Allplex™ 2019-nCoV Assay (version 2.3; December 13th, 2022)

(Cat no. RP10250X / RP10252W)

Instructions for Use

For *in vitro* diagnostic use

For use under Emergency Use Authorization (EUA) Only

Prescription Use only

Seegene

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■ CHAPTER 1: Intended Use

The Allplex™ 2019-nCoV Assay is an *in vitro* diagnostic (IVD) real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of SARS-CoV-2 viral nucleic acids in human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, bronchoalveolar lavage (BAL) and sputum specimens from individuals who are suspected of COVID-19 by their health care 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 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 the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.
Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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

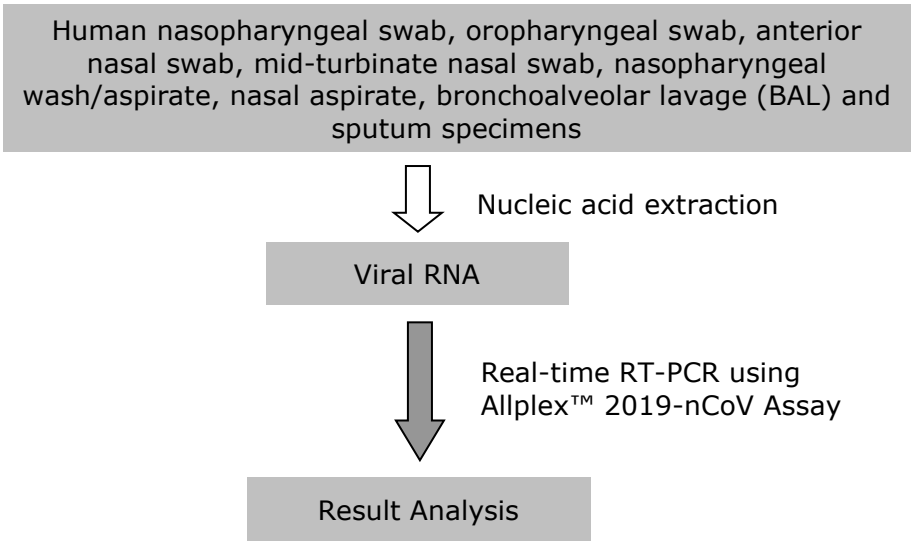
The Allplex™ 2019-nCoV Assay is intended for use by qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures. The Allplex™ 2019-nCoV Assay is only for use under the Food and Drug Administration's Emergency Use Authorization (EUA).

CHAPTER 2: Summary and Explanation of the Test

The technology of the Allplex™ 2019-nCoV Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The 2019-nCoV primer and probe set(s) is designed to detect viral RNA of SARS-CoV-2 in non-pooled human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, bronchoalveolar lavage (BAL) and sputum specimens.

CHAPTER 3: Principle of the Procedure

Nucleic acids are isolated and purified from specimen using a manual or an automated nucleic acid extraction system. 10 µL of Internal Control (RP-V IC) must be added before the extraction. Follow detailed extraction procedures in manufacturer's instructions. 8 µL of purified nucleic acid is reverse transcribed using 5X Real-time One-step Buffer/Real-time One-step Enzyme into cDNA which is then subsequently amplified in a CFX96™, CFX96™ Dx, CFX96 Touch™, CFX96 Opus 96 Dx, Applied Biosystems™ 7500, or Applied Biosystems™ 7500 Fast Dx real-time PCR systems. During 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 CFX96™, CFX96™ Dx, CFX96 Touch™, CFX96 Opus 96 Dx, Applied Biosystems™ 7500 or Applied Biosystems™ 7500 Fast Dx real-time PCR detection systems. The result of amplification is reported through 'Seegene viewer' analysis. The 'Seegene viewer' shows whether the exported data is 2019-nCoV Detected, Presumptive positive, or Negative for easy retrieval of result by the user.



■ CHAPTER 4: Assay Materials

Materials provided

The reagents contained in one Allplex™ 2019-nCoV Assay kit are sufficient for 100/124 reactions.

Table 1. Allplex™ 2019-nCoV Assay Composition

Contents	Volume (RP10250X/ RP10252W)	Description
2019-nCoV MOM	500 µL / 620 µL	MuDT* Oligo Mix (MOM): - Amplification and detection reagent *MuDT is the brand name of Seegene's oligo mixture
Real-time One-step Enzyme	200 µL	Enzyme mix for one-step RT-PCR
5X Real-time One-step Buffer	500 µL	Buffer for one-step RT-PCR - Buffer containing dNTPs
2019-nCoV PC	80 µL	Positive Control (PC) for PCR control: - Mixture of pathogen and IC clones
RP-V IC	1,000 µL	Exogenous Internal Control (IC) of Allplex™ 2019-nCoV Assay
RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade RNase-free Water provided for: 1. Negative Control (NC) for PCR control 2. RT-PCR Mastermix (Refer to Table 6)

Materials required but not provided

Additional materials and equipment required

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Clean bench
- Ice
- Desktop centrifuge
  (1.5 mL microcentrifuge and 96 well plate centrifuge)
- Vortex mixer
- Instruments and kits for nucleic acid extraction

Manufacturer	Instrument	Extraction Kit	Catalog No./Reaction No.
Seegene	Seegene STARlet (67930-03)	STARMag 96 X 4 Universal Cartridge Kit	384 reactions (744300.4.UC384)
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384 reactions (EX00013C)
Hamilton	Microlab STARlet IVD (173000-075)	STARMag 96 X 4 Universal Cartridge Kit	384 reactions (744300.4.UC384)
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384 reactions (EX00013C)
Seegene	Seegene NIMBUS (65415-03)	STARMag 96 X 4 Universal Cartridge Kit	384 reactions (744300.4.UC384)
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384 reactions (EX00013C)
Hamilton	Microlab NIMBUS IVD (65415-02)	STARMag 96 X 4 Universal Cartridge Kit	384 reactions (744300.4.UC384)
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384 reactions (EX00013C)
LG Chem	AdvanSure E3 System (YETS0001EG)	AdvanSure NA EX Kit	96 reactions Reagent Plate (RPE0001K01)
			Proteinase K Tube (RPK0001K01)
			Strip (E3 System) (YSTP0500KG)
GeneAll	N/A (Manual)	Ribospin vRD (Viral RNA/DNA Extraction Kit)	50 extractions (302-150 SG1701)
QIAGEN	N/A (Manual)	QIAamp® DSP Viral RNA Mini Kit	50 extractions (61904)
Roche	Roche MagNA Pure 96 (MP96)	DNA and Viral NA Small Volume Kit	576 extractions (06 543 588 001)
ThermoFisher Scientific	KingFisher Flex automated extraction	MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	200 extractions (A42352) 2,000 extractions (A48310)

NOTE:
(1) All extraction options are commercially available.
(2) The Seegene, Hamilton, LG Chem, and GeneAll extraction reagents/instrumentation can be purchased through Seegene Technologies (CA, US), support@seegenetech.com
(3) The Seegene and Hamilton extraction reagents/instrumentation are validated with Seegene Launcher V6 software.

- PCR Instrument & Consumables
  - ⊖ Applied Biosystems™ 7500 (ThermoFisher Scientific), Applied Biosystems™ 7500 Fast Dx (ThermoFisher Scientific)

<table>
<tr><td colspan="2">Consumables (Cat. No.)</td></tr>
<tr><td colspan="2">For Applied Biosystems™ 7500;
• MicroAmp™ Optical 96-Well Reaction Plate (Cat. No. N8010560, ThermoFisher Scientific)
• MicroAmp™ Optical 96-Well Reaction Plate with Barcode (Cat. No. 4306737, ThermoFisher Scientific)
For Applied Biosystems™ 7500 Fast Dx.
• MicroAmp™ Fast 96-Well Reaction Plate (0.1mL) (Cat. No. 4346907, ThermoFisher Scientific)
• MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL (Cat. No. 4346906, ThermoFisher Scientific)
For both Applied Biosystems™ 7500/7500 Fast Dx;
• Optical Adhesive Covers (Cat No. 4360954, ThermoFisher Scientific)</td></tr>
<tr><td colspan="2">Software</td></tr>
<tr><td>Applied Biosystems™ 7500</td><td>Applied Biosystems™ 7500 Fast Dx</td></tr>
<tr><td>SDS software v2.0.5</td><td>SDS software v1.4.1 (Windows XP) / 1.5.1 (Windows 7 64-bit)</td></tr>
</table>

⊖ CFX96 ™ Real-time PCR Detection System-IVD (Bio-Rad),
CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
CFX96™ Dx System (Bio-Rad),
CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad),
CFX Opus 96 Dx Real-Time PCR Detection System (Bio-Rad)

Consumables (Cat. No.)
• Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Cat. No. HSP9655, Bio-Rad) • Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Cat. No. HSP9955, Bio-Rad) • Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad) • Low Tube Strip, WHT (Cat. No. TLS0851, Bio-Rad) • Optically Clear Heat Seal (Cat. No. 1814030, Bio-Rad) • Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)*** • PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)*** • MicroAmp™ Optical 8-Cap Strip (Cat. No. 4323032, ThermoFisher Scientific) • MicroAmp™ Optical 8-Tube Strip (0.2 mL) (Cat. No. 4316567, ThermoFisher Scientific) • EU 8-Single Attachable Indented Cap (Cat. No. B79501, BIOplastics) • EU 0.2 ml Thin-wall 8-Tube Strip (Cat. No. B77009, BIOplastics) • Mini-centrifuge (Cat. No. Mini-6K, Protagen) • PCR plate centrifuge (Cat. No. MPC-P25, Powerlab) • 0.1ml 96-Well PCR plate Half Skirt (Cat. YPP-0.1-HSW, YongYue) • Sealing film (Cat. YPF-QPC, YongYue) *** The Permanent Clear Heat Seal must be used with the PX1 PCR Plate Sealer when running the Allplex™ assay
Software
CFX Manager™ Software V3.1 or CFX Maestro™ Software V1 CFX Manager™ Software V3.1 for use with CFX96-IVD and CFX96™ Dx, CFX Maestro™ Software V1 for use with CFX96 Touch, or CFX Maestro™ V2.0 Software for use with the CFX Opus 96 Dx

NOTE: All consumables for CFX96™ Real-time PCR Detection System-IVD, CFX96™ Dx System, CFX96 Touch™ Real-Time PCR Detection System, and CFX Opus Dx Real-time PCR Detection System can be purchased through Seegene Technologies (California, US).

CHAPTER 5: Warnings and Precautions

The Allplex™ 2019-nCoV Assay should be performed by qualified, trained personnel.

- For *in vitro* diagnostic use.
- For use under Emergency Use Authorization (EUA) Only
- For Prescription Use Only
- 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.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This 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. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- This test has not been validated for any other types of specimens other than those indicated in the intended use.
- If not tested immediately, store extracted RNA at ≤ -70℃ until use and keep on ice during testing.
- Workflow in the laboratory should proceed in a unidirectional manner.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink, or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. Use of sterilized aerosol resistant disposable pipette tips is recommended.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use the product after its expiry date.
- Do not reuse any disposable items.

- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.
- Avoid possible contamination of reagents with extracted nucleic acids, PCR products, and positive control. To prevent contamination of reagents, use of filter-tips is recommended.
- Use separated and segregated working areas for each test run.
- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only in designated working areas after amplification.
- Store positive materials separated from the kit's reagents.
- Handle all specimens as if infectious. Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components (product residuals, packaging) can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Manipulation of potentially infected specimens should be performed in a certified Class II BSC in a BSL-2 facility or higher. This includes aliquoting and/or diluting specimens and nucleic acid extraction procedures involving potentially infected specimens.
- Use appropriate personal protective equipment including but not limited to disposable gloves, laboratory coat/gown, and eye protection when handling specimens, reagents, pipettes, and other equipment.
- Keep extracted RNA on cold block or on ice during reaction set-up.
- Keep PCR reagents on cold block or on ice during reaction set-up.
- Expiry date is 8 months from the date of manufacture when product is stored at ≤ -20℃. Please refer to label for expiry date.
- Seegene STARlet and Seegene NIMBUS are private label devices and are the same as the Microlab STARlet IVD and Microlab NIMBUS IVD. There is no change in the device other than labeling. All four devices can be used interchangeably and generate equivalent results. Instruments indicated share the same software application ("Seegene Launcher") and extraction kit ("STARMag 96 X 4 Universal Cartridge Kit" and "STARMag 96 X 4 Viral DNA/RNA 200 C Kit").
- This Allplex™ 2019-nCoV Assay is a qualitative *in vitro* test for the single or multiple detection of 3 target genes (E gene, RdRP gene, and N gene)
- The brand name of "CFX96™ Real-time PCR Detection System" is changed to "CFX96™ Dx system". Since there are no hardware changes to the systems, it is expected that performance of the Allplex™ 2019-nCoV Assay will be the same with both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software v1.6". The upgraded software includes

enhancements to the “Run” menu. These enhancements do not impact the results of data analysis; therefore, results will be the same.

CHAPTER 6: Storage and Handling Conditions

Reagent storage and handling

- Expiration date is 8 months from the date of manufacture when product is stored at -20 °C or below.
- Completely thaw all reagents on ice prior to use
- Do not store reagents in a frost-free freezer.
- Do not use kits or reagents beyond indicated expiry date.
- Always check the expiry date on the reagent tubes prior to use.

NOTE: The performance of kit components is unaffected for up to 7 cycles of freeze and thaw. If the reagents are used only intermittently, they should be stored in aliquots.

Specimen storage and transport

- Specimen types: human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, bronchoalveolar lavage (BAL) and sputum specimens

NOTE: Sample collection devices are not provided with the assay. All testing for COVID-19 should be conducted in consultation with a healthcare provider. Refer to CDC guidelines for sample collection (Nasopharyngeal swab (NP) /oropharyngeal swab (OP) / anterior nasal swab / mid-turbinate nasal swab and sputum) and storage at:
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Once the swabs have been collected in accordance with CDC guidelines, it is recommended to use Universal Transport Medium (UTM) for collection of nasopharyngeal, oropharyngeal anterior nasal and mid-turbinate nasal swabs.

- After collection, the specimen should be stored at 2-8°C and processed within 72 hours.
- If delivery and processing exceed 72 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder.

NOTE:
(1) Performance may be affected by prolonged storage of specimens.
(2) Specimens transport should adhere to local and national instructions for transport of pathogenic material.
(3) Specimens should be collected and handled according to the swab collection device manufacturer's recommended procedures.

CHAPTER 7: Assay Control Material(s)

PCR controls

The PCR controls below are provided with the Allplex™ 2019-nCoV Assay to confirm the validity of each PCR run on the same plate.

Prior to determining the validity of each PCR run, the user must confirm the results of the negative control and positive control on the 'Well Plate' on the upper left corner of the Seegene viewer.

The results of the negative control and positive control are displayed under the 'Auto Interpretation' column on the bottom half of the Seegene viewer. If the positive and/or negative control results are invalid, the corresponding PCR run must be repeated.

1. **Negative Control (NC)** is used as a PCR control to confirm test validity, and the absence of any contaminants during testing. The "No template" control is prepared using RNase-free Water added to the Master Mix prior to PCR. NC must be included in each test run. No signal should be detected with the NC.

2. **Positive Control (PC)** is used to confirm test validity, and functions as the validation control for PCR amplification and the test detection steps. The PC is constructed using plasmids encoding Allplex™ 2019-nCoV Assay target sequences and must be included in each test run.

NOTE: The Positive Control included in this kit is a high concentration PCR control. Dilute the PC with TE buffer by 1:10 before use.

The real-time PCR results of the positive and negative control can be viewed from the Seegene Viewer as shown in Picture 1 and Picture 2.

Picture 1. Example of valid positive/negative control results

Well	Name	Type	FAM		Cal Red 610		Quasar 670		HEX		Auto Interpretation	Comment
			E gene	C(t)	RdRP ...	C(t)	N gene	C(t)	IC	C(t)		
B11		NC	-	N/A	-	N/A	-	N/A	-	N/A	Negative Control(-)	
B12		PC	+	20,64	+	20,97	+	19,09	+	19,96	Positive Control(+)	

Picture 2. Example of invalid positive/negative control results

Well	Name	Type	FAM		Cal Red 610		Quasar 670		HEX		Auto Interpretation	Comment
			E gene	C(t)	RdRP ...	C(t)	N gene	C(t)	IC	C(t)		
F01		PC	+	38,99	+	38,77	+	39,05	-	N/A	Positive Control(Invalid)	
F02		NC	-	N/A	+	37,23	+	36,85	-	N/A	Negative Control(Invalid)	

Table 2. Allplex™ 2019-nCoV Assay; Control Acceptance Criteria

Control	Seegene Viewer Result (Ct value)				
	IC (HEX)	E gene (FAM)	RdRP gene (CalRed 610)	N gene (Quasar 670)	Auto Interpretation
2019-nCoV Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control (+)
	>40 or N/A	>40 or N/A	>40 or N/A	>40 or N/A	Positive Control (Invalid)
Negative Control	>40 or N/A	>40 or N/A	>40 or N/A	>40 or N/A	Negative Control (-)
	≤ 40	≤ 40	≤ 40	≤ 40	Negative Control (Invalid)

Internal Control

The Allplex™ 2019-nCoV Assay includes a full process Internal Control (RP-V IC) which is composed of MS2 phage genome. This Internal Control material verifies all steps of the analysis process, including sample extraction, reverse transcription, and PCR to demonstrate proper specimen processing and test validity of each specimen.

A positive signal for the Internal Control indicates that all processing steps performed by the Allplex™ 2019-nCoV Assay were successful.

A negative signal of all targets including the Internal Control invalidates all negative results in the analysis. Repeat testing if an invalid result is reported. Refer to section 'Interpretation of Results' for more details.

External Control

External controls are not provided with the Allplex™ 2019-nCoV Assay. Quality control requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

The following external controls are available:
AccuPlex™ SARS-COV-2 reference material (Seracare Life Sciences, Inc., Cat no. 0505-0126; this kit includes positive & negative reference material.)
The positive reference material may be used as an external extraction control.

■ CHAPTER 8: Procedure

Sample collection, transport, and storage

Collect Nasopharyngeal swab (NP) /oropharyngeal swab (OP)/nasal swab/mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, bronchoalveolar lavage (BAL) and sputum according to CDC guidelines and/or manufacturer's protocol for sample collection, storage and handling.

Nucleic acid extraction

The assay was validated with the extraction options listed below. Perform the RNA extraction on samples according to the manufacturer's instructions for use. For the Microlab STARlet IVD, Seegene STARlet, Microlab NIMBUS IVD and Seegene NIMBUS extraction methods, follow the detailed instructions provided in the section of 'Preparation on Microlab STARlet IVD, Seegene STARlet, Microlab NIMBUS IVD and Seegene NIMBUS'.

Seegene STARlet / Seegene NIMBUS / Microlab STARlet IVD / Microlab NIMBUS IVD (STARMag 96 X 4 Universal Cartridge Kit; Cat No. 744300.4.UC384) See Operation Manual of each instrument or the section under 'preparation' for details. - Sample volume: 300 µL, Elution volume: 100 µL
Seegene STARlet / Seegene NIMBUS / Microlab STARlet IVD / Microlab NIMBUS IVD (STARMag 96 X 4 Viral DNA/RNA 200 C Kit; Cat No.EX00013C) See Operation Manual of each instrument or the section under 'preparation' for details. - Sample volume: 300 µL, Elution volume: 100 µL
AdvanSure E3 System **(AdvanSure NA EX Kit; Cat No.RPE0001K01,RPK0001K01)** See AdvanSure NA EX Kit User Manual for details. - Sample volume: 200 µL, Elution volume: 100 µL
QIAamp® DSP Virus Spin Kit **(Cat No.61704) (QIAGEN)** Follow the 'Protocol Purification of viral nucleic acids from plasma or serum' of the QIAamp® DSP Virus Spin Kit Handbook. - Specimen volume: 190 µL, Elution volume: 40 µL
Ribospin™ vRD **(Viral RNA/DNA Extraction Kit (Cat No.302-150, SG1701) (GeneAll)** See Ribospin™ vRD Manual for details. - Specimen volume: 290 µL, Elution volume: 40 µL
KingFisher™ Flex automated extraction **(MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, Cat No. A42352)** See MagMAX Viral/Pathogen Nucleic Acid Isolation Kit Manual for details. - Specimen volume: 200 µL, Elution volume: 50 µL
Roche MagNA Pure 96 (MP96) **(DNA and Viral NA Small Volume Kit; Cat No.06 543 588 001)** See DNA and Viral NA Small Volume Kit Manual for details. - Specimen volume: 200 µL, Elution volume: 50 µL

Preparation on Microlab STARlet IVD, Seegene STARlet, Microlab NIMBUS IVD and Seegene NIMBUS:

Hardware installation, Seegene Launcher software for operation and customer training (on-site and/or video tutorial) are provided by Seegene Technologies (California, US), support@seegenetech.com.
The Seegene Launcher is an application software that controls functions and protocols of the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS.
The user manual of 'Seegene Launcher V6' containing detailed descriptions of instrument maintenance and experimental procedures of nucleic acid extraction using Microlab STARlet IVD, Seegene STARlet, Microlab NIMBUS IVD and Seegene NIMBUS will be provided.

The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples and comprises of 4 steps: sample lysis, nucleic acids binding to magnetic beads, debris washing and elution of purified nucleic acids.

The instructions below describe the procedures for operating the Microlab STARlet IVD and Seegene STARlet. For Microlab NIMBUS IVD and Seegene NIMBUS, the same Seegene Launcher software is used. Please follow exactly the same procedure as below after selecting NIMBUS in the setting during installation of the launcher.

