Instructions for LabGun™ COVID-19 RT-PCR Kit

For prescription use only.
For in vitro diagnostic use only.
For Emergency Use Authorization only.

This version of the LabGun™ COVID-19 RT-PCR Kit User's Manual was published in September, 2020.

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Product Name

LabGun™ COVID-19 RT-PCR Kit

Intended Use

LabGun™ COVID-19 RT-PCR Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal, or oropharyngeal, anterior nasal and mid-turbinate nasal swabs, as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum, from individuals who are 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, to perform high complexity tests.

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

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The LabGun™ COVID-19 RT-PCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The LabGun™COVID-19 RT-PCR Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principles of the Assay

The LabGun™ COVID-19 RT-PCR Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer/probe set is designed to detect RNA from the SARS-CoV-2 in nasopharyngeal, or oropharyngeal, anterior nasal and mid-turbinate nasal swabs, as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum, from patients with signs and symptoms of infection who are suspected of COVID-19. The primer/probe set for detection of Sarbecovirus is also included.

Kit Components and Descriptions (Cat. No. CV9032B)

Component	Description	Amount (100samples)	Storage
2X One-step buffer	One-step real-time RT-PCR buffer	2 x 1000 µL	≤ -20 ℃
One-step enzyme	DNA polymerase and reverse transcriptase	200 µL	≤ -20 ℃
Assay 1 (RdRp gene)	Primers and probe mix for RdRp + Rox Dye	400 µL	≤ -20 ℃
Assay 2 (E gene)	Primers and probe mix for E + Rox Dye	400 µL	≤ -20 ℃
MS2 Internal control	MS2 bacteriophage	1000 µL	≤ -20 ℃
Positive control	Cloned plasmid DNAs of RdRp and E gene	250 µL	≤ -20 ℃

Materials Required but Not Provided

- Thermal Cycler: Applied Biosystems™ 7500 Real-time PCR Instrument system or Bio-Rad CFX96™ Real-time PCR detection system
- RNA extraction kits: QIAamp Viral RNA Mini kit (QIAGEN, Cat. No. 52904)
- DEPC-water
- PBS, 1X
- Bench-top centrifuge
- Serological Pipet (Pipette Aid)
- P-10, P-20, P-200, P-1000 Pipettes
- Multi-channel pipette (Mettler Toledo, Cat No. 17013794)
- P-10, P-20, P-200, P-1000 ART Plugged Tips
- 1.5 mL microcentrifuge tubes
- 96-Well Optical Reaction Plates
- Optical Adhesive Cover
- Vortex
- Microcentrifuge
- Refrigerator
- Freezers (-20°C and -80°C)

Storage and Handling Requirement

1. Store all reagents at -25 to -15°C.
2. Use the reagents within 30 days once opened.
3. Completely thaw the reagents before use.
4. Avoid repeated freeze/thaw cycles for reagents.

Warning and Precautions

1. For in vitro diagnostic use under Emergency Use Authorization only.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
4. Keep the kit upright during storage and transportation.
5. Before using the kit, check tubes for leakage or damage. Each component in the kit should be thawed at room temperature, thoroughly mixed, and centrifuged before use.
6. Cross-contamination may occur when inappropriate handling of reference materials and specimens, which will cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
7. All specimens to be tested and the reference materials of the kits should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Sterile centrifuge tubes and filter-tips should be used. After use, the tips should be disposed into a waste bin containing a 10% sodium hypochlorite solution. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 75% ethanol or pure water. Finally, turn on UV light to disinfect working surfaces for 30 minutes.
8. The PCR instrument used for this assay should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.
9. This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Access to each area must be in strict accordance with a single flow direction, namely the specimen preparation area → reagent preparation area → amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.

Instruments

- Applied Biosystems™ 7500 Real-time PCR Instrument system with software version 2.3
- Bio-Rad CFX96™ Real-time PCR detection system with Bio-Rad CFX Manager 3.1

Specimen Collection, Handling, and Storage

1. Specimen Collection
   Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing viral transport media. Sputum is collected in a sterile sputum collection container.
2. Storage
   If specimens are not shipped or processed immediately, it is acceptable to store

specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected to exceed 72 hours, specimens can be stored at -70°C or below until processing can proceed.

3. Shipping

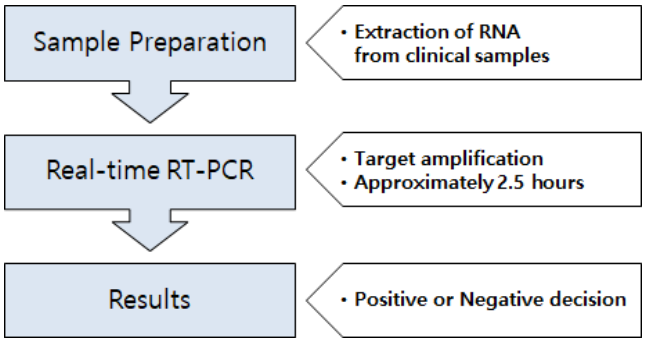
Specimens PUI's must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation External Icon. Store specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70°C, ship overnight 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).

For more information, refer to:

Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/SARS-CoV-2/guidelines-clinical-specimens.html
Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/SARS-CoV-2/lab-biosafety-guidelines.html

Assay Procedure

Nucleic acids are isolated and purified from nasopharyngeal/oropharyngeal swabs, anterior nasal and mid-turbinate nasal swabs, as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and, sputa specimens using the QIAamp Viral RNA Mini extraction kit. The purified nucleic acid is directly amplified using the LabGun™COVID-19 RT-PCR Kit on either the Applied Biosystems™ 7500 Real-time PCR Instrument system or CFX96™ Real-time PCR detection system. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Real-time PCR Instrument system.



A) RNA Extraction

The IC is provided in the kit to confirm nucleic acid extraction and identify any PCR

inhibition. Before nucleic acid extraction, 10 µL of MS2 phage IC should be added to each specimen. Isolate and purify RNA using the QIAamp Viral RNA Mini kit (QIAGEN, Cat. No. 52904) according to manufacturer's manual.

B) Preparation of Real-time PCR reagents

1) Prepare the reagents according to the table below. The final volume is calculated by multiplying the number of samples by the volume of each component (Table 1).

Table 1.Components and volume of the reagents for PCR reaction

Component	Volume **(㎕)** per reaction
2X One-step Buffer	10
One-step Enzyme	1
Assay 1 (or 2)	4
Template RNA	5
Total volume	20

2) Mix the reaction master mix except the template, and spin-down briefly. Aliquot 15㎕ of the master mix into each well of 96-well plate, and add the template RNA. For positive control, use the "Positive Control" included in the kit instead of template RNA. For negative control, use the RNase-free water (DEPC-water) instead of template RNA.

3) Spin-down the plate briefly, and run the PCR reaction immediately.

C) Running Real-time RT-PCR instrument

1) CFX96 Real-time PCR instrument set up

- The set-up of the CFX96 Real-time PCR System for the detection of COVID-19 and IC can be divided into the following steps: Protocol Setup, Plate Setup, and Start run.

