Allplex™ SARS-CoV-2/ FluA/FluB/RSV Assay

(Cat no. RV10259X, RV10349Z)

Instructions for Use

For *in vitro* diagnostic use

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■ CHAPTER 1: Intended Use

The Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is an in vitro diagnostic (IVD) real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the simultaneous qualitative multiple detection and differentiation of nucleic acid from acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV) in human nasopharyngeal swab specimen from individuals with signs and symptoms of who are suspected of COVID-19, Flu, RSV by their health care provider.

Results are for the identification of SARS-CoV-2, Flu A, Flu B and RSV RNA, which are generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the single- or multiple-infection (s) of SARS-CoV-2, Flu A, Flu B and/or RSV; 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 positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, Flu A, Flu B or RSV 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™ SARS-CoV-2/FluA/FluB/RSV 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™ SARS-CoV-2/FluA/FluB/RSV Assay is only for use under the Health Canada's expedited authorization pathways for COVID-19 medical devices.

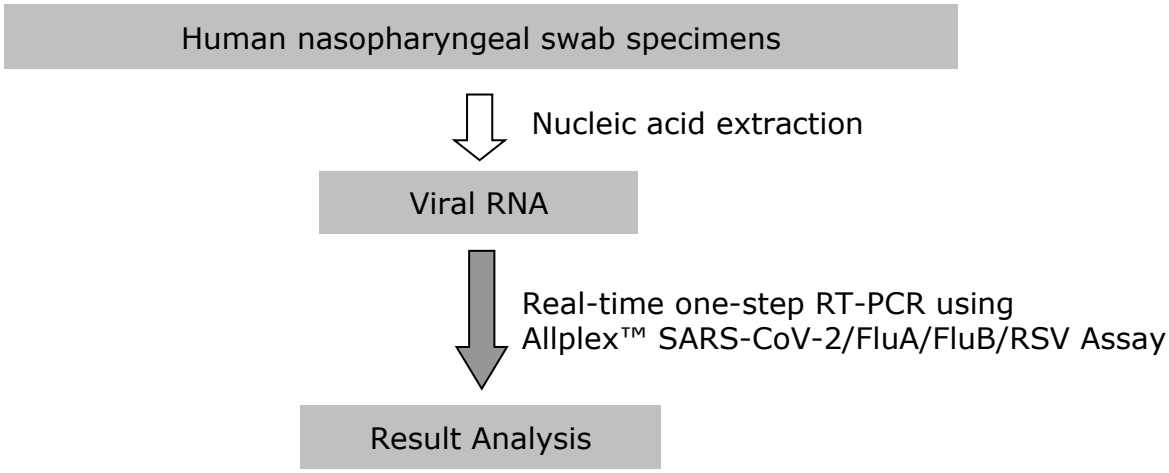
CHAPTER 2: Summary and Explanation of the Test

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is a qualitative multiplex real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay enables simultaneous amplification and differentiation of target nucleic acids of S gene, RdRP gene and N gene of SARS-CoV-2, Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV) in human nasopharyngeal swab specimen from individuals with signs and symptoms of who are suspected of COVID-19, Flu, RSV by their health care provider. The assay is designed to target three genes (S gene, RdRP gene and N gene) of the SARS-CoV-2 RNA, M gene of Flu A RNA and NS2 gene of Flu B RNA as well as M gene of RSV RNA. This assay also uses two internal controls (Exogenous and Endogenous) to monitor all steps of the analysis process, including sample collection from a patient, RNA extraction, reverse transcription, and PCR to demonstrate proper sample collection and test validity of each specimen in a single reaction.

To prevent amplification product from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay. The UDG-dUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond.

■ CHAPTER 3: Principle of the Procedure

Nucleic acids are isolated and purified from a specimen using an automated nucleic acid extraction system. 10 µL of Exogenous Internal Control (RP-V IC 2) must be added before the extraction. Follow detailed extraction procedures in manufacturer's instructions. 10 µL of purified nucleic acid is reverse transcribed using SC2FabR MOM (oligo mix)/EM8 (RTase, DNA polymerase, UDG and buffer containing dNTPs) into cDNA which is then subsequently amplified by a CFX96™ IVD, CFX96™ Dx or CFX96 Touch™ real-time PCR system. To perform the multiple target amplification and detection with superior accuracy in a single reaction well, this assay kit employ Seegene's innovative proprietary DPO™, TOCE™ and MuDT™ technologies. During the process, the TOCE™-Pitcher probe anneals to a specific target sequence located between the DPO™-forward and DPO™-reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase cleaves target-bound Pitcher probe and releases the unlabeled extender in the Pitcher probe which specially serves as a primer for an artificial template, a quenched-florescent molecule, the TOCE™-Catcher. Annealing and extension of the extender on the Catcher generates Duplex Catcher, resulting in a fluorescence signal that is directly correlated to the quantity of the target DNA (Ref and figure adding to draw PCR). Fluorescence intensity is monitored at each PCR cycle by the CFX96™ IVD, CFX96™ Dx or CFX96 Touch™ real-time PCR detection systems. The result of amplification is reported through 'Seegene Viewer' analysis. The 'Seegene Viewer' shows whether the exported data is SARS-CoV-2, Flu A, Flu B and/or RSV detected, invalid or negative for easy retrieval of result by the user.



■ CHAPTER 4: Assay Materials

Materials provided

The reagents contained in one Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay kit are sufficient for 100/25 reactions.

Table 1-1. Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay Composition (100 rxn)

Contents	Volume (RV10259X)	Description
SC2FabR MOM	500 µL	MuDT* Oligo Mix (MOM): - Amplification and detection reagent *MuDT is the brand name of Seegene's novel analytical technology.
EM8	500 µL	Enzyme mix and buffer for one-step RT-PCR
SC2FabR PC	100 µL	Positive Control (PC) for PCR control: - Mixture of pathogen and IC clones
RP-V IC 2	1,000 µL	Exogenous Internal Control (IC)
RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade -Negative Control (NC) for PCR control

Table 1-2. Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay Composition (25 rxn)

Contents	Volume (RV10349Z)	Description
SC2FabR MOM	125 µL	MuDT* Oligo Mix (MOM): - Amplification and detection reagent *MuDT is the brand name of Seegene's novel analytical technology.
EM8	125 µL	Enzyme mix and buffer for one-step RT-PCR
SC2FabR PC	100 µL	Positive Control (PC) for PCR control: - Mixture of pathogen and IC clones
RP-V IC 2	250 µL	Exogenous Internal Control (IC)
RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade -Negative Control (NC) for PCR control

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
- Sterile TE buffer
- Instruments and kits for nucleic acid extraction

Manufacturer	Instrument (Cat. No.)	Extraction Kit	Reaction No. (Cat. 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 (67415-03)	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)
Roche	MagNA Pure 96 (06541089001)	MagNA Pure 96 DNA and Viral NA Small Volume Kit	576 extractions (06543588001)
ThermoFisher Scientific	KingFisher Flex Purification System (5400630)	MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	200 extractions (A42352)

NOTE:
(1) All extraction options are commercially available.
(2) The Seegene and Hamilton reagents/ instruments can be purchased through Seegene CANADA (Toronto, CA), canada@seegene.com.

(3) The Seegene and Hamilton extraction reagents/instruments are validated with Seegene Launcher software.

- PCR Instrument & Consumables
  ① CFX96™ Dx System (Bio-Rad, CFX Manager™ Dx Software v3.1)
  ② CFX96™ Real-time PCR Detection System-IVD (Bio-Rad, CFX Manager™ Software-IVD v1.6)
  ③ CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, CFX Maestro™ Software v2)

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) • 0.2 mL 8-Tube PCR Strips without Caps, low profile, white (Cat. No. TLS0851, Bio-Rad) • Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad) • Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)* • PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)* • EU 0.1ml 8-tube strip, LP, W. Extra Robust (Cat. No. B72719, BIOplastics)** • EU Optical Wide area 8-Cap Strip (Cat. No. B57801, BIOplastics)** • 96 x 0.1ml Plate, LP, W, FULL, 96 well plate (Cat. No. B70679, BIOplastics)*** • Opti-Seal Optical Sealing Sheet (Cat. No. 157300, BIOplastics)*** • Mini-centrifuge (Cat. No. Mini-6K, Protagen) • PCR plate centrifuge (Cat. No. MPC-P25, Powerlab) * The Permanent Clear Heat Seal must be used with the PX1 PCR Plate Sealer when running the Allplex™ assay. ** Make sure to use only 8-tube strip with 8-Cap Strip as listed above together. *** Make sure to use only 96 well plate with sealing sheet as listed above together.

NOTE: All consumables can be purchased through Seegene CANADA (Toronto, CA), canada@seegene.com.

■ CHAPTER 5: Warnings and Precautions

The Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay should be performed by qualified, trained personnel.

- For *in vitro* diagnostic use only.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, Influenza A and B virus as well as RSV, not for any other viruses or pathogens.
- The performance of this device has not been assessed in a population vaccinated against covid-19.
- 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.
- Sensitivity of the assay may decrease if samples are repeatedly frozen and thawed for more than 5 times.
- 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 reagent prep area and specimen processing area.
- 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 13 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 interactively 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”).
- Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is intended for the qualitative multiple detection and differentiation of the target pathogen infections; SARS-CoV-2, Influenza A and B virus (Flu A/B) as well as Human respiratory syncytial virus (RSV).

■ CHAPTER 6: Storage and Handling Conditions

Reagent storage and handling

- All reagents of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay kit must be 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 5 cycles of freeze and thaw. If the reagents are used only intermittently, they should be stored in aliquots.

Specimen storage and transport

- Specimen type: human nasopharyngeal swab specimen

NOTE: Sample collection devices are not provided with the assay. All testing for COVID-19 should be conducted in consultation with a healthcare provider. Always treat human biospecimens as potentially infectious. Follow your biosafety protocols. Refer to CDC guidelines for sample collection 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 swab specimens.

- After collection, the specimen should be stored at 2~8°C and processed within 72 hours.

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™ SARS-CoV-2/FluA/FluB/RSV Assay to confirm the validity of each PCR run on the same plate.

In prior to determining of 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™ SARS-CoV-2/FluA/FluB/RSV 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 sterile TE buffer by 1:100 before use. The sterile TE buffer for dilution of the Positive Control is required but not provided with the assay. The diluted Positive Control is for single use only and should not be reused.