For STARMag 96 X 4 Universal Cartridge Kit:

1. Take out 1 cartridge from the STARMag 96 X 4 Universal Cartridge Kit. 1 cartridge contains reagents for 96 tests, and the STARMag 96 X 4 Universal Cartridge Kit contains 4 cartridges (384 tests).

Picture 3. 1 cartridge from the STARMag 96 X 4 Universal Cartridge Kit

Table 3. Components of STARMag 96 X 4 Universal Cartridge Kit

Reagents	Volume
Lysis Buffer Universal LB	4 X 23 mL

Reagents	Volume
Binding Buffer Universal BB	4 X 68 mL
Wash Buffer 1 Universal WB1	4 X 55 mL
Wash Buffer 2 Universal WB2	4 X 10 mL
Wash Buffer 3 Universal WB3	4 X 55 mL
Elution Buffer Universal EB	4 X 18 mL
Universal Magnetic Beads	4 X 1.8 mL
Lysis Buffer Universal LB	200 mL
Universal Proteinase K (lyophilized)	4 X 75 mg
Proteinase Buffer Universal PB	4 X 3 mL
Tub Cover	25 ea.
User Manual	2 ea.

NOTE:

(1) Lysis Buffer (LB), Binding Buffer (BB), and Wash Buffer 1 (WB1) contain chaotropic salt. Wear gloves and goggles always when handling buffers.
(2) Store all the components of extraction reagent kit at room temperature (18 – 25 ℃). In case of dissolved Proteinase K, store at -20 ℃.
(3) The expiration date of the product is indicated on the label. The cartridge remains effective for up to 15 months prior to its opening and for up to 4 months after its opening.
(4) All buffers are delivered ready-to-use.
(5) Lysis Buffer (LB) may form a salt precipitate during storage. To re-dissolve the precipitate, incubate the buffer bottle at 40 ℃ until the precipitate is re-dissolved completely.

2. Before placing the cartridge on the Microlab STARlet IVD, Seegene STARlet, Microlab NIMBUS IVD or Seegene NIMBUS, prepare the following:

- Proteinase K: When using the kit for the first time, add 2.6 mL Proteinase Buffer Universal PB to the lyophilized Proteinase K. Dissolved Proteinase K solution is stable at – 20 °C for at least 6 months. Transfer the Proteinase K solution into a 1.5 mL microtube according to the number of samples. The volume of Proteinase K solution is automatically calculated by the Launcher software if the number of samples is entered into the software.
- Wash Buffer 2 Universal WB2: Prepare 48 mL of absolute ethanol (Cat. No. 1.00983.1011, Merck). After removing the film from the WB2 tub, add 48 mL of absolute ethanol into the WB2 tub. The WB2 tub should be covered after use and should be stored at room temperature (18 – 25 °C).

- Magnetic Bead: Suspend the magnetic beads by manually tapping the tube followed by a quick vortex.

For STARMag 96 X 4 viral DNA/RNA 200 C Kit;

1. Take out 1 cartridge from the STARMag 96 X 4 viral DNA/RNA 200 C Kit. 1 cartridge contains reagents for 96 tests, and the STARMag 96 X 4 viral DNA/RNA 200 C Kit contains 4 cartridges (384 tests).

Picture 4. 1 cartridge from the STARMag 96 X 4 viral DNA/RNA 200 C Kit

Table 4. Components of STARMag 96 X 4 viral DNA/RNA 200 C Kit

Reagents	Volume
Lysis Buffer LB	4 X 23 mL
Binding Buffer BB	4 X 68 mL
Wash Buffer 1 WB1	4 X 55 mL
Wash Buffer 2 WB2	4 X 10 mL
Wash Buffer 3 WB3	4 X 55 mL
Elution Buffer EB	4 X 18 mL
Magnetic Beads	8 mL
Bead Tube (2 mL tube)	4 ea.
Tub Cover	25 ea.
User Manual	1 ea.

NOTE:

(1) Store all the components of the extraction reagent kit at room temperature (18 – 25 ℃).
(2) The expiration date of STARMag 96 X 4 Viral DNA/RNA 200 C Kit is indicated on the box label and store up to 1 month after its opening.

(3) All buffers are delivered ready-to-use.

2. Before placing the cartridge on the Microlab STARlet IVD or Seegene STARlet, prepare the following:

- Add 48 mL of absolute ethanol into WB2 tub before use. WB2 tub should be covered with Tub Cover after using and stored at room temperature (18 - 25 °C).
- After sufficiently vortexing the Magnetic beads in the bottle, transfer 1.8 ml of Magnetic beads to bead tube (2 mL tube) before use.

Table 5. Materials required, but not provided

Basic Item
Absolute EtOH
Disposable powder free gloves (latex or nitrile)
Desktop centrifuge
Ice or cooler box
Pipettes (adjustable) and sterile aerosol resistant pipette tips
Vortex mixer

Purchasing Item	Cat. No.	Manufacturer
SMP-CAR-24-Tube Carrier Set-4 (24 sample carrier)	173440	Hamilton
SMP CAR 12 D35 (12 sample carrier)	185052	Hamilton
1.5 mL sterile microtubes	MCT-150-C	Axygen
96 Deep Well Micro Plate	SDP0096	Supercon
Deep well plate, 96 wells with Barcode label	SDP0096B	Supercon
MicroAmp® Optical 8-Tube Strip (0.2 mL)	4316567	Applied Biosystems
EU 0.2 ml Thin-wall 8-Tube Strip	B77009	BIOplastics
Hard-Shell® PCR plates 96-well WHT/WHT	HSP9655	Bio-Rad
Hard-Shell® PCR plates 96-well WHT/WHT,	HSP9955	Bio-Rad
0.1ml 96-Well PCR plate Half Skirt	YPP-0.1-	YongYue
Low Tube Strip, WHT	TLS0851	Bio-Rad
MicroAmp® Optical 8-Cap Strip	4323032	Applied Biosystems
EU 8-Single Attachable Indented Cap	B79501	BIOplastics
Optical Flat 8-Cap Strips	TCS0803	Bio-Rad
Sealing film	YPF-QPC	YongYue
Optically Clear Heat Seal	1814030	Bio-Rad
Permanent Clear Heat Seal	1814035	Bio-Rad
PX1 PCR plate sealer (auto-sealer)	1814000	Bio-Rad

Mini-centrifuge	Mini-6K	Protagen
PCR plate centrifuge	MPC-P25	Powerlab
UPS	HP 910	Sampoongpower

NOTE: All purchasing items listed above can be purchased through Seegene Technologies (California, US).

Operation

NOTE:

(1) Prior to running the Seegene launcher, inspect the deck and carriers for cleanliness and empty the tip waste/liquid waste if there are any.

(2) A minimum of 300 µL specimen volume is required to ensure 200 µL of specimen pipetting by Microlab STARlet/Seegene STARlet. This will result in 100 µL elution volume of nucleic acids (RNA) necessary to run the Allplex™ 2019-nCoV Assay.

(3) Only 12mm tubes, 16mm tubes and 1.5 mL micro centrifuge tubes can be directly loaded to the Microlab STARlet/Seegene STARlet.

(4) For information on maintenance, refer to the Seegene Launcher V6 manual.

1. Open the Seegene launcher software installed on the laptop connected to the Microlab STARlet IVD/Seegene STARlet for operation of the Microlab STARlet IVD/Seegene STARlet.

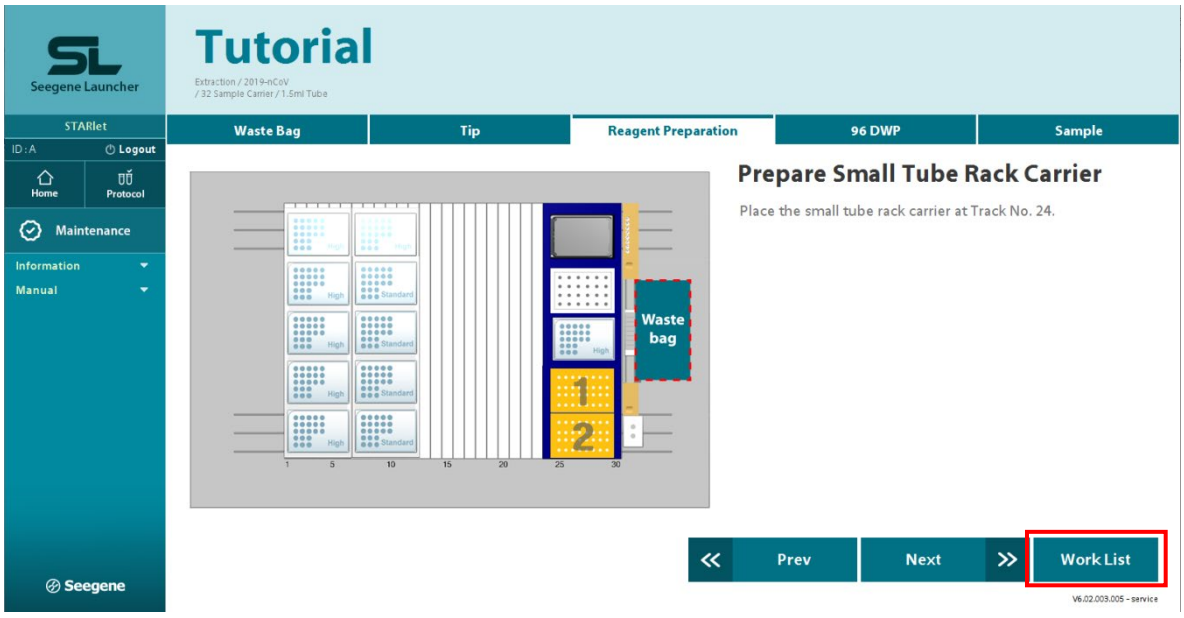
2019-nCoV
Launcher

2. Click on **LAUNCHER RUN** on the main page.

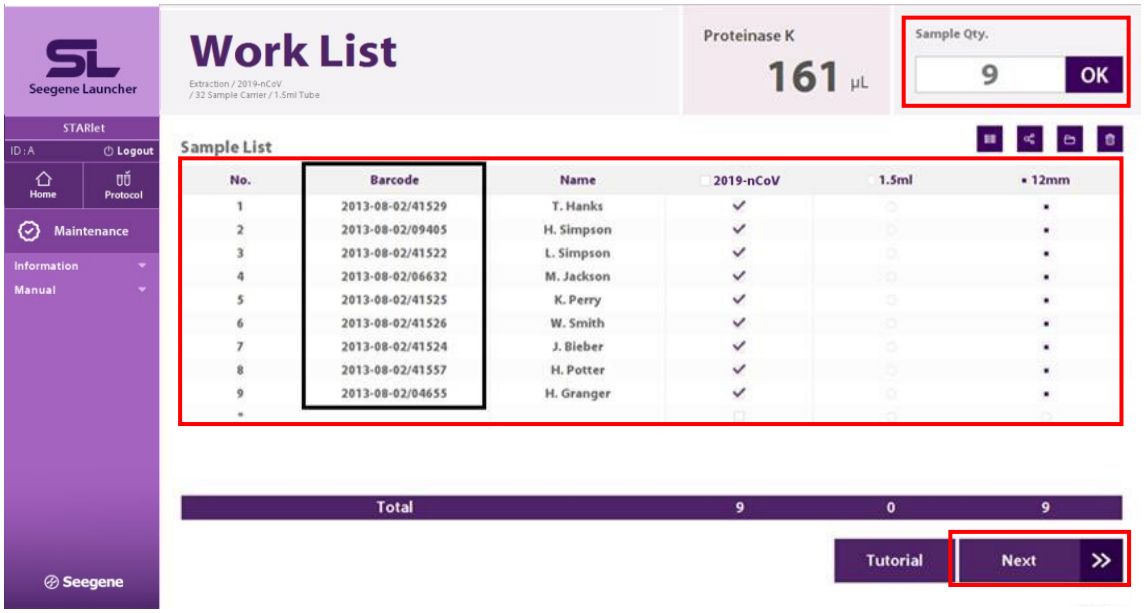
3. Select **2019-nCoV** (protocol for Allplex™ 2019-nCoV Assay) to begin the protocol. All following steps are included in a step by step instruction included in the software.

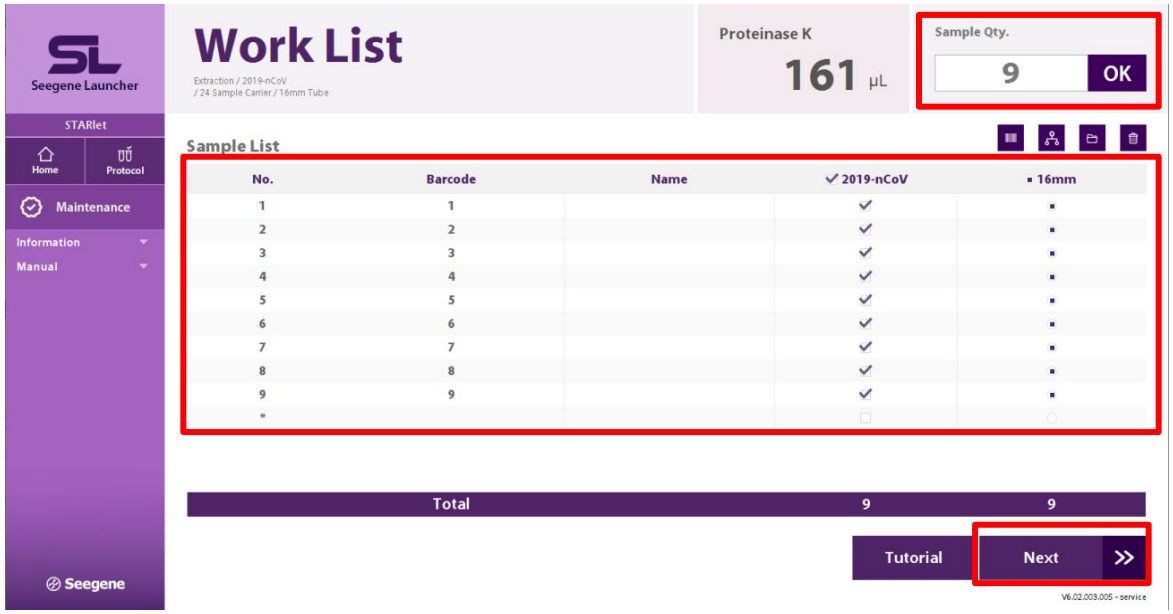
4. Check and follow the instructions carefully and then click on **Work List**. Samples, Internal Control, consumables, and 1 cartridge from the STARMag 96 X 4 Universal Cartridge Kit are placed on the Microlab STARlet IVD/Seegene STARlet while following step by step instructions guided by the Seegene Launcher software.

   NOTE: After equilibrating specimens to room temperature, vortex each specimen briefly.

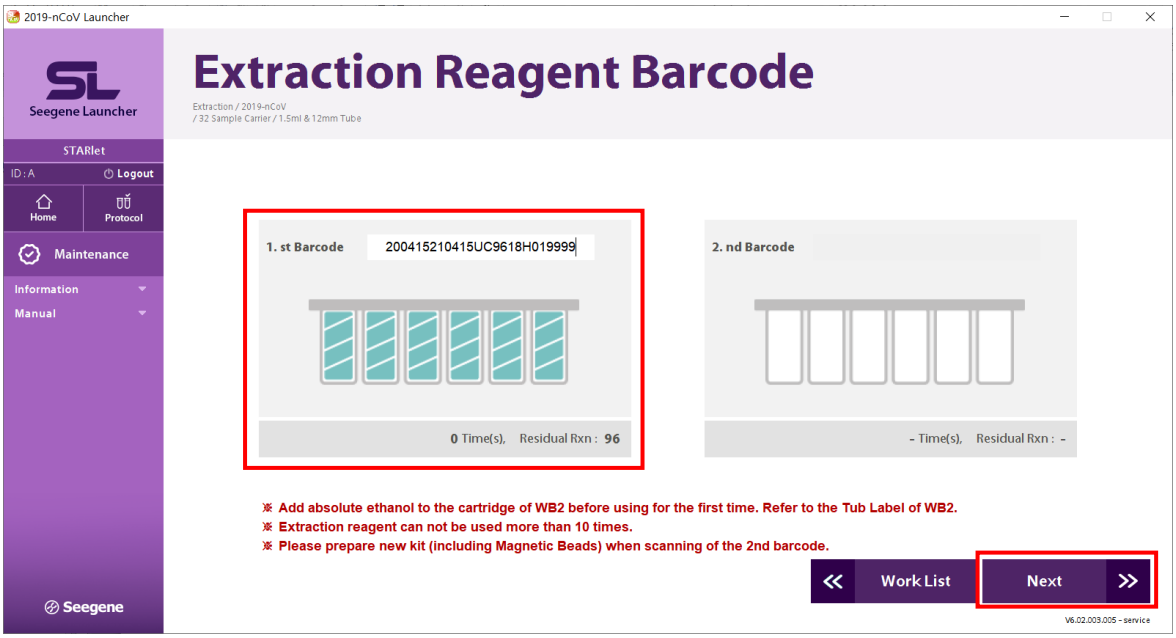


5. A barcode reader installed inside the Microlab STARlet IVD/Seegene STARlet automatically reads sample information. The sample information can also be manually entered, if necessary.
   Click on **Next**, once **Sample Quantity, Barcode, Name** (optional) and labware (1.5 ml,12 mm or 16 mm) information are entered correctly.

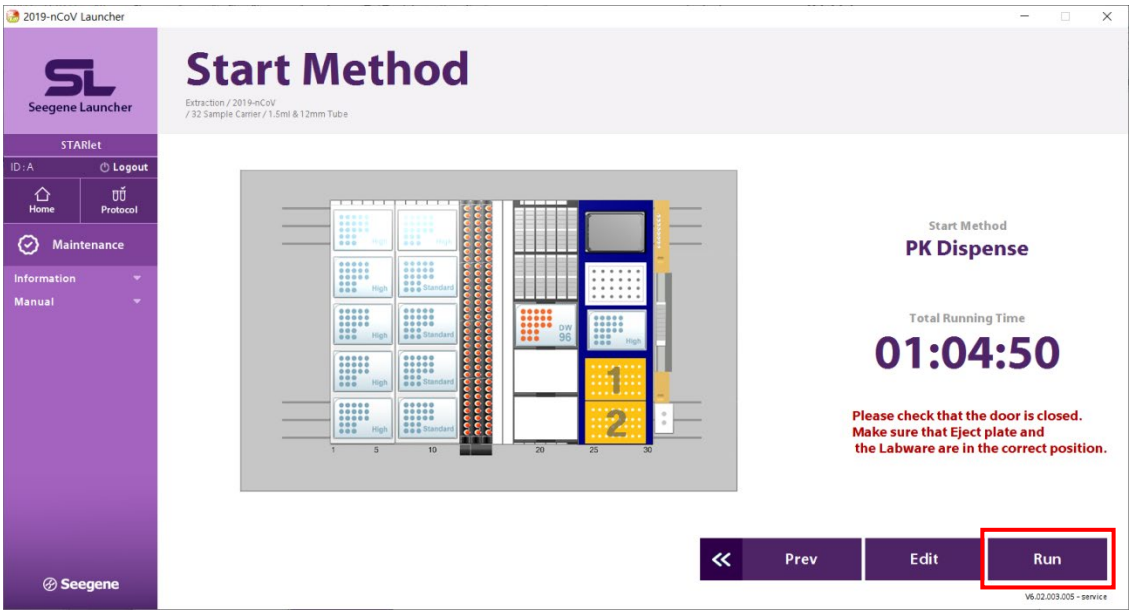




6. Using a hand-held barcode reader provided with the Microlab STARlet IVD/Seegene STARlet, read barcode label attached on the side of the cartridge. After the **Extraction Reagent Barcode** information is entered, click on **Next**. If the remaining volume of the existing cartridge is insufficient to run the desired number of samples, a second cartridge needs to be barcoded and placed.

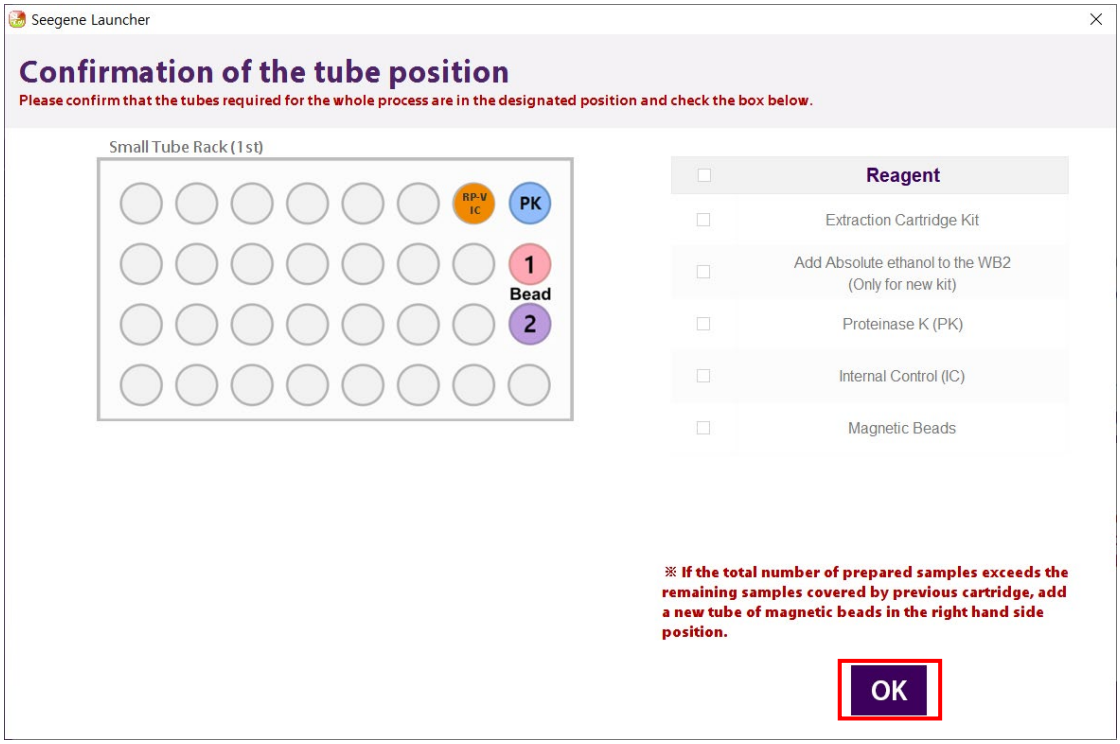


7. Ensure that the Microlab STARlet IVD/Seegene STARlet door is firmly closed, and that the eject plate and labware are in their correct positions as shown below. Click on **Run** after all preparations are done.
Do not open the door of the Microlab STARlet IVD/Seegene STARlet during operation.