Protocol Set up

- In the main menu, clink "Protocol" to open the File

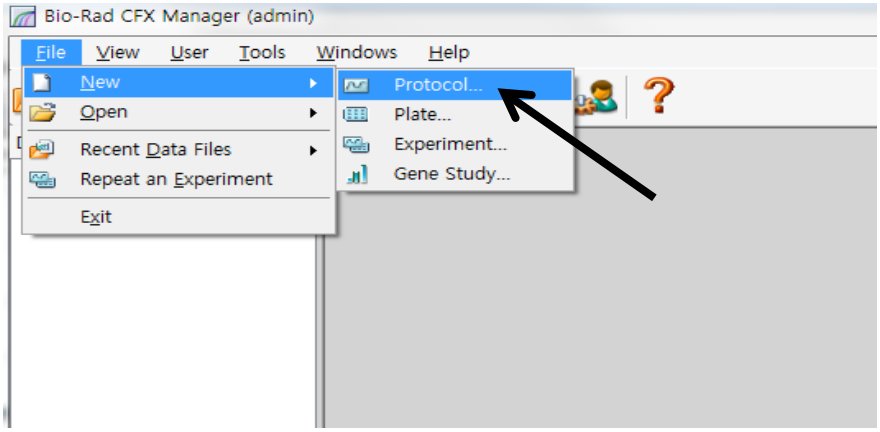


Fig 1. Protocol set up

- Create a new protocol or load an existing protocol for the experiment.
- In Protocol Editor, define the thermal profile as follows:

Segment	Temperature	Time	Cycles
1	50 ℃	30 min	1
2	95 ℃	15 min	1
3	95 ℃	15 sec	45
4*	60 ℃	1 min	

*collect the fluorescence data

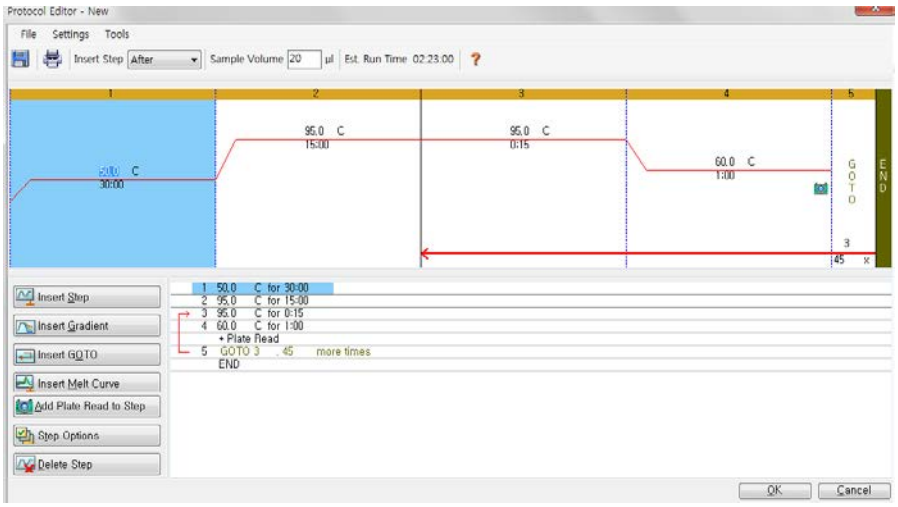


Fig 2. Protocol Editor

- Click on Sample Volume to directly edit the “20uL”
- Click “OK” and Click “Next”

Plate Set up

- In “Experiment Setup plate”, click “Create New” to open the “Plate Editor” to create a new plate.

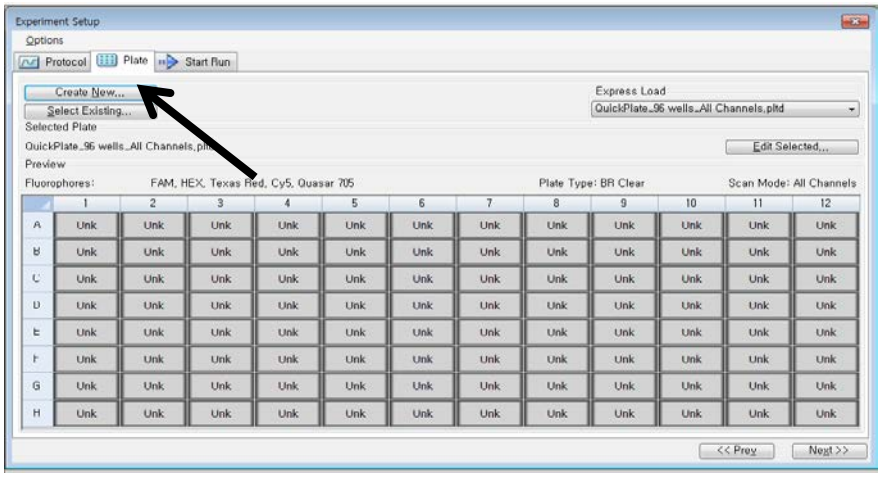


Fig 3. Plate Editor “Create a new” plate or load an existing plate for the experiment.

- Click “Select Fluorophores” to indicate the fluorophores (FAM, Cy5 and HEX(VIC)) that will be used in the experiment.

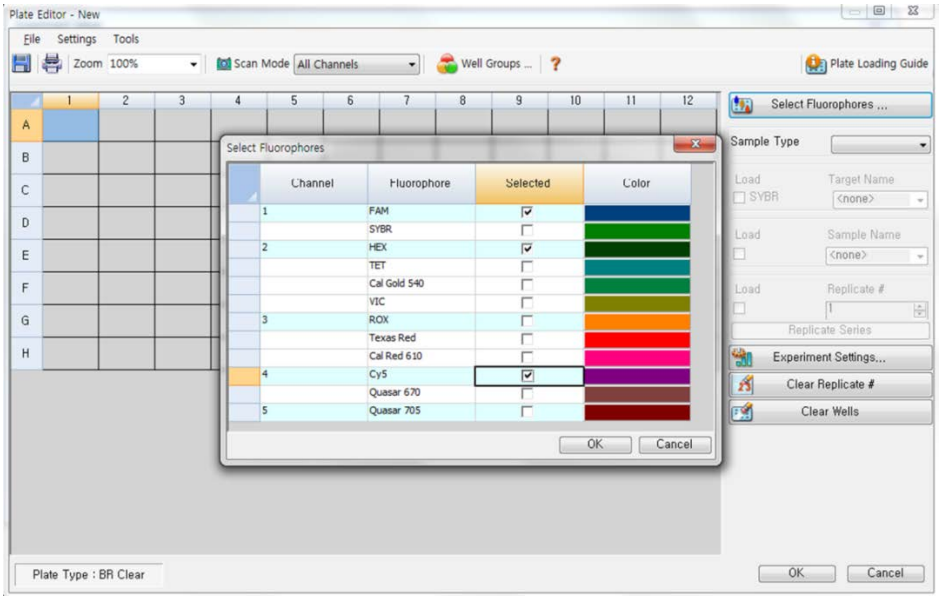


Fig 4. “Select Fluorophores” (FAM, Cy5 and HEX(VIC))

- Choose the appropriate well and then click the “Sample Type” from drop-down menu.

Note: Unknown : Clinical Sample, Negative control, Positive control

- Click the appropriate checkboxes [Assay 1:FAM and HEX(VIC), Assay 2: Cy5 and HEX(VIC)] to specify the fluorophores in the selected wells.

