The kit also uses a dUTP/UNG carryover preventation system to avoid cross and carryover contamination of PCR and subsequent false positive results.

3. Kit Components and Packaging Specifications

The kit is composed of 2X Reaction Buffer, Enzyme Mix, Reaction Mix, Positive and Negative Control.



No.	label	Cap	Volume / quantity
1	2X Reaction Buffer		750㎕ / 2ea
2	Enzyme Mix		100㎕ / 1ea
3	Reaction Mix		400㎕ / 1ea
4	Positive Control		200㎕ / 1ea
5	Negative Control		200㎕ / 1ea

4. Storage and Handling Requirements

- Store all reagents at -25 to -15°C.
- Use the reagents within 3 months once opened
- Completely thaw the reagents before each use
- Avoid excessive freeze/thaw cycles
- Vortex and spin down briefly the reagents before each use

5. Additional Materials and Equipment

U-TOP™ COVID-19 Detection Kit includes reagents for nucleic acid amplification and detection. The kit does not include reagents for extracting viral RNA. Please prepare RNA extraction kit or reagents and additional materials in below before the test.

- RNA extraction reagents for extracting RNA from sputum, bronchoalveolar lavage, oropharyngeal or nasopharyngeal smears (We recommend to use a RNA extraction reagent with license of in vitro diagnostic medical device)
- Real-time PCR system: CFX96 real-time PCR detection system (Bio-Rad) or ABI 7500 Real-time PCR System (Thermo Fisher Scientific)
- 96 well White plate or Low-Profile 0.2ml PCR Tubes, white
- Sealing Film or Optical flat Cap Strips for 0.2ml tube strips/plates
- Roller or Film sealing paddle
- 1.5ml micro tubes
- Vortex and micro-centrifuge
- Sterilized pipette tips with filters
- Centrifugation of micro plate

6. Warnings and Precautions

- For in vitro diagnostic use only
- Use under the guidance of physicians and specialists.
- Please read this user guide carefully before first use.
- Sensitivity may lowered with prolonged exposure to room temperature or light.
- Keep refrigerated between -15℃ ~ -20℃ away from UV/sunlight.
- Avoid to use if the kit is contaminated with test sample.
- Keep clear the external environment, always use it in a clean place.
- Only use sterilized single-use micro filter tip.
- Strong external impact may damage the screw tube.
- If any abnormality is observed, stop the experiment, contact the manufacturer.

7. Specimen Collection, Handling and Storage

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.

- Collecting specimen : Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV). https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
  Follow specimen collection device manufacturer instructions for proper collection methods. Swab specimens should be collected using only swabs with a synthetic tip, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

- Shipping : Specimens must be packaged, and transported according to the current edition of the International Air Transport Association (IATA)

Dangerous Goods Regulation. Store

specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70° C ship overnight to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

8. How to Use

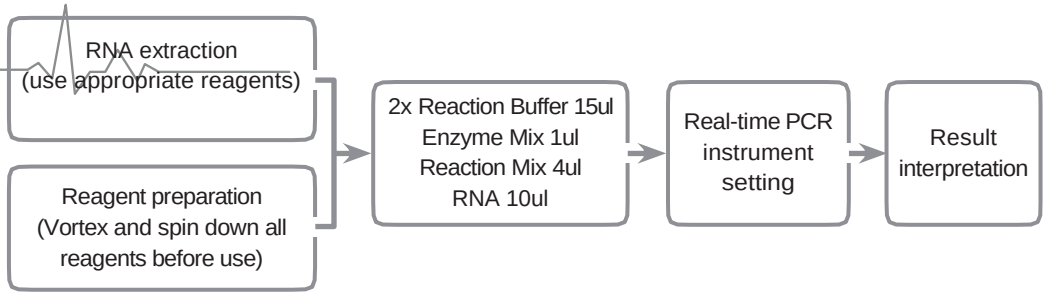
Preparation beforetest

U-TOP™ COVID-19 Detection Kit uses RNA extracted from Sputum, Bronchoalveolar lavage, Oropharyngeal or Nasopharyngeal smears as template for PCR detection. This kit does not include reagents for extracting viral RNA. The nucleic acid extraction systems tested with the U-TOP™ COVID-19 Detection Kit were shown below. In addition to the kits shown below, it is possible to use other RNA extraction kits or reagents available for using sputum, bronchoalveolar lavage, oropharyngeal or nasopharyngeal smears. RNA Extraction reagents have different names and characteristics for each manufacturer, the reagents should be used following the manufacturer's instructions. Experimental result may varied depended on the extraction methods and reagents.

Specimen	Manufacturer	Model
sputum, bronchoalveolar lavage, oropharyngeal or nasopharyngeal smears	QIAGEN	QIAamp DSP virus kit
	PANAGENE	Equipment: PANAMAX™ 48 Reagent: PANAMAX™ Viral DNA extraction kit

* RNA extraction proceeds according to the manufacturer's protocol.

Summary of Preparation and Testing Process



< Work flow of 2019-nCoV detection >

PCR mixturepreparation

Clean and decontaminate all work areas, and equipment as well as all supplements e.g. pipette, vortex, micro centrifuge prior to use to minimize the risk of nucleic acid cross- contamination.

PCR mixture preparation step should be performed on ice or cold-block, if possible. Place the Enzyme mix always on ice or cold-block during the test.

Pipetting of Positive control is recommended to be done in a separate area, from preparation of PCR Mixture. Always change pipette tips in-between patient sample elution and after pipetting each component. Add the Positive Control in PCR plate at last, to avoid the contamination. Positive control contains high concentration of viral nucleic acid.

Prepare PCR mixture according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers. Be sure to calculate 2 extra reactions for a Positive and a Negative Control.

Reaction mixture

Reagent	Volume(ul)
2X Reaction Buffer	15
Enzyme Mix	1
Reaction Mix	4
Template RNA	10

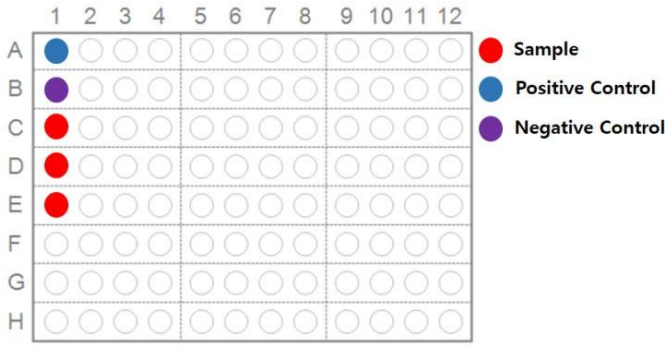
Reaction mixture

- 2X Reaction Buffer = 15 X N X 1.1* = ul
- Enzyme Mix = 1 X N X 1.1* = ul
- Reaction Mix = 4 X N X 1.1* = ul

< PCR mixture >

①The PCR mixture shown in above table is for one reaction.

②When more than one specimen are tested, the reagents (2X Reaction Buffer, Enzyme Mix, Reaction Mix) except the extracted RNA of the specimen are prepared with number of the specimens.



*Dispensing Sample, Positive and Negative control in 96 plate are irrelevant (No fixed position)

③Add the PCR mixture as 20μl/well in the PCR plate in the same order as the well information, and add 10μl of RNA extracted from the specimen. (Up to 94 samples can be analyzed, Be sure to pay attention to Positive control (1well) and Negative control (1well) dispensing)

④This kit is a product that qualitatively examines the presence or absence of a 2019- nCoV (COVID-19) infection. The infection status is shown with PCR Threshold Cycle (Ct) value. Ct for amplification curve is detected if viral RNA is present and amplified. If the Ct value does not appear, check an amplification curve regards to Internal Positive Control (IPC) to confirm the PCR is working properly.

⑤Seal the PCR plate with 8-Cap Strips, completely absorb it using a roller, and spin down using a micro plate centrifuge to collect the contents downward and remove extra air bubbles.

* If flat 8-cap strips are not used, it can be replaced with PCR plate sealing film.
* When using a sealing film, evaporation may occur during PCR process if it is not completely adsorbed, which may affect the analysis of the results.