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				HEX				Cal Red 610				Quasar 670				Auto Interpretation	Comment
			S gene	C(t)	RSV	C(t)	RdRP gene	C(t)	Flu B	C(t)	N gene	C(t)	Flu A	C(t)	Endo IC	C(t)	Exo IC	C(t)		
C12		PC	+	21,44	+	20,92	+	22,95	+	20,65	+	21,58	+	21,85	+	22,97	+	20,18	Positive Control(+)	
F12		NC	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	Negative Control(-)	

Picture 2. Example of invalid positive/negative control results

Well	Name	Type	FAM				HEX				Cal Red 610				Quasar 670				Auto Interpretation	Comment
			S gene	C(t)	RSV	C(t)	RdRP gene	C(t)	Flu B	C(t)	N gene	C(t)	Flu A	C(t)	Endo IC	C(t)	Exo IC	C(t)		
D07		PC	-	N/A	+	35,78	-	N/A	-	N/A	+	37,33	+	36,88	+	37,83	-	N/A	Positive Control(Invalid)	
E05		NC	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	37,27	-	N/A	-	N/A	Negative Control(Invalid)	

Table 2. Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay; Control Acceptance Criteria

Control	Seegene Viewer Result (Ct value)								
	FAM		HEX		Cal Red 610		Quasar 670		Auto Interpretation
	S gene	RSV	RdRP gene	Flu B	N gene	Flu A	Endo IC	Exo IC	
SC2FabR Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control (+)
	> 40 or N/A	> 40 or N/A	> 40 or N/A	> 40 or N/A	> 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	> 40 or N/A	> 40 or N/A	> 40 or N/A	> 40 or N/A	Negative Control (-)
	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Negative Control (Invalid)

Internal Controls

The Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay contains two internal controls (Endogenous IC and Exogenous IC). Both of endogenous gene and exogenous gene are used as internal controls. Endogenous internal control targeting for the human RNase P gene is used to confirm sample quality. Exogenous internal control (RP-V IC 2) should be added to clinical samples before nucleic acids extraction. Exogenous internal control is used to monitor all steps of the analysis process from nucleic acid extraction through the RT-PCR process and will identify possible PCR inhibitors.

Negative signals of two internal controls invalidate all results regardless of positive or negative target signal(s) in the analysis. Repeat testing if an invalid result is reported. Refer to section 'Interpretation of Results' for more details. A positive signal for two Internal Controls indicates that all processing steps performed by the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay were successful.

External Control

External controls are not provided with the Allplex™ SARS-CoV-2/FluA/ FluB/RSV Assay. Viral transport medium can be used as the negative control. Previously characterized positive samples or viral transport medium spiked with well characterized organism can be used as the external positive control. 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.

■ CHAPTER 8: Procedure

Sample collection, transport, and storage

Collect Nasopharyngeal swab (NP) 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 nucleic acid extraction on samples according to the manufacturer's instructions for use. For the Microlab STARlet IVD, Seegene STARlet, Microlab NIMBUS IVD and Seegene NIMBUS, follow the detailed instruction 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
KingFisher™ Flex Purification System **(MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, Cat No. A42352)** See MagMAX Viral/Pathogen Nucleic Acid Isolation Kit User Manual for details. - Specimen volume: 200 µL, Elution volume: 80 µL
MagNA Pure 96 **(MagNA Pure 96 DNA and Viral NA Small Volume Kit; Cat No.06543588001)** See MagNA Pure 96 DNA and Viral NA Small Volume Kit User Manual for details. - Specimen volume: 200 µL, Elution volume: 100 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 CANADA (Toronto, CA), canada@seegene.com.

The Seegene Launcher is 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' containing detailed descriptions on 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.

Below instructions describe the procedures for 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
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 and 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 1.5mL 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 48mL of absolute ethanol (Cat. No. 1.00983.1011, Merck). After removing the film on 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 bead by manually tapping the tube, and then quick vortexing.

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 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, Seegene STARlet, Microlab NIMBUS IVD and Seegene NIMBUS, prepare the following:

- Add 48 mL of absolute ethanol into WB2 tub before use. WB2 tub should to 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
5-Sample Rack	741-6560	Seegene
5 X 12 Sample rack plate	A6061-T6-1	Seegene
5 X 18 Sample rack plate (For Nimbus 72 system)	A6061-T6-1 New	Seegene
Sample rack fixing block	A6061-T6-2	Seegene
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
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 CANADA (Toronto, CA), canada@seegene.com.

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 300µL specimen volume is required to ensure 200µL of specimen pipetting by Microlab STARlet IVD/Seegene STARlet. This will result in 100µL elution volume of nucleic acids (RNA) necessary to run the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay.

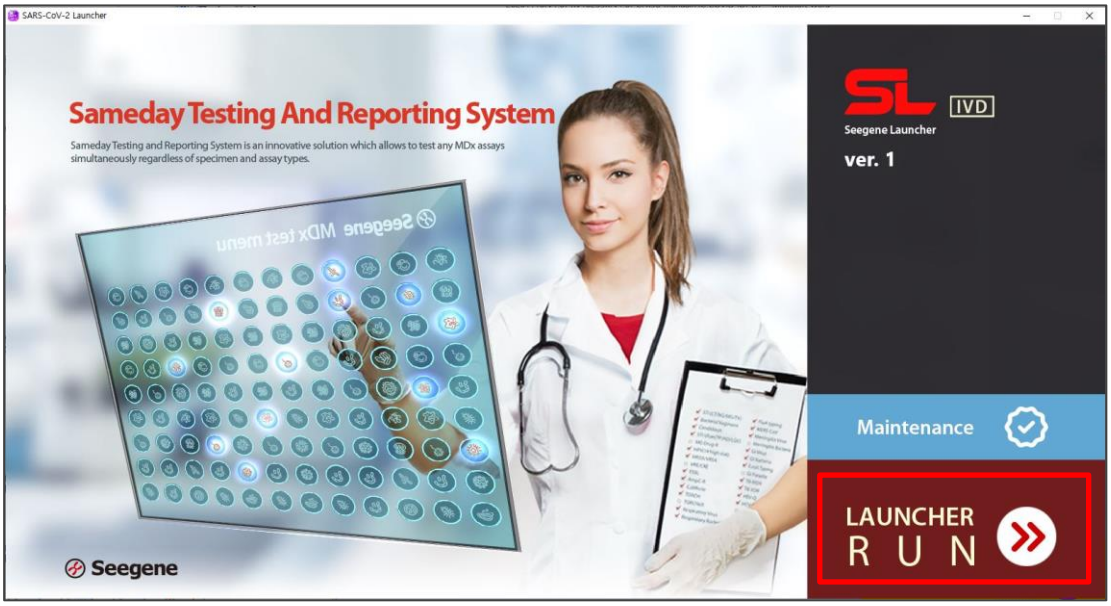
(3) Only 12mm tubes, 16mm tubes and 1.5mL micro centrifuge tubes can be directly loaded to the Microlab STARlet IVD/Seegene STARlet.

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

(5) Available Seegene Launcher (SARS-CoV-2 Launcher) version is 1.00 or higher.

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.

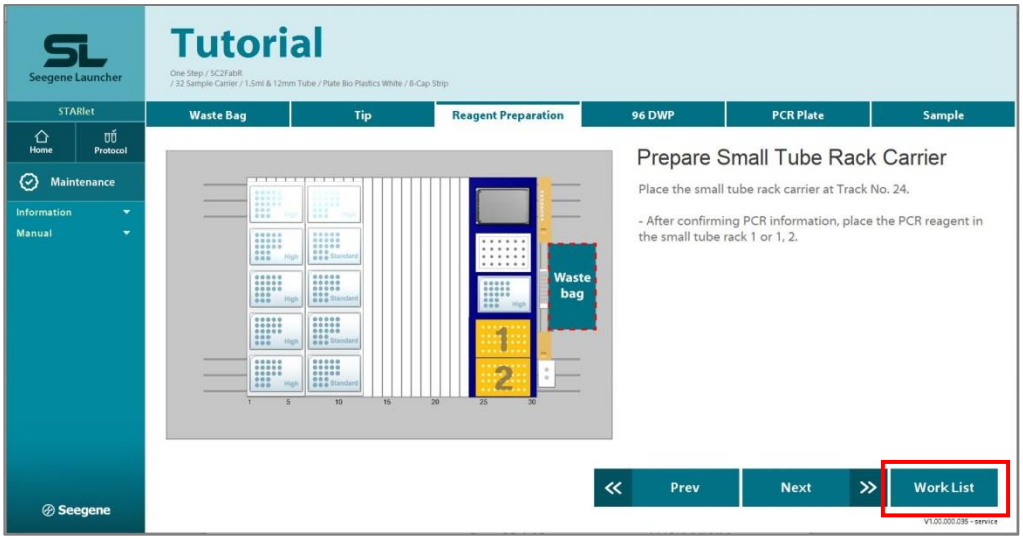
2. Click on **LAUNCHER RUN** on the main page.



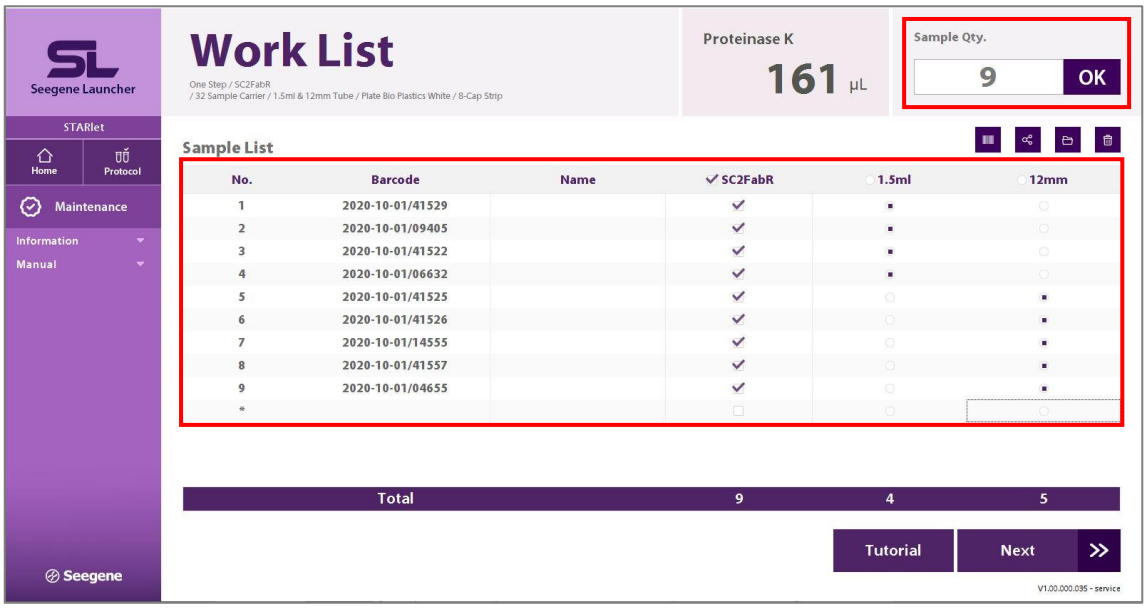
3. Select **SC2FabR** (protocol for Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay) to begin the protocol. All following steps are included in a step by step instruction included in the software.