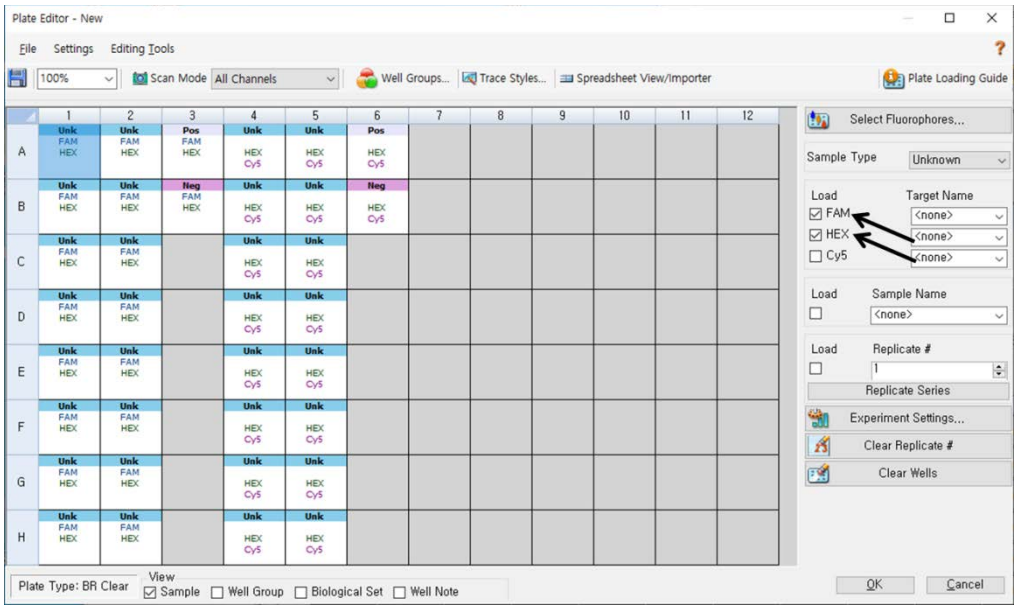


Fig 5.1. Plate Setup for Assay 1

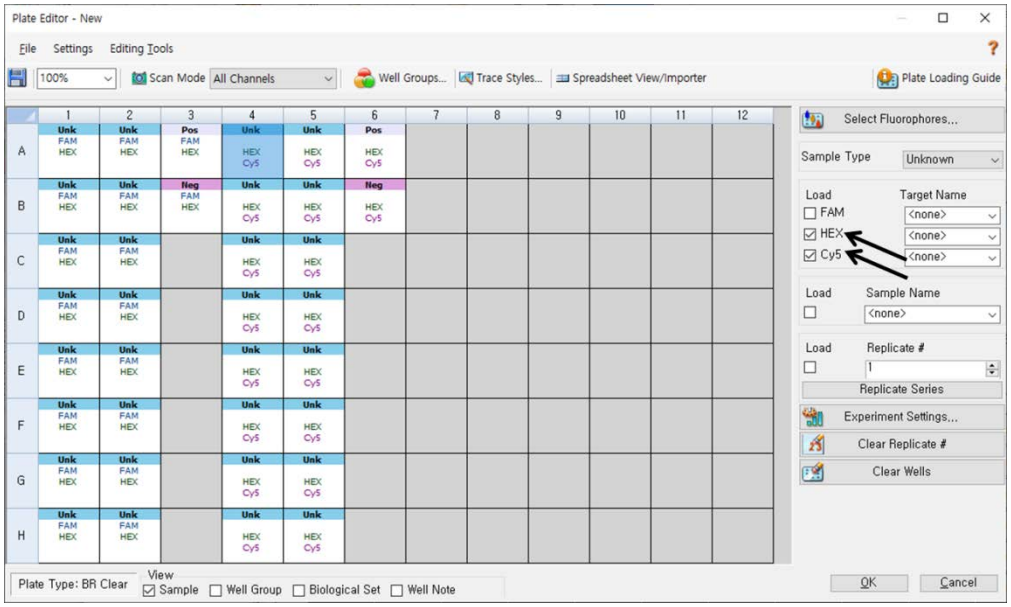


Fig 5.2. Plate Setup for Assay 2

- Type the “Sample Name” and press enter key.
- In “Setting” of the “Plate Editor” main menu, choose the “Plate Size” and “Plate Type (BR White)”

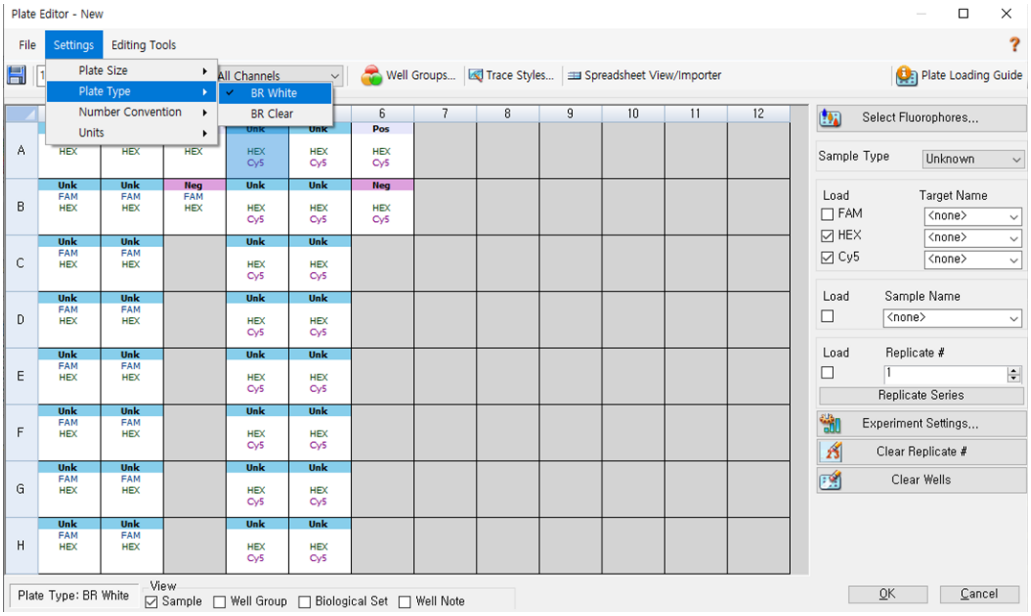


Fig 6. Plate Type

- Click “OK” and save a new plate set up file.
- Finally, “Experiment set up” window will open.