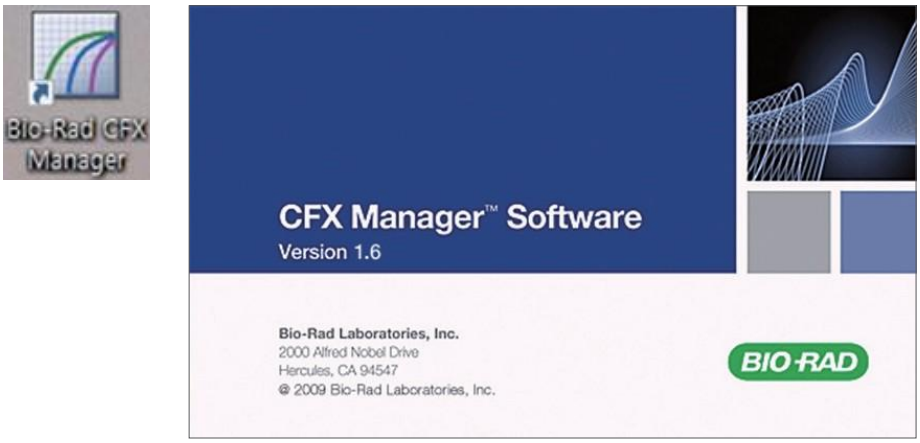
CFX96 and Software Operation - 1 (New experiment)

①Turn on a computer and CFX96 with power switch of the instrument located on the back. At the same time as the start, the CFX96 performs a self-check automatically.

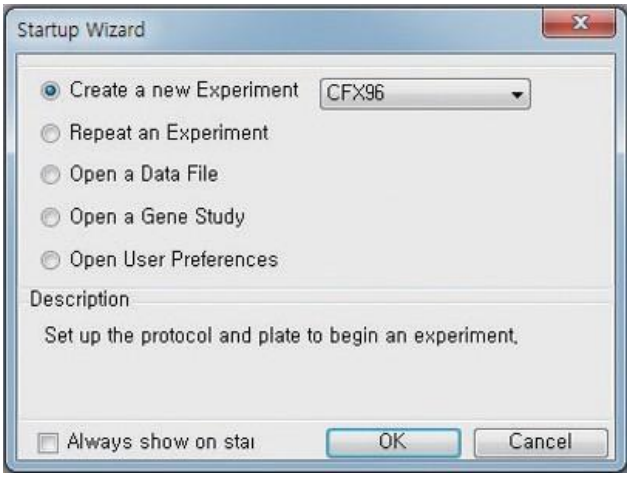
Figure 1. Front view of CFX96 Real-time PCR System

②Press the Door button on the front of machine to display the 96 well thermal block. Place the 96 well white plate required for the reaction in the well of the thermal block.

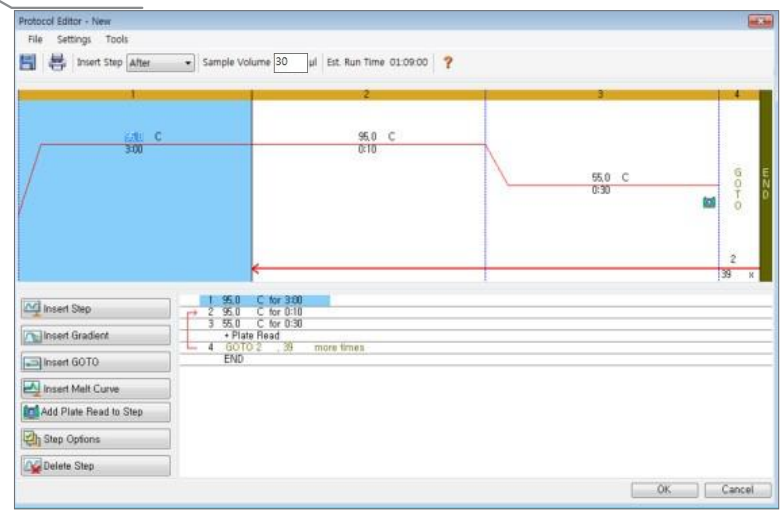
③Run the CFX Manager software of the computer connected to the CFX96.



④Select 'Create a new Experiment' (This step proceed to the initial assay and refer to Section
for repeated afterwards)



⑤Select the 'Edit Selected' button on the right side of the window to open the window shown below



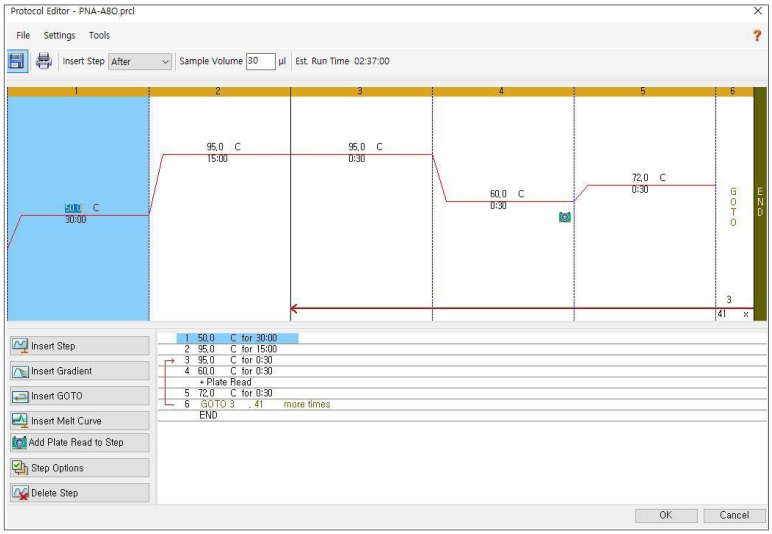
⑥When the Run setup window appears, set the conditions for each item. Set "Sample volume" to 30 ul.

- Insert Step: Input reaction condition, set temperature and time
- Insert GOTO: Specify the cycle to repeat

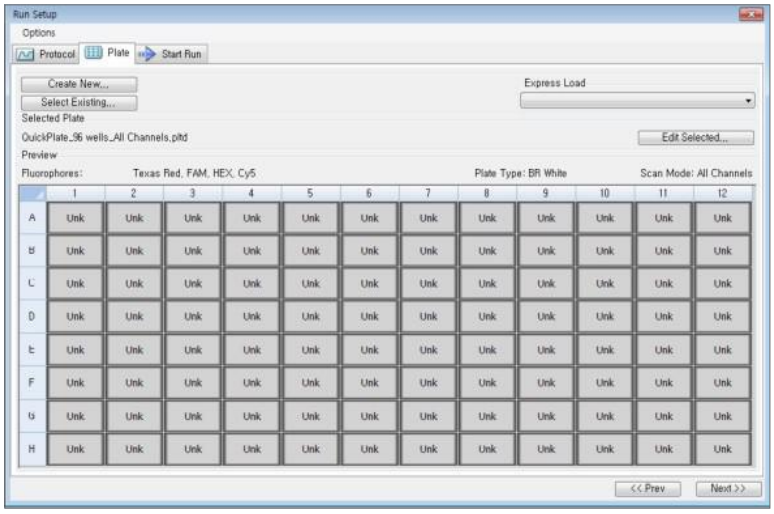
Step	Temp	Time	Cycle (repeat)
cDNA synthesis	50°C	30 min	1
Amplification	95°C	15 min	1
	95°C	30 sec	42
	60°C	30 sec*	
	72°C	30 sec	

* Collect fluorescence signal after reaction for 60°C / 30 sec

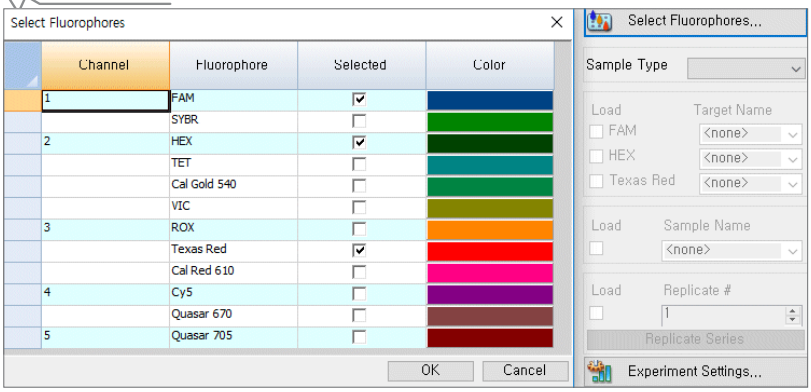
⑦Press the OK button to save the protocol.