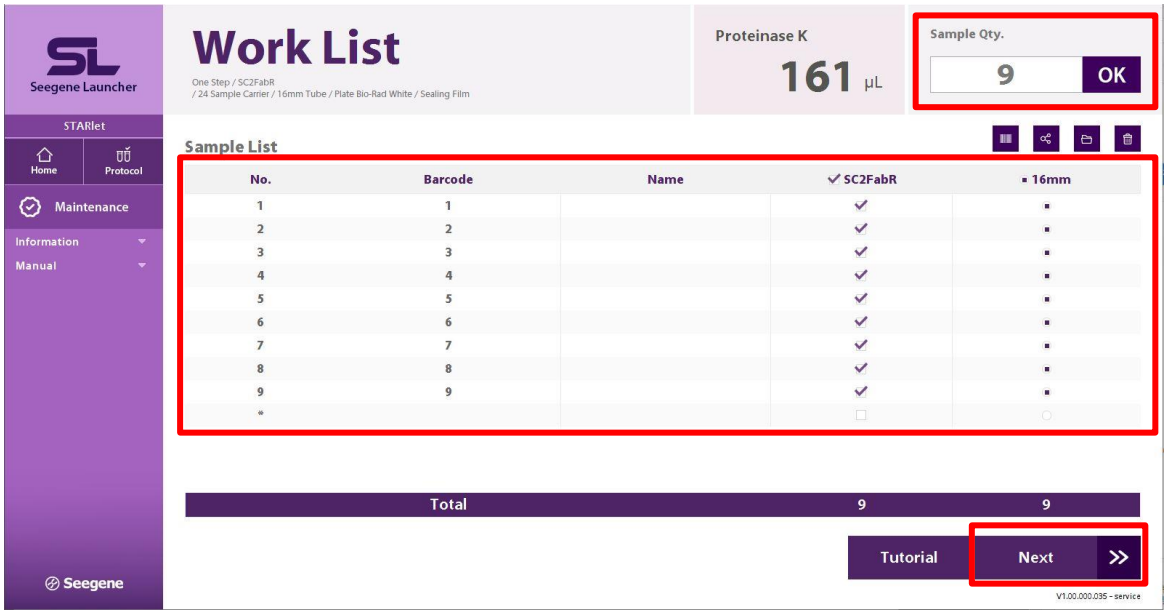
4. Using a hand-held barcode reader provided with the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS, read barcode label attached on the side of the cartridge. After the **Extraction Reagent Barcode** information is filled in, hit **Enter**.

5. 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 or STAMag 96 X 4 Viral DNA/RNA 200 C Kit are placed on the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS while following step by step instructions guided by the Seegene Launcher software.
NOTE: After equilibrating specimens to room temperature, vortex each specimen briefly.

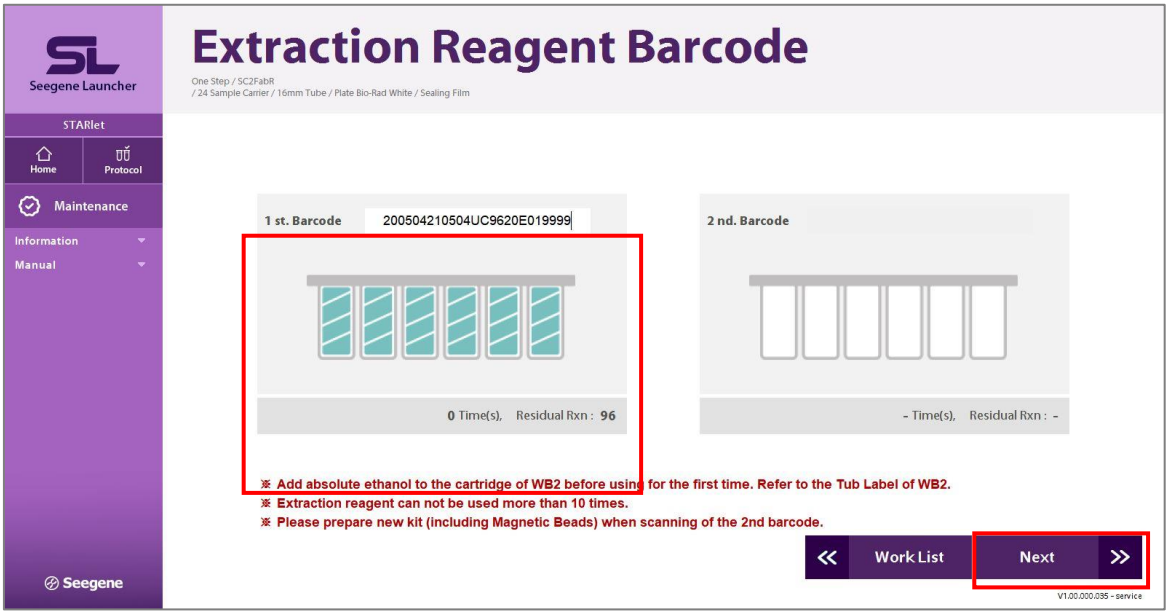


6. A barcode reader installed inside the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS automatically reads sample information. The sample information can also be manually entered, if necessary. Click on **Next**, once **Sample Quantity, Barcode, Name** (optional) and labware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

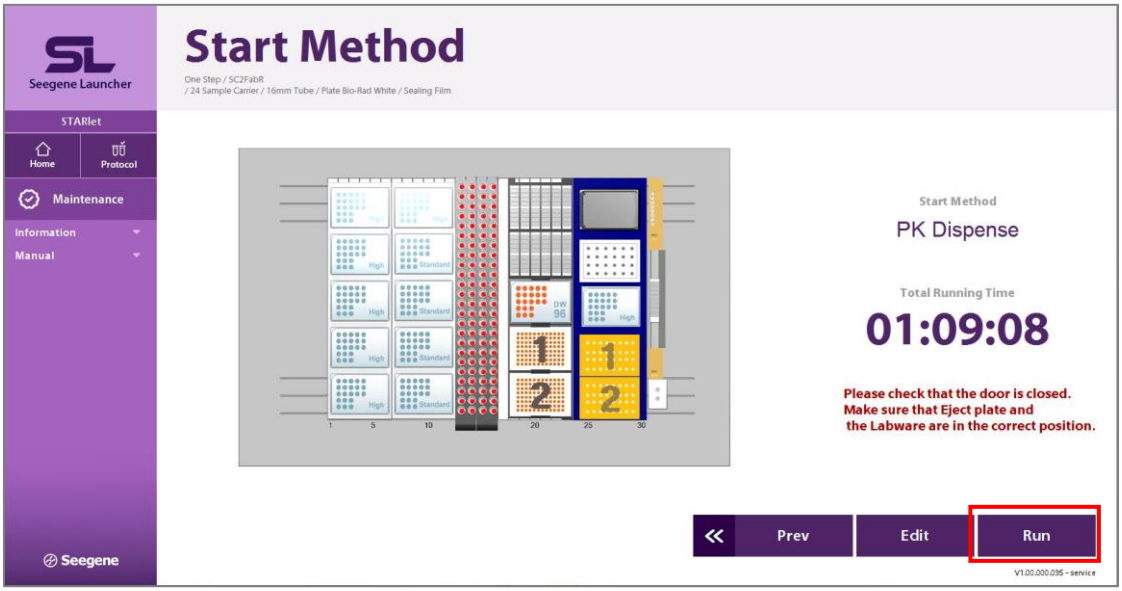




7. Using a hand-held barcode reader provided with the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS, read barcode label attached on the side of the cartridge. After the **Extraction Reagent Barcode** information is entered, click on **Next**. If the remaining volume of the existing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



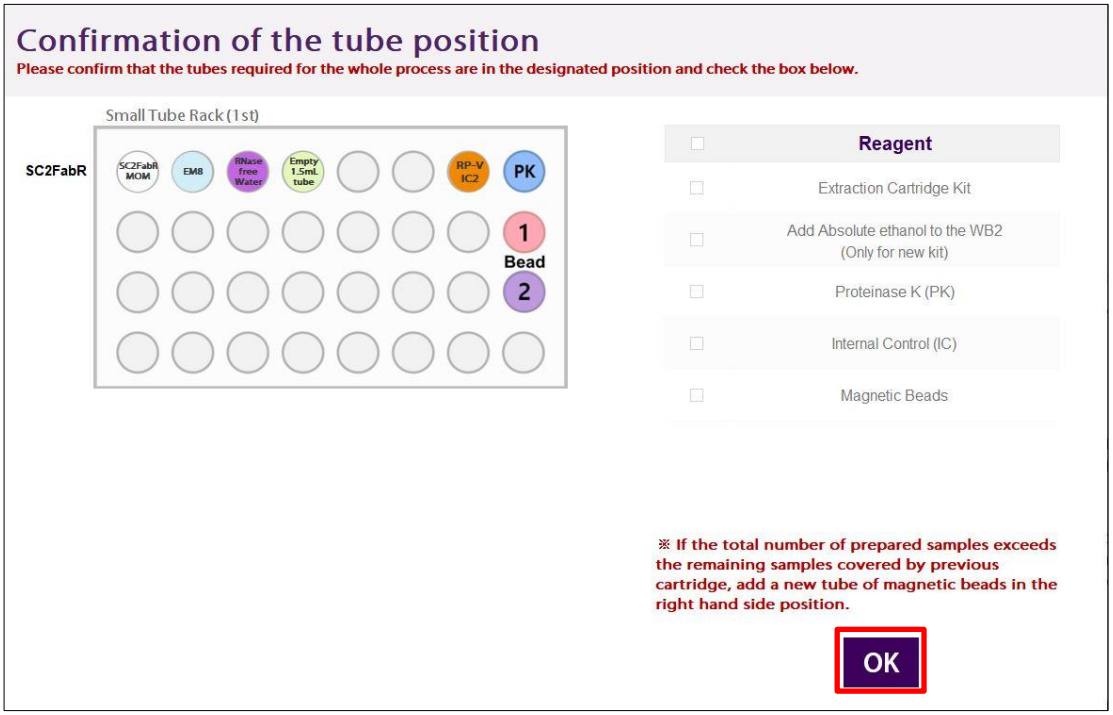
8. Ensure that the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS door is firmly closed, and that the eject plate and labware are in their correct positions as shown below. Click on **Run** after all preparations are done.