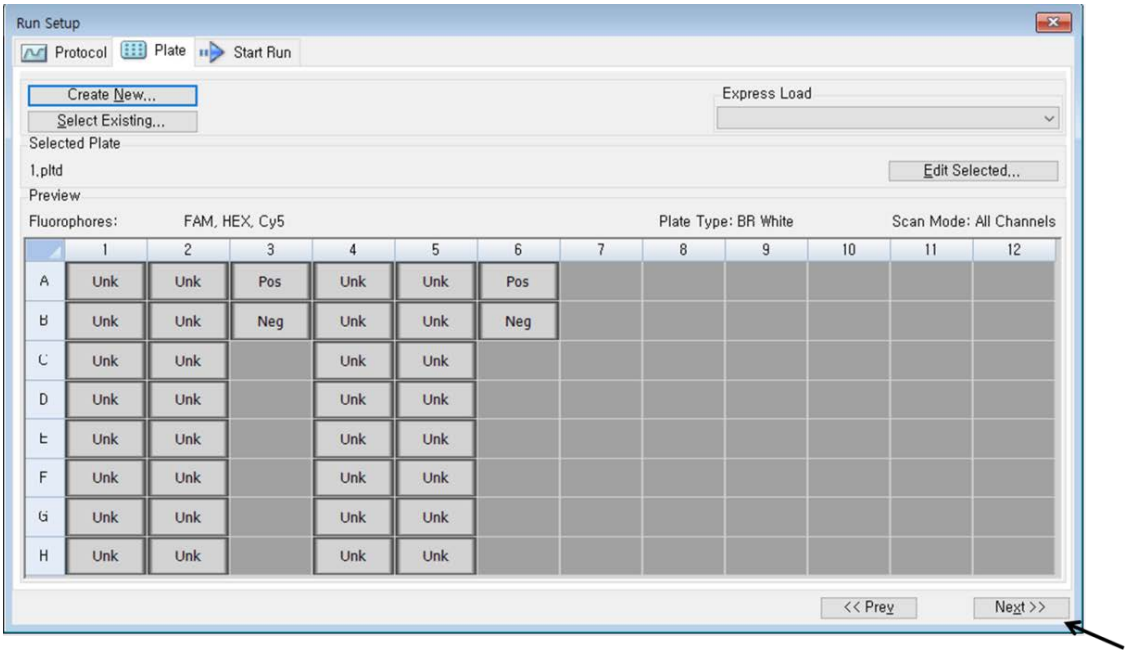


Fig 7. Experiment setup Plate

Start Run

- In “Experiment set up start run”, after the test plate on the device, click “Close Lid” to close the lid and click "Start Run"

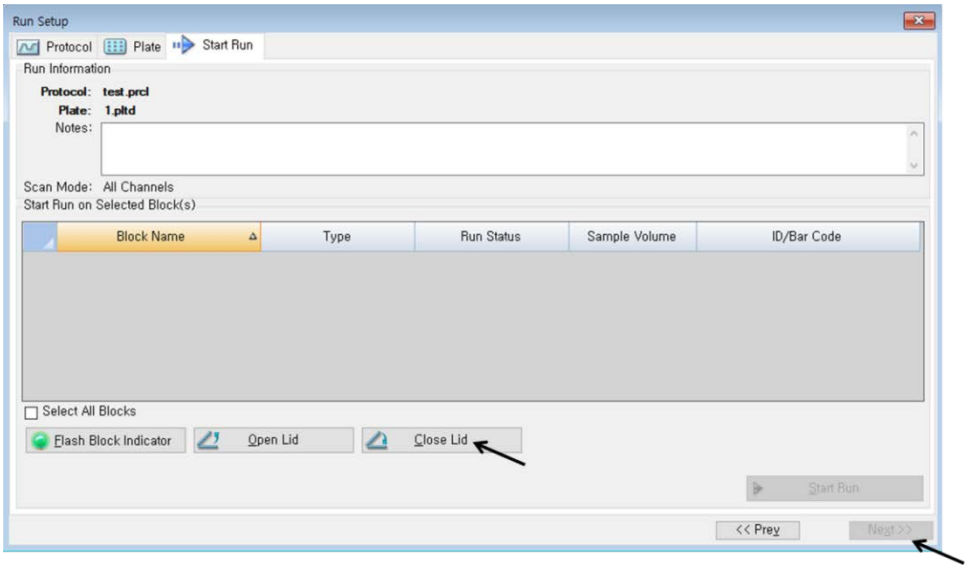


Fig 8. Close Lid and Start Run

- After the reaction is completed, verify the amplification curve. Please refer to the instruction manual for your real time PCR instrument to read about analytical methods and set threshold for each real-time PCR machine in its management program following the table below.

Instrument	Threshold
CFX96™	Auto threshold

- Store the run file in New folder. Fill in the file name, click the “SAVE”, and machine will start.

2) Applied Biosystems™ 7500 Real-time PCR Instrument system set up

- The set-up of the ABI 7500 Real-time PCR Instrument for the detection of COVID-19 and IC can be divided into the following steps: Experiment Properties, Plate Setup, and Run Method.

Experiment Properties

- In the Home screen, click “Advanced Setup” to open the setup stage.

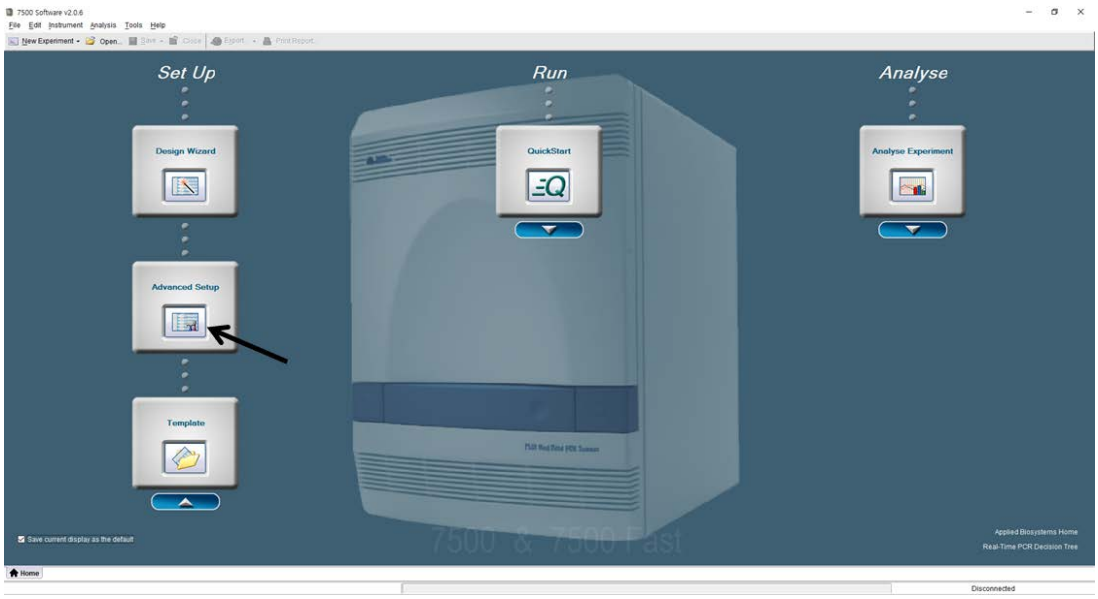


Fig 9. Advanced Setup

- Give the “Experiment Name” and click “Quantitation – Comparative Ct”