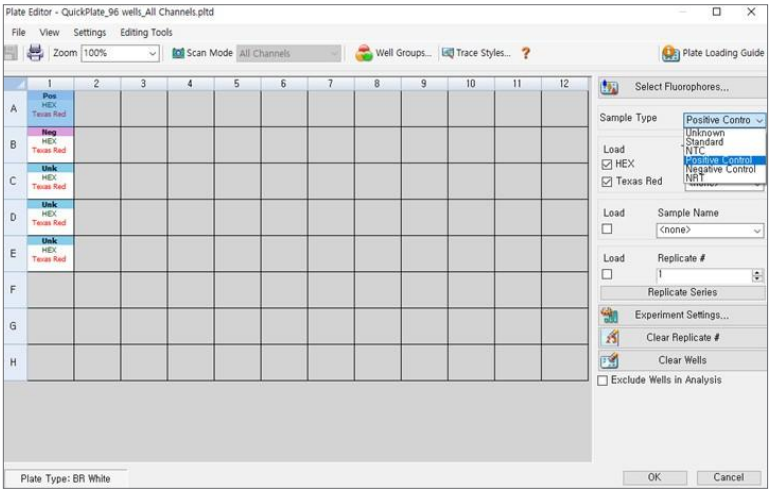
⑧Select 'Edit Selected' on the Plate tab.



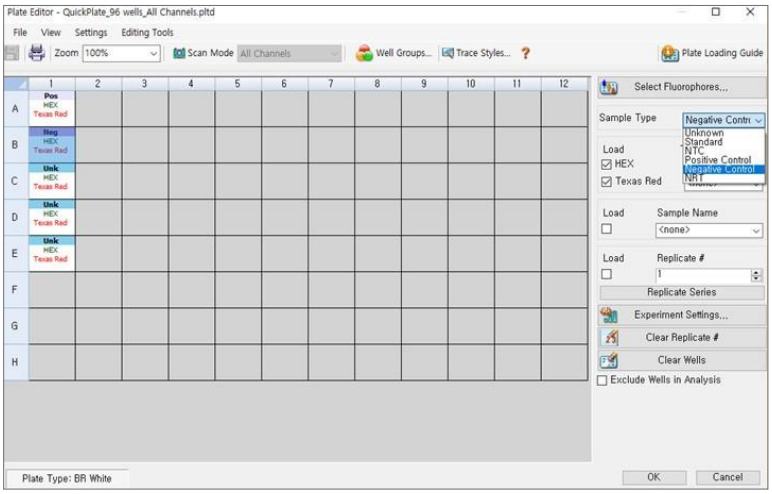
⑨Select 3 fluorescence (FAM, HEX, and Texas Red) and the wells to be analyzed and then click OK.



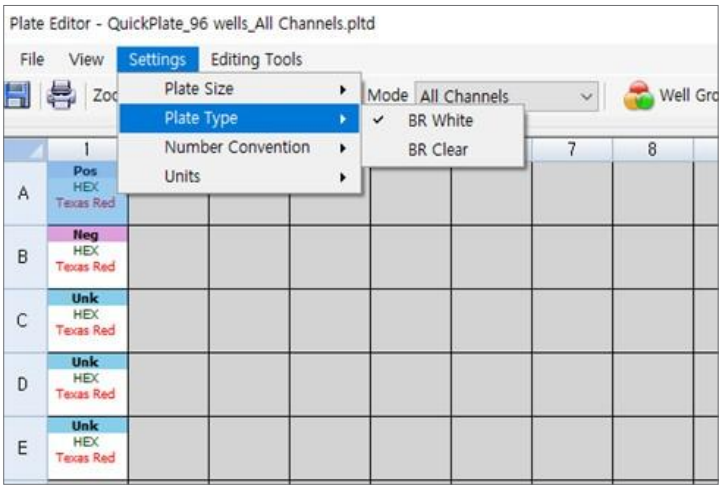
⑩Specify the positive control well, select "Positive Control" from sample type, and input 3 detection fluorescences in the load (Select FAM, HEX, Texas Red).



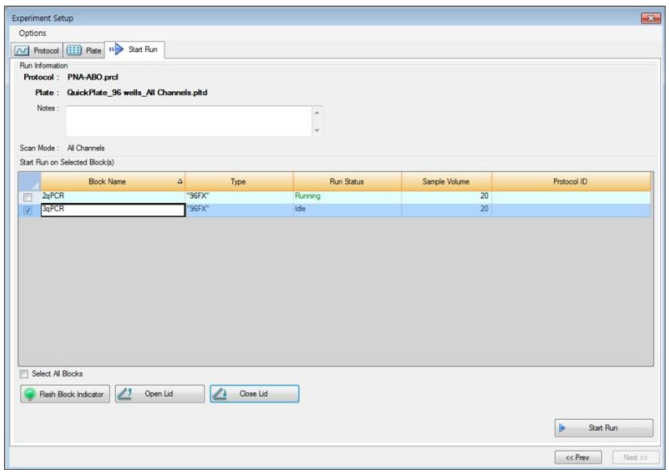
⑪Specify the negative control well, select "Negative Control" from sample type, and input 3 detection fluorescences in the load (Select FAM, HEX, Texas Red).



⑫Select BR white from Plate Type < Settings

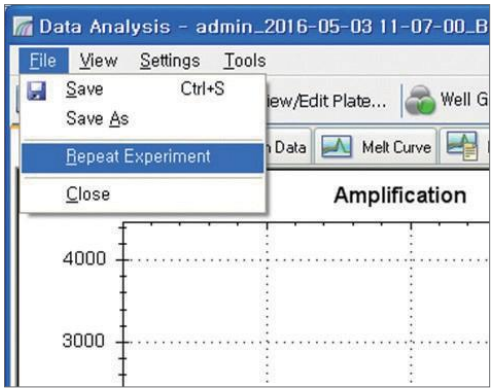


⑬Select a block (PCR machine) to use and press the 'Close Lid' button to close cover of the CFX96 machine. After the cover is completely closed, press 'Start Run' to start the amplification with selecting the folder to save the test result.
* Store a file in an easy-to-find location.



8.5 CFX96 and Software Operation - 2 (Existing experiment)

① If you have an existing analysis file, you can easily open it and re–use for further. Double click on an existing file to open it and select sequentially 'File > Repeat Experiment'.



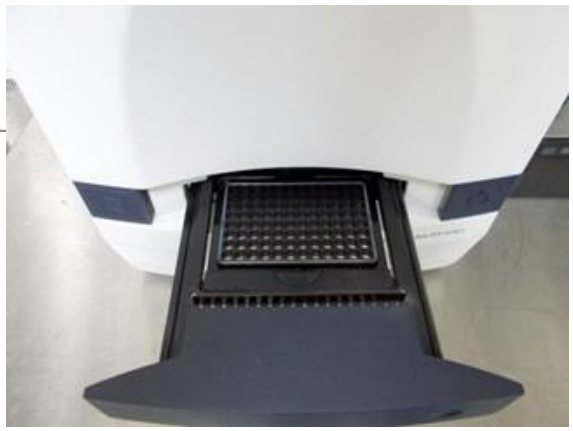
②Select 'Next' to proceed to the next step.
③In the Plate tab, select 'Edit Selected' to select the well containing the specimen.
The fluorescence channel, plate type, and volume are already selected and are therefore can passed.
④Finally, select 'Start run' to select the location where the analyzed file will be saved.
* Store files in easy-to-find locations.