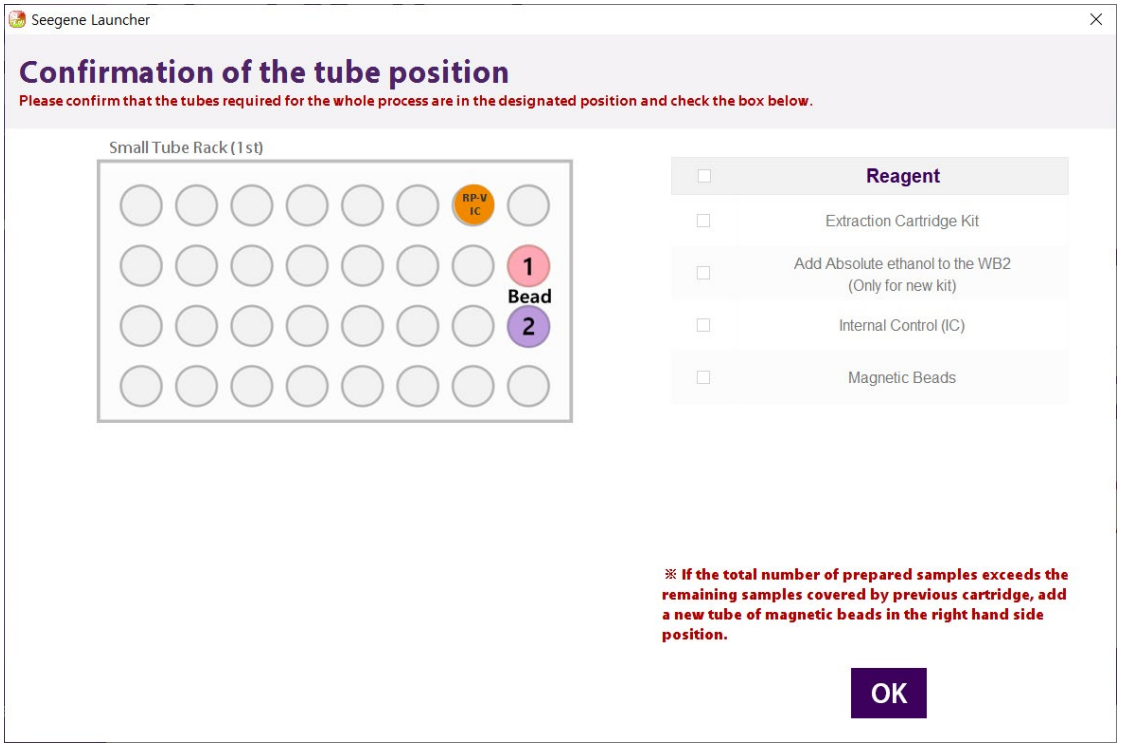


8. Check that the reagents are in the right position and click on **OK** to start the run.

For STARMag 96 X 4 Universal Cartridge Kit;



For STARMag 96 X 4 viral DNA/RNA 200 C Kit;



For further inquiries regarding the extraction procedure, contact Seegene Technologies (California, US) at support@seegenetech.com.

Please refer to the user manual of 'Seegene Launcher V6' for detailed descriptions of experimental procedures of nucleic acid extraction using Microlab STARlet IVD and Seegene STARlet.

Amplification and detection: Bio-Rad CFX Systems

A video tutorial is available upon request to Seegene Technologies (California, US, support@seegenetech.com) for training on all experimental procedures related to amplification and detection under this section. Seegene Viewer v 3.20 or higher for auto-interpretation of results is provided by Seegene Technologies (California, US), support@seegenetech.com.

Preparation for real-time PCR

NOTE:

(1) To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
(2) Extracted RNA handling and PCR reagent preparation must be performed at different areas.
(3) Remove all reagents from ≤ -20℃ storage. After thawing them completely, spin down each reagent for quick spin.
(4) The provided positive control (PC, PCR control) and clinical sample RNA extracts require special caution in handling to avoid carry-over contamination.
(5) Include one Positive Control and one Negative Control on each plate.

1. Prepare the following reagents in a labeled sterile 1.5 mL tube. Set up all reagents on ice.

Table 6. One-step RT-PCR Mastermix for different number of reactions (unit: µL)

No. of Reactions	1	2	3	4	5
2019-nCoV MOM	5	10	15	20	25
RNase-free Water	5	10	15	20	25
5X Real-time One-step Buffer	5	10	15	20	25
Real-time One-step Enzyme	2	4	6	8	10

2. Mix by inverting each reagent tube 5 times or quick vortex, and briefly centrifuge.

   In 96-well PCR plate, Aliquot 17 µL of the One-step RT-PCR Mastermix into PCR tubes. NOTE: Prior to adding specimen extract/positive controls

to PCR plate, move from the reagent prep area to a specimen processing area.

3. Add 8 µL of each sample's extracted nucleic acids, 2019-nCoV PC and NC (RNase-free Water; Negative Control (NC) for PCR control) into the tubes containing aliquot of the One-step RT-PCR Mastermix.

4. Cover with Permanent Clear Heat seal for 96-Well Skirted PCR Plates on PX1™ PCR Plate sealer, and briefly centrifuge the PCR tubes.

   NOTE: The PCR tubes must be centrifuged before running the PCR reaction in order to force the liquid to the bottom and eliminate air bubbles.

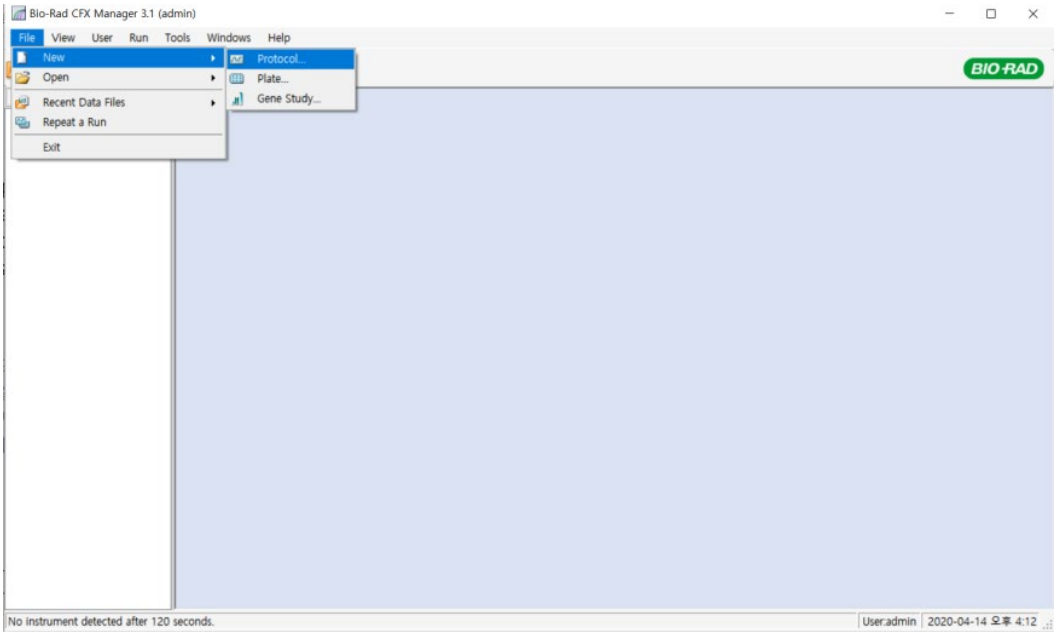
5. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher RPM and for a longer time.

6. Immediately initiate the PCR on the Bio-Rad CFX96 instruments. See details on PCR instrumentation set-up below.

Real-time PCR Instrument Set Up

Protocol Setup

1. In the main menu, select File → New → Protocol to open Protocol Editor.



2. In Protocol Editor, define the thermal profile as table below.

Step	No. of cycles	Temperature	Duration
1	1	50℃	20 min
2	1	95℃	15 min
3	45	94℃	15 sec
4		58℃	30 sec
5	GOTO Step 3, 44 more times		

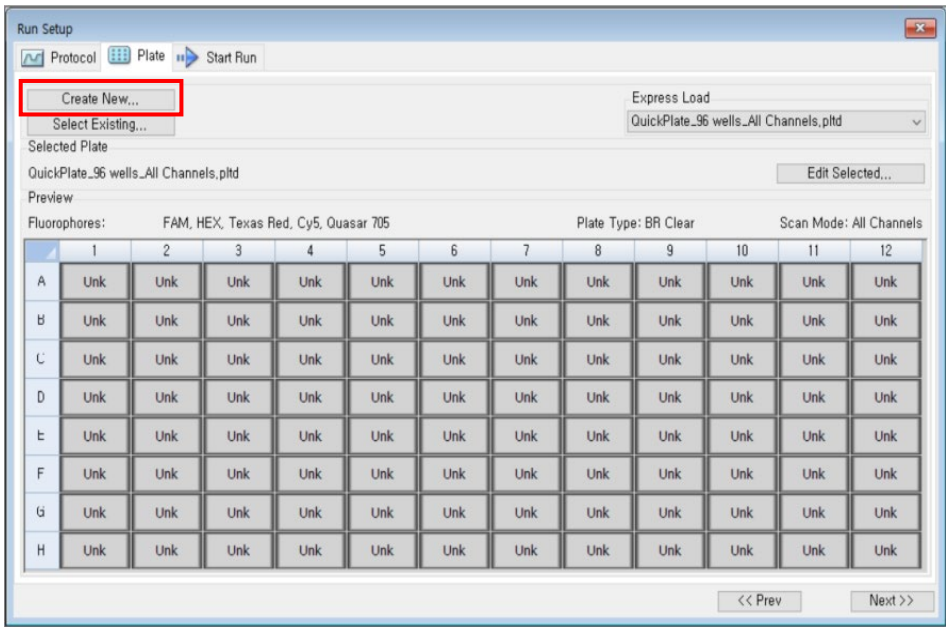
NOTE: Plate Read at Step 4. Fluorescence is detected at 58°C.

3. Click the box next to Sample Volume to directly input 25 µL.

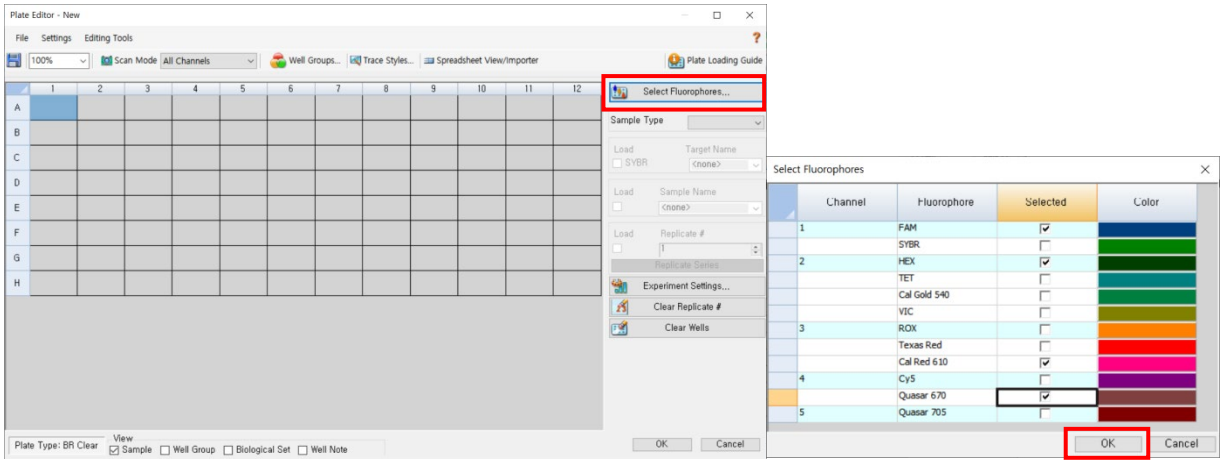
4. Click **OK** and save the protocol to open the Experiment Setup window.

Plate Setup

1. From **Plate** tab in **Experiment Setup**, click **Create New** to open **Plate Editor** window.

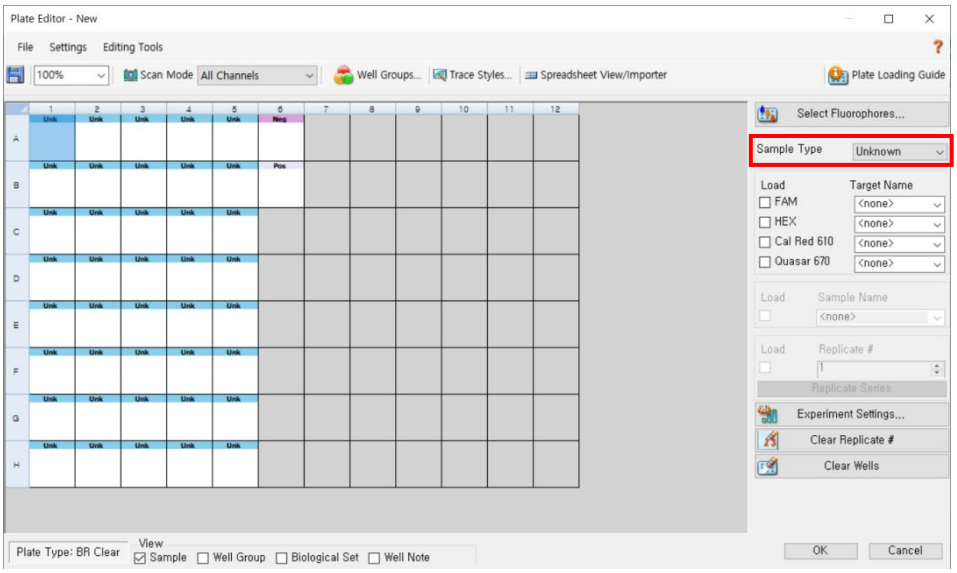


2. Click **Select Fluorophores** to indicate the fluorophores **(FAM, HEX, Cal Red 610** and **Quasar 670)** that will be used and click **OK**.

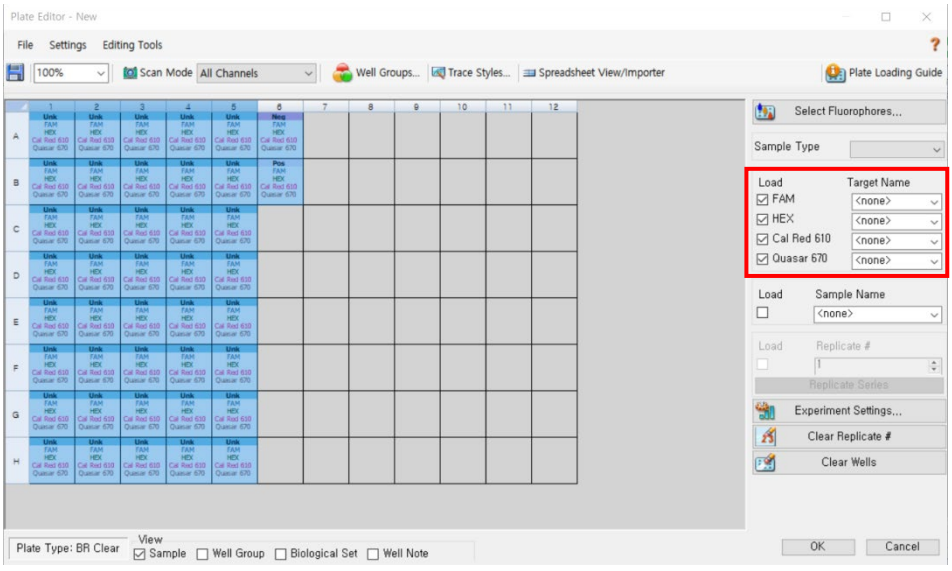


3. Select the desired well(s) and then its sample type from the **Sample Type** drop-down menu.

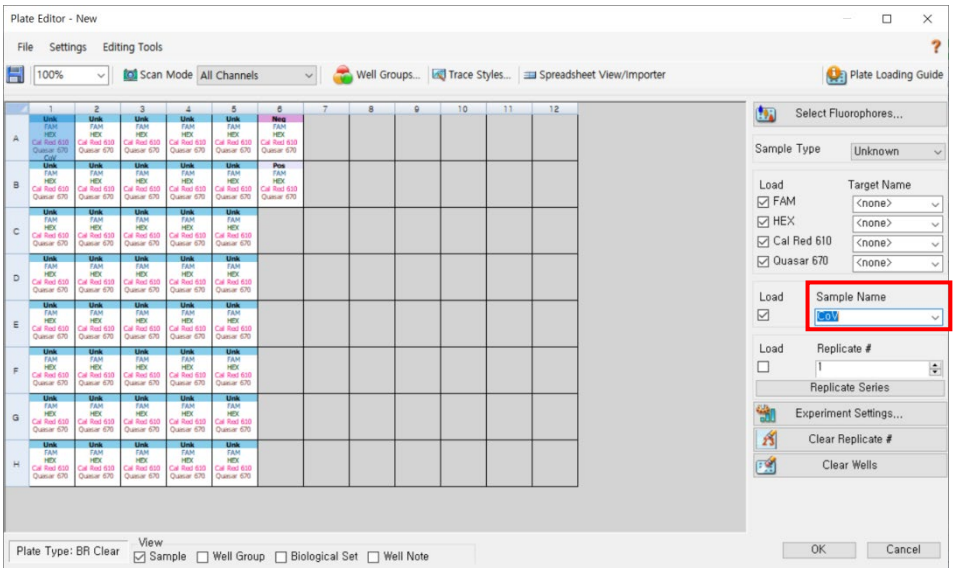
- **Unknown**: Clinical samples
- **Negative Control**
- **Positive Control**



4. Click on the appropriate checkboxes (**FAM, HEX, Cal Red 610** and **Quasar 670**) to specify the fluorophores to be detected in the selected wells.

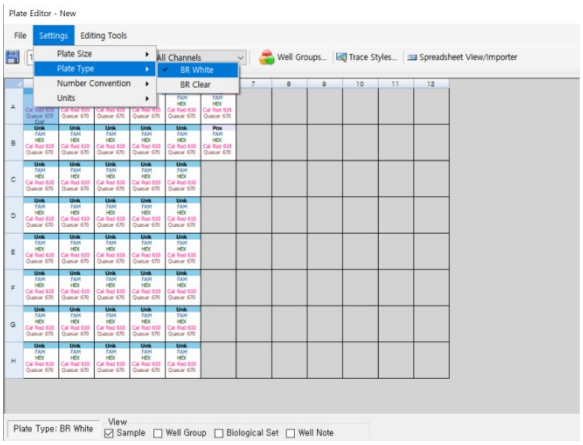


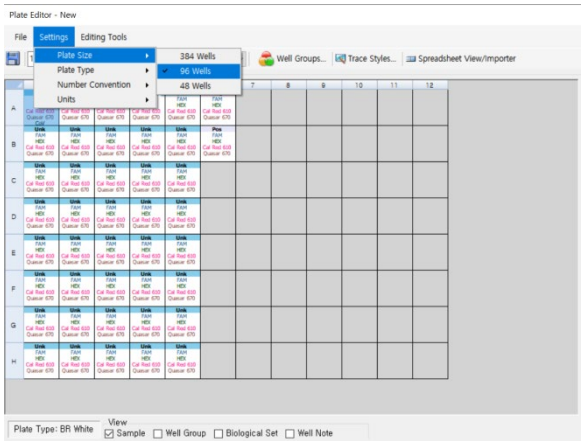
5. Type in **Sample Name** and press enter key.