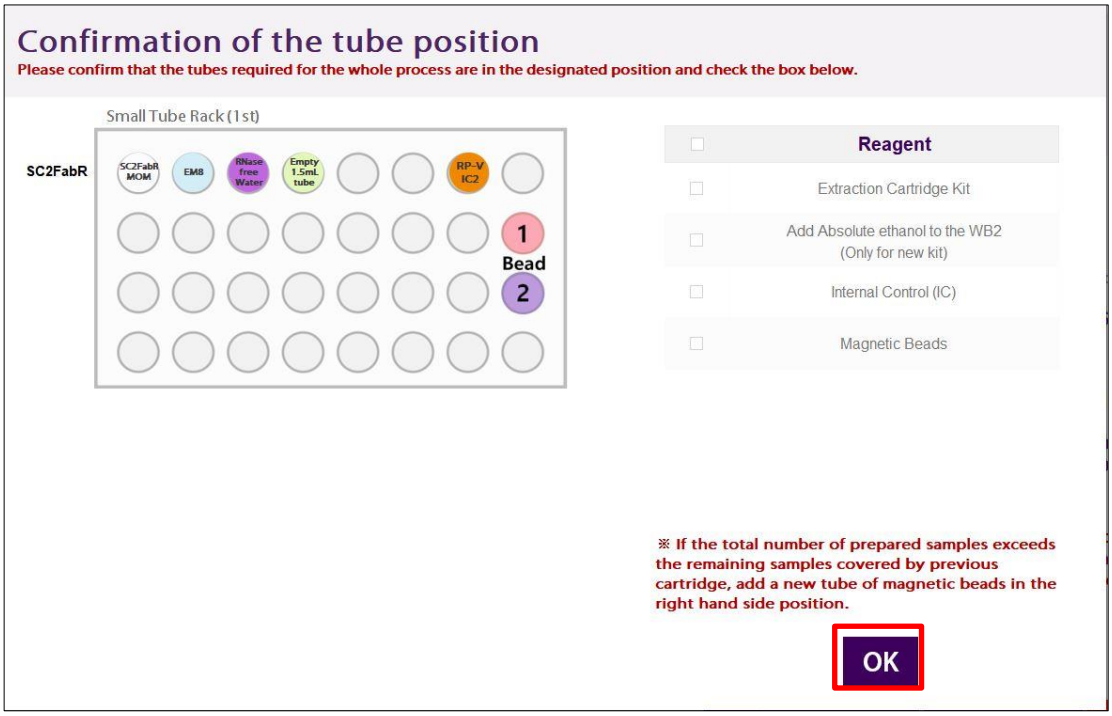
Do not open the door of the Microlab STARlet IVD/Seegene STARlet/Microlab NIMBUS IVD/Seegene NIMBUS during operation.

9. Check that the reagents are in the right position and click on **OK** to start 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 CANADA (Toronto, CA) at canada@seegene.com.

Please refer to the user manual of 'Seegene Launcher' for detailed description on experimental procedures of nucleic acid extraction using Microlab STARlet IVD, Seegene STARlet, NIMBUS IVD and Seegene NIMBUS.

Amplification and detection

A video tutorial is available upon request to Seegene CANADA (Toronto, CA, canada@seegene.com) for training on all experimental procedures related to amplification and detection under this section. Seegene Viewer (Seegene Viewer for Real time Instruments) for auto-interpretation of results is provided by Seegene CANADA (Toronto, CA), canada@seegene.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 run.

1. Prepare 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
SC2FabR MOM	5	10	15	20	25
EM8	5	10	15	20	25

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

3. Aliquot 10 µL of the One-step RT-PCR Mastermix into PCR tubes.

   NOTE: Prior to adding sample's extracted nucleic acids/PC to PCR tubes, move from the reagent prep area to a specimen processing area.

4. Add 10 µL of each sample's extracted nucleic acids, SC2FabR PC and NC (RNase-free Water; Negative Control (NC) for PCR control) into the PCR tubes containing aliquot of the One-step RT-PCR Mastermix.

5. Close the PCR tubes with cap or film, and briefly centrifuge the PCR tubes

PCR tube	Applicable Cover
8-Tube Strip (Bio-Rad)	8-Cap Strip (Bio-Rad)
96-Well PCR Plates (Bio-Rad)	8-Cap Strip (Bio-Rad)
	Permanent Clear Heat Seal (Bio-Rad)
8-tube strip (BIOplastics)	8-Cap Strip (BIOplastics)
96 well plates (BIOplastics)	Opti-Seal Optical Sealing Sheet (BIOplastics)

NOTE: Refer to table of PCR consumables in “Materials required but not provided” section for detailed information.

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

6. 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.

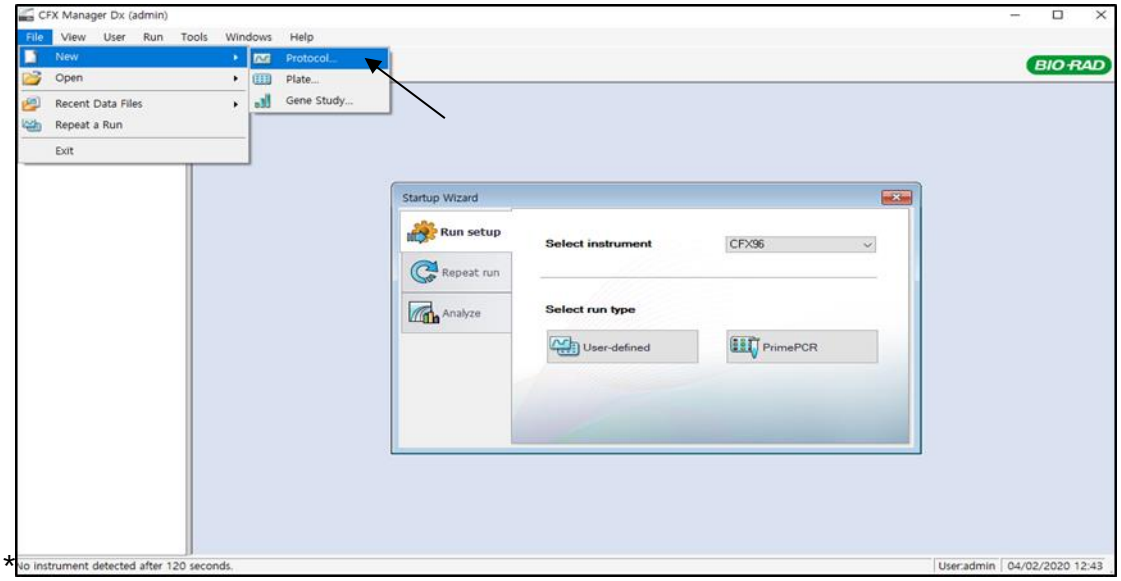
7. Immediately initiate the PCR on the Bio-Rad CFX96™ Dx, CFX96™ IVD or CFX96 Touch™. See details on PCR instrumentation set-up below.

Real-time PCR Instrument Set Up

Below instructions describe the procedures for CFX96™ Dx. For CFX96™ IVD and CFX96 Touch™, titles of some menus are different. However set up processes of all PCR instrument are the same, please follow the procedure as below.

Protocol Setup

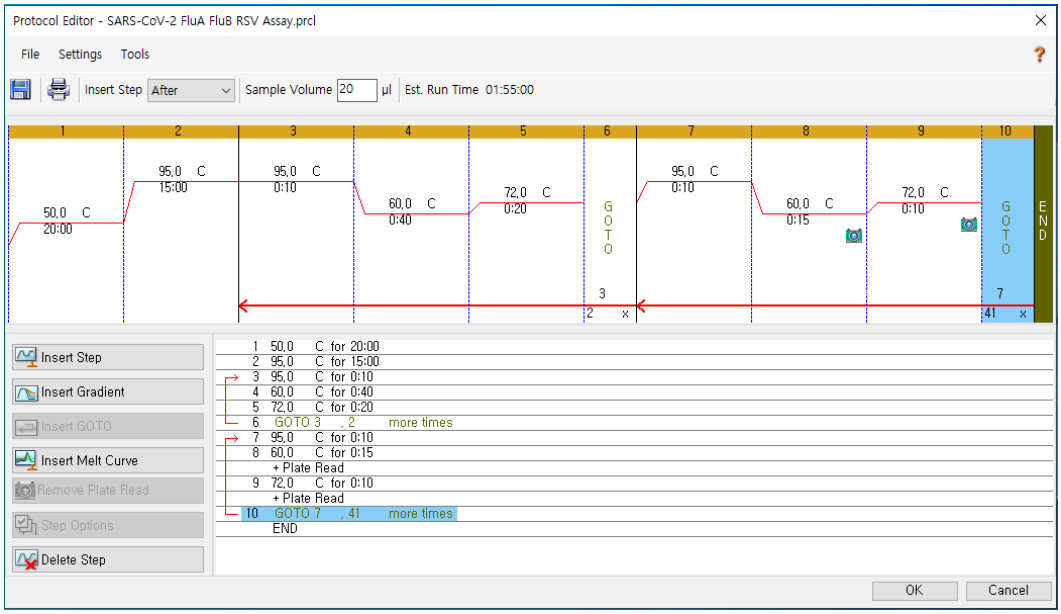
1. In the main menu, select **File** → **New** → **Protocol** to open **Protocol Editor**.



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	3	95℃	10 sec
4		60℃	40 sec
5		72℃	20 sec
6	GOTO Step 3, 2 more times		
7	42	95℃	10 sec
8		60℃ (D)	15 sec
9		72℃ (D)	10 sec
10	GOTO Step 7, 41 more times		

NOTE: Fluorescence is detected at 60 and 72°C (Step 8 and 9).