ABI7500 and Software Operation - 1 (New experiment)

①Turn on a computer and ABI7500 with power switch of the instrument located on the back. At the same time as the start, the ABI7500 performs a self-check.

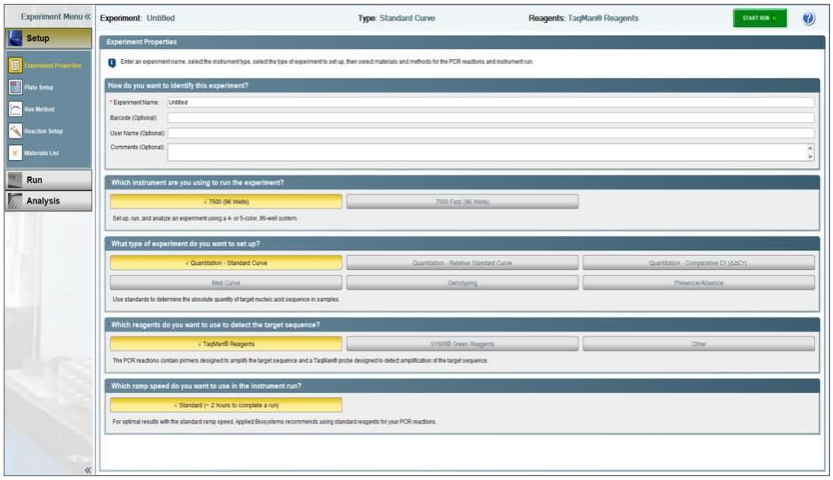
Figure. Front view of ABI7500 Real-time PCR System

②Press the Door button to display the 96 well thermal block. Be sure to input the 96-well PCR plate in proper direction.



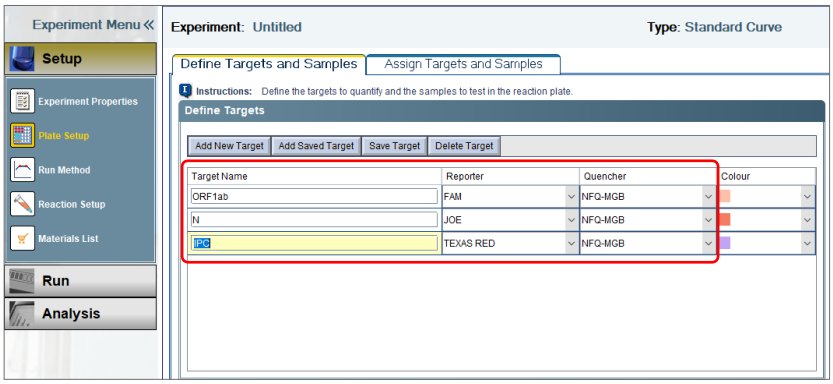
③Run 7500 Software of the computer connected to the instrument and select New Experiment.

④Input the options shown in below "7500 (96well) > Quantitation-Standard curve > TaqMan@ Reagents > Standard (2 hours to complete a run)" and press Plate Setup.

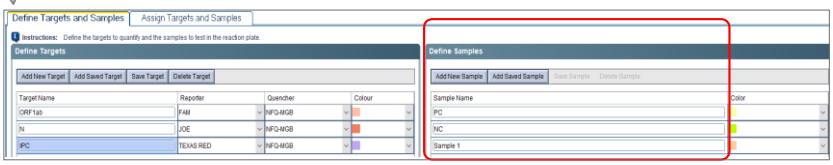


⑤Click "Add New Target" and set Target Name and Reporter as shown below

1. ORF1ab: Reporter FAM, Quencher NFQ-MGB
2. N gene: Reporter JOE, Quencher NFQ-MGB
3. IPC: Reporter TEXAS RED, Quencher NFQ-MGB

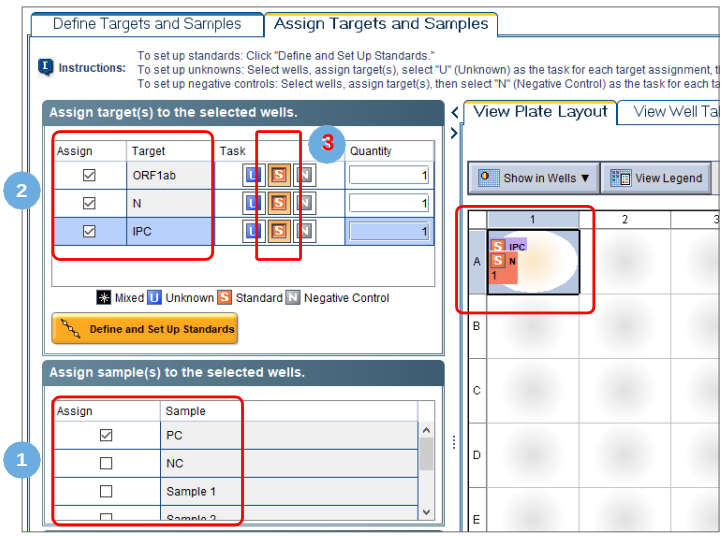


⑥Click "Add New Sample" and set sample number. Be sure to input "Positive Control (PC)", "Negative Control (NC)".

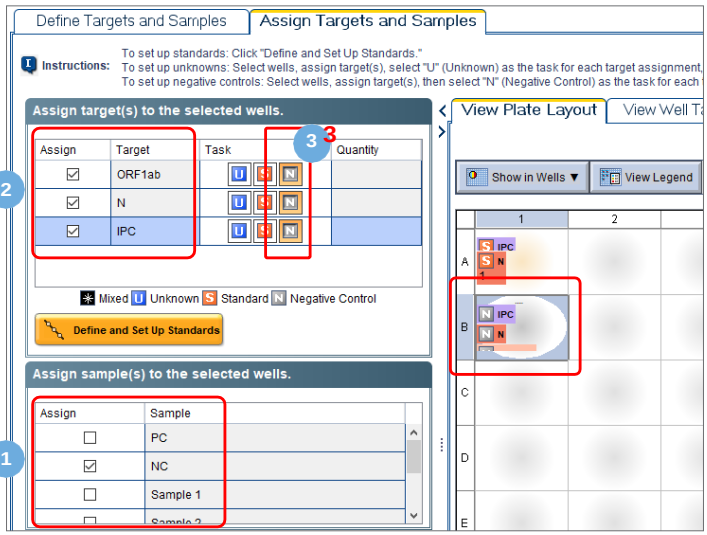


⑦Go to "Assign Target and Samples", set targets and well positions for PC, NC and Samples to analyze.

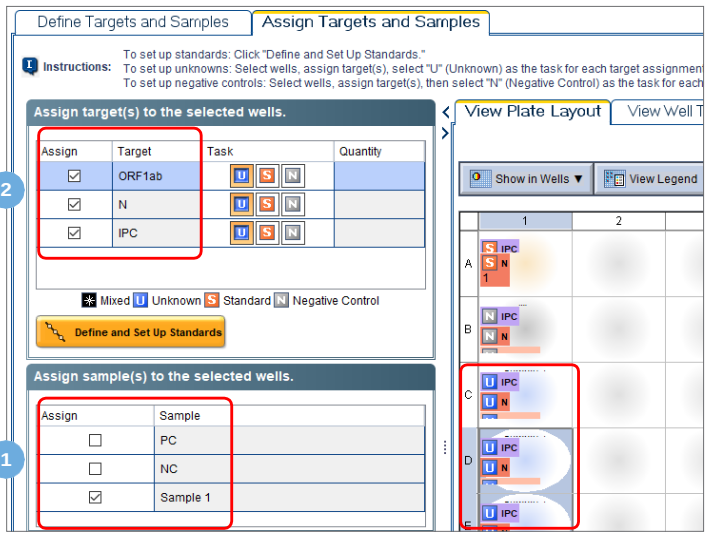
1. Positive Control: Click Positive Control Well from "View Plate Layout". Select "PC" from (1) (shown in figure below) and activate all (2) targets from "S" for all 3 targets from (3) (shown in figure below) then activate