6. In **Settings** of the **Plate Editor** main menu, choose **Plate Size (96 wells)** and **Plate Type (BR White)**.

7. Click **OK** to save the new plate.





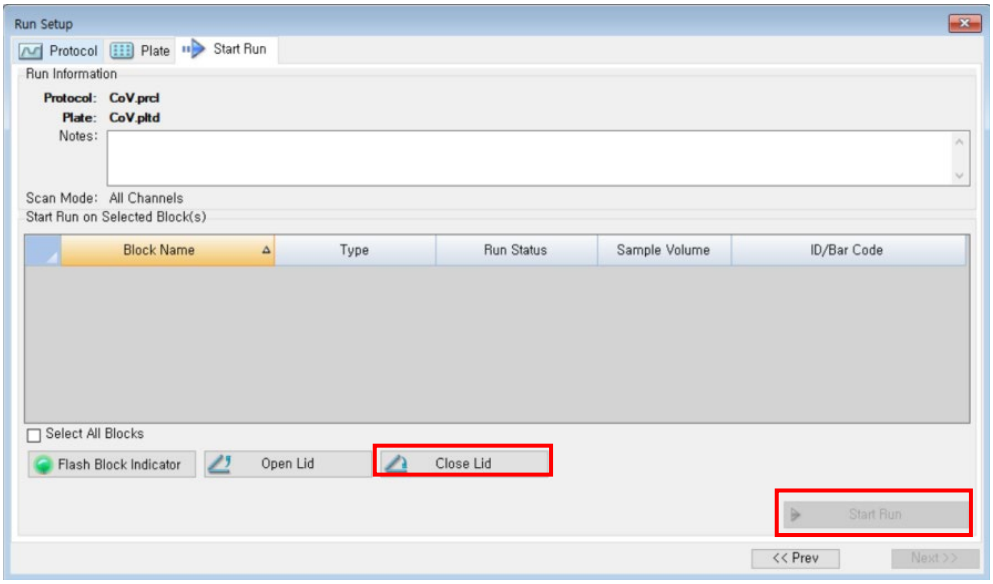
8. You will be returned to the Experiment Setup window.

Real-time PCR run

Start Run

1. From **Start Run** tab in **Experiment Setup**, click **Close Lid** to close the instrument lid.

2. Click **Start Run**.



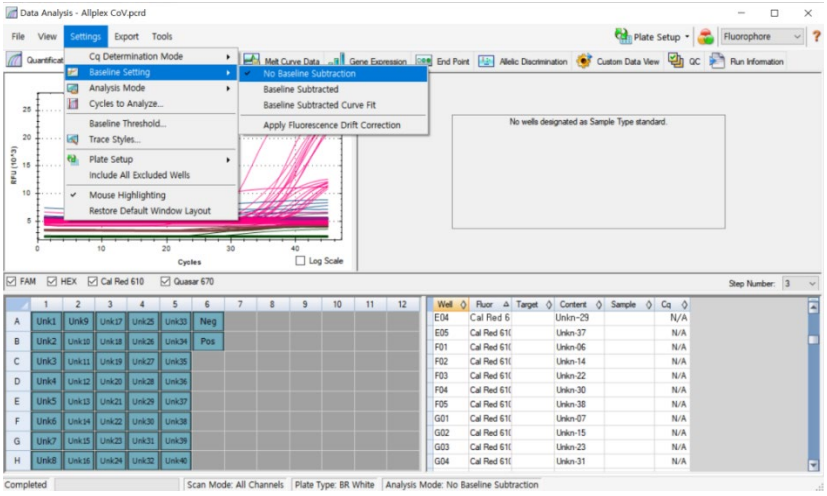
3. Store the run file either in My Documents or in a designated folder. Enter the file name, click **SAVE**, and the run will start.

Data export and analysis

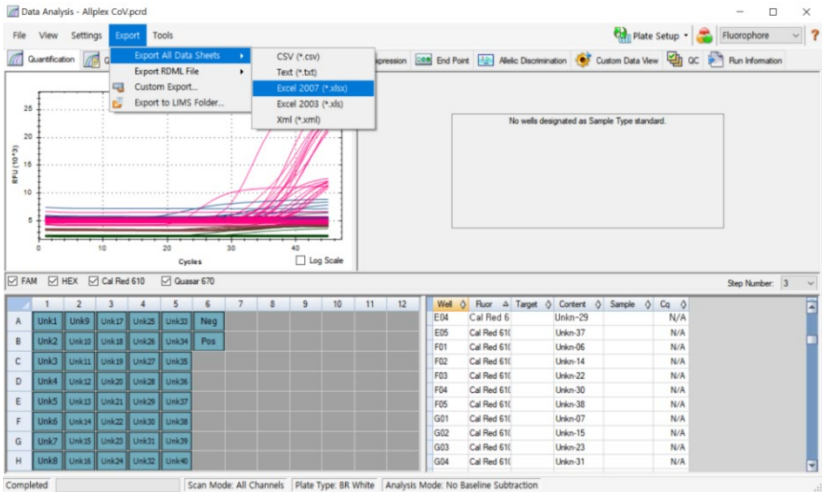
Data export
(CFX96 Touch™, CFX Manager™ Software V3.1 & CFX Maestro™ Software)
(CFX96™ and CFX96™ Dx, CFX Manager™ Dx Software V3.1)
(CFX96 Touch™, CFX Maestro™ Software V1)
(CFX Opus 96 Dx, CFX Maestro™ Software V2.0)

1. Create folders for data export
   - Create a folder to save amplification curve detection results.
   - The location and name of the folder are specified by user, but in case of using 'Seegene Export' function, the folder named "QuantStep4" is created automatically in the selected location.

2. After the PCR reaction, select **No Baseline Subtraction** from **Baseline Setting** of **Settings** menu.



3. Select **Excel 2007** from **Export All Data Sheets** from **Export** menu.

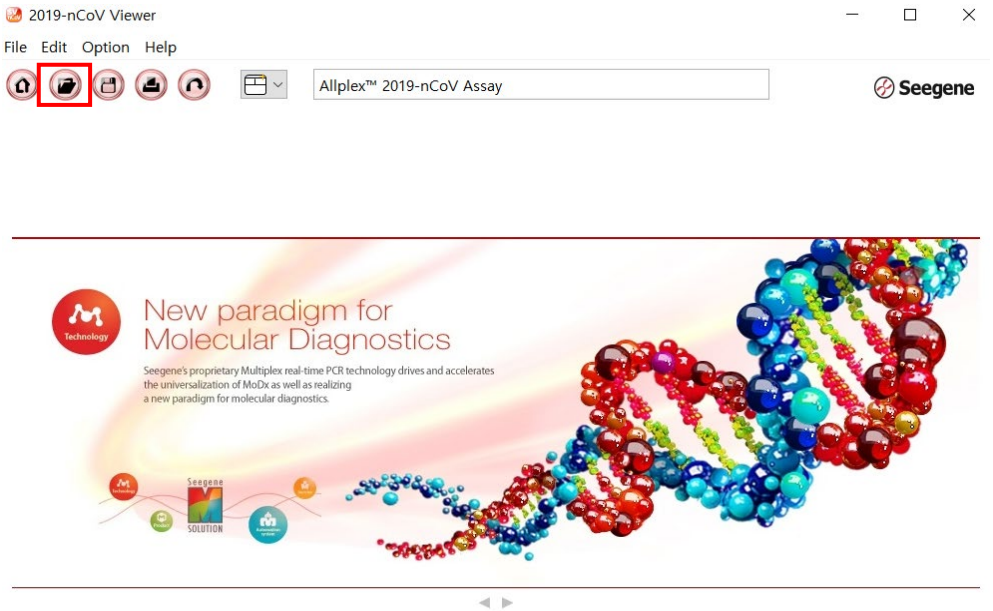


4. Choose a location to save data and click **OK**.

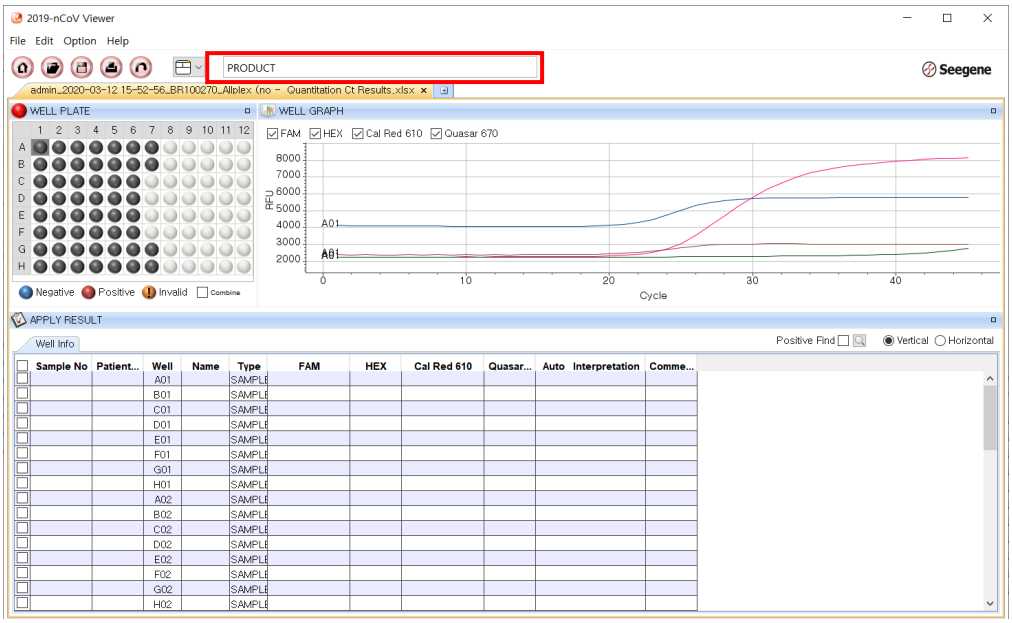
Data analysis

1. Open the **Seegene Viewer** software installed on the laptop connected to the Bio-Rad CFX96™.

2. Click on the Open icon and find CFX96™ export data on the location where CFX96™ data was saved.



3. After opening the results file, select '**Allplex™ 2019-nCoV Assay (FDA EUA only)**' from the **PRODUCT** menu.



4. View test results. The results for each sample can be viewed by clicking on each well.

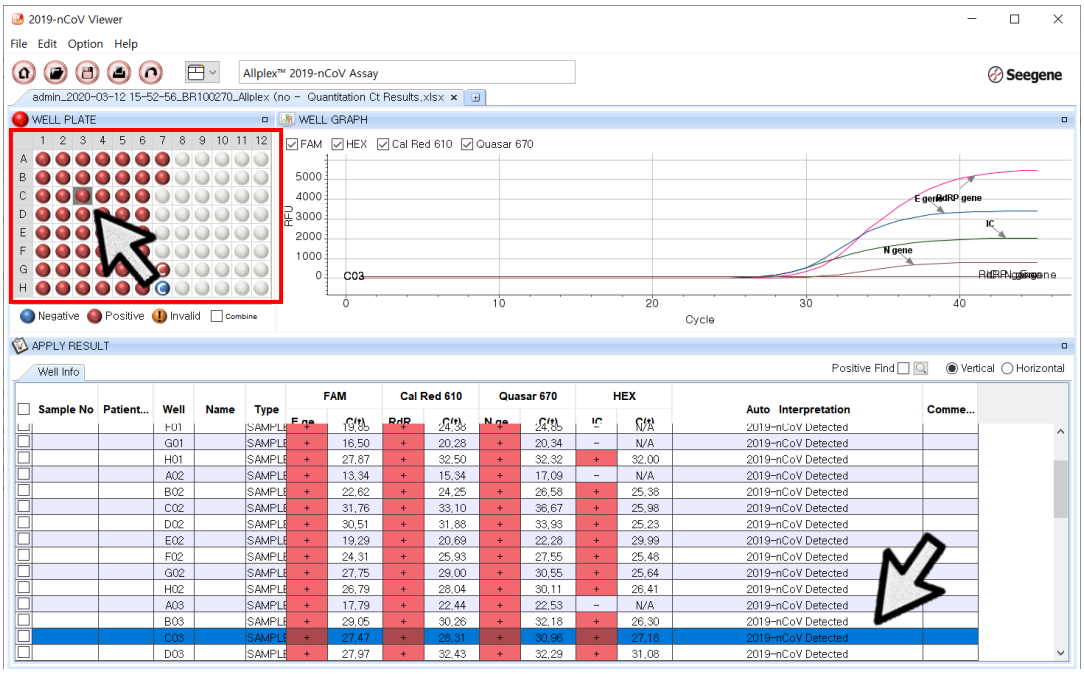


Table 7. Analytes of the Allplex™ 2019-nCoV Assay

Fluorophore	Analyte
FAM	E gene
HEX	Internal Control (IC)
Cal Red 610	RdRP gene
Quasar 670	N gene

Amplification and detection: Applied Biosystems™ 7500

Preparation for real-time PCR

NOTE:

(1) To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
(2) Extracted RNA handling and PCR reagent preparation must be performed at different areas.
(3) Remove all reagents from ≤ -20℃ storage. After thawing them completely, spin down each reagent for quick spin.
(4) The provided positive control (PC, PCR control) and clinical sample RNA extracts require special caution in handling to avoid carry-over contamination.
(5) Include one Positive Control and one Negative Control on each plate.

1. Prepare the following reagents in a labeled sterile 1.5 mL tube. Set up all reagents on ice.

Table 6. One-step RT-PCR Mastermix for different number of reactions (unit: µL)

No. of Reactions	1	2	3	4	5
2019-nCoV MOM	5	10	15	20	25
RNase-free Water	5	10	15	20	25
5X Real-time One-step Buffer	5	10	15	20	25
Real-time One-step Enzyme	2	4	6	8	10

2. Mix by inverting each reagent tube 5 times or quick vortex, and briefly centrifuge. In 96-well PCR plate, Aliquot 17 µL of the One-step RT-PCR Mastermix into PCR tubes.

   NOTE: Prior to adding specimen extract/positive controls to the PCR plate, move from the reagent prep area to a specimen processing area.

3. Add 8 µL of each sample's extracted nucleic acids, 2019-nCoV PC and NC (RNase-free Water; Negative Control (NC) for PCR control) into the tubes containing aliquot of the One-step RT-PCR Mastermix.

4. Cover with adhesive covers for 96-Well PCR Plates, and briefly centrifuge the PCR tubes.

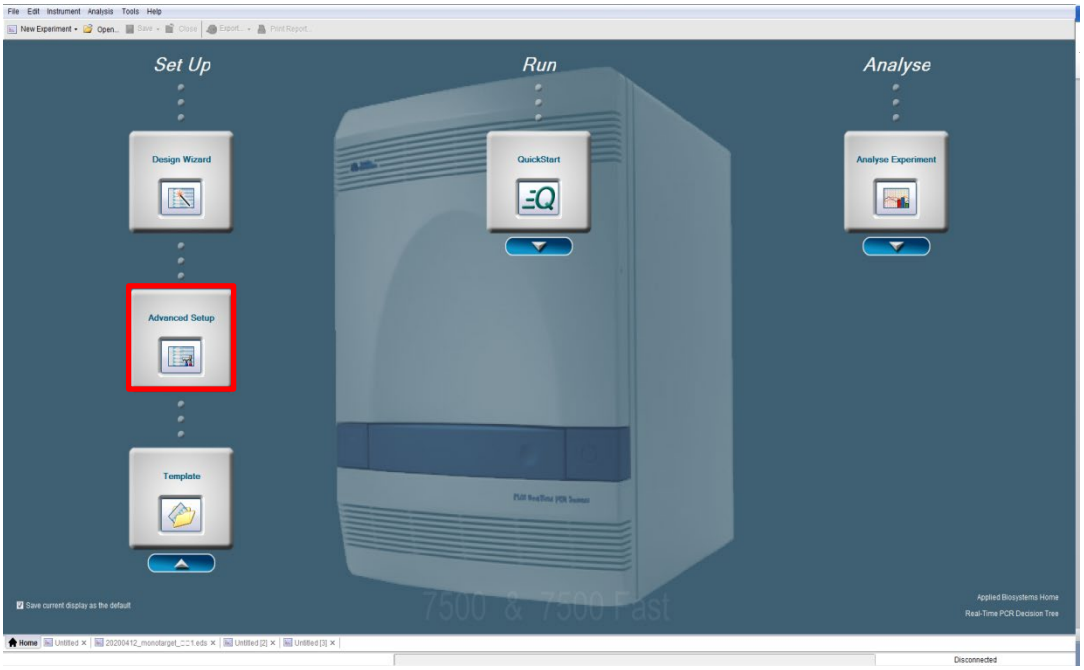
5. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher rpm and for a longer time.

6. Immediately initiate the PCR on the Applied Biosystems™. See details on PCR instrumentation set-up below.

Real-time PCR Instrument set up

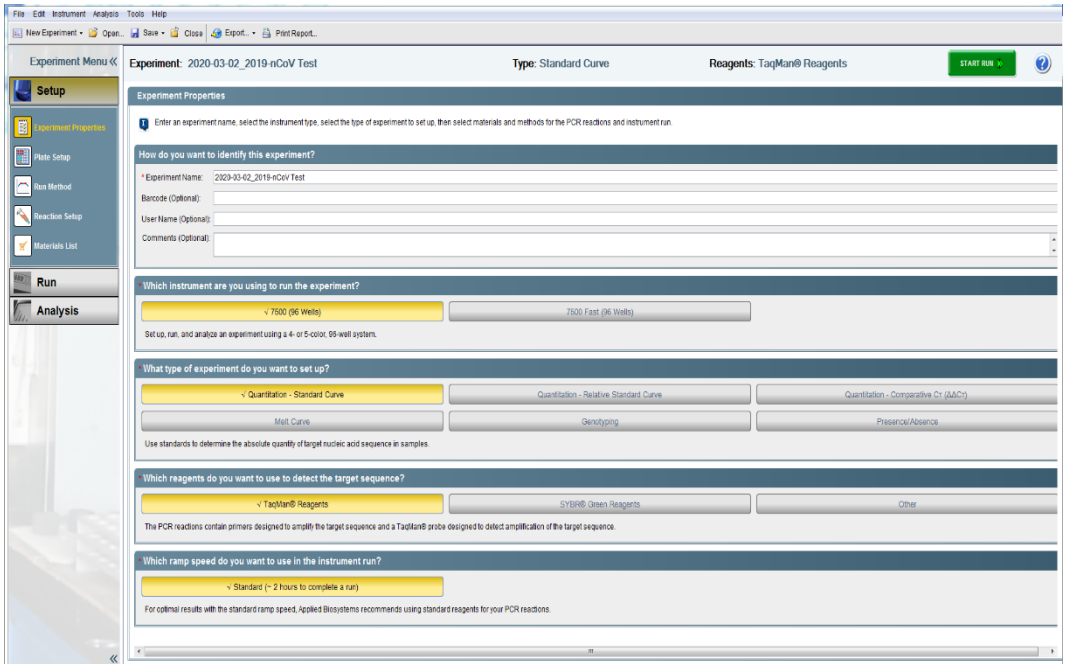
NOTE: The instrument must be calibrated before use.

1. In the Applied Biosystems™ 7500 software, click on **Setup** → **Advanced set up**.



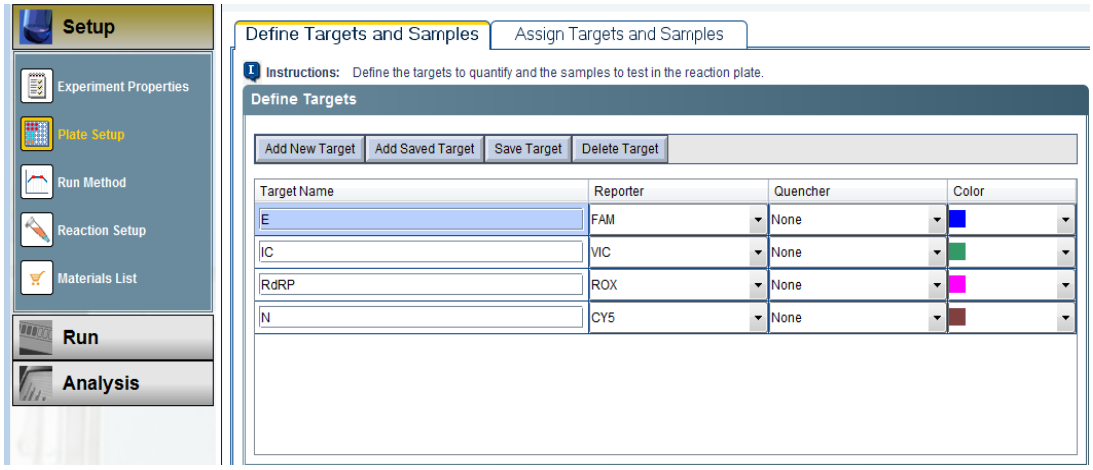
2. In the **Experiment properties tab**, enter **Experiment Name** and select **Instrument**, **Experiment type**, **Reagents**, and **Ramp speed** as follows.

Instrument	7500 (96 Wells)
Experiment type	Quantitation – Standard Curve
Reagents	Taqman® Reagents
Ramp speed	Standard



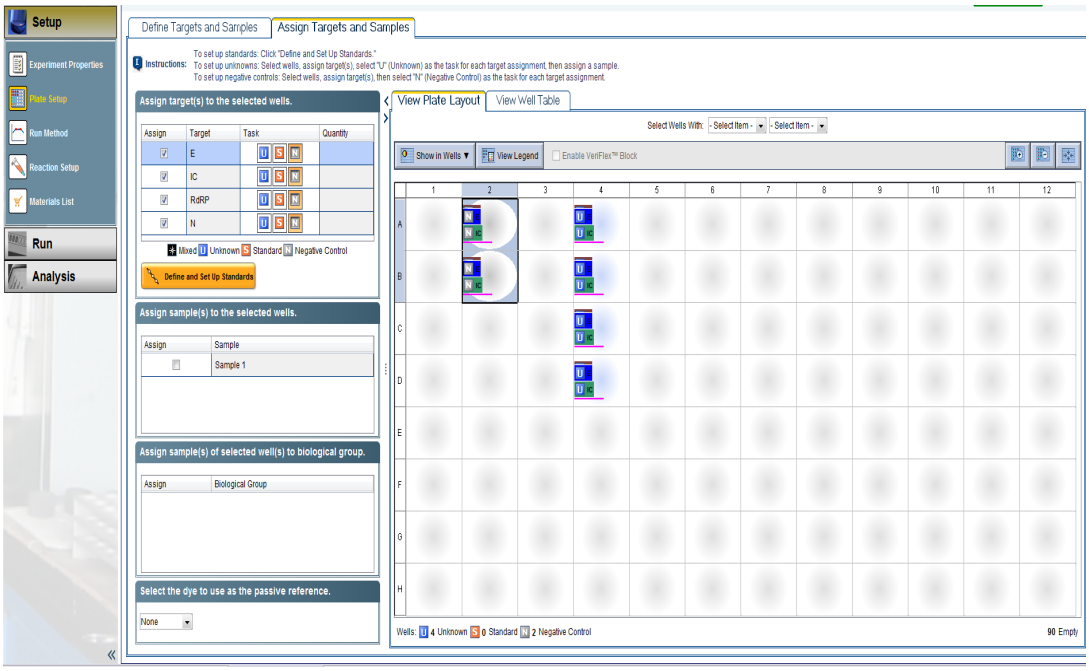
3. Click on **Plate setup** tab. In the **Define Targets** and **Samples** tab, enter **Target Name** and select **Reporter** and **Quencher** as follows.