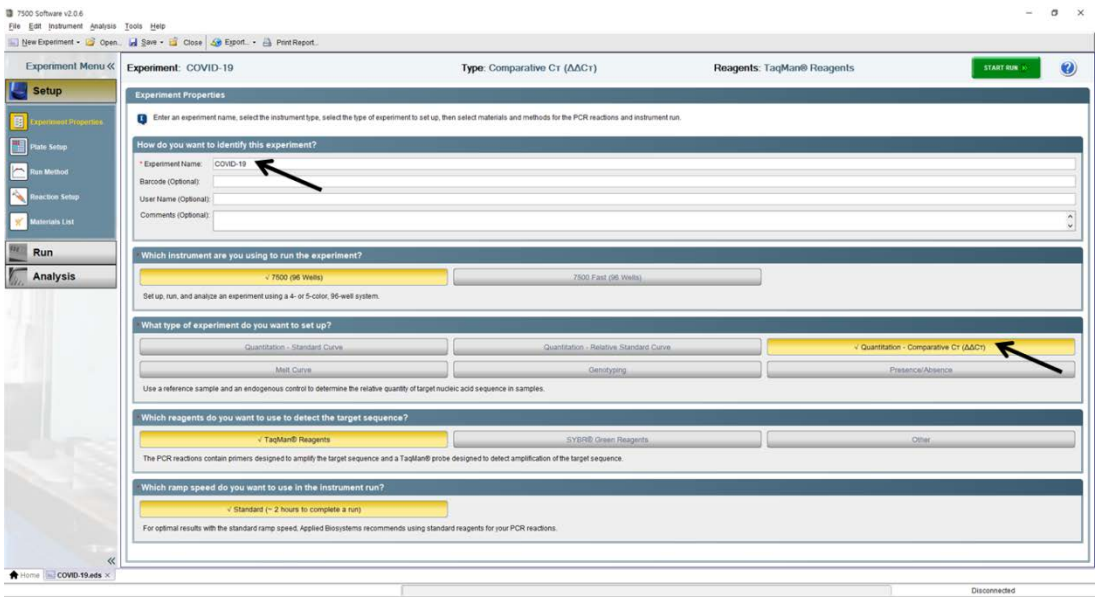


Fig 10. Experiment Properties

Plate Setup

- Add new target for RdRp gene, E gene and IC, then specify Reporter, Quencher and Color.

- Add New Sample by the number of samples containing the Positive Control and Negative Control, and then give the sample name.

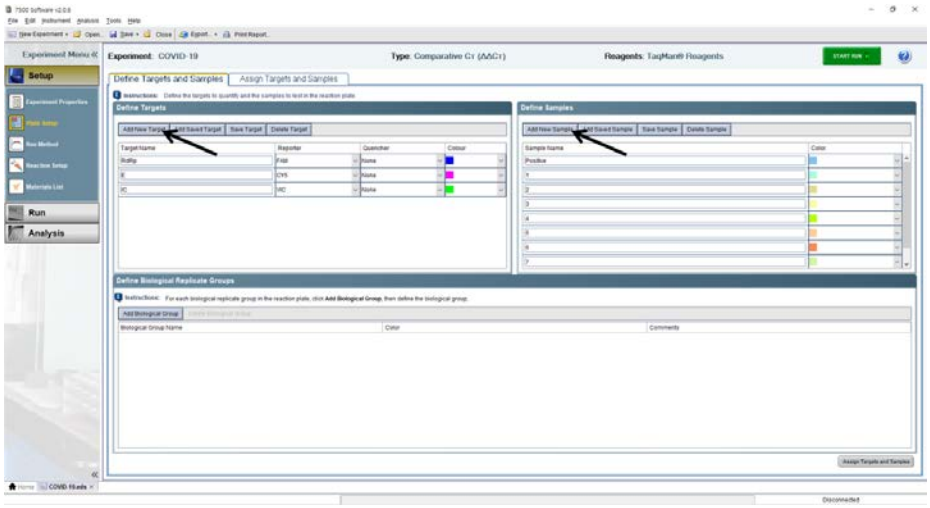


Fig 11. Set three targets and Sample name

- At the Assign Targets and Samples Tab, specify the sample location on the well and the assay target.

  Note: Assay 1 target :RdRp and IC, Assay 2 target : E and IC

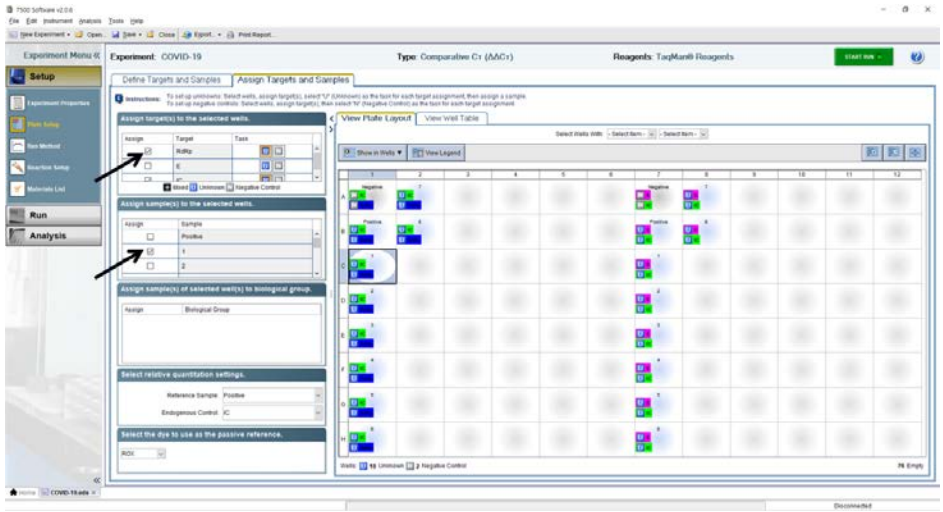


Fig 12. Set the sample location on the well and target

Run Method

- Add new target for RdRp gene, E gene and IC, then specify Reporter, Quencher and Color.
- In Graphical View, define the thermal profile as follows:

Segment	Temperature	Time	Cycles
1	50 ℃	30 min	1
2	95 ℃	15 min	1
3	95 ℃	15 sec	45
4*	60 ℃	1 min	

*collect the fluorescence data

- Click on Reaction Volume Per Well to directly edit the “20 uL”

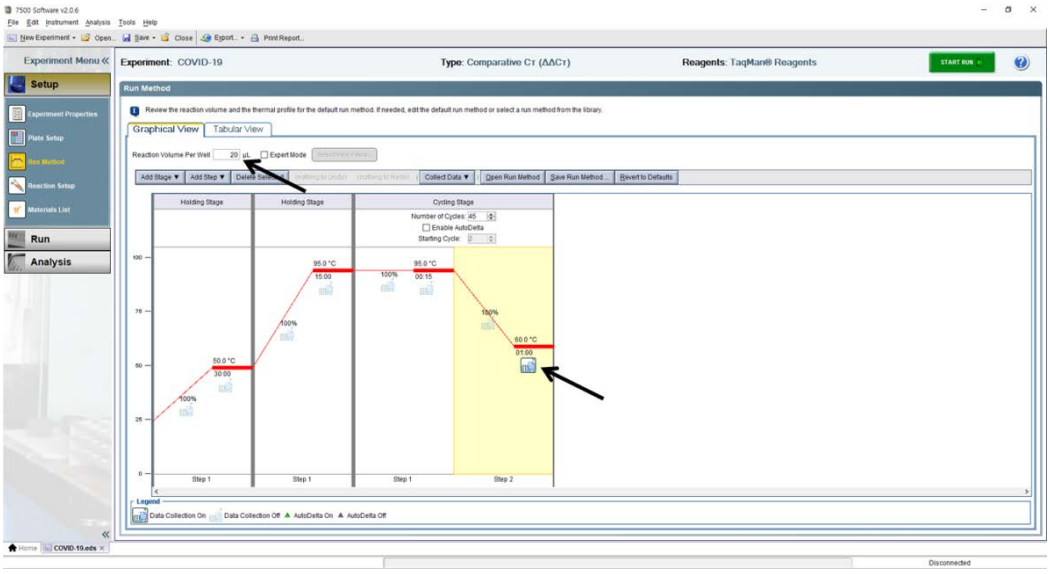


Fig 13. Protocol Design

Note: Plate Read On Last Segment.