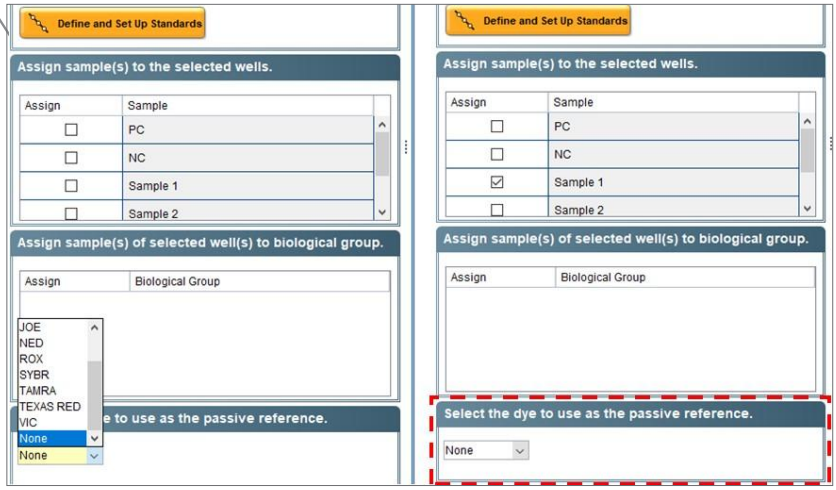
2. Negative Control: Click Negative Control Well from "View Plate Layout". Select "NC" from ① (shown in figure below) and activate all ② (shown in figure below) then targets from "N" for all 3 targets from ③ activate (shown in figure below)



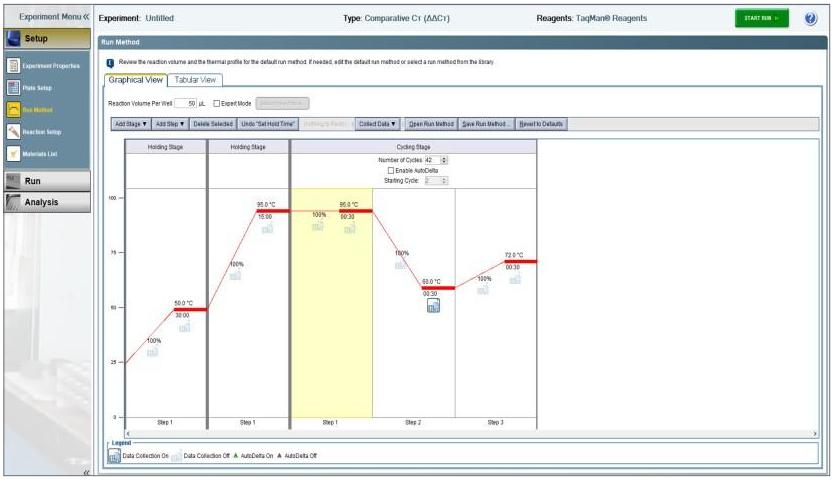
3. Sample: Click Wells with samples from "View Plate Layout". Select "Sample" from ① (shown in figure below) and activate all targets from ② (shown in figure below).



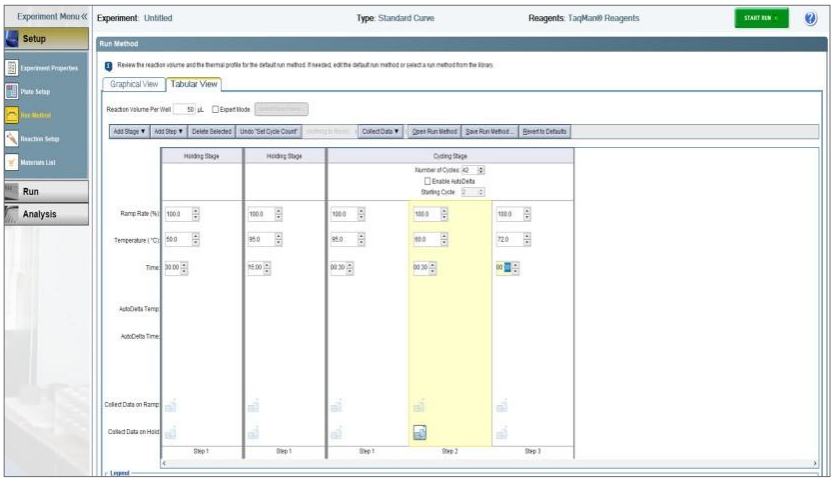
⑧Select "None" from "Select the dye to use as the passive reference"



⑨Selecting the "Run Method" button open a window as shown below.



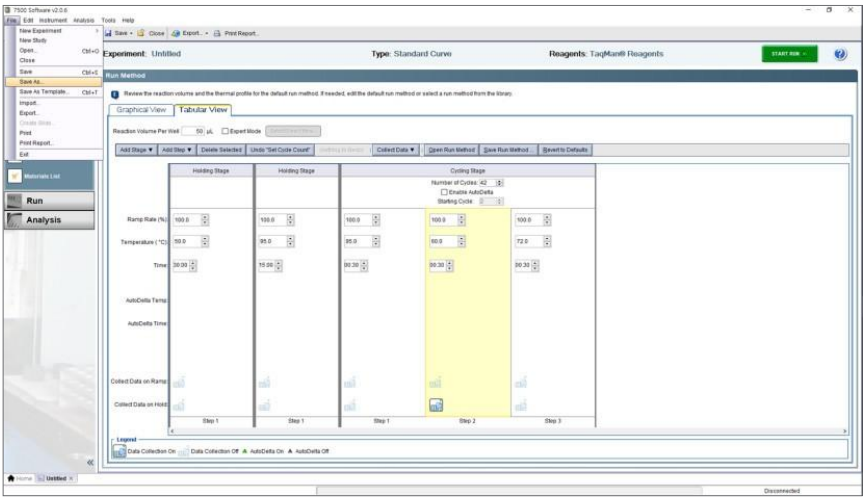
⑩Go to "Tabular View". Set the PCR condition as shown in below and set "Reaction volume Per Well" to 30ul.



Step	Temp	Time	Cycle (repeat)
cDNA synthesis	50°C	30 min	1
Amplification	95°C	15 min	1
	95°C	30 sec	42
	60°C	30 sec*	
	72°C	30 sec	

* Collect fluorescence signal on Hold for 60°C / 30 sec

⑪Press "File" > Press "Save As" and choose a location to save the file then click "START RUN" to start amplification.
* Store files in easy-to-find locations.



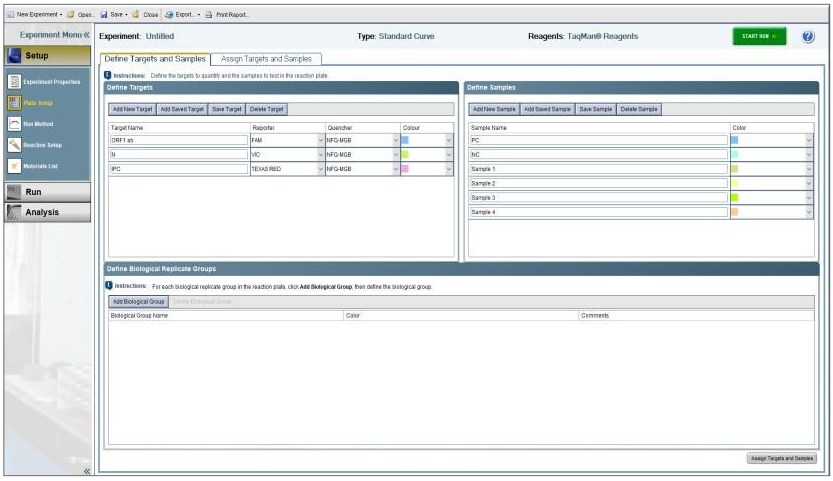
8.7 ABI7500 and Software Operation - 2 (Existing experiment)

①If you have an existing analysis file, it can be used for further test easily. Click 'File> Open.

②Select the "COVID19.edt" file provided.

②Input the sample information in the "Plate setup" and proceed in the same order as above.

Proceed with reference to ⑦in 5.3.