Target Name	Reporter	Quencher
E	FAM	None
IC	VIC	None
RdRP	ROX	None
N	CY5	None



4. Click on **Assign Targets and Samples** tab, select wells where the PCR tube will be placed and assign targets. Select None for Passive reference.

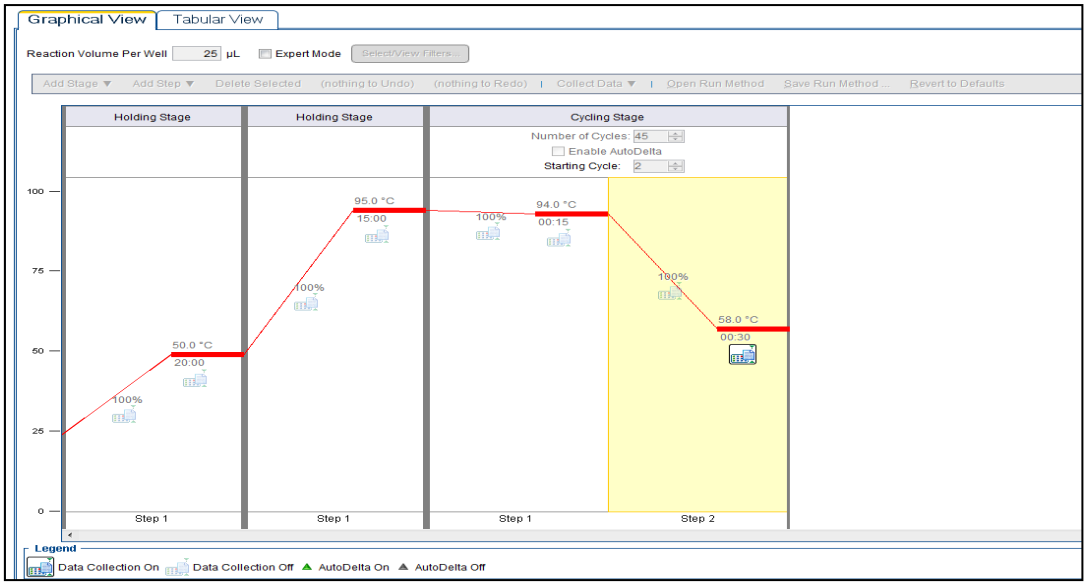
NOTE: If a well without sample or Mastermix is selected, signal noise may be observed. Ensure that only wells containing samples or Mastermix are selected.



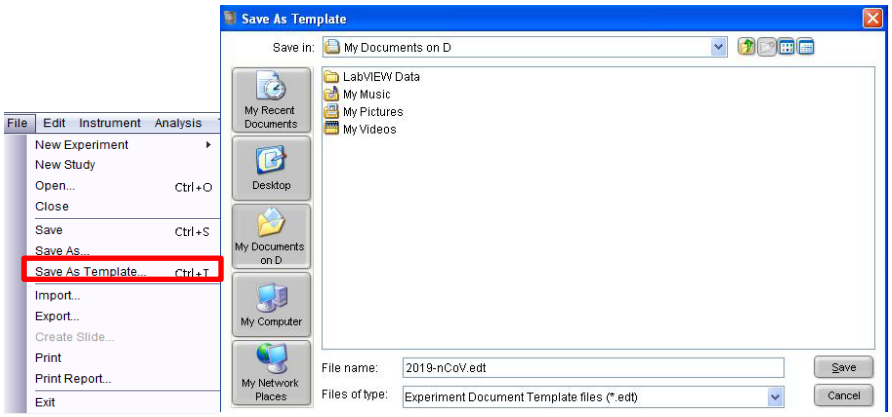
5. Click on Run Method. In the Graphical View or Tabular View tab, enter **25 µL** as the **Reaction Volume** per Well field. Define the thermal profile as table below.

Step	No. of cycles	Temperature	Duration
1	1	50 °C	20 min
2		95 °C	15 min
3	45	94 °C	15 sec
4		58 °C	30 sec
5	GOTO Step 3, 44 more times		

NOTE: Plate Read at Step 4. Fluorescence is detected at 58°C.



6. Click on **File** → **Save as Template** to save the new template file in **.edt** format. Enter the file name, select a location for the template, then click **Save**. The saved template can be used for future testing.



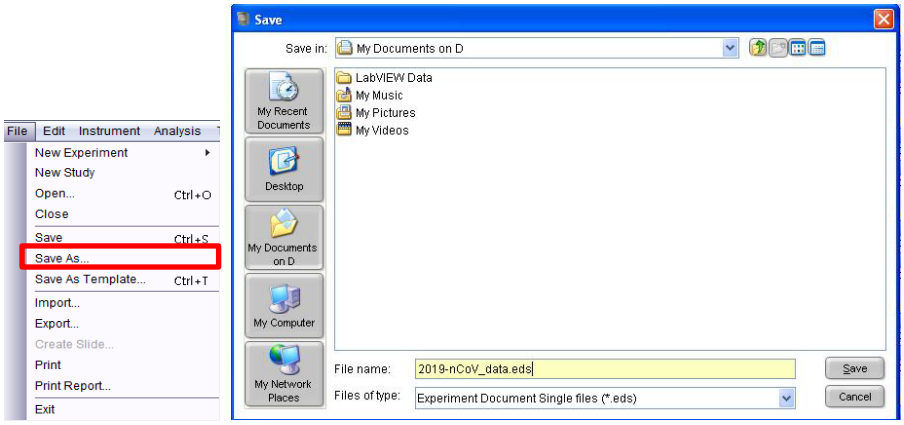
Start the Run

1. Turn on the laptop and Applied Biosystems™ 7500 real-time PCR system. Ensure that the laptop is connected to the instrument.

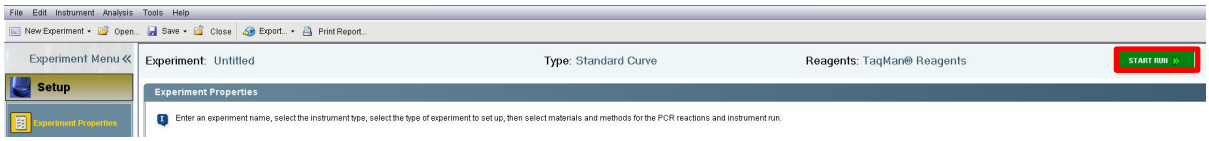
2. Push the tray door to open the instrument. Load the PCR plate onto the plate holder of the instrument.

3. Push the tray door to close the instrument.

4. Click on **File** → **Save As** to save experiments in .eds format.



5. Click **START RUN**.



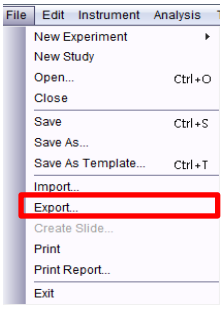
Data export and analysis

Create folders for data export

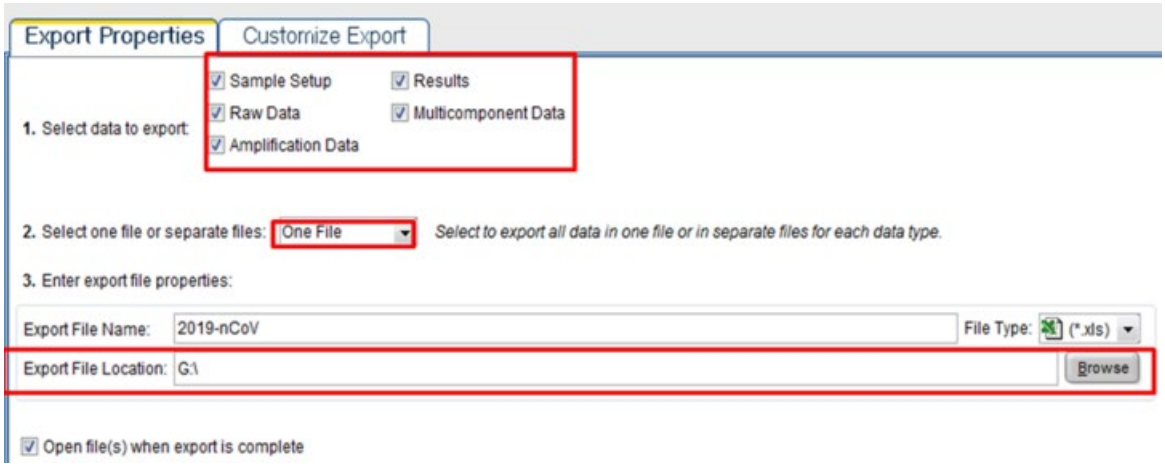
1. Create a folder to save data for all of the amplification curve detection steps from the result file.
2. Enter folder name as necessary.

Data export

1. Click on **File** → **Export**



2. Click on the Export Properties tab (default) and select Sample Setup, Raw data, Amplification Data, Results, and Multicomponent Data under 1. Select data to export.

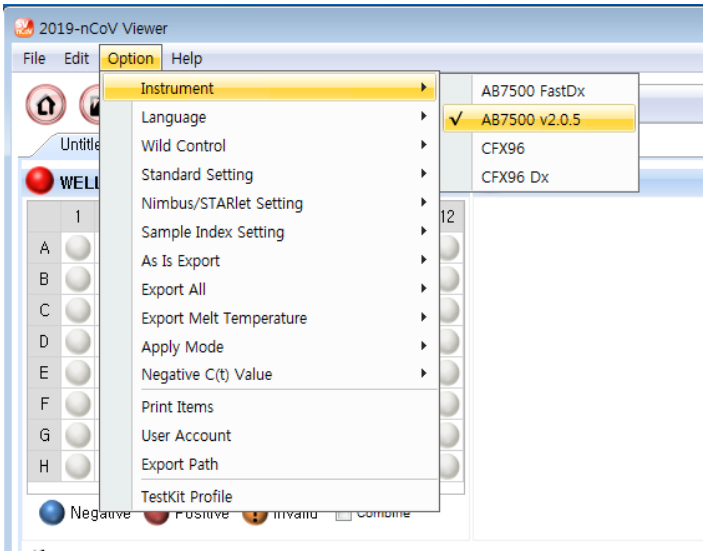


3. Select 'One File' under 2. Select one or separate files:
4. Enter Export File Name, then select Export File Location.
5. Select .xls in the File Type drop-down list.
6. Click Start Export.

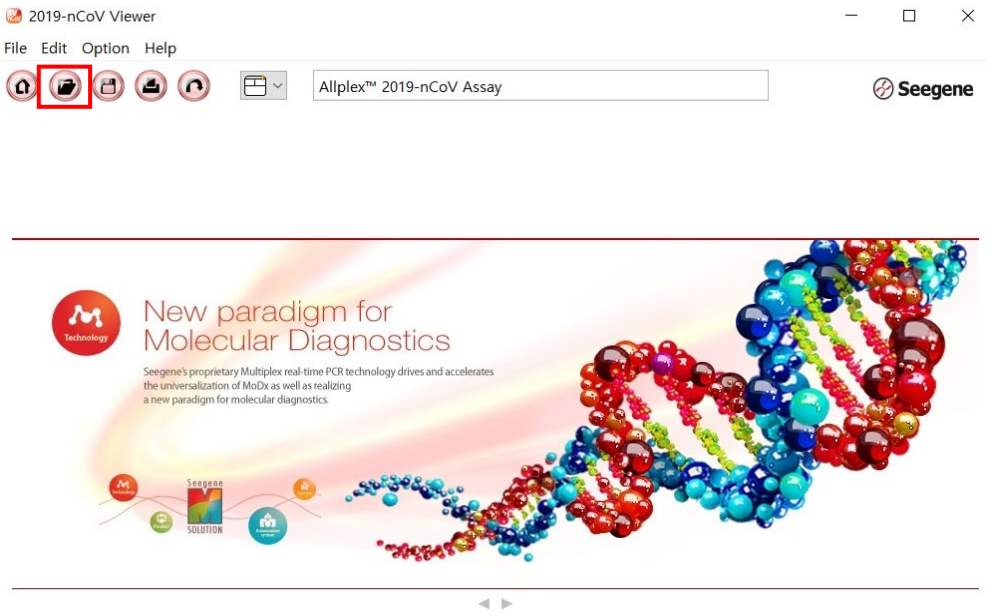
Data analysis

1. Open the Seegene Viewer software installed on the laptop connected to the Applied Biosystems™ 7500.

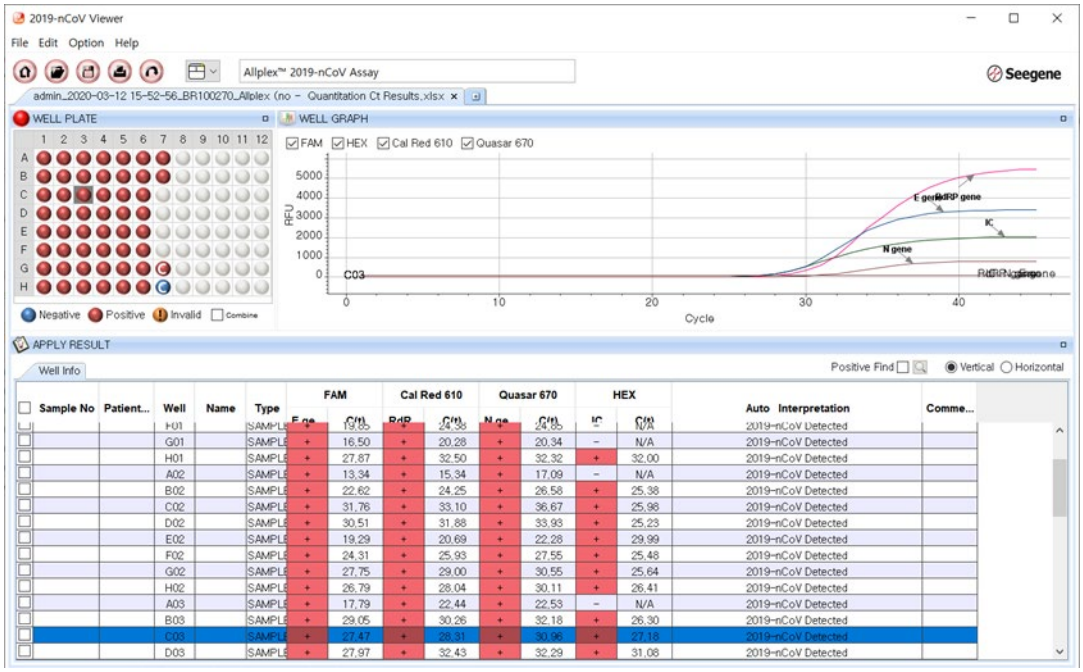
2. Click on **Option** to select AB7500 v2.0.5 from the **Instrument** menu.



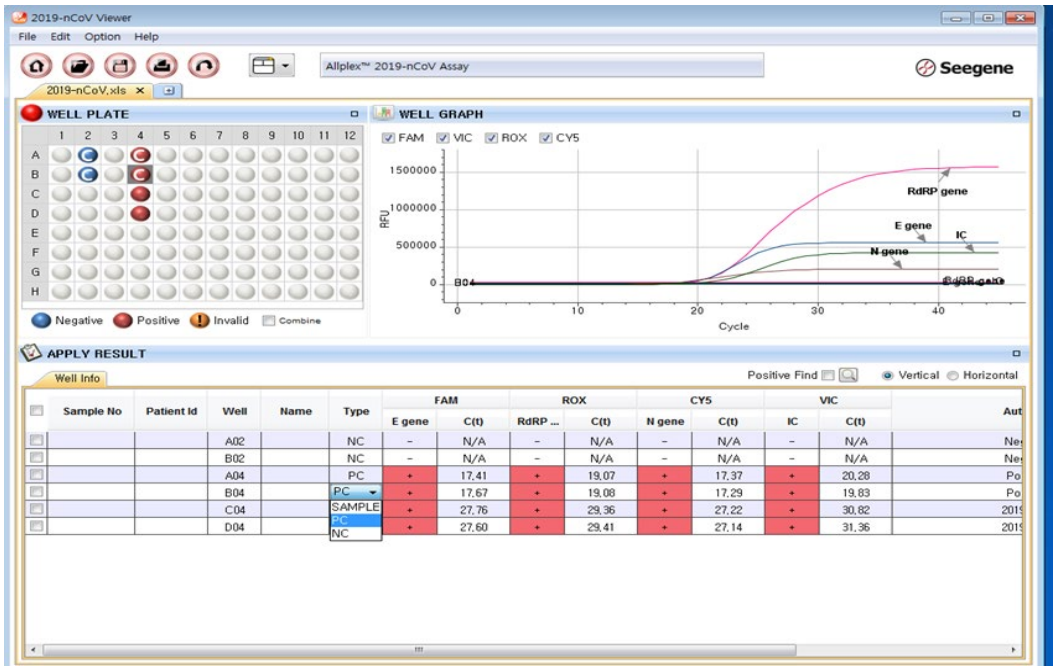
3. Click on the **Open** icon and locate the Applied Biosystems™ 7500 export data where the Applied Biosystems™ 7500 data was saved.



4. After opening the results file, select 'Allplex™ 2019-nCoV Assay (FDA EUA only)' from the PRODUCT menu.



5. Assign Positive and Negative control accordingly by selecting PC and NC under the Type drop-down menu.



6. View test results. The auto-interpreted results for each sample can be viewed by clicking on each well.

Amplification and detection: Applied Biosystems™ 7500 Fast Dx

Preparation for real-time PCR

NOTE:

(1) To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.
(2) Extracted RNA handling and PCR reagent preparation must be performed at different areas.
(3) Remove all reagents from ≤ -20℃ storage. After thawing them completely, spin down each reagent for quick spin.
(4) The provided positive control (PC, PCR control) and clinical sample RNA extracts require special caution in handling to avoid carry-over contamination.
(5) Include one Positive Control and one Negative Control on each plate.

1. Prepare the following reagents in a labeled sterile 1.5 mL tube. Set up all reagents on ice.

Table 6. One-step RT-PCR Mastermix for different number of reactions (unit: µL)

No. of Reactions	1	2	3	4	5
2019-nCoV MOM	5	10	15	20	25
RNase-free Water	5	10	15	20	25
5X Real-time One-step Buffer	5	10	15	20	25
Real-time One-step Enzyme	2	4	6	8	10

2. Mix by inverting each reagent tube 5 times or quick vortex, and briefly centrifuge.

   In 96-well PCR plate, Aliquot 17 µL of the One-step RT-PCR Mastermix into PCR tubes. NOTE: Prior to adding specimen extract/positive controls to the PCR plate, move from the reagent prep area to a specimen processing area.

3. Add 8 µL of each sample's extracted nucleic acids, 2019-nCoV PC and NC (RNase-free Water; Negative Control (NC) for PCR control) into the tubes containing aliquot of the One-step RT-PCR Mastermix.

4. Cover with adhesive covers for 96-Well PCR plates, and briefly centrifuge the PCR tubes.

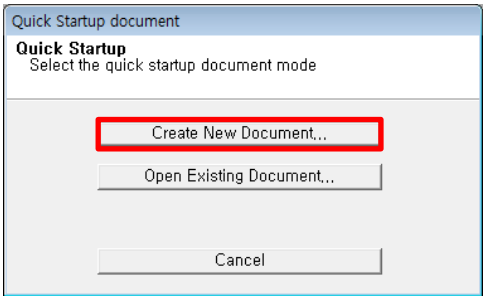
5. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher rpm and for a longer time.

6. Immediately initiate the PCR on the Applied Biosystems™ 7500. See details on PCR instrumentation set-up below.

Real-time PCR Instrument set up

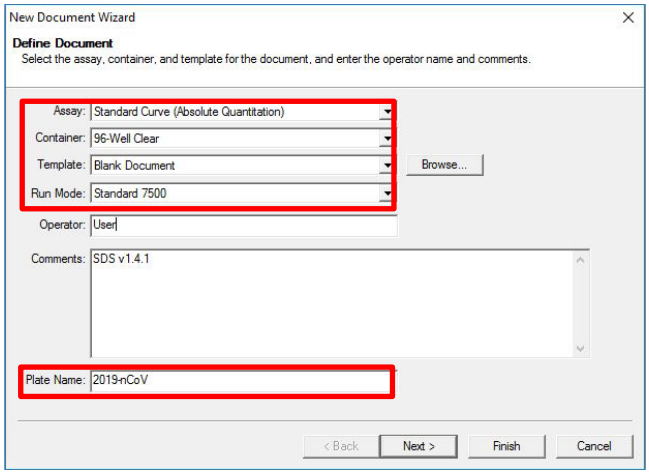
NOTE: The instrument must be calibrated before use.