3. Click the box next to **Sample Volume** to directly input 20µL.

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

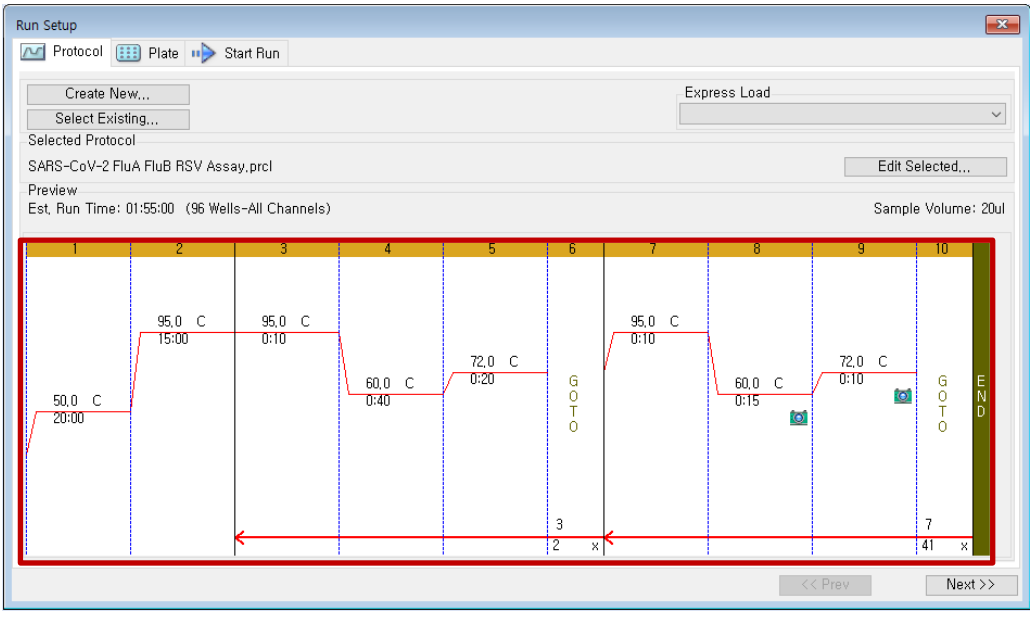
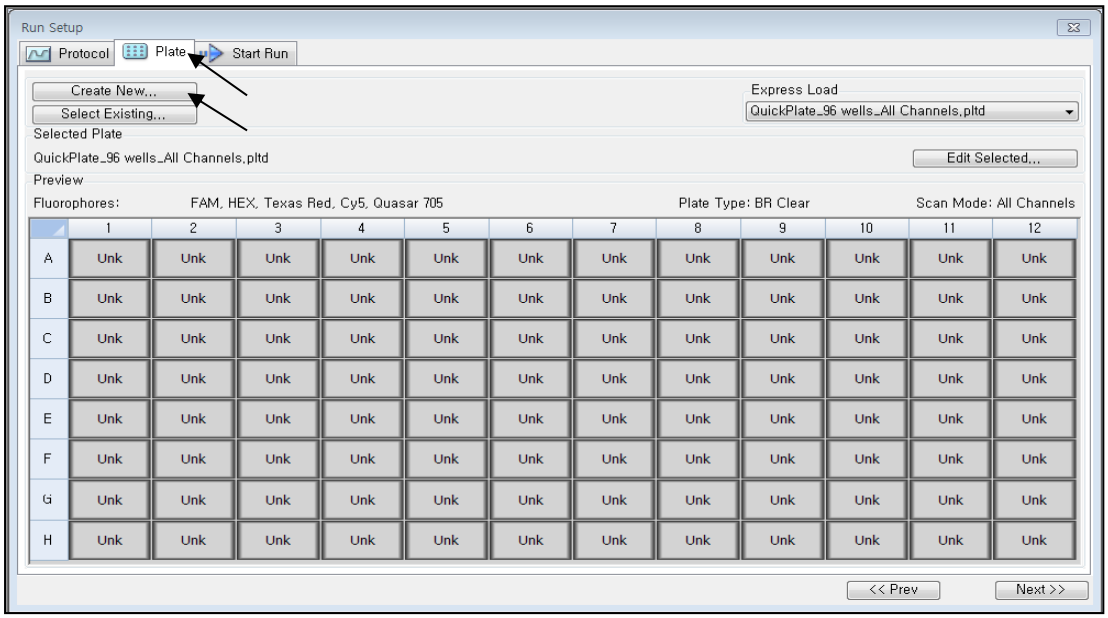
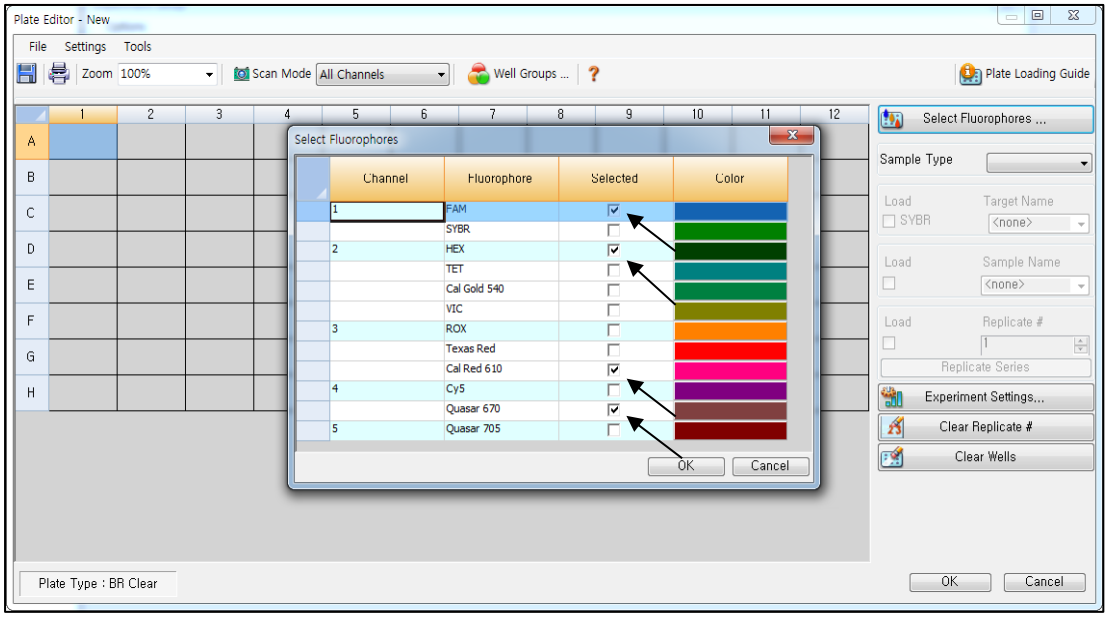


Plate Setup

1. From **Plate** tab in **Run 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**.



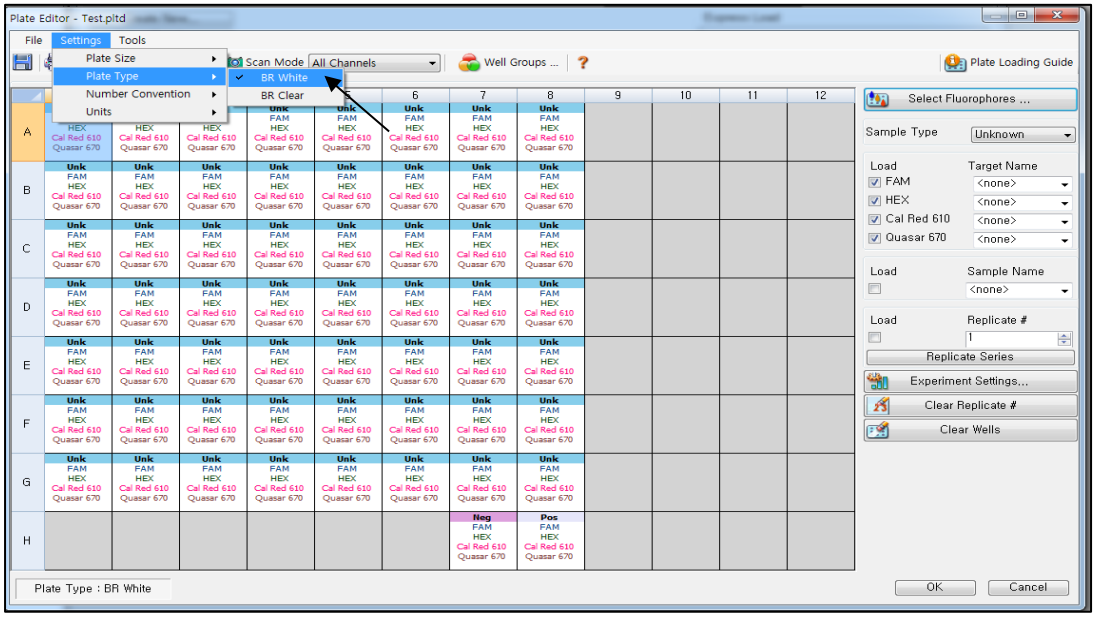
3. Select the desired well(s) and then its sample type from the **Sample Type** drop-down menu.

- **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.

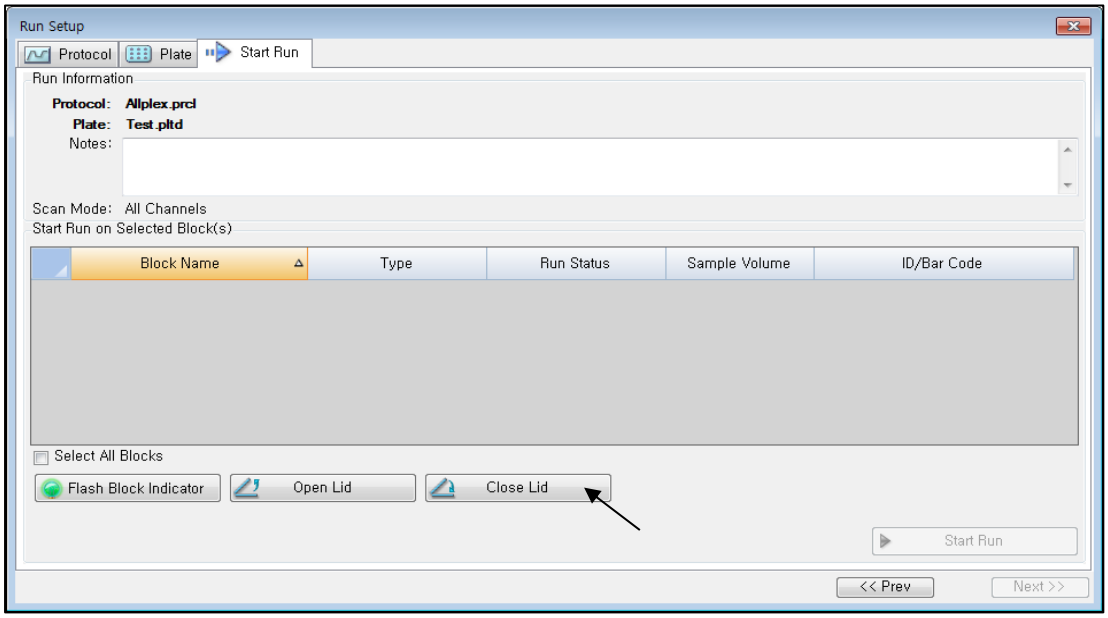
8. You will be returned to the **Run Setup** window.