9. Result Interpretation

Base line and threshold setting

We recommend to use the baseline setting which is automatically adjusted by the instrument. However, the baseline setting can be adjusted manually in case of production of background noise signal in PCR initiation phase. For adjusting manually, be sure the Thresholds always should be set to fall within exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities.

Quality Control

- Negative Control : Both ORF1ab and N of 2019-nCoV must not be detected, and the Ct value of IPC should be ≤38.
- Positive Control : Both ORF1ab and N of 2019-nCoV must be detected, and the Ct value of IPC should be ≤38.
  If negative and positive control results are not as described above, the test results of the entire batch are invalid
- Internal Control : If the result for a specimen is 2019-nCoV RNA not detected, the Ct value of the IPC must be ≤38, otherwise the result of that specimen is inconclusive; if the result for a specimen is 2019-nCoV RNA detected, the Ct value of the IPC is not required to be considered valid. Positive and Negative controls should meet the requirements listed in the below table to ensure valid results.

Control	Results (Ct ≤ 38)	
	ORF1ab (FAM)	N (HEX)
Negative	–	–
Positive	+	+

Interpretation ofResults

If the values of the controls are conclusive, refer to the table below to determine the infection status.

Results (Ct ≤ 38)			Interpretation
ORF1ab (FAM)	N (HEX)	IPC (TexasRed)	
–	–	+	Negative (Absence of 2019-nCoV RNA)
+	–	+/-	Positive (Presence of 2019-nCoV RNA)*
–	+	+/-	Positive (Presence of 2019-nCoV RNA)*
+	+	+/-	Positive (Presence of 2019-

			nCoV RNA)
–	–	–	Invalid**

* Result is suggestive of: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in one of the target regions, or 3) other factors

** Re test after confirmation of PCR mixture preparation step, PCR protocol and Kit storage condition and validity.

10. Limitations

- For in vitro use only.
- For professional use only.
- This test is a qualitative test and does not provide the quantitative value of viral load in the original specimens.
- The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- Amplification and detection of 2019-nCoV with the this kit Detection Kit has only been validated with the CFX-96 Real-time PCR Detection system and Applied Biosystems® 7500 Real-Time PCR instrument. Use of other instrument systems may cause inaccurate results.
- False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
- This kit uses an UNG/dUTP PCR products carryover prevention system which can prevent contamination caused by PCR products. However, in the actual operation process, the amplicon contamination can be avoided only by strictly following the instructions of PCR laboratories.

11. Assay Performance

Limit ofDetection

- Limit of detection (LoD) was determined as the lowest concentration of 2019-nCoV that at which the U-TOP™ COVID-19 Detection Kit can detect at a ≥95% positive rate. The LoD was determined by limiting dilution studies using characterized samples.
- The test designed for detection in vitro transcribed RNA (ORF1ab and N) of known titer (RNA copies/μL) spiked into pooled sputum samples.
- A preliminary LoDs for each target were determined testing triplicate samples with 10- fold serial dilutions (Table 11.1).
- A confirmation of the LoDs were determined using 3-fold serial dilution samples with 20 replicates (Table 11.2).
- The LoD was determined as 10 copies/reaction as the lowest concentration where ≥ 95% of the replicates were positive.

Table 11.1. A preliminary LoD for each target

	ORF1_ab				
Concentration	1X10^5	1X10^4	1X10^3	1X10^2	1X10
Positive total	3/3	3/3	3/3	3/3	3/3
Mean Ct	25.4	29.6	32.7	35.5	37.2
Standard Deviation (Ct)	0.4	0.1	0.2	0.3	0.1

	N gene				
Concentration	1X10^5	1X10^4	1X10^3	1X10^2	1X10
Positive total	3/3	3/3	3/3	3/3	3/3
Mean Ct	25.3	28.5	32.5	35.4	37.0
Standard Deviation (Ct)	0.1	0.3	0.3	0.2	0.1

Table 11.2. A confirmation LoD for each target

	ORF1_ab			N gene		
Concentration	10^1	10^0.5	10^0.1	10^1	10^0.5	10^0.1
Positive total	20/20	16/20	6/20	20/20	18/20	11/20
Mean Ct	36.88	NA	NA	36.89	NA	NA
SD (Ct)	0.5	NA	NA	0.38	NA	NA

Inclusivity (analyticalsensitivity)

- All primer as well the detection probe sequences are selected based on the research papers and nucleic acid sequences for 2019-nCoV comparing with other microorganisms and viruses include SARS CoV, MERS CoV and human Coronavirus sequences in a public database National Center for Biotechnology Information (NCBI) as of February, 2020.
- All the alignments of 2019-nCoV show 100% identity of the oligonucleotide composed in the kit.
- In summary, the 2019-nCoV rRT-PCR assay ORF1ab and N, designed for the specific detection of 2019-nCoV only, showed no significant combined homologies with other coronaviruses, or human microflora that would predict potential false positive results.

Specificity (Cross reactivity, Interfering substances)

- Evaluation of analytical specificity of the kit was conducted using both in silico analysis and wet testing against pathogenic organisms mainly found in the human respiratory tract.
- As a result of BLASTn analysis of the primers and probes included in this kit against gene bank /blast.ncbi.nlm.nih.gov/ with default settings, only the 2019-nCoV isolates including Bat coronavirus RaTG13, complete genome were found that have 100% of sequence homologies with the queries.
- In addition to the in silico analysis, the nucleic acid extracted from the pathogens found in human respiratory tract were tested with U-TOP™ COVID-19 Detection Kit. Each pathogen shown in the table below was tested in triplicate with the nucleic acid concentrations of 15ng/ul.
- In addition to the cross reactivity test, the kit was evaluated with the presence of PCR interfere substances in addition to the target RNA of 2019-nCoV to test the PCR interfere as well.
- All results of the cross reactivity test using the non-specific nucleic acids were negative, and no PCR inhibition and non-specific reaction was appeared with addition of PCR interfere substances.

< Substances used in specificity (Cross reactivity, PCR interfere) test >

Target	Nonspecific substances
Nucleic acids	Human coronavirus OC43 Human coronavirus HKU1 Human coronavirus NL63 Human coronavirus 229E SARS-coronavirus MERS-coronavirus Adenovirus 1 KBPV-VR-1 DNA Adenovirus 3 KBPV-VR-2 DNA Adenovirus 8 KBPV-VR-3 DNA Enterovirus, Coxsackievirus B1 KBPV-VR-13 RNA Enterovirus Coxsackievirus B2 KBPV-VR-14 RNA Enterovirus Coxsackievirus B3 KBPV-VR-15 RNA Influenza A virus H3N2 KBPV-VR-32 RNA Influenza A virus H1N1 KBPV-VR-33 RNA Parainfluenza virus KBPV-VR-44 RNA Parainfluenza virus KBPV-VR-45 RNA Human metapneumovirus Chlamydia pneumoniae Haemophilus influenzae Listeria monocytogenes Neisseria meningitidis Streptococcus agalactiae Streptococcus pneumoniae Legionella pneumophila Mycobacterium tuberculosis Streptococcus pyogenes Bordetella pertussis Mycoplasma pneumoniae Pneumocystis jirovecii (PJP) Candida albicans

	Pseudomonas aeruginosa Staphylococcus epidermis Staphylococcus salivarius Human gDNA (Hela gDNA)
PCR interfere	Human nasal wash Human genomic RNA Mucin (60ug/ml) Human blood 2% Tobramycin (4ug/ml)

Clinical evaluation

Clinical specimens were tested with the U-TOP™ COVID-19 Detection kit at three different experimental sites by using total 90 nucleic acid extracts. For each of the sites, an established comparator was used. The calculated Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were shown in table below.