1. In the SDS software, click on **Quick Startup → Create New Document.**

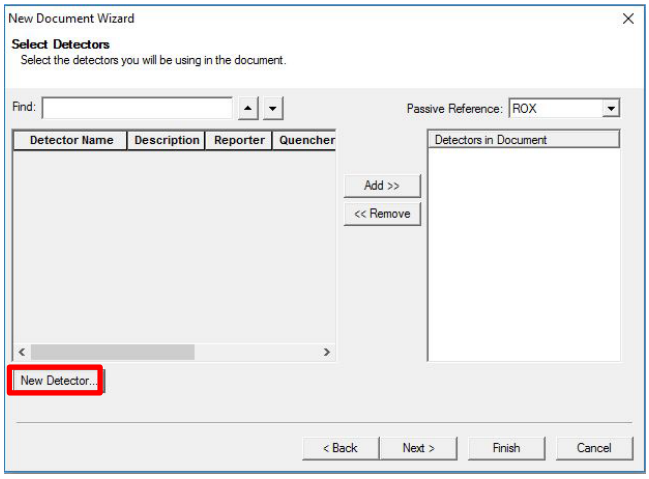


2. In **New Document Wizard**, select **Assay**, **Container**, **Template**, and **Run mode** as below then enter **Plate Name**.

Assay	Standard Curve (Absolute Quantitation)
Container	96-Well Clear
Template	Blank Document
Run mode	Standard 7500

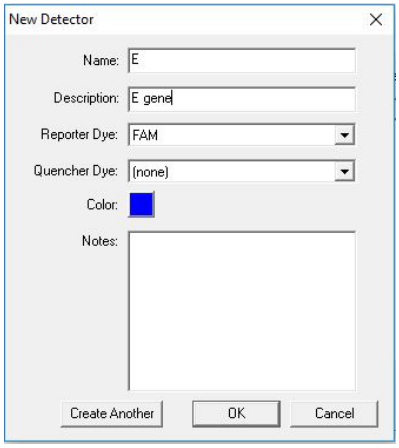


3. In **Select Detectors**, click on **New Detector** to add reporter and quencher information of analytes.

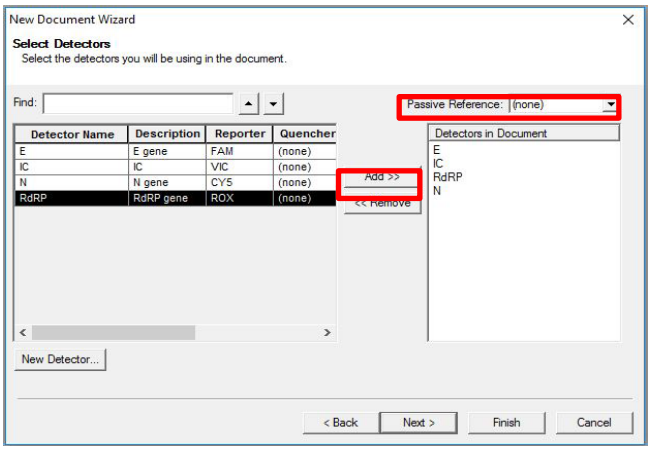


4. In **New Detector**, enter **Detector Name**, **Description** (optional) and select **Reporter, Quencher** as table below.

Detector Name	Reporter	Quencher
E	FAM	None
IC	VIC	None
RdRP	ROX	None
N	CY5	None

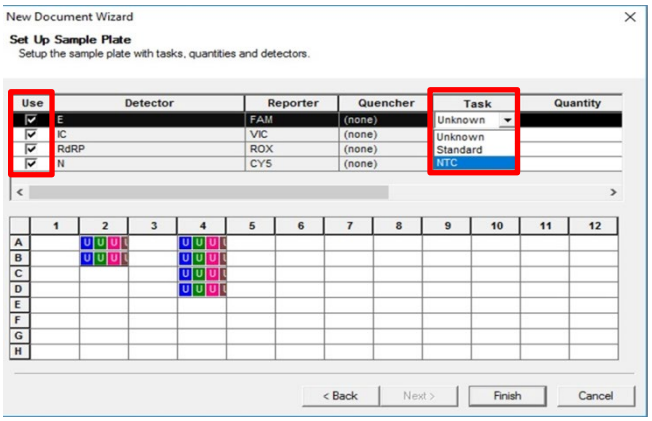


5. In **Select Detectors**, add E, IC, RdRP, and N to **Detectors in Document** field by clicking on **Add >>**. Select **none** for **Passive Reference.**

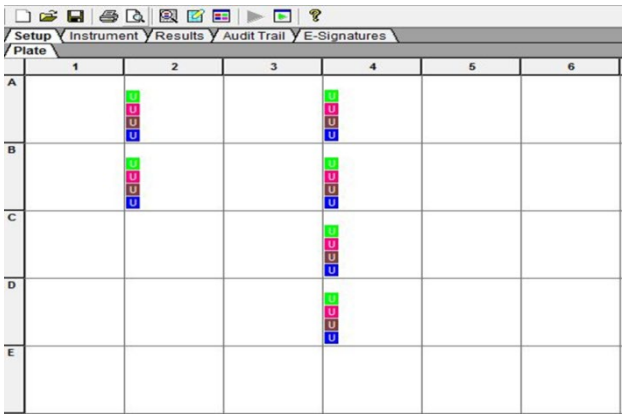


6. In **Set Up Sample Plate**, drag and select the wells where the PCR tube will be placed and assign targets by clicking on the check boxes next to each **Detector** and select **Unknown** from **Task**.

NOTE: If a well without sample or Mastermix is selected, signal noise may be observed. Ensure that only wells containing samples or Mastermix are selected.



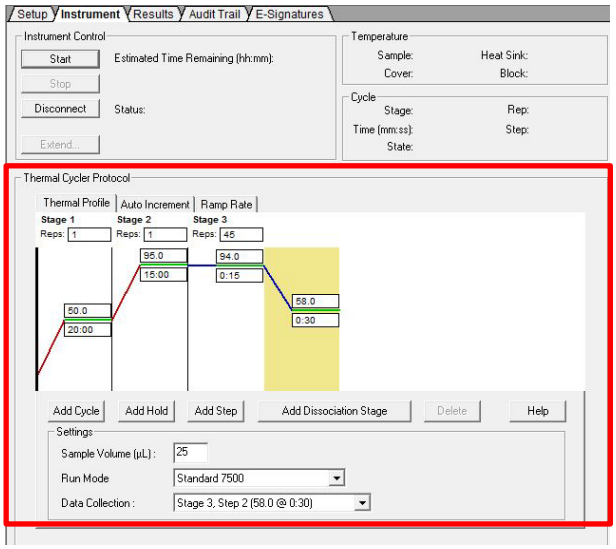
7. In the **Setup** – **Plate** tab, confirm the run plate information.



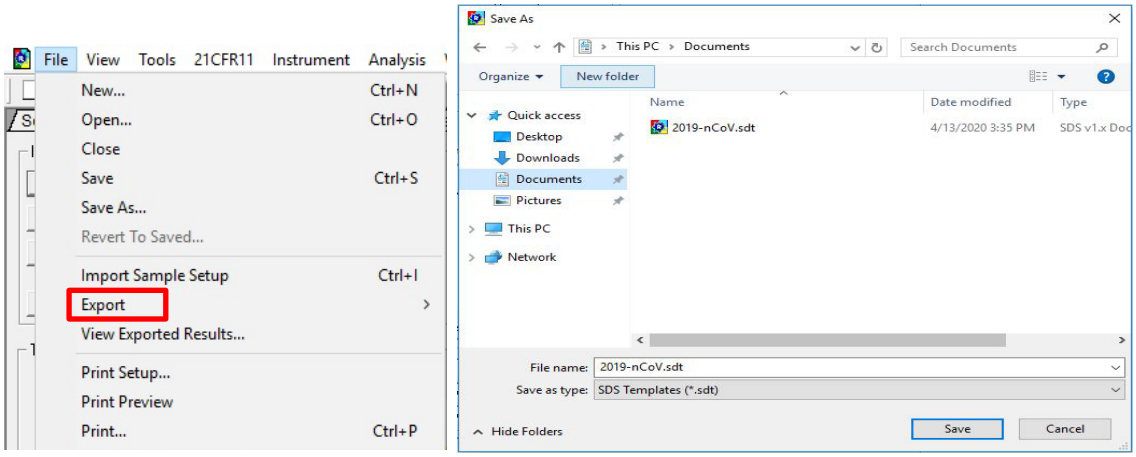
8. In the **Instrument** tab – **Thermal Cycler Protocol**, define the thermal profile as below. Enter **25 µL** in the **Sample Volume (µL)** field, select Stage 3, Step 2 [58.0°C @ 0:30] for **Data Collection** (Plate Read)

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	45	94°C	15 sec
4		58°C	30 sec
5	GOTO Step 3, 44 more times		

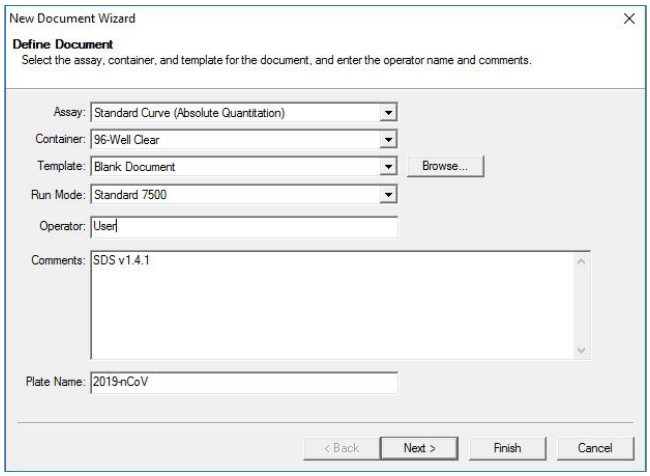
NOTE: Plate Read at Step 4. Fluorescence is detected at 58°C.

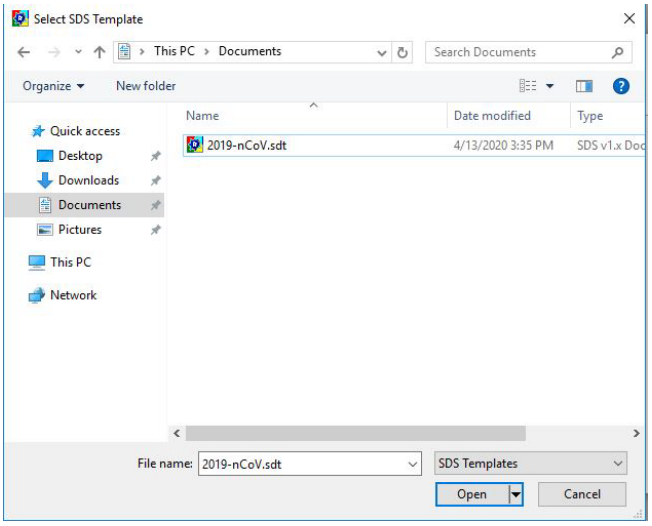


9. Click on **File → Save As** to save the new template file in **.sdt** format. Enter **File name**, select a location for the template, then click on **Save**. The saved template can be used for future testing.



10. To open the saved run protocol file, click on **Browse** in the **Template** field to open the template file.





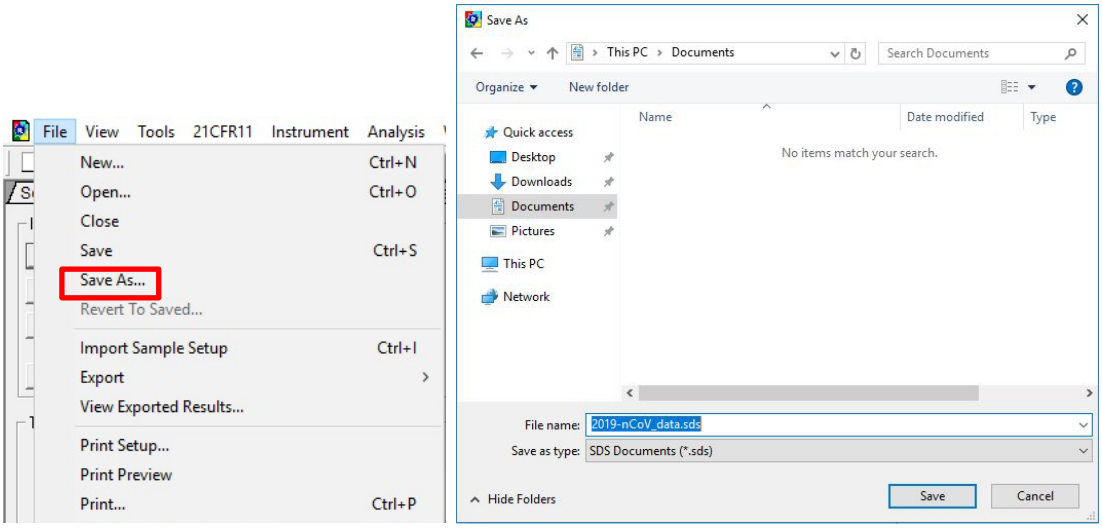
Start the Run

1. Turn on the laptop and Applied Biosystems™ 7500 Fast Dx real-time PCR system. Ensure that the laptop is connected to the instrument.

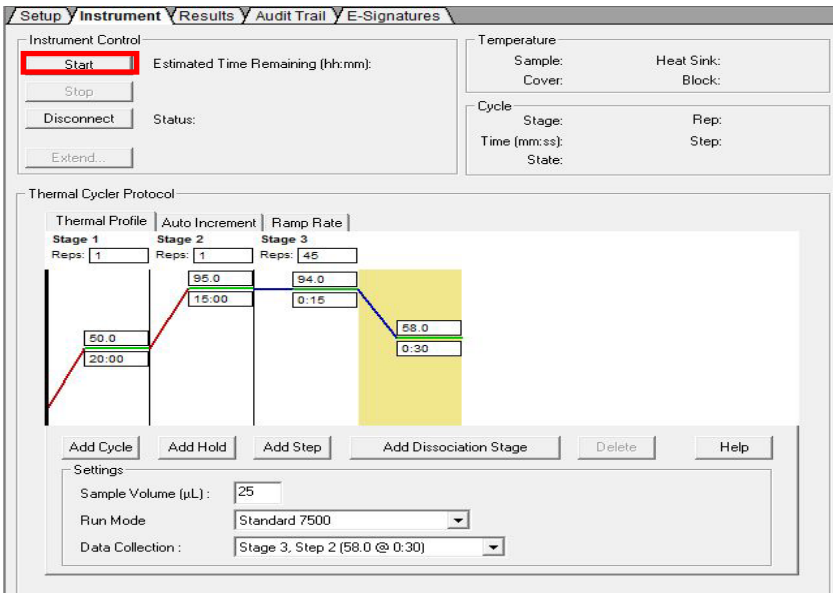
2. Push the tray door to open the instrument. Load the PCR plate onto the plate holder of the instrument.

3. Push the tray door to close the instrument.

4. Click on **File → Save As** to save the experiment in **.sds** format.



5. Click on **Start**.



Data export and analysis

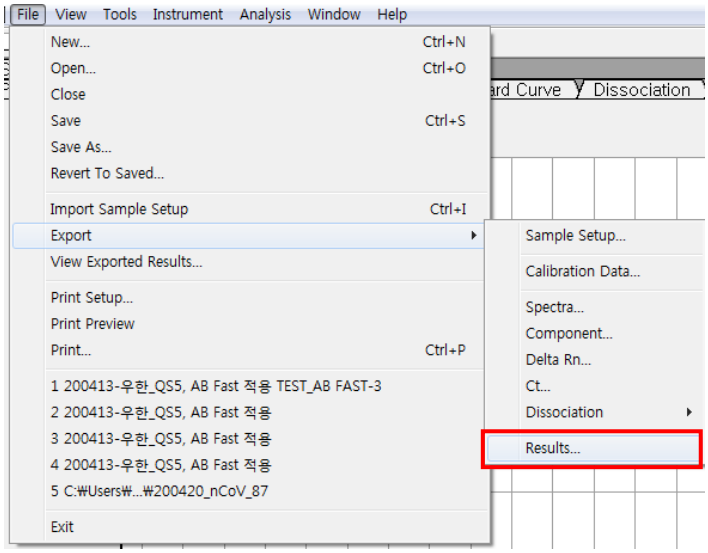
Create folders for data export

1. Create a folder to save data for all of the amplification curve detection steps from the result file.

2. Enter folder name as necessary.

Data export

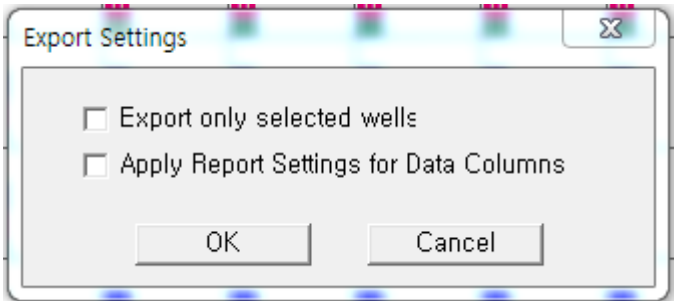
1. Click on **File** → **Export** → **Results** and select the data file to export.



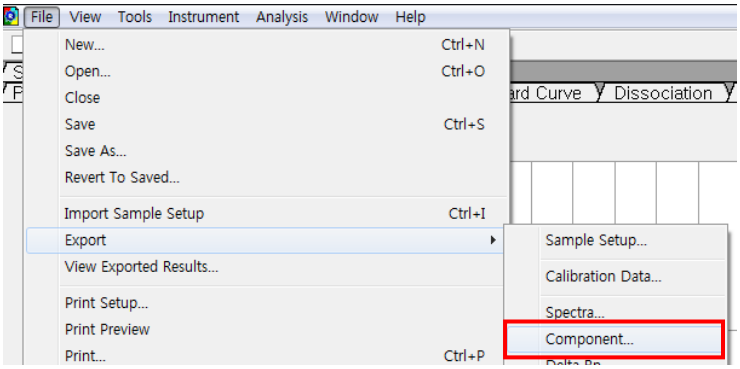
2. Select the location to save exported files and enter '_Result' at the end of the file name. Then click on **Save**.

e.g. 200420-Allplex 2019-nCoV Assay_AB FAST_Result

3. In Export Settings, click on **OK** without checking the boxes.



4. After exporting the Ct file, export data containing graphs by clicking on **File → Export → Component.**



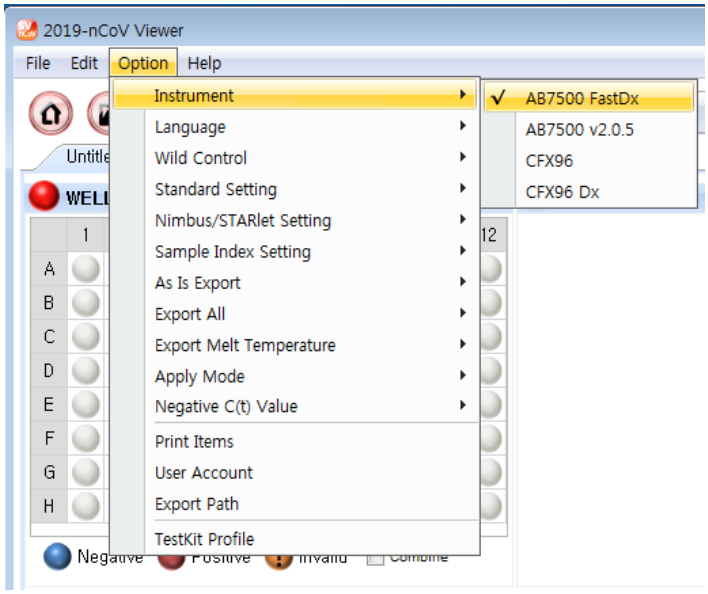
5. Select the location to save exported files, and enter '_Result-g' at the end of the file name. Then click on **Save.**

e.g. 200420-Allplex 2019-nCoV Assay_AB FAST_Result-g

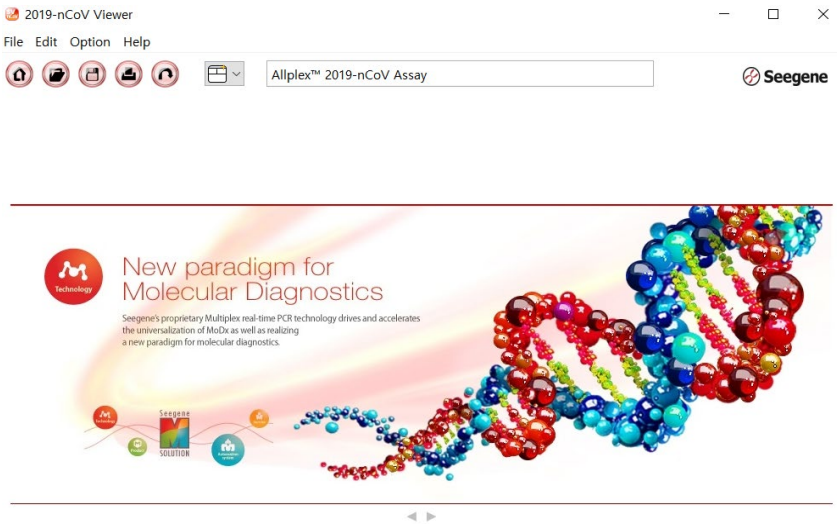
Data analysis

1. Open the Seegene Viewer software installed on the laptop connected to the Applied Biosystems™ 7500 Fast Dx

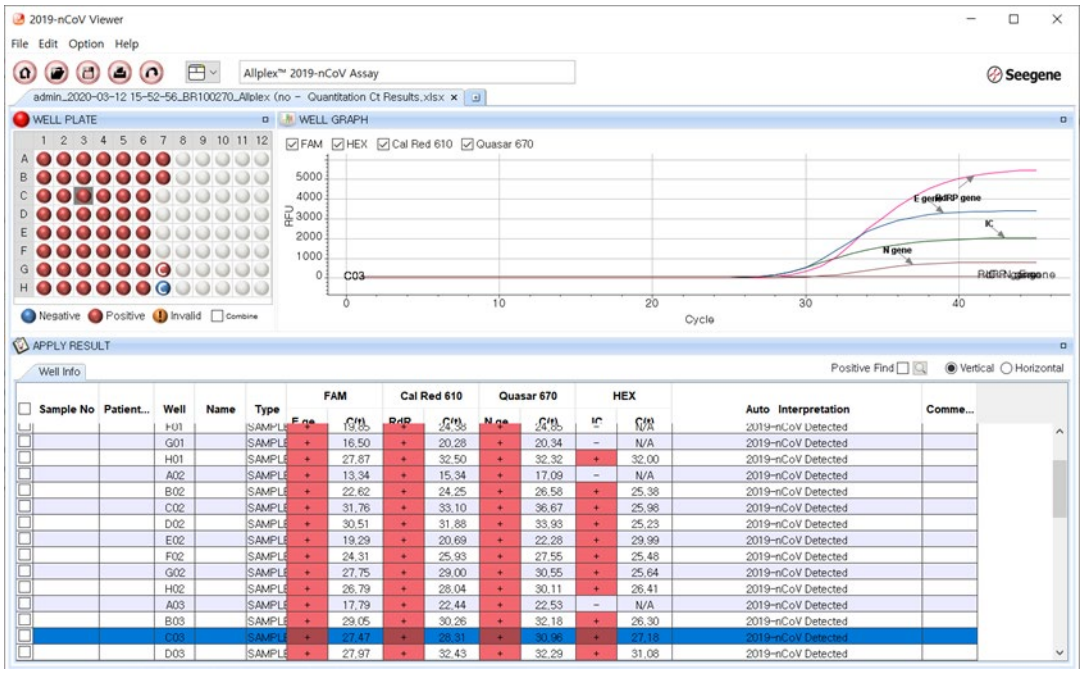
2. Click on **Option** to select AB7500 FastDx from the **Instrument** menu.