9. Click **Next** to Start Run.

Real-time PCR run

Start Run

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



2. Click **Start Run**.

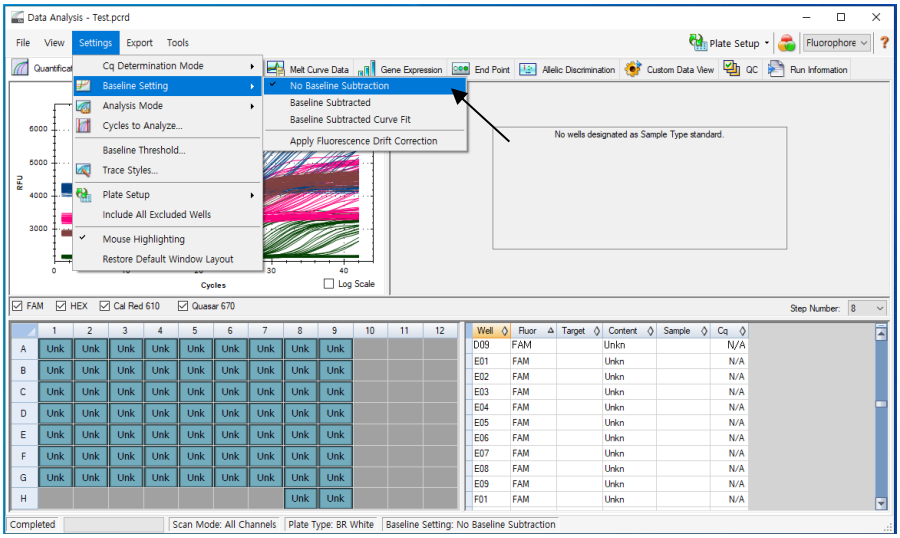
3. Store the run file either in My Documents or in a designated folder. Enter the file name, click **SAVE**, and the run will start.

Data export and analysis

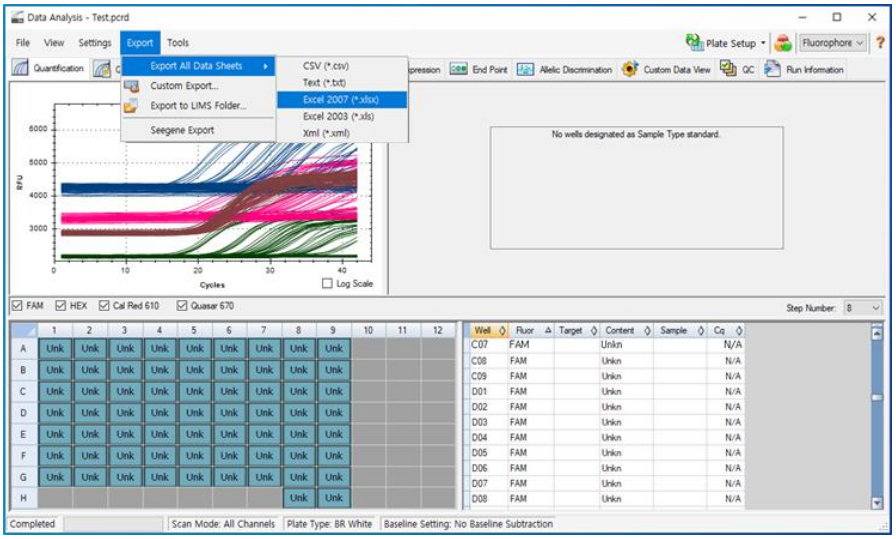
Data export

1. Create folders for data export
   - Create a folder to save amplification curve detection results.
   - The location and name of the folder is specified by user, but in case of using ‘Seegene Export’ function, folders named “QuantStep8” and “QuantStep8” are created automatically in 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.



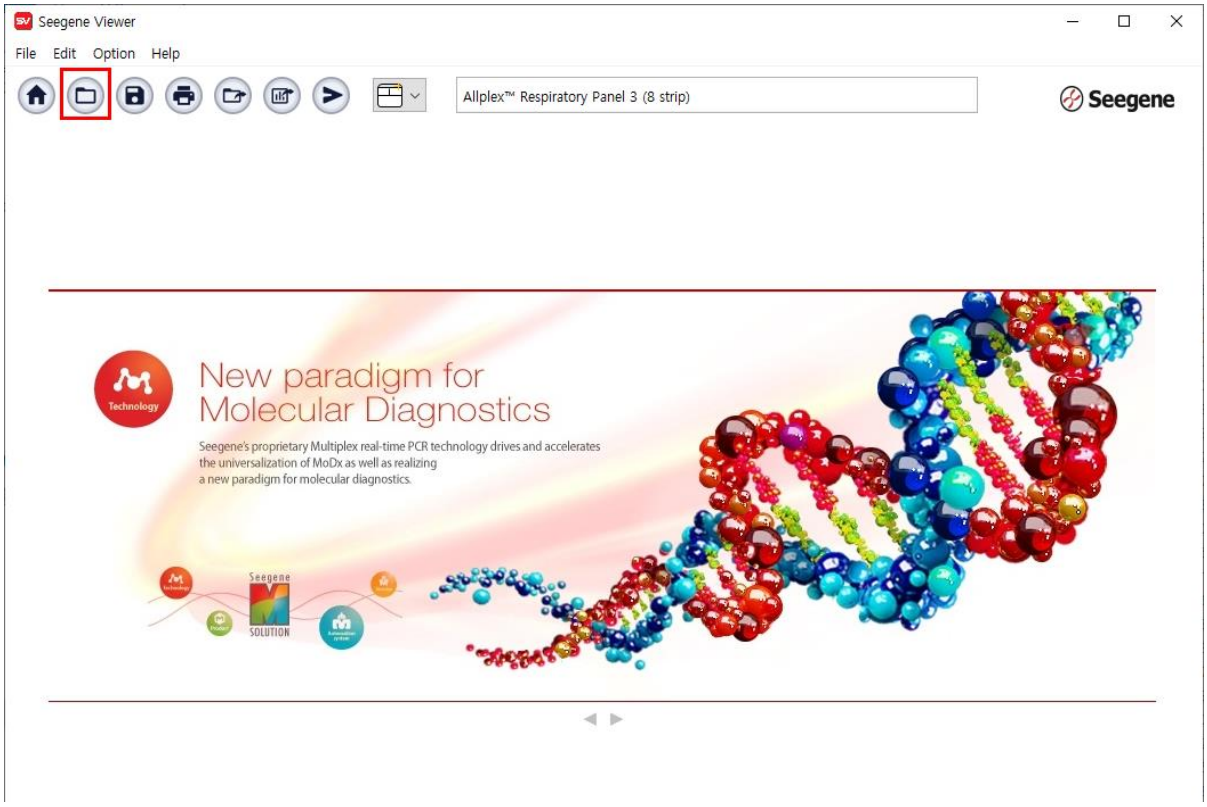
4. Choose a location to save data and click **OK**.

Data analysis

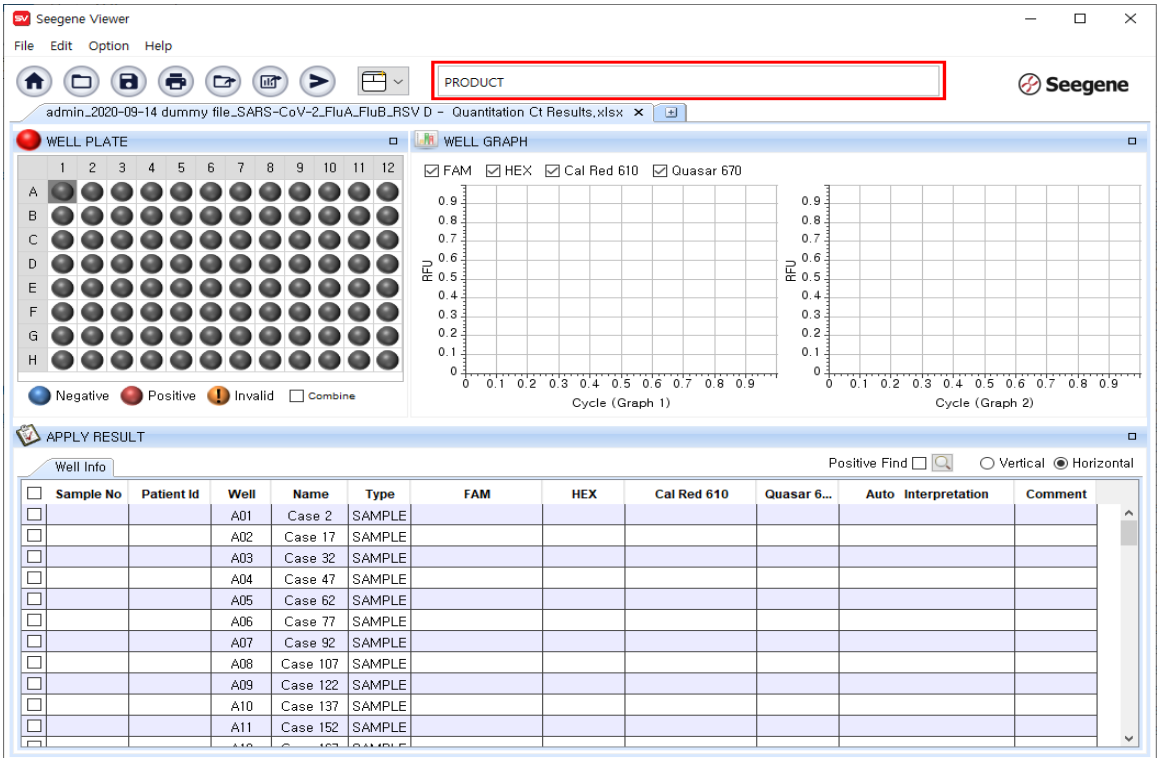
NOTE:
(1) Available **Seegene Viewer** (Seegene Viewer for Real time Instruments) version is 1.00 or higher.

1. Open the **Seegene Viewer** (Seegene Viewer for Real time Instruments) software installed on the laptop connected to the Bio-Rad CFX96™.