Clinical evaluation			Comparator		Agreement (%)
			Negative	Positive	
U-TOP™ COVID-19	Site 1 (SH)	Negative	7	–	NPA: 7/7 (100%)
		Positive	–	3	PPA: 3/3 (100%)
	Site 2 (GC)	Negative	10	–	NPA: 10/10 (100%)
		Positive	–	10	PPA: 10/10 (100%)
	Site 3 (SB)	Negative	30	–	NPA: 30/30 (100%)
		Positive	–	30	PPA: 30/30 (100%)
Total		Negative	47	-	NPA: 47/47 (100%)
		Positive	-	43	PPA: 43/43 (100%)

NPA: Negative Percent Agreement, PPA: Positive Percent Agreement

Analytical Reproducibility

The analytical reproducibilities of the kit were evaluated with triplicates using 3 different PCR instrument, 3 different Lot of the kit in 3 different days by 3 technicians. The results are summarized in table below. Briefly the reproducibilities were between 98.4 – 99.7% while estimated standard deviations were 0.1-0.4.

Step	Total reaction	Reproducibility (CV%)	Standard Deviation
3 Lot	9	99.1-99.7%	0.1-0.2
3 Technician	27	98.9-99.6%	0.1-0.3

3 Instrument	27	98.4-99.6%	0.1-0.4
3 Day	27	98.9-99.5%	0.1-0.3

12. Result interpretation using U-TOP™ COVID-19 Analyzer

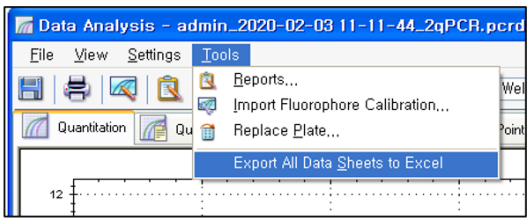
Data export from CFX 96 (Bio-Rad) and ABI 7500 (ThermoScientific)

<CFX 96 real-time PCR system>

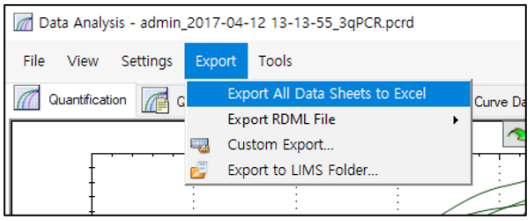
① After the PCR is completed, export the data as shown in figure below.

- CFX Manager 1.6: Tools → Export All Data Sheet to Excel → Save the data in a desired folder
- CFX Manager 3.0: Export → Export All Data Sheet to Excel → Save the data in a desired folder
- CFX Manager 3.1: Export → Export All Data Sheets → Excel 2007 (*xlsx) → Save the data in a desired folder

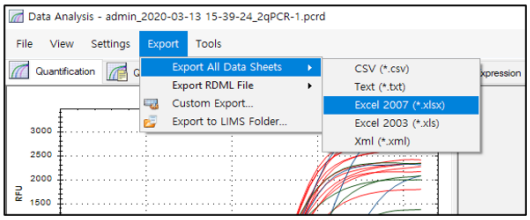
Biorad CFX Manager 1.6



Biorad CFX Manager 3.0

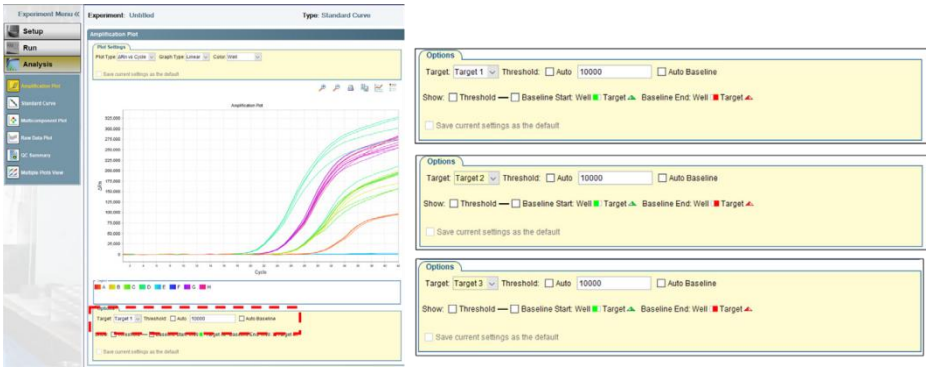


Biorad CFX Manager 3.1



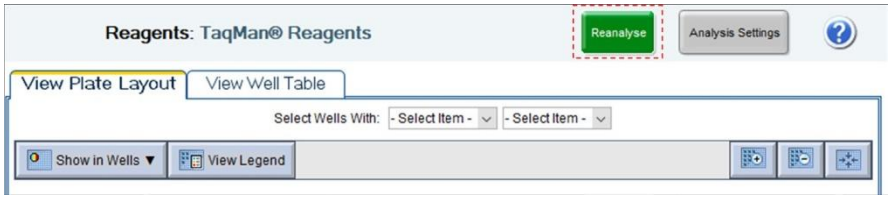
<ABI 7500>

① Adjust target threshold values to 10000 from Amplification plot "Options". Target 1, 2 and 3 should be selected individually for the adjusting.



* If you have background noise signal on Negative control or on the samples, the baseline setting can be adjusted manually. Please be sure the Thresholds always should be set to fall within exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities.

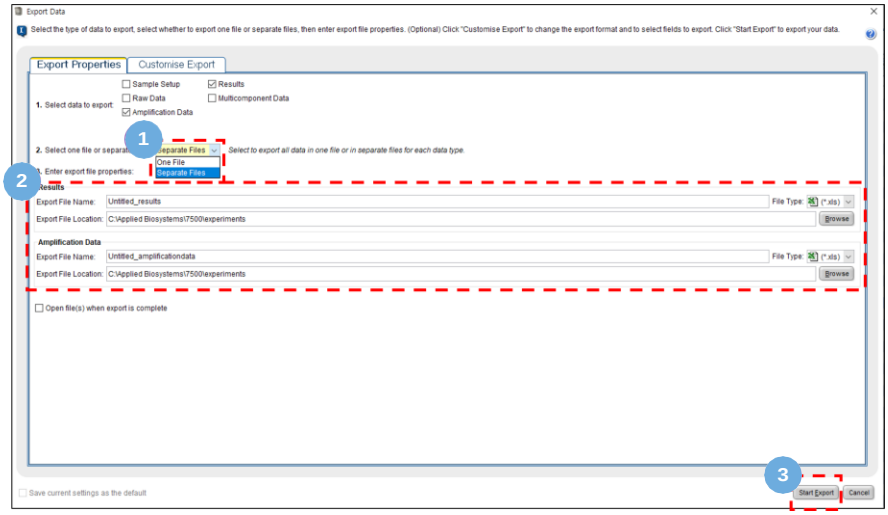
② After adjusting the threshold, click "Re analyse" to complete the analysis



③ Select all wells to be analyzed (shown in 1) and click "Export" (shown in 2) to export data in excel.

④ Check the box in front of "Results", "Amplification data".

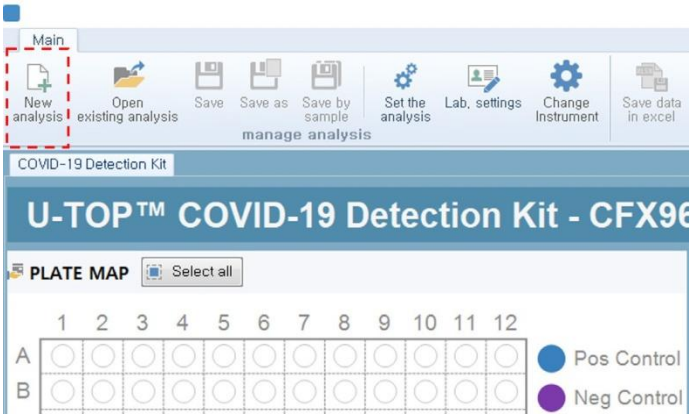
⑤ Select "Separate Files" (Shown in 1) and choose the location to save "Results", "Amplification data" data (Save the files in a same folder). "Start Export" to save the files (Shown in 3).
*Save the files in an easy to find location.