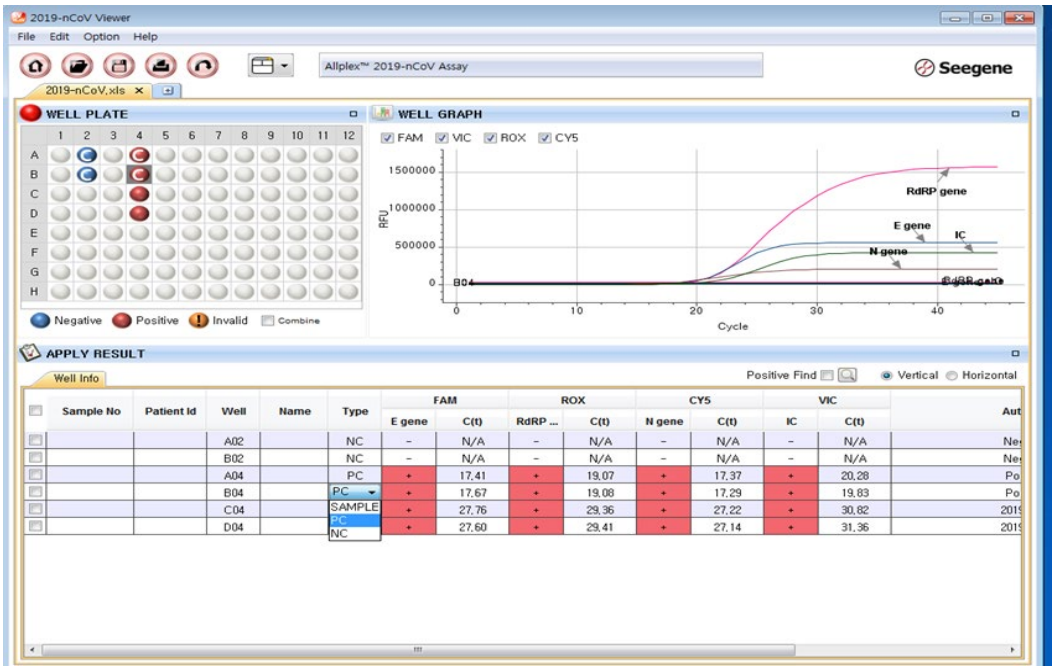
3. Click on the **Open** icon and locate the Applied Biosystems™ 7500 Fast Dx export data where the Applied Biosystems™ 7500 data was saved. The name of the file should end in '_Result'



4. After opening the results file, select 'Allplex™ 2019-nCoV Assay (FDA EUA only)' from the PRODUCT menu.



5. Assign Positive and Negative Control accordingly by selecting PC and NC under the Type drop-down menu.



6. View test results. The auto-interpreted results for each sample can be viewed by clicking on each well.

CHAPTER 9: Interpretation of Results

All PCR controls should be examined prior to interpretation of patient results. If the controls are invalid, the patient results cannot be interpreted and reported.

One Negative Control and one Positive Control are processed with each run.

The results are analyzed by the Seegene Viewer software. Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. The results are validated using the Seegene Viewer auto-interpretive software based on performance of the Positive Control and Negative Control. In cases of validity failure, the sample results should not be interpreted or reported, and the run must be repeated.

The Seegene Viewer software is installed on a separate computer that is interfaced with the Bio-Rad CFX96™ or Thermofisher Scientific Applied Biosystems™ 7500/7500 Fast Dx. The results are exported and transferred to the Seegene Viewer according to instructions under the section of 'Procedure: application and detection' provided for each instrument.

The auto-interpreted results can be exported to obtain a report in a preferred format (such as excel or pdf).

Seegene Viewer software (V 3.20 or higher) is provided by Seegene Technologies (California, US), support@seegenetech.com.

Result interpretation for clinical specimens is presented in Table 8.

Table 8. Result interpretation, clinical specimens

Ct value	Result
≤ 40	Detected (+)
> 40 or N/A	Not detected (-)

Potential Result Type	IC (HEX)	E gene (FAM)	RdRP gene (CalRed 610)	N gene (Quasar 670)	Auto-Interpretation	Interpretation/Further Actions
Case 1	+/-	+	+	+	2019-nCoV Positive	All Target Results are valid. 2019-nCoV (SARS-CoV-2) RNA is Detected.
Case 2	+/-	+	-	+	2019-nCoV Positive	All Target Results are valid. 2019-nCoV (SARS-CoV-2) RNA is Detected. Missing amplification of individual targets may be due to: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors.
Case 3	+/-	+	+	-		
Case 4	+/-	-	+	+		
Case 5	+/-	-	-	+		
Case 6	+/-	-	+	-		
Case 7	+/-	+	-	-	Presumptive positive for 2019-nCoV	All Target Results are valid. Sarbecovirus RNA is detected but 2019-nCoV (SARS-CoV-2) specific RNA targets are not detected. Repeat testing. For samples with the same result on a repeated test, additional confirmatory testing may be conducted, if it is necessary to differentiate between 2019-nCoV (SARS-CoV-2) and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. Missing amplification of the 2019-nCoV (SARS-CoV-2) specific targets may be due to: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors.
Case 8	+	-	-	-	Negative	All Target Results are valid. 2019-nCoV (SARS-CoV-2) RNA is Not Detected.
Case 9	-	-	-	-	Invalid	Results are invalid. Repeat test. If the result is still invalid, a new specimen should be obtained.

CHAPTER 10: Assay Limitations

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

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

- The performance of the Allplex™ 2019-nCoVAssay was established using nasopharyngeal swab, oropharyngeal swab and sputum samples. Anterior nasal swabs and mid-turbinate nasal swabs are also considered acceptable specimen types for use with the Allplex™ 2019-nCoV Assay but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
  https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2

- SARS-CoV-2 may mutate in one or more of the target regions of the Allplex™ 2019-nCoV Assay. If this occurs, then SARS-CoV-2 may not be detected.

- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the Allplex™ 2019-nCoV Assay. SARS-CoV is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.

- Samples must be collected, transported, and stored using appropriate procedures and conditions.

- False negative results may arise from improper specimen collection, handling, and degradation of the viral RNA during shipping/storage.

- Detection of viral RNA may not indicate the presence of the infectious virus or that 2019-nCoV is the causative agent for clinical symptoms.

- 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.

- Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

- False positive results may happen from cross- contamination between patient samples, specimen mix-up and RNA contamination during product handling.

- False-negative results may arise from:
    - Improper sample collection
    - Degradation of the viral RNA during shipping/storage
    - Specimen collection after nucleic acid can no longer be found in the specimen matrix
    - The presence of RT-PCR inhibitors
    - Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use

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

    A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

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

CHAPTER 11: Conditions of Authorization for Laboratory

The Allplex™ 2019-nCoV Assay 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-molecular-diagnostic-tests-sars-cov-2. However, to assist clinical laboratories using the Allplex™ 2019-nCoV Assay, the relevant Conditions of Authorization are listed below.

1. Authorized laboratories[1] using the Allplex™ 2019-nCoV Assay must include with result reports of the Allplex™ 2019-nCoV Assay, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

2. Authorized laboratories using the Allplex™ 2019-nCoV Assay must perform the Allplex™ 2019-nCoV Assay as outlined in the Allplex™ 2019-nCoV Assay Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Allplex™ 2019-nCoV Assay are not permitted.

3. Authorized laboratories that receive the Allplex™ 2019-nCoV Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

4. Authorized laboratories using the Allplex™ 2019-nCoV Assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

5. Authorized laboratories must collect information on the performance of Allplex™ 2019-nCoV Assay and report to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Seegene Technologies (support@seegenetech.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 Allplex™ 219-nCoV Assay must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the Allplex™ 2019-nCoV Assay in accordance with the authorized labeling.

7. Seegene Inc., its authorized distributor(s) and authorized laboratories using the Allplex™ 2019-nCoV Assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

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

CHAPTER 12: Performance Evaluation

Limit of Detection (LoD) - Analytical Sensitivity

1. A study was conducted to evaluate the LoD of the Allplex™ 2019-nCoV Assay on different real time PCR instruments using SARS-CoV-2 reference RNA material (AccuPlex SARS-COV-2 Reference Material Kit, Seracare Life Sciences, Inc., Cat no. 0505-0126). All sample replicates were prepared by spiking the reference RNA material into negative clinical sputum matrix. An initial- range-finding study was performed and included five replicates at each of four different analyte concentrations (i.e., 1.2X LoD, 1X LoD, 0.1X LoD, and 0.01X LoD based on preliminary LoD testing using an alternate RNA material). An additional 20 replicates were evaluated at a concentration level where all targets were detected in the range finding study as well as at a 3-fold lower concentration to establish the LoD. The final LoD for each target was confirmed to be the lowest concentration for which at least 19/20 replicates were detected.

2. Specimen extraction was performed using the STARMag 96 X 4 Universal Cartridge Kit and the Microlab STARlet IVD instrument. Real-time RT-PCR was performed using the CFX96™, and CFX96 Touch™ Real-time PCR Detection Systems (Bio-Rad), and the Applied Biosystems™ 7500 and 7500 Fast Dx (ThermoFisher Scientific) real-time PCR systems. The LoD of each SARS-CoV-2 target is shown in Table 9.

Table 9. LoD of each target gene

PCR instrument	Target	Positive Rate	Limit of Detection	Unit
CFX96™	E gene	20/20	4,167	Copies/mL
	RdRP gene	19/20	1,250	Copies/mL
	N gene	20/20	4,167	Copies/mL
CFX96 Touch™	E gene	20/20	4,167	Copies/mL
	RdRP gene	20/20	4,167	Copies/mL
	N gene	20/20	4,167	Copies/mL
Applied Biosystems™ 7500	E gene	20/20	4,167	Copies/mL
	RdRP gene	20/20	4,167	Copies/mL
	N gene	20/20	4,167	Copies/mL
	E gene	20/20	4,167	Copies/mL

PCR instrument	Target	Positive Rate	Limit of Detection	Unit
Applied Biosystems™ 7500 Fast Dx	RdRP gene	20/20	4,167	Copies/mL
	N gene	20/20	4,167	Copies/mL

3. The final LoD of the Allplex™ 2019-nCoV Assay on CFX96™, and CFX96 Touch™ Real-time PCR Detection Systems (Bio-Rad), Applied Biosystems™ 7500, and Applied Biosystems™ 7500 Fast Dx (ThermoFisher Scientific) is confirmed as in Table 10 following the result interpretation criteria in Table 8.

Table 10. LoD Summary of the Allplex™ 2019-nCoV Assay on different real time PCR instruments

PCR instrument	Limit of Detection	Unit
CFX96™	4,167	Copies/mL
CFX96 Touch™	4,167	Copies/mL
Applied Biosystems™ 7500	4,167	Copies/mL
Applied Biosystems™ 7500 Fast Dx	4,167	Copies/mL

4. A study was conducted to evaluate the LoD of the Allplex™ 2019-nCoV Assay using additional extraction methods. Samples were prepared by spiking the same Accuplex SARS-CoV-2 reference material (catalog no. 0505-0126) into pooled lower (sputum) respiratory negative sample matrix. As the RNA target concentration of the Seracare material is 5,000 copies/mL, an initial- range-finding study was performed and included five replicates at each of four different analyte concentrations (i.e., 1.2X LoD, 1X LoD, 0.1X LoD, and 0.01X LoD). The tentative LoD was determined followed by confirmatory LoD evaluation (20 replicates spiked at 1X LoD) for lower (sputum) respiratory negative sample matrix. If 20/20 replicates were detected in the confirmatory LoD testing, the next lower concentration, using 3-fold dilution, was tested until <100% detection was observed. The confirmed LoD, defined as the lowest SARS-CoV-2 target concentration with ≥95% detection, is presented in Table 11 for each extraction method.

Table 11. LoD Summary of the Allplex™ 2019-nCoV Assay using different extraction methods

Manufacturer	Instrument	Extraction Kit	Limit of Detection (Copies/mL)
Seegene	Seegene STARlet (65415-03)	STARMag 96 X 4 Universal Cartridge Kit	4,167
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	4,167
Hamilton	Microlab STARlet IVD (173000-075)	STARMag 96 X 4 Universal Cartridge Kit	4,167
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	4,167
Seegene	Seegene NIMBUS (67930-03)	STARMag 96 X 4 Universal Cartridge Kit	4,167
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	4,167
Hamilton	Microlab NIMBUS IVD (65415-02)	STARMag 96 X 4 Universal Cartridge Kit	4,167
		STARMag 96 X 4 Viral DNA/RNA 200 C Kit	4,167
LG Chem	AdvanSure E3 System (YETS0001EG)	AdvanSure NA EX Kit	4,167
GeneAll	N/A (Manual)	Ribospin vRD (Viral RNA/DNA Extraction Kit)	4,167
QIAGEN	N/A (Manual)	QIAmp DSP Viral RNA Mini Kit	4,167
Roche	MagNA Pure 96 (MP96)	DNA and Viral NA Small Volume Kit	4,167
ThermoFisher Scientific	KingFisher Flex automated extraction	MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (200uL of sample is used)	4,167

5. FDA SARS-CoV-2 Reference Panel

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using the 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 corroborate the LoD of the Allplex™ 2019-nCoV assay. The extraction method and instrument used were the Seegene STARlet with CFX96 Touch. The results are summarized in Table 12.

Table 12: 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	6,000 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not Applicable

ND: Not Detected

6. LOD Study with WHO International Standard for SARS-CoV-2 RNA and Additional Consumable/PCR Instrument Equivalency

An LOD study was conducted to re-establish the reagent performance for the current assay system and consumables described in the table below using the First WHO International Standard for SARS-CoV-2 RNA (Cat# 20/146), acid-heat inactivated England/02/2020 isolate of SARS-CoV-2, spiked in the most challenging claimed matrix, clinical sputum matrix.

The preliminary LoD study was conducted using three-fold serial dilution panels made with inactivated SARS-CoV-2 virus in sputum. Triplicate of each panel member were extracted using Seegene STARlet and tested on the CFX96 Touch™. The preliminary LoD study results are presented in the table below:

Sample Matrix	Sample Concentration (IU/ml)	Replicate	C_t Results				SARS-CoV-2 Final Interpretation	Result percentile % (No. of positive / No. of replicate)
			E gene	RdRP gene	N gene	Exo IC		
Sputum	48600	1	28.41	30.48	32.87	26.85	Detected	100% (3/3)
		2	27.81	30.10	32.15	26.23	Detected	
		3	28.55	30.71	32.47	27.02	Detected	
	16200	1	29.30	31.72	33.83	26.16	Detected	100% (3/3)
		2	29.34	31.50	33.87	26.40	Detected	
		3	29.51	32.41	34.76	27.21	Detected	
	5400	1	30.98	34.05	35.32	26.45	Detected	100% (3/3)
		2	31.02	33.97	35.74	26.27	Detected	
		3	32.44	35.92	36.49	26.99	Detected	
	1800	1	32.48	34.43	38.68	26.13	Detected	100% (3/3)
		2	35.14	34.57	37.61	26.07	Detected	
		3	35.58	N/A	39.69	26.96	Detected	
	600	1	N/A	N/A	N/A	26.25	Not Detected	66% (2/3)
		2	N/A	N/A	38.52	26.54	Detected	
		3	N/A	37.15	N/A	27.50	Detected	
	200	1	N/A	N/A	N/A	26.50	Not Detected	66% (2/3)
		2	N/A	35.92	N/A	26.18	Detected	
		3	N/A	N/A	39.64	26.50	Detected	
-	Positive Control	1	19.99	19.48	19.55	18.34	Valid	-
		2	19.91	19.39	19.42	18.30	Valid	
		3	19.92	19.33	19.32	18.29	Valid	
	Negative Control	1	N/A	N/A	N/A	N/A	Valid	-

The LoD was confirmed by testing twenty (20) replicates of the lowest three-fold dilution that produced 100% (3/3) positive results for all 3 genes in the preliminary LoD study. If all twenty (20) replicate results were positive in the confirmatory LoD testing, the next lower concentration, using a two-fold dilution, was tested until <100% detection was observed. The lowest concentration with ≥95% positive result was reported as the confirmed LoD. The confirmed LoD was also tested with other instruments and consumables. The worst LoD across all instruments and consumables was 1350 IU/ml. The confirmatory LoD study results are presented in the table below:

Instrument	Consumables	Sample Concentration (IU/mL)	Result Percentile % (No. of positive / No. of replicates
CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)	Hard-Shell® PCR plates 96-well WHT/WHT (Bio-Rad) + Permanent Clear Heat Seal (Bio-Rad)	5400	100% (20/20)
		2700	100% (20/20)
		1350	**100% (20/20)**
		675	75% (15/20)[1]
	Hard-Shell® PCR plates 96-well WHT/WHT (Bio-Rad) + Optical Flat 8-Cap Strips (Bio-Rad)	5400	100% (20/20)
		2700	100% (20/20)
		1350	**100% (20/20)**
		675	70% (14/20)[1]
	0.1ml 96-well PCR Plate Half Skirt (YongYue) + Sealing Film (YongYue)	5400	95% (19/20)[1]
		2700	100% (20/20)
		1350	100% (20/20)
		675	**95% (19/20)**
		338	55% (11/20)[1]
CFX96™ Dx Real-Time PCR Detection System (Bio-Rad)	Hard-Shell® PCR plates 96-well WHT/WHT (Bio-Rad) + Permanent Clear Heat Seal (Bio-Rad)	5400	100% (20/20)
		2700	100% (20/20)
		1350	**100% (20/20)**
		675	70% (14/20)[1]
CFX Opus 96 Dx Real-Time PCR Detection System (Bio-Rad)	Hard-Shell® PCR plates 96-well WHT/WHT (Bio-Rad) + Permanent Clear Heat Seal (Bio-Rad)	5400	100% (20/20)
		2700	100% (20/20)
		1350	100% (20/20)
		675	**100% (20/20)**
		338	85% (17/20)[1]

[1] Presumptive positive tests were not repeated and were considered as negative for the analysis.