2. Click on Open icon and find export data on location saved.



3. After opening the results file, select '**Allplex™ SARS-CoV-2/FluA/FluB/ RSV Assay**' from the **PRODUCT** menu.



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

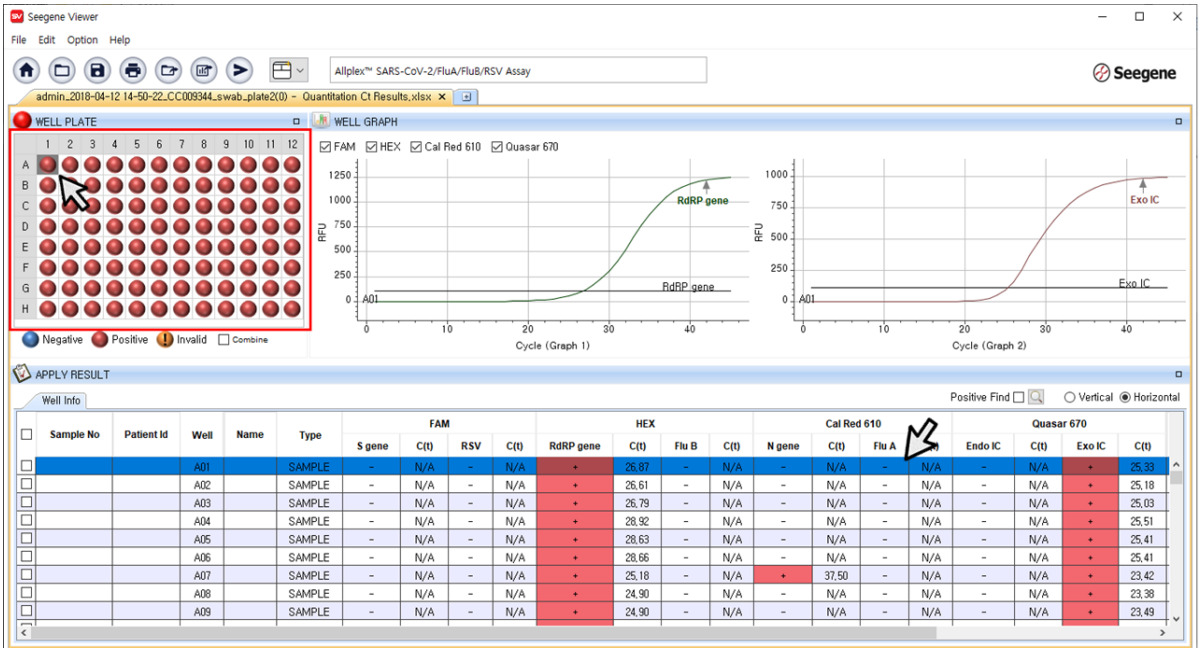


Table 7. Analytes of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay

Fluorophore	Analyte	
	Graph 1	Graph 2
FAM	S gene (of SARS-CoV-2)	RSV
HEX	RdRP gene (of SARS-CoV-2)	Flu B
Cal Red 610	N gene (of SARS-CoV-2)	Flu A
Quasar 670	Endo IC	Exo IC

■ 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™. The results are exported and transferred to the Seegene Viewer according to instructions under the section of 'Procedure: application and detection'.

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

Seegene Viewer (Seegene Viewer for Real time Instruments) software is provided by Seegene CANADA (Toronto, CA), canada@seegene.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 (-)

Overall Interpretation

Targets	Endo IC	Exo IC	Interpretation of Validity
+	+	+	Valid
-	+	+	
+	-	+	Valid Endo IC amplification may have been inhibited by high titer of target pathogen or presence of PCR inhibitor. Negative for Endo IC does not indicate that positive results for targets are invalid.
+	+	-	Valid Exo IC amplification may have been inhibited by high titer of target pathogen or presence of PCR inhibitor. Negative for Exo IC does not indicate that positive results for targets are invalid.
-	-	+	Invalid 1) Sample collection may be incorrect. Repeat from the sample collection.
-	+	-	Invalid 2) Extraction or PCR could be inhibited. Repeat from the nucleic acid extraction.
+	-	-	Invalid 3) Repeat from the nucleic acid extraction. If the same result is shown, repeat from the same collection.
-	-	-	

Interpretation of Results

SARS-CoV-2*	Flu A	Flu B	RSV	Interpretation
-	-	-	-	**Target RNA, Not detected**
+	-	-	-	**SARS-CoV-2 RNA**, Detected
-	+	-	-	**Influenza A RNA**, Detected
-	-	+	-	**Influenza B RNA**, Detected
-	-	-	+	**RSV RNA**, Detected
-	+	+	-	**Influenza A RNA** and **Influenza B RNA**, Detected
-	+	-	+	**Influenza A RNA** and **RSV RNA**, Detected
-	-	+	+	**Influenza B RNA** and **RSV RNA**, Detected
-	+	+	+	**Influenza A RNA** and **Influenza B RNA** and **RSV RNA**, Detected
+	+	-	-	**SARS-CoV-2 RNA** and **Influenza A RNA**, Detected
+	-	+	-	**SARS-CoV-2 RNA** and **Influenza B RNA**, Detected
+	-	-	+	**SARS-CoV-2 RNA** and **RSV RNA**, Detected
+	+	+	-	**SARS-CoV-2 RNA** and **Influenza A RNA** and **Influenza B RNA**, Detected
+	+	-	+	**SARS-CoV-2 RNA** and **Influenza A RNA** and **RSV RNA**, Detected
+	-	+	+	**SARS-CoV-2 RNA** and **Influenza B RNA** and **RSV RNA,** Detected
+	+	+	+	**SARS-CoV-2 RNA** and **Influenza A RNA** and **Influenza B RNA** and **RSV RNA**, Detected

* SARS-CoV-2 is considered as “detected” when one or more of the three target genes are positive. Negative result for one or two target genes for SARS-CoV-2 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.

■ CHAPTER 10: Assay Limitations

- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- SARS-CoV-2, Flu A/B or RSV may mutate in one or more of the target regions of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay. If this occurs, then these viruses may not be detected.
- 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 infectious virus or that these viruses are the causative agents for clinical symptoms.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- 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 target viruses
    - Failure to follow instructions for use
- Negative results do not preclude infection with SARS-CoV-2, Flu A/B, and/or RSV 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.

■ CHAPTER 11: Performance Evaluation

Analytical Sensitivity

Limit of detection (LoD) for each target of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was determined for multiple automated extraction methods/kits, two PCR instruments, multiple product lots, and PCR consumables. Each viral strain (3influenza A strains, 2 influenza B strains, RSV A, RSV B, and gamma-irradiated SARS-CoV-2) was serially diluted into negative NP swab/UTM matrix. LoD was defined as the lowest concentration that gives ≥ 95% positive results of the time. The results showed that the LoD for each target was equivalent across multiple extraction methods, PCR instruments, product lots, and consumable sets. Overall LoD results and final LoD of each analyte are summarized in Table 9 and Table 10, respectively.

Table 9. Overall LoD Study Results

Target		Conc. ($TCID_{50}$/mL)	NIMBUS (Universal Cartridge Kit)*	NIMBUS (Viral DNA/RNA 200 C kit)	STARlet (Universal Cartridge Kit)	STARlet (Viral DNA/RNA 200 C kit)	MagNA Pure 96	King Fisher
SARS-CoV-2	S gene	0.23	160/160	20/20	20/20	20/20	20/20	20/20
		0.077	39/40	20/20	19/20	20/20	20/20	20/20
		0.023	33/40	12/20	18/20	16/20	15/20	15/20
	RdRP gene	0.23	160/160	20/20	20/20	20/20	20/20	20/20
		0.077	36/40	20/20	18/20	18/20	20/20	19/20
		0.023	24/40	13/20	15/20	13/20	13/20	9/20
	N gene	0.23	160/160	20/20	20/20	20/20	20/20	20/20
		0.077	26/40	13/20	14/20	14/20	9/20	10/20
		0.023	17/40	8/20	14/20	5/20	8/20	8/20
RSV A (4/2015 Isolate #1)		0.17	159/160	20/20	20/20	20/20	20/20	20/20
		0.057	29/40	13/20	13/20	13/20	16/20	16/20
RSV B (12/2014 Isolate #1)		0.39	158/160	20/20	20/20	20/20	20/20	20/20
		0.13	26/40	10/20	9/20	11/20	14/20	11/20
Flu B (Brisbane/60/08)		0.09	159/160	20/20	20/20	20/20	20/20	20/20
		0.03	26/40	14/20	16/20	12/20	11/20	14/20
Flu B (Wisconsin/1/10)		0.26	119/120	-	-	-	-	-
		0.087	-	-	-	-	-	-
Flu A H1N1 (New Cal/20/99)		4.96	120/120	-	-	-	-	-
		1.65	-	-	-	-	-	-
Flu A H1N1pdm (California/07/09)		0.80	119/120	-	-	-	-	-
		0.27	-	-	-	-	-	-
Flu A H3N2 (Victoria/361/11)		0.07	160/160	20/20	20/20	20/20	20/20	20/20
		0.023	24/40	15/20	11/20	12/20	13/20	11/20

*The data shows overall positive rate for each extraction method, including tests conducted using multiple PCR instruments, consumables and product lots.

Table 10. Final LoD

Detection Target		Source	Strain	LoD ($TCID_{50}$/mL)
SARS-CoV-2*	S gene	BEI (Cat. NR-52287)	USA-WA1/2020	0.23
	RdRP gene			0.23
	N gene			0.23
RSV A		Zeptometrix (Cat. 0810481CF)	4/2015	0.17
RSV B		Zeptometrix (Cat. 0810450CF)	12/2014	0.39
Flu B		Zeptometrix (Cat. 0810254CF)	Brisbane/60/08	0.09
Flu B		Zeptometrix (Cat. 0810241CF)	Wisconsin/1/10	0.26
Flu A H1N1		Zeptometrix (Cat. 0810036CF)	New Cal/20/99	4.96
Flu A H1N1pdm		Zeptometrix (Cat. 0810165CF)	California/07/09	0.80
Flu A H3N2		Zeptometrix (Cat. 0810240CF)	Victoria/361/11	0.07

* LoD of SARS-CoV-2 is determinated as the concentration at which all 3 targets of SARS-CoV-2 are detected by more than 95%.

Analytical Specificity

The high specificity of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is ensured by the oligos designed specifically for the targets of interest. Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was tested for cross-reactivity to 124 different pathogens, and PCR amplification and detection were only identified for the specified targets.