- After the reaction is completed, verify the amplification curve. Please refer to the instruction manual for your real time PCR instrument to read about analytical methods and set threshold for each real-time PCR machine in its management program following the table below.

Instrument	Threshold
AB7500	FAM: 1, Cy5: 0.5
	HEX (VIC) : Auto threshold

- Save the Protocol and then Click “Start Run”
- From the next run, recall the stored protocol and modify sample location/ targets and proceed.

Interpretation of Results

The result analysis is confirmed using the Ct value measured for each target. It reads positive when Ct value ≤40 for each target among 45 cycles, and negative when Ct value> 40.

1. LabGun^{TM}COVID-19 RT-PCR Kit Controls – Positive, Negative, and Internal

The Ct value should be ≤40 for the positive control, and not detectable for the negative control. The Ct value of the internal control (MS2) should be ≤40. All test controls should be examined prior to interpretation of patient results (Table 4). If the controls are not valid, the patient results cannot be interpreted.

Table 4. Summary of the interpretation for control results

Control Type/Name	Used to monitor	RdRp gene (FAM)	E gene (Cy5)	MS2 phage (VIC/HEX)	Expected Ct values
Positive Control (PC)	Substantial reagent failure including primer and probe integrity	+	+	–	≤40
Negative Control (NC)	Reagent and/or environmental contamination	–	–	–	None detected
MS2 RNA Internal Control (IC)	Failure in lysis and extraction procedure	–	–	+	≤40

If the controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

2. Examination and Interpretation of Patient Specimen Results:

The interpretation of patient specimen results is determined by reading the Ct value of each sample and judging if the value is below or above 40. The summary of the interpretation is below (Table 5).

Table 5. Summary of the interpretation for patient specimen results

RdRp gene (FAM)	E gene (Cy5)	MS2 (VIC/HEX)	Result Interpretation	Action
+	+	±	SARS-CoV-2 detected	Report results to healthcare provider and appropriate public health authorities.
+	–*	±		
–	+	±	Inconclusive result	Repeat testing using residual nucleic acid first. If the repeated test result is inconclusive, re-extract nucleic acid from the remaining sample and repeat rRT-PCR. If the repeated result remains inconclusive, additional confirmatory testing should be conducted if clinically indicated.
–	–	+	SARS-CoV-2 not detected	Report results to healthcare provider.
–	–	–	Invalid result	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

***- Ct Not Detected**

Limitations

1. The use of this assay as an in vitro diagnostic under 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.
2. This kit is used for qualitative detection of SARS-CoV-2 RNA from human nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swab as well as nasopharyngeal wash/aspirate or nasal aspirate specimens and sputum specimens. The results do not reflect the viral load in the original specimens.
3. The performance of the LabGun™ COVID-19 RT-PCR Kit was established using contrived nasopharyngeal swab and sputum specimens. Anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with the LabGun™ COVID-19 RT-PCR Kit. 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.
4. The LabGun™ COVID-19 RT-PCR Kit performance has only been established with nasopharyngeal swabs and sputum specimens.
5. 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.

6. 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.
7. Amplification and detection of SARS-CoV-2 with the LabGun™ COVID-19 RT-PCR Kit has only been validated with the Applied Biosystems® 7500 Real-Time PCR instrument and the CFX96™ Real-time PCR detection system. Use of other instrument systems may cause inaccurate results.
8. The limit of detection (LoD) is determined based on a 95% confidence of detection. When SARS-CoV-2 presents at the LoD concentration in the test specimen, there will be a low probability that SARS-CoV-2 is not detected. When SARS-CoV-2 presents below the LoD concentration in the test specimen, there will also be a certain probability that SARS-CoV-2 can be detected.
9. Primers and probes for this kit target highly conserved regions within the genome of SARS- CoV-2. Mutations occurred in these highly conserved regions (although rare) may result in RNA being undetectable.
10. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
11. Laboratories are required to report all positive results to the appropriate public health authorities.

Conditions of Authorization for the Laboratory

The LabGun™ COVID-19 RT-PCR Kit's 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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd. However, to assist clinical laboratories using the LabGun™ COVID-19 RT-PCR Kit, the relevant Conditions of Authorization are listed below:

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

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

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

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

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-14-EUA-reporting@fda.hhs.gov) and You (via email: COVID-19.TechnicalSupport@labgenomics.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

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

G. You, authorized distributors, and authorized laboratories using your product will 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, to perform high complexity tests" as "authorized laboratories."

Performance Characteristics

Limit of Detection (LoD)

The analytical sensitivity (limit of detection or LoD) experiments were performed to determine the lowest concentration of SARS-CoV-2 detected at which approximately 95% of all (true positive) replicates tested positive.

To determine the LoD of the LabGun™ COVID-19 RT-PCR Kit, SARS-CoV-2 genomic RNA obtained from National Culture Collection for Pathogens of Korea (NCCP No.43326, Lot: 012669-T0013, Concentration: 10μg/μL) was spiked into negative clinical nasopharyngeal swab or sputum matrices, and pre-mixed with lysis buffer from the extraction kit (QIAamp Viral RNA Mini kit, QIAGEN) at six different concentrations. The viral genomic RNA extracted from each dilution was tested on the BioRadCFX96™ PCR instrument and the Applied Biosystems® 7500 Real-Time PCR instrument for SARS-CoV-2 (RdRP gene, Assay 1) and pan-Sarbecovirus (E gene, Assay 2).

The LoD was determined to be 20 genomic RNA copies/μL and subsequently confirmed by testing 20 replicates of the extracted RNA after spiking into each specimen type.