The following table includes LoD result details, including mean Ct values for each assay target when the Allplex 2019-nCoV Assay is run on the CFX96™ Touch system using the Hard-Shell PCR plates 96-well WHT/WHT (Bio-Rad) with Permanent Clear Heat Seal (Bio-Rad);

Concentration (copies/mL)	E gene Detection Rate (Mean Ct)	RdRp Gene Detection Rate (Mean Ct)	N gene Detection Rate (Mean Ct)	IC Detection Rate (Mean Ct)	Detection Rate for SARS-CoV-2
10,800	20/20 (29.6)	20/20 (31.3)	20/20 (33.6)	20/20 (25.9)	100% (20/20)
5,400	20/20 (30.4)	20/20 (32.2)	20/20 (34.4)	20/20 (25.7)	100% (20/20)
2,700	20/20 (31.7)	20/20 (33.3)	20/20 (35.5)	20/20 (25.8)	100% (20/20)
1350	**18/20 (33.1)**	**17/20 (34.4)**	**20/20 (36.7)**	**20/20 (26.0)**	**100% (20/20)**
675	5/20 (35.7)	7/20 (35.5)	13/20 (38.8)	20/20 (27.9)	75% (15/20)*
338	2/20 (35.9)	10/20 (35.7)	11/20 (39.4)	20/20 (27.9)	75% (15/20)**

*One presumptive positive, four negatives
**Five negatives

The following table includes LoD result details, including mean Ct values for each assay target when the Allplex 2019-nCoV Assay is run on the CFX96™ Touch system using the Hard-Shell PCR plates 96-well WHT/WHT (Bio-Rad) with Optical Flat 8-Cap Strips (Bio-Rad);

Concentration (copies/mL)	E gene Detection Rate (Mean Ct)	RdRp Gene Detection Rate (Mean Ct)	N gene Detection Rate (Mean Ct)	IC Detection Rate (Mean Ct)	Detection Rate for SARS-CoV-2
10,800	20/20 (29.8)	20/20 (31.8)	20/20 (33.8)	20/20 (26.4)	100% (20/20)
5,400	20/20 (30.6)	20/20 (32.8)	20/20 (34.7)	20/20 (26.3)	95% (19/20)
2,700	20/20 (31.7)	20/20 (33.6)	20/20 (35.8)	20/20 (26.3)	100% (20/20)
1350	**20/20 (33.2)**	**19/20 (34.5)**	**20/20 (37.3)**	**20/20 (26.7)**	**100% (20/20)**
675	11/20 (35.8)	9/20 (35.8)	11/20 (38.9)	20/20 (28.6)	70% (14/20)*
338	3/20 (36.5)	5/20 (36.1)	7/20 (39.3)	20/20 (28.6)	55% (11/20)**

*Three presumptive positives, three negatives
**Two presumptive positives, seven negatives

The following table includes LoD result details, including mean Ct values for each assay target when the Allplex 2019-nCoV Assay is run on the CFX96™ Touch system using the the 0.1 ml 96-well PCR plate Half Skirt (YongYue)+ Sealing film (YongYue):

Concentration (copies/mL)	E gene Detection Rate (Mean Ct)	RdRp Gene Detection Rate (Mean Ct)	N gene Detection Rate (Mean Ct)	IC Detection Rate (Mean Ct)	Detection Rate for SARS-CoV-2
10,800	19/20 (30.7)	19/20 (33.2)	20/20 (34.9)	20/20 (26.2)	100% (20/20)
5,400	20/20 (31.3)	18/20 (33.9)	19/20 (35.6)	20/20 (26.7)	95% (19/20)
2,700	20/20 (31.0)	20/20 (33.6)	20/20 (35.3)	20/20 (26.3)	100% (20/20)
1,350	20/20 (31.3)	20/20 (33.7)	20/20 (35.7)	20/20 (26.2)	100% (20/20)
675	**11/20 (35.4)**	**8/20 (35.2)**	**18/20 (38.5)**	**20/20 (27.1)**	**95% (19/20)***
338	4/20 (35.8)	3/20 (35.7)	9/20 (38.7)	20/20 (27.3)	55% (11/20)**

*One negative
** One presumptive positive, eight negatives

The following table includes LoD result details, including mean Ct values for each assay target when the Allplex 2019-nCoV Assay is run on the CFX96™ Dx system

using the Hard-Shell PCR plates 96-well WHT/WHT (Bio-Rad) with Permanent Clear Heat Seal (Bio-Rad);

Concentration (copies/mL)	E gene Detection Rate (Mean Ct)	RdRp Gene Detection Rate (Mean Ct)	N gene Detection Rate (Mean Ct)	IC Detection Rate (Mean Ct)	Detection Rate for SARS-CoV-2
10,800	20/20 (29.8)	20/20 (32.1)	20/20 (33.8)	20/20 (26.3)	100% (20/20)
5,400	20/20 (30.6)	20/20 (33.1)	20/20 (34.8)	20/20 (26.3)	100% (20/20)
2,700	20/20 (32.1)	20/20 (33.9)	20/20 (36.3)	20/20 (26.5)	100% (20/20)
1,350	**19/20 (33.7)**	**19/20 (34.9)**	**20/20 (37.5)**	**20/20 (26.5)**	**100% (20/20)**
675	6/20 (36.6)	6/20 (36.5)	12/20 (38.8)	20/20 (28.0)	70% (14/20)*
338	3/20 (36.6)	4/20 (36.7)	4/20 (38.9)	20/20 (28.1)	35% (7/20)**

* Two presumptive positives, four negatives
**Three presumptive positives, ten negatives

The following table includes LoD result details, including mean Ct values for each assay target when the Allplex 2019-nCoV Assay is run on the CFX Opus 96 Dx system using the Hard-Shell PCR plates 96-well WHT/WHT (Bio-Rad) with Permanent Clear Heat Seal (Bio-Rad);

Concentration (copies/mL)	E gene Detection Rate (Mean Ct)	RdRp Gene Detection Rate (Mean Ct)	N gene Detection Rate (Mean Ct)	IC Detection Rate (Mean Ct)	Detection Rate for SARS-CoV-2
10,800	20/20 (30.6)	20/20 (31.9)	20/20 (33.4)	20/20 (26.2)	100% (20/20)
5,400	20/20 (31.1)	20/20 (32.6)	20/20 (33.8)	20/20 (26.0)	100% (20/20)
2,700	20/20 (32.5)	20/20 (33.4)	20/20 (34.7)	20/20 (26.1)	100% (20/20)
1,350	18/20 (34.0)	19/20 (34.3)	20/20 (35.7)	20/20 (26.3)	100% (20/20)
675	**11/20 (33.5)**	**16/20 (34.9)**	**20/20 (36.7)**	**20/20 (26.3)**	**100% (20/20)**
338	12/20 (34.8)	10/20 (35.2)	15/20 (37.3)	20/20 (26.6)	85% (17/20)*

* One presumptive positive, two negatives

Inclusivity (Analytical Sensitivity)

Inclusivity was evaluated using in silico analysis to analyze the sequences of the RdRP, N, and E gene primer/probe sets for homology with 16,667 full length sequences available in the GISAID database (http://gisaid.org) and 3490 full length sequences available in the NCBI database (https://ncbi.nhm.nih.gov/datasets/coronavirus/genomes/) as of May 13, 2020. The *in-silico* analysis generated 9 cases where there were less than 100% homology in the primer/probe region. All cases had mismatches in only one of the target regions and is predicted to not impact the detection of SARS-CoV-2. All 9 cases were wet-tested using *in vitro* transcribed RNA containing the specific variant/mismatch sequences and demonstrated that all 9 cases were detected at 3x LoD.

During the omicron surge, an additional *in-silico* analysis was performed based on the GISAID database added in the US for the time period of February 23, 2022 through March 25, 2022. The sequences of the RdRP, N, and E gene primer/probe sets were analyzed for homology with the 33,388 SARS-CoV-2 sequences. The result of the *in-silico* analysis found two sequences with less than 100% homology with greater than 5% prevalence in the time-period (one 1-mer mismatch in the N gene forward primer, one 1-mer mismatch in the E gene forward primer). Both sequences were wet tested using *in vitro* transcribed RNA containing the specific variant/mismatch sequences and demonstrated that both cases were detected at 3x LoD. In all cases where mismatches were observed in the *in-silico* analysis, the mismatches were in only one of the target regions and is predicted to not impact the detection of SARS-CoV-2.

In silico analysis was also performed on three Omicron sub-lineage sequences (BA.4, BA.5, and BA.275) in the GISAID database as of August 25, 2022. The sequences of the RdRP, N, and E gene primer/probe sets were analyzed for homology with the 73,770 SARS-CoV-2 sequences (BA.2.75, n = 3,680; BA.4, n=41,063; BA.5, n=29,027). In all cases where mismatches were observed in the *in-silico* analysis, the mismatches were in only one of the target regions, or the mismatch was demonstrated to be detected through wet-testing. Therefore, all sequences analyzed are predicted to not impact the detection of SARS-CoV-2.

Cross-reactivity (Analytical Specificity)

Evaluation of Cross-reactivity with high priority pathogens

In silico analysis was performed to evaluate the potential for cross-reactivity of the Allplex™2019-nCoV Assay targets with pathogens listed in Table 13 that may be encountered in clinical respiratory specimens. In addition, the pathogens listed in Table 15, were also wet tested.

Table 13. List of pathogens analyzed *in silico*

Other high priority pathogens from the same genetic family	High priority pathogens likely in the circulating area
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

In silico analysis test results

Cross-reactivity of the Allplex™ 2019-nCoV Assay was evaluated by *in silico* analysis and cross-reactivity was defined as greater than 80% homology between 'oligo set' and any sequence present in the targeted microorganism as table above. Cross-reaction is likely to occur when first, the amplicon size is below 500 bp, and second, when the homology of the binding site between the oligo set (forward primer, reverse primer, and probe) and the microorganism is greater or equal to 80% (Table 14. *In silico* analysis results of targeted pathogens).

Table 14. *In silico* analysis results of targeted pathogens

Pathogen	RdRP gene	E gene	N gene	Complex
Human coronavirus 229E	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human coronavirus OC43	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human coronavirus HKU1	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human coronavirus NL63	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
SARS-coronavirus	Amp. Mis. #	100% Match*	Amp. Mis. #	Amp. Mis. #
MERS-coronavirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Adenovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human Metapneumovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 1	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 2	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 3	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 4	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Influenza A virus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Influenza B virus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Enterovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Respiratory syncytial virus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Rhinovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Chlamydia pneumoniae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #

Pathogen	RdRP gene	E gene	N gene	Complex
Hemophilus influenzae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Legionella pneumophila	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Mycobacterium tuberculosis	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Streptococcus pneumoniae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Streptococcus pyogenes	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Bordetella pertussis	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Mycoplasma pneumoniae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Pneumocystis gynoecia (PJP)	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Candida albicans	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Pseudomonas aeruginosa	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Staphylococcus epidermis	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Streptococcus salivarius	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #

NOTE:
(*) E gene covers 100% of SARS-coronavirus (taxonomy ID: 694009)
(#) Amp. Mis: Amplicon mismatch. Amplicon is not predicted to be formed. The combination of assay oligos with each microorganism did not achieve above 80% homology.

As a result of the analysis, there were no microorganisms with potential non-specific or cross-reactive sequences except for E gene target sequences that showed a 100% match with SARS-coronavirus. E gene is a target gene for Sarbecovirus, so the results of the *in silico* analysis are expected (see Table 8 for result interpretation).

The Allplex™ 2019-nCoV Assay was further evaluated for potential cross-reactivity by wet-testing a total of 60 pathogens as well as pooled human nasal wash (Table 15). The bacterial species were tested at ≥ 1 x 10^6 CFU/mL, and viral species at ≥ 1 x 10^5 PFU/mL or 1 x 10^6 genome copies/rxn.

Testing with the Allplex™ 2019-nCoV Assay was performed in triplicate for each organism under the same conditions. None of the 60 pathogens or the

pooled human nasal wash generated detectable signals with SARS-CoV-2 targets of the Allplex™ 2019-nCoV Assay.

Table 15. List of pathogens evaluated by wet testing

No.	Usage	Pathogen	Source	Isolate No.
1	Exclusivity	human coronavirus HKU1	Korean isolate	
2	Exclusivity	human coronavirus OC43	ATCC	VR-1558
3	Exclusivity	human coronavirus NL63	Korean isolate	
4	Exclusivity	human coronavirus 229E	ATCC	VR-740
5	Exclusivity	human Severe Acute Respiratory Syndrome, SARS	Korean isolate	
6	Exclusivity	human Middle East Respiratory Syndrome Coronavirus: MERS-CoV	Korean isolate	
7	Exclusivity	influenza A virus (H1N1)	ATCC	VR-95
8	Exclusivity	Influenza A virus (H3N2)	ATCC	VR-547
9	Exclusivity	influenza B virus	ATCC	VR-523
10	Exclusivity	Human Rhinovirus 1	KBPV	VR-81
11	Exclusivity	Rhinovirus 21	KBPV	VR-40
12	Exclusivity	Human rhinovirus type 90	ATCC	VR-1291
13	Exclusivity	Human rhinovirus type 16	ATCC	VR-283
14	Exclusivity	Human rhinovirus type 42	ATCC	VR-338
15	Exclusivity	Human rhinovirus type 8	ATCC	VR-488
16	Exclusivity	Human rhinovirus type 14	ATCC	VR-284
17	Exclusivity	Human enterovirus type 68	ATCC	VR-1826
18	Exclusivity	Human enterovirus type 70	ATCC	VR-836
19	Exclusivity	Human enterovirus type 71	ATCC	VR-784
20	Exclusivity	human respiratory syncytial virus A	ATCC	VR-26
21	Exclusivity	human respiratory syncytial virus B	ATCC	VR-955
22	Exclusivity	Parainfluenza 1 virus	ATCC	VR-1380
23	Exclusivity	Human parainfluenza virus 2	ATCC	VR-92
24	Exclusivity	Human parainfluenza virus 3	ATCC	VR-93
25	Exclusivity	human parainfluenza 4 virus 4a	ATCC	VR-1378
26	Exclusivity	Human parainfluenza virus 4b	ATCC	VR-1377
27	Exclusivity	Human Metapneumovirus (MPV)	KBPV	VR-87
28	Exclusivity	Human adenovirus 1	ATCC	VR-1
29	Exclusivity	Human adenovirus 11	KBPV	VR-63
30	Exclusivity	Human adenovirus 18	ATCC	VR-1095
31	Exclusivity	Human adenovirus 23	ATCC	VR-1101
32	Exclusivity	Human adenovirus 3	ATCC	VR-3
33	Exclusivity	Human adenovirus 4	ATCC	VR-1572

No.	Usage	Pathogen	Source	Isolate No.
34	Exclusivity	Human adenovirus 8	ATCC	VR-1368
35	Exclusivity	Human adenovirus type 31	ATCC	VR-1109
36	Exclusivity	Human adenovirus type 40	ATCC	VR-931
37	Exclusivity	Human adenovirus type 5	KBPV	VR-61
38	Exclusivity	Human adenovirus type 35	ATCC	VR-718
39	Exclusivity	*Legionella pneumophila Serotype 2*	ATCC	33154
40	Exclusivity	*Legionella pneumophila subsp. fraseri Serotype 4*	ATCC	33156
41	Exclusivity	*Legionella pneumophila Serotype 7*	ATCC	33823
42	Exclusivity	*Legionella pneumophila Serotype 10*	ATCC	43283
43	Exclusivity	*Legionella pneumophila Serotype 11*	ATCC	43130
44	Exclusivity	*Legionella pneumophila Serotype 12*	ATCC	43290
45	Exclusivity	*Legionella pneumophila Serotype 13*	ATCC	43736
46	Exclusivity	*Legionella pneumophila Serotype 14*	ATCC	43703
47	Exclusivity	*Legionella pneumophila subsp. fraseri Serotype 15*	ATCC	35251
48	Exclusivity	*Mycoplasma pneumoniae*	ATCC	15293
49	Exclusivity	*Mycoplasma pneumoniae M129-B7*	ATCC	29342
50	Exclusivity	*Chlamydophila pneumoniae*	ATCC	53592
51	Exclusivity	*Bordetella pertussis*	ATCC	BAA-589
52	Exclusivity	*Pseudomonas aeruginosa (Z139; VIM-1)*	Zeptometrix	801908
53	Exclusivity	*Mycobacterium tuberculosis*	ATCC	25177
54	Exclusivity	*Haemophilus influenzae*	ATCC	51907
55	Exclusivity	*Streptococcus pneumoniae*	KCCM	40410
56	Exclusivity	*Streptococcus pyogenes*	ATCC	19615
57	Exclusivity	*Staphylococcus epidermidis*	KCCM	40416
58	Exclusivity	*Candida albicans*	KCCM	11282
59	Exclusivity	*Pneumocystis pneumonia jirovecii (PJP)*	Korean isolate	
60	Exclusivity	*Staphylococcus salivarius*	Korean isolate	
61	Exclusivity	Pooled human nasal wash	Clinical sample	

Clinical Evaluation

In the clinical evaluation study, selected left-over archived samples from symptomatic patients suspected of COVID-19 infection were used. Specimens were previously subjected for SARS-CoV-2 testing and then stored at a clinical laboratory in South Korea prior to being included in this study. A total of 300 samples (150 upper respiratory samples, 150 lower respiratory samples); 100 positive samples (50 upper respiratory samples (NP/OP swabs in UTM), 50 sputum samples) and 200 negative samples (100 upper respiratory samples (NP/OP swabs in UTM), 100 sputum samples) were tested. The purpose of this clinical study was to assess the clinical performance of Seegene's Allplex™ 2019-nCoV Assay.

For this study, extraction was performed using the STARMag 96 X 4 Universal Cartridge Kit and the Microlab STARlet IVD instrument. Real-time RT-PCR was performed using the CFX96 Real-time PCR Detection System (Bio-Rad).

All specimens were evaluated with the Allplex™ 2019-nCoV Assay and a validated real-time PCR comparator assay. The comparator assay primers and probes were identical to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel but used alternate extraction and PCR instrumentation. The LoD of the comparator assay was shown to be equivalent to the CDC assay and therefore adequate for evaluation of clinical performance for the Allplex™ 2019-nCoV Assay.

The results from testing upper respiratory specimens including nasopharyngeal + oropharyngeal swabs shown in Table 16 generated a Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% - 100.00%], and a Negative Percent Agreement (NPA): 93.07% (94/101) [95% CI: 85.76% - 96.93%].

The results from testing lower respiratory specimens (sputum) shown in Table 17, generated Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% - 100.00%], and a Negative Percent Agreement (NPA): 96.84% (92/95) [95% CI: 90.39% - 99.18%]

Table 16. Upper respiratory samples (nasopharyngeal + oropharyngeal swab) n=150

Final results		Comparator assay			
		2019-nCoV Detected	Inconclusive	2019-nCoV Not Detected	Total
Allplex™ 2019-nCoV Assay	SARS-CoV-2 Positive	49	1[1)]	6[2)]	56
	Presumptive Positive for SARS-CoV-2	0	0	0	0
	Negative	0	0	94	94
	Total	49	1	100	150

NOTE: (1) Sequencing result was SARS-CoV-2 positive (Comparator assay: N1 positive / N2 negative)
(2) Sequencing results were SARS-CoV-2 positive for 5 cases, and SARS-CoV-2 negative for 1 remaining case.

A. Positive Percent Agreement (PPA): 100.00% (49/49)
[95% CI: 92.75% - 100.00%]
B. Negative Percent Agreement (NPA): 93.07% (94/101)
[95% CI: 85.76% - 96.93%]

Table 17. Lower Respiratory samples (Sputum) n=150

Final results		Comparator assay			
		2019-nCoV Detected	Inconclusive	2019-nCoV Not Detected	Total
Allplex™ 2019-nCoV Assay	SARS-CoV-2 Positive	49	1[1)]	2[2)]	52
	Presumptive Positive for SARS-CoV-2	0	0	0	0
	Negative	0	0	92	92
	Total	49	1	94	144

NOTE: (1) Sequencing result was SARS-CoV-2 positive. (Comparator assay: N1 negative / N2 positive)
(2) Sequencing results were all SARS-CoV-2 positive for 2 cases.

A. Positive Percent Agreement (PPA): 100.00% (49/49)
[95% CI: 92.75% - 100.00%]
B. Negative Percent Agreement (NPA): 96.84% (92/95)
[95% CI: 90.39% - 99.18%]

■ CHAPTER 13: Key to Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalog number
	Use-by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
ENZYME	Enzyme Mix
BUFFER	Buffer
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	Consult instructions for use
	Manufacturer
	Date of manufacture
	Caution
	Contains sufficient for <n> tests
Rx Only	Prescription Use only
EUA	Emergency Use Authorization
UDI	Unique Device Identifier
rxns	Reaction barcode for automated extraction system

■ CHAPTER 14: Ordering Information

The product will be distributed by Seegene Inc., located at Taewon Bldg., 91. Ogeum-ro, Songpa-gu, Seoul, Republic of Korea, 05548, and Seegene Technologies located at 325 N Wiget Ln #140, Walnut Creek, CA 94598 U.S.A.

■ CHAPTER 15: EUO Label For RUO Instruments

For the Applied BioSystems 7500, Bio-Rad CFX96 Real-Time PCR Detection System, and CFX96 Touch Real-Time PCR Detection System, affix the Emergency Use Only (EUO) label on each instrument and retain this labeling throughout the Emergency Use Authorization (EUA) use of the claimed Real-Time PCR instruments.

1. Print the following EUO label:

Emergency Use Only

This instrument is authorized for use with Seegene, Inc. Allplex™ 2019-nCoV Assay that have received Emergency Use Authorization.

Visible affix the EUO instrument verification label on your instrument. If the instrument includes labeling indicating "For Research Use Only", cover with the EUO instrument label.

Seegene Inc., Taewon Bldg., 91. Ogeum-ro, Songpa-gu, Seoul, Republic of Korea, 05548

Customer Support & Technical Support: support@seegenetech.com

For more contact information visit www.seegene.com

Rx Only | IVD

For use under Emergency Use Authorization (EUA) only

Allplex™ 2019-nCoV Assay

Product Information Card (PIC)

- This product information card does not contain the full instructions.
- Please contact support@seegenetech.com if you require a printed copy free of charge.
- Healthcare Provider (HCP) and Patient Fact Sheets and Instructions for Use can be found at the following link: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-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.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of 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. § 360bbb-3 (b)(1), unless the declaration is terminated or authorization is revoked sooner.

Rev.0, December 13 2022