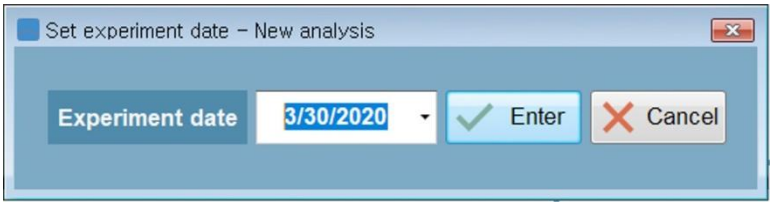
Result Interpreting with U-TOP™ COVID-19 Analyzer
① Run the U-TOP™ COVID-19 Analyzer.

② Select your PCR platform.

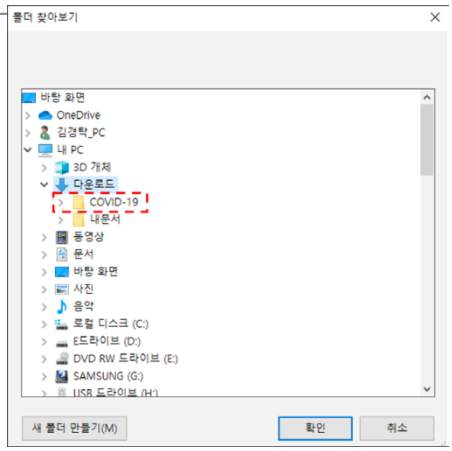
③ Click “New analysis”.



④ Set your test date.



⑤ Import the previously exported data from "CFX96" or "ABI 7500". Files can be imported as folder type (e.g. "COVID-19" shown in figure below).



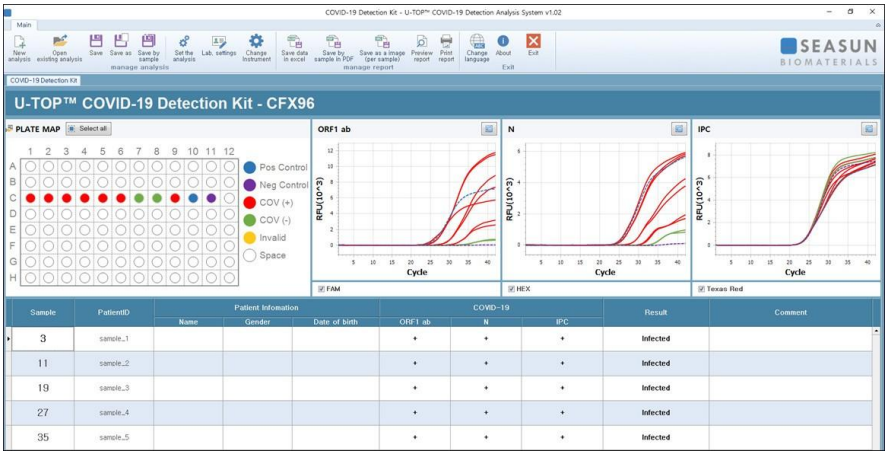
⑥ Positive(●) and Negative Control(●)s are automatically selected on the first screen.

⑦ The infection status can be confirmed by selecting the wells to analyze from the PLATE MAP.

● in PLATE MAP indicates COVID-19 Positive ● in PLATE MAP indicates COVID-19 Negative

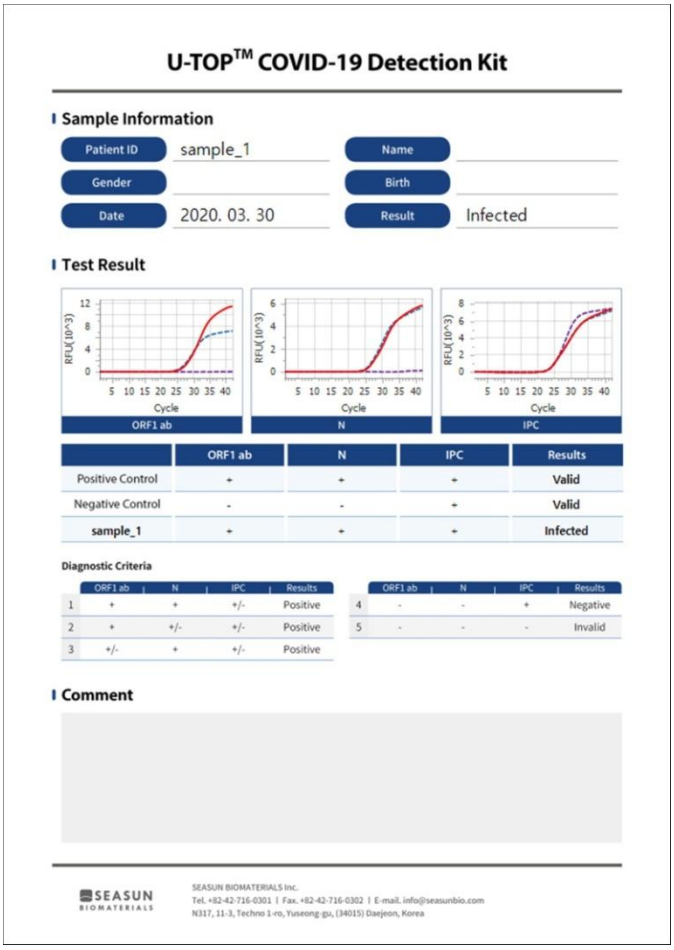
● in PLATE MAP indicates Invalid result (See TROUBLE SHOOTING)

"Sample name/#, Patient ID, Name, Gender, Date of Birth, Comment" can be entered additionally. The entered informations are reflected in the report.



⑧ See the Report with "Preview report".

⑨ Use the print button to print the report.



U-TOP™ COVID-19 Detection Kit

Sample Information

Patient ID	sample_1	Name	
Gender		Birth	
Date	2020. 03. 30	Result	Infected

Test Result

	ORF1 ab	N	IPC	Results
Positive Control	+	+	+	Valid
Negative Control	-	-	+	Valid
sample_1	+	+	+	Infected

Diagnostic Criteria

	ORF1 ab	N	IPC	Results
1	+	+	+/-	Positive
2	+	+/-	+/-	Positive
3	+/-	+	+/-	Positive
4	-	-	+	Negative
5	-	-	-	Invalid

Comment

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13. Trouble shooting

Problem	Cause	Solution
Fluorescence signal is not detected in all samples	Error of the PCR reaction	Review if anything is missing during the preparation process..
	If the storage conditions of the kit are not appropriate, or the expiration date has expired	Repeat the test after checking the storage conditions and expiration date
Fluorescent signal is low in all samples	If the PCR reagents were not mixed correctly	Proceed the test after review of PCR mix
	Long storage at room temperature or light	Dispose the kit.
	If the expiration date has passed	Check the expiration date of the kit.
Signal detection in Negative Control	If the PCR mixture is contaminated	Discard the PCR mixture.
	If the experiment place or the tool is contaminated	Check whether the test site or tool is not contaminated. Repeat the experiment with new aliquots of all reagents
If there are different	Pipetting error	Check the pipette
	Cross contamination	Be careful with DNA splitting and repeat the test.

results in the same sample	Contaminated 96-well plate	Test with a new 96-well plate.

14. Reference

1. Victor M Corman et al., Diagnostic detection of 2019-nCoV by real-time RT-PCR. Euro Surveill 2020. 25(3): 2000045
2. Leo Poon et al., Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR. www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf.
3. Mary Johnson. Wuhan 2019 Novel Coronavirus – 2019-nCoV. MATER METHODS. 2020. 10:2867
4. Zheng-Li Shi et al., Discovery of a novel coronavirus associated with the recent pneumonia outbreak in 2 humans and its potential bat origin. Nature. 2020 579(7798): 270-273
5. Naganori Nao et alk., Detection of second case of 2019-nCoV infection in Japan. NIID. 2020.
6. 2019-Novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. 2020. US Centers for Disease Control and Prevention.
7. 2019-nCoV detection real-time RT-PCR protocol. 2020. KCDC v1.5

15. Symbols

1	RE	Catalog number	2	2	Do Not Reuse (For single use only)
3		Expiration Date	4		Manufacturer
5		Temperature limitation (Storage temperature)	6	LO	Medical
7	IVD	In Vitro Diagnostic			

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