Table 6. LoD test from six different concentrations of SARS-CoV-2 RNA in nasopharyngeal swab on CFX96™ PCR instrument

Concentration (Genomic RNA copies/µL)	Assay 1 (RdRp gene)			Assay 2 (E gene)		
	Mean Ct	Standard Deviation	Positive rate	Mean Ct	Standard Deviation	Positive rate
$2x10^4$	28.0	0.39	3/3	29.4	0.1	3/3
$2x10^3$	30.5	0.01	3/3	31.8	0.15	3/3
$2x10^2$	33.7	0.26	3/3	35.5	0.34	3/3
$1x10^2$	35.1	0.29	3/3	36.1	0.23	3/3
$2x10^1$	37.3	0.83	3/3	39.0	1.38	3/3
$2x10^0$	NA	NA	0/3	41.4	NA	1/3

Table 7. Preliminary LoD test from six different concentrations of SARS-CoV-2 RNA in sputum on CFX96™ PCR instrument

Concentration (Genomic RNA copies/µL)	Assay 1 (RdRp gene)			Assay 2 (E gene)		
	Mean Ct	Standard Deviation	Positive rate	Mean Ct	Standard Deviation	Positive rate
$2x10^4$	28.1	0.18	3/3	29.2	0.27	3/3
$2x10^3$	31.0	0.02	3/3	31.8	0.12	3/3
$2x10^2$	34.4	0.55	3/3	35.3	0.14	3/3
$1x10^2$	35.4	0.11	3/3	36.5	0.63	3/3
$2x10^1$	37.6	0.38	3/3	40.0	NA	2/3
$2x10^0$	39.6	NA	2/3	43.3	NA	0/3

Table 8. Summary of LOD confirmation tests with clinical matrices on CFX96™ PCR instrument

Concentration (Genomic RNA copies/µL)	Specimen	Positive rate		Mean Ct			
		RdRp gene	E gene	RdRp gene	MS2	E gene	MS2
$2x10^1$	Nasopharyngeal swab	100% (20/20)	100% (20/20)	37.23	26.94	37.29	26.98
	Sputum	100% (20/20)	100% (20/20)	36.23	26.87	35.88	26.95

Table 9. Preliminary LoD test from six different concentrations of SARS-CoV-2 RNA In nasopharyngeal swab on AB7500 PCR instrument

Concentration (Genomic RNA copies/µL)	Assay 1 (RdRp gene)			Assay 2 (E gene)		
	Mean Ct	Standard Deviation	Positive rate	Mean Ct	Standard Deviation	Positive rate
$2x10^4$	28.8	0.15	3/3	29.0	0.08	3/3
$2x10^3$	31.4	0.11	3/3	31.3	0.04	3/3
$2x10^2$	34.6	0.16	3/3	34.6	0.16	3/3
$1x10^2$	35.5	0.43	3/3	35.9	0.84	3/3
$2x10^1$	38.5	0.55	3/3	39.1	NA	2/3
$2x10^0$	40.9	NA	1/3	40.6	NA	1/3

Table 10. Preliminary LoD test from six different concentrations of SARS-CoV-2 RNA in sputum on AB7500 PCR instrument

Concentration (Genomic RNA copies/µL)	Assay 1 (RdRp gene)			Assay 2 (E gene)		
	Mean Ct	Standard Deviation	Positive rate	Mean Ct	Standard Deviation	Positive rate
$2x10^4$	28.7	0.13	3/3	28.9	0.11	3/3
$2x10^3$	31.2	0.05	3/3	31.5	0.06	3/3
$2x10^2$	34.7	0.18	3/3	34.9	0.24	3/3
$1x10^2$	35.6	0.36	3/3	35.8	0.51	3/3
$2x10^1$	38.3	0.21	3/3	38.5	0.48	3/3
$2x10^0$	40.0	NA	2/3	40.3	NA	1/3

Table 11. Summary of LOD confirmation tests with clinical matrices on AB7500 PCR Instrument

Concentration (genomic RNA copies/µL)	Specimen	Positive rate		Mean Ct			
		RdRp gene	E gene	RdRp gene	MS2	E gene	MS2
2×10^1	Nasopharyngeal swab	100% (20/20)	100% (20/20)	37.24	28.80	37.98	28.57
	Sputum	100% (20/20)	100% (20/20)	37.07	28.73	37.79	28.54

Inclusivity

For specific detection of SARS-CoV-2, the available sequences published from Germany, China, and Hong Kong were first identified, and aligned. As a result of alignment, primers and probes used in the LabGun™COVID-19 RT-PCR Kit were designed for a region of the RdRp gene without mutations between strains.

In silico analysis showed that the primers/probe sequences of the LabGun™COVID-19 RT-PCR Kit detect all analyzed SARS-CoV-2 sequences in NCBI and in GISAID databases as of April 14, 2020. The primers/probe for SARS-CoV-2 (RdRp gene) of the LabGun™COVID-19 RT-PCR Kit showed 100% homology to all but one sequence of RdRp gene in 43 NCBI sequences and 659 GISAID sequences. A single base mismatch of one sequence in NCBI sequences was located in the probe sequence for the RdRp gene. This mismatch is not predicted to impact assay performance. The primers/probe for Sarbecovirus (E gene) of the LabGun™COVID-19 RT-PCR Kit showed 100% homology to all sequences of the E gene in 44 NCBI and 659 GISAID sequences.

Cross-Reactivity - Wet Testing

1. Cross-reactivity with other infectious strains

Wet testing against normal and pathogenic organisms of the respiratory tract was performed to assess the potential cross-reactivity of the assay's primers and probes. Each organism identified in the table below was tested in triplicate with the LabGunTM COVID-19 RT-PCR Kit at the concentrations indicated. Either RNA or DNA of each viral or bacterial organism was spiked into negative clinical nasopharyngeal swab matrix, extracted using QIAamp Viral RNA Mini kit, and tested on the CFX96 PCR instrument for SARS-CoV-2 (RdRP gene, Assay 1) and pan-Sarbecovirus (E gene, Assay 2). All results were negative (Table 12).

Table 12. Infectious strains tested for cross-reactivity and their concentrations

Viral strains	**Concentration (genome copies/ µL)**	**Bacterial strains**	**Concentration (genome copies/ µL)**
Enterovirus	1.5x 10^8	*Neisseria meningitidis*	2.56 x 10^5
Influenza A virus	4.7x 10^8	*Acinetobacter baumannii*	1.28 x 10^5
Influenza B virus	4.68x 10^8	*Bacillus subtilis*	7.32 x 10^4
Parainfluenza virus type 1	7.12x 10^7	*Campylobacter jejuni*	2.7 x 10^5
Parainfluenza virus type 2	1.19x 10^8	*Candida glabrata*	3.82 x 10^5
Parainfluenza virus type 3	7.1x 10^7	*Citrobacter freundii*	1.05 x 10^5
Parainfluenza virus type 4	6.38x 10^7	*Escherichia coli*	9.48 x 10^4
Adenovirus	3.16x 10^7	*Enterococcus faecium*	1.47 x 10^5
Respiratory syncytial virus A	7.24x 10^7	*Listeria monocytogenes*	1.78 x 10^5
Respiratory syncytial virus B	7.24x 10^7	*Pseudomonas aeruginosa*	7.74 x 10^4
Dengue virus 2	1x 10^8	*Shigella boydii*	1 x 10^5
Dengue virus 3	1 x 10^8	*Shigella sonnei*	1 x 10^5
Dengue virus 4	1 x 10^8	*Staphylococcus haemolyticus*	1.89 x 10^5
Dengue virus 1	9.9 x 10^7	*Streptococcus agalactiae*	2.26 x 10^5
		Haemophilus haemolyticus	2.68 x 10^5
		Salmonella typhi	1 x 10^5
		Neisseria cinerea	2.9 x 10^6
		Bacillus cereus	7.96 x 10^4

		Klebsiella pneumoniae	8.48 x 10^4
		Candida parapsilosis	1.83 x 10^5
		Enterobacter cloacae	9.92 x 10^4
		Klebsiella oxytoca	7.86 x 10^4
		Morganella morganii	1.23 x 10^5
		Shigella dysenteriae	1 x 10^5
		Shigella flexneri	1.07 x 10^5
		Staphylococcus epidermidis	1.87 x 10^5
		Streptococcus pyogenes	2.58 x 10^5
		Yersinia enterocolitica	9.92 x 10^4
		Haemophilus influenzae	2.68 x 10^5

2. Cross-Reactivity *In silico* analysis

The primers/probe of the LabGun™ COVID-19 RT-PCR Kit specific for SARS-CoV-2 and Sarbecovirus were analyzed in silico for cross-reactivity with other organisms using NCBI BLASTn version 2.10.0+ under the default option (Table 13). The database search parameters were as follows:

- The match and mismatch scores were 1 and -3, respectively.
- The penalty to create and extend a gap in an alignment was 5 and 2, respectively.
- The search parameters automatically adjusted for short input sequences and the expected threshold was 1000 (Table 13).

In summary, no organisms other than related SARS-coronaviruses, exhibited >70% homology to the forward primer, reverse primer, and probe for either the RdRp or E target. The results of the in silico analysis suggest the LabGun™ COVID-19 RT-PCR Kit is designed for the specific detection of SARS-CoV-2, with no expected cross reactivity to the human genome, other coronaviruses, or human microflora that would predict potential false positive RT-PCR results.

Table 13. Organisms tested for cross-reactivity by in silico analysis

Strain	% Identity to SARS-CoV-2 (RdRp gene)	% Identity to Sarbecovirus (E gene)
CoV 229E	70.0	Not reactive
SARS-CoV	79.2	98.7
CoV HKU1	Not reactive	Not reactive
CoV NL63	Not reactive	Not reactive
CoV OC43	Not reactive	Not reactive
MERS	Not reactive	Not reactive
AdV	Not reactive	Not reactive
HMPV	Not reactive	Not reactive
HPIV1	Not reactive	Not reactive
HPIV2	Not reactive	Not reactive
HPIV3	Not reactive	Not reactive
HPIV4	Not reactive	Not reactive
Flu A	Not reactive	Not reactive
Flu B	Not reactive	Not reactive
EV	Not reactive	Not reactive
HRSV	Not reactive	Not reactive
HRV	Not reactive	Not reactive
Influenza C	Not reactive	Not reactive
Parechovirus	Not reactive	Not reactive
Chlamydia pneumoniae	Not reactive	Not reactive
Legionella pneumophila	Not reactive	Not reactive
Mycobacterium tuberculosis	Not reactive	Not reactive
Streptococcus pneumoniae	Not reactive	Not reactive
Bordetella pertussis	Not reactive	Not reactive
Mycoplasma pneumoniae	Not reactive	Not reactive
Pneumocystis jirovecii	Not reactive	Not reactive
Candida albicans	Not reactive	Not reactive
Corynebacterium diphtheriae	Not reactive	Not reactive
Legionell non-pneumophila	Not reactive	Not reactive
Bacillus anthracis	Not reactive	Not reactive
Moraxella catarrhalis	Not reactive	Not reactive
Neisseria elongata subsp. glycolytica ATCC 29315	Not reactive	Not reactive
Neisseria meningitidis	Not reactive	Not reactive
Pseudomonas aeruginosa	Not reactive	Not reactive
Streptococcus salivarius	Not reactive	Not reactive
Leptospiraalstonii	Not reactive	Not reactive
Chlamydia psittaci	Not reactive	Not reactive
Coxiellaburneti	Not reactive	Not reactive
Staphylococcus aureus	Not reactive	Not reactive

Clinical Study

The clinical performance of the LabGun™COVID-19 RT-PCR Kit was evaluated using SARS-CoV-2 genomic RNA spiked into individual negative, nasopharyngeal/oropharyngeal swab and sputum matrices. For each respective specimen, 100 negative samples and 50 contrived positive samples were tested. Samples were contrived by spiking known concentrations of SARS-CoV-2 viral genomic RNA, which was obtained from National Culture Collection for Pathogens of Korea (NCCP No.43326, Lot:012669-T0013, Concentration: 10 μg/μL), relative to the product LoD, into each specimen matrix that were determined to be negative by the LabGun™COVID-19 RT-PCR Kit before spiking in the genomic RNA. The spiking concentrations of SARS-CoV-2 viral genomic RNA into each respective specimen matrix were low positive (1x LOD and 2x LOD) and moderate positive (5x LOD) concentrations. The prepared samples were randomized and single-blinded and RNA was extracted using QIAamp Viral RNA Mini kit (QIAGEN). Testing was performed on the BioRad CFX96 PCR instrument in triplicate RT-PCR runs with one positive and one negative control included per run.

Results for the tests are shown in the tables below:

Table 14. Clinical evaluation with nasopharyngeal/oropharyngeal swab samples

	RdRp gene			E gene		
	% positive	**Mean Ct**	**% CV**	**% positive**	**Mean Ct**	**% CV**
unspiked	0/100	NA	NA	0/100	NA	NA
1x LOD	100% (20/20)	38.64	1.73%	100% (20/20)	38.63	1.87%
2x LOD	100% (20/20)	37.39	1.47%	100% (20/20)	37.89	1.81%
5x LOD	100% (10/10)	36.35	0.85%	100% (10/10)	36.79	1.34%

Table 15. Clinical evaluation with sputum samples

	RdRp gene			E gene		
	% positive	**Mean Ct**	**% CV**	**% positive**	**Mean Ct**	**% CV**
unspiked	0 /100	NA	NA	0/100	NA	NA
1x LOD	100% (20/20)	38.84	1.60%	100% (20/20)	38.63	1.80%
2x LOD	100% (20/20)	37.29	1.20%	100% (20/20)	38.00	1.90%
5x LOD	100% (10/10)	36.28	0.85%	100% (10/10)	36.73	1.29%

Table 16.LabGun™COVID-19 RT-PCR Kit performance relative to the expected results of the contrived samples with the respective nasopharyngeal/oropharyngeal swab and sputum specimen

		Contrived samples expected results	
		positive	**negative**
LabGun™ COVID-19 RT-PCR Kit	**Positive**	50	0
	negative	0	100

Positive Percent Agreement: 100% (95% CI, 92.89% - 100%)
Negative Percent Agreement: 100% (95% CI, 96.38% – 100%)

Reference Panel Testing

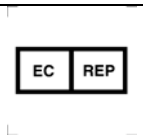
The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method used was manual spin protocol by QIAamp Viral RNA Mini Kit (QIAGEN, Cat No. 52904). The instrument used was CFX96^{TM} with CFX manager version 3.1 (Bio-Rad). The results are summarized in Table 17.

Table 17. 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 swab	1.8 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

Symbols	**Explanation**

	Batch code Reference No[2492]
	Catalogue number Reference No[2493]
	Use by Reference no [2607]
	Temperature limitation Reference No [0533]
	Caution Reference no [0434A]
	Operator's Manual; Operating instructions Reference No [1641]
	Manufacturer Reference No [3082]
	Date of manufacture Reference no [2497]
	Authorized representative in the European Community
	In vitro diagnostic medical device
	Conformite Europeenne Mark

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