NO.	Organism	Source	Isolate No.	Result†
1	Influenza A H1N1 (Brisbane/59/07)	ZMC	0810244CF	Flu A Detected
2	Influenza A H1N1 (New Cal/20/99)	ZMC	0810036CF	Flu A Detected
3	Influenza A H1N1 A/PR/8/34	ATCC	VR-95	Flu A Detected
4	Influenza A H1N1 (Singapore/63/04)	ZMC	0810246CF	Flu A Detected
5	Influenza A H1N1 (Solomon Islands/03/06)	ZMC	0810036CFN	Flu A Detected
6	Influenza A H1N1 (Taiwan/42/06)	ZMC	0810247CF	Flu A Detected
7	Influenza A virus (H1N1) (A/FM/1/47)	ATCC	VR-97	Flu A Detected
8	Influenza A virus (H1N1) (A/NWS/33)	ATCC	VR-219	Flu A Detected
9	Influenza A virus (H1N1) (A/WS/33)	ATCC	VR-825	Flu A Detected
10	Influenza A H1N1pdm (California/07/09)	ZMC	0810165CF	Flu A Detected
11	Influenza A H1N1pdm (Mexico/4108/09)	ZMC	0810166CF	Flu A Detected
12	Influenza A H1N1pdm (NY/01/09)	ZMC	0810248CF	Flu A Detected
13	Influenza A H1N1pdm (NY/02/09)	ZMC	0810109CFN	Flu A Detected
14	Influenza A H1N1pdm (NY/03/09)	ZMC	0810249CF	Flu A Detected
15	Influenza A H1N1pdm Virus (Michigan/45/15)	ZMC	0810538CF	Flu A Detected
16	Influenza A virus (H1N1-pdm09) (A/Virginia/ATCC1/2009)	ATCC	VR-1736	Flu A Detected
17	Influenza A H3N2 (Brisbane/10/07)	ZMC	0810138CF	Flu A Detected
18	Influenza A H3N2 A/Hong Kong/8/68	ATCC	VR-544	Flu A Detected
19	Influenza A H3N2 (Perth/16/09)	ZMC	0810251CF	Flu A Detected
20	Influenza A H3N2 (Texas/50/12)	ZMC	0810238CF	Flu A Detected
21	Influenza A H3N2 (Victoria/361/11)	ZMC	0810240CF	Flu A Detected
22	Influenza A H3N2 (Wisconsin/67/05)	ZMC	0810252CF	Flu A Detected
23	Influenza A H3N2 Virus (Hong Kong/4801/14)	ZMC	0810526CF	Flu A Detected
24	Influenza A virus (H3N2) (A/Aichi/2/68)	ATCC	VR-547	Flu A Detected
25	Influenza A virus (H3N2) (A/Port Chalmers/1/73)	ATCC	VR-810	Flu A Detected
26	Influenza B (Brisbane/33/08)	ZMC	0810253CF	Flu A Detected
27	Influenza B (Brisbane/60/08)	ZMC	0810254CF	Flu B Detected
28	Influenza B (Florida/02/06)	ZMC	0810037CF	Flu B Detected
29	Influenza B virus B/Florida/4/2006	ATCC	VR-1804	Flu B Detected
30	Influenza B (Florida/07/04)	ZMC	0810256CF	Flu B Detected
31	Influenza B (Lee/40)	ZMC	0810257CF	Flu B Detected

NO.	Organism	Source	Isolate No.	Result†
32	Influenza B (Malaysia/2506/04)	ZMC	0810258CF	Flu B Detected
33	Influenza B (Massachusetts/2/12)	ZMC	0810239CF	Flu B Detected
34	Influenza B (Panama/45/90)	ZMC	0810259CF	Flu B Detected
35	Influenza B (Texas/6/11)	ZMC	0810242CF	Flu B Detected
36	Influenza B (Wisconsin/1/10)	ZMC	0810241CF	Flu B Detected
37	Respiratory Syncytial Virus Type A (12/2014 Isolate #2)	ZMC	0810452CF	Flu B Detected
38	Respiratory Syncytial Virus Type A (3/2015 Isolate #3)	ZMC	0810482CF	RSV Detected
39	Respiratory Syncytial Virus Type A (2006 isolate)	ZMC	0810040ACF	RSV Detected
40	Respiratory Syncytial Virus Type A (2014 Isolate 341)	ZMC	0810290CF	RSV Detected
41	Respiratory Syncytial Virus Type A (2014 Isolate 342)	ZMC	0810291CF	RSV Detected
42	Respiratory Syncytial Virus Type A (2/2015 Isolate #2)	ZMC	0810474CF	RSV Detected
43	Respiratory Syncytial Virus Type A (2/2015 Isolate #3)	ZMC	0810475CF	RSV Detected
44	Respiratory Syncytial Virus Type A (4/2015 Isolate #1)	ZMC	0810481CF	RSV Detected
45	Respiratory Syncytial Virus Type A (2013 Isolate)	ZMC	0810299CF	RSV Detected
46	Human respiratory syncytial virus A (Long)	ATCC	VR-26	RSV Detected
47	Respiratory Syncytial Virus Type B (CH93(18)-18)	ZMC	0810040CF	RSV Detected
48	Respiratory Syncytial Virus Type B (12/2014 Isolate #1)	ZMC	0810450CF	RSV Detected
49	Respiratory Syncytial Virus Type B (11/2014 Isolate #2)	ZMC	0810451CF	RSV Detected
50	Respiratory Syncytial Virus Type B (3/2015 Isolate #1)	ZMC	0810479CF	RSV Detected
51	Respiratory Syncytial Virus Type B (3/2015 Isolate #2)	ZMC	0810480CF	RSV Detected
52	Human respiratory syncytial virus B (Strain: 9320)	ATCC	VR-955	RSV Detected
53	SARS-CoV-2 isolate Australia/VIC01	TWIST BIOSCIENCE	102019	RSV Detected
54	SARS-CoV-2 isolate Wuhan-Hu-1	TWIST BIOSCIENCE	102024	SARS-CoV-2 Detected
55	SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated	BEI	NR-52287	SARS-CoV-2 Detected
56	First WHO International Standard for SARS-CoV-2 RNA	NIBSC	20/146	SARS-CoV-2 Detected
57	Human adenovirus 1	ATCC	VR-1	Not Detected
58	Human adenovirus 18	ATCC	VR-1095	Not Detected
59	Human adenovirus 2	KBPV	VR-58	Not Detected
60	Human adenovirus 23	ATCC	VR-1101	Not Detected
61	Human adenovirus 3	ATCC	VR-3	Not Detected
62	Human adenovirus 4	ATCC	VR-1572	Not Detected

NO.	Organism	Source	Isolate No.	Result†
63	Human adenovirus 5	KBPV	VR-61	Not Detected
64	Human adenovirus 8	ATCC	VR-1368	Not Detected
65	Human coronavirus 229E	ATCC	VR-740	Not Detected
66	Human coronavirus NL63	ZMC	0810228CF	Not Detected
67	Human coronavirus HKU1	Korean isolated sample		Not Detected
68	Human coronavirus OC43	ATCC	VR-1558	Not Detected
69	Human Metapneumovirus (MPV)	ZMC	NATHMPV-ST	Not Detected
70	Human coxsackievirus A24	ATCC	VR-583	Not Detected
71	Human coxsackievirus A9	KBPV	VR-11	Not Detected
72	Human coxsackievirus B1	KBPV	VR-13	Not Detected
73	Human coxsackievirus B2	KBPV	VR-14	Not Detected
74	Human coxsackievirus B3	KBPV	VR-15	Not Detected
75	Human coxsackievirus B4	KBPV	VR-16	Not Detected
76	Human coxsackievirus B5	KBPV	VR-17	Not Detected
77	Human coxsackievirus B6	KBPV	VR-18	Not Detected
78	Human echovirus 11	KBPV	VR-22	Not Detected
79	Human Echovirus 22 (Parechovirus)	KBPV	VR-23	Not Detected
80	Human echovirus 25	KBPV	VR-24	Not Detected
81	Human echovirus 30	KBPV	VR-25	Not Detected
82	Human echovirus 6	KBPV	VR-19	Not Detected
83	Human echovirus 7	KBPV	VR-20	Not Detected
84	Human echovirus 9	ATCC	VR-39	Not Detected
85	Human enterovirus 70	ATCC	VR-836	Not Detected
86	Human enterovirus 71	ATCC	VR-784	Not Detected
87	Human herpesvirus 1	ATCC	VR-260	Not Detected
88	Human herpesvirus 2	ATCC	VR-734	Not Detected
89	Human parainfluenza virus 1	ATCC	VR-1380	Not Detected
90	Human parainfluenza virus 2	ATCC	VR-92	Not Detected
91	Human parainfluenza virus 3	ATCC	VR-93	Not Detected
92	Human parainfluenza virus 4A	ATCC	VR-1378	Not Detected
93	Human parainfluenza virus 4B	ATCC	VR-1377	Not Detected
94	Human rhinovirus 14	ATCC	VR-284	Not Detected
95	Human rhinovirus 16	ATCC	VR-283	Not Detected
96	Human rhinovirus 8	ATCC	VR-488	Not Detected
97	Human Rhinovirus A90	ATCC	VR-1291	Not Detected
98	*Pseudomonas aeruginosa*	ZMC	0801908	Not Detected
99	*Streptococcus pneumoniae*	KCCM	40410	Not Detected
100	*Proteus mirabilis*	ZMC	0801544	Not Detected

NO.	Organism	Source	Isolate No.	Result†
101	*Candida albicans*	KCCM	11282	Not Detected
102	*Streptococcus pyrogenes*	KCCM	11873	Not Detected
103	*Streptococcus mitis*	KCCM	42898	Not Detected
104	*Bordetella pertussis*	ATCC	9797	Not Detected
105	*Staphylococcus epidermidis*	KCCM	40416	Not Detected
106	*Chlamydophila pneumoniae*	ATCC	53592	Not Detected
107	*Enterobacter aerogenes*	KCTC	2190	Not Detected
108	*Enterobacter cloacae*	ZMC	0801830	Not Detected
109	*Klebsiella pneumoniae*	ATCC	BAA-1706	Not Detected
110	*Legionella pneumophila*	KCTC	12009	Not Detected
111	*Mycoplasma pneumoniae M129*	ZMC	0801579	Not Detected
112	SARS-coronavirus, Tor2	ZMC	NATSARS-ST	Not Detected
113	MERS-coronavirus, EMC/2012	ZMC	NATMERS-ST	Not Detected
114	*Streptococcus salivarius*	KCTC	5512	Not Detected
115	*Corynebacterium diphtheriae*	KCTC	3075	Not Detected
116	*Escherichia coli*	NCCP	13718	Not Detected
117	*Lactobacillus acidophilus*	KCTC	3140	Not Detected
118	*Legionella longbeachae*	ATCC	33462	Not Detected
119	*Moraxella catarrhalis*	ATCC	25238	Not Detected
120	*Neisseria meningitidis*	KCCM	41562	Not Detected
121	*Cytomegalovirus*	KBPV	VR-7	Not Detected
122	*Epstein-Barr virus*	ATCC	VR-1491	Not Detected
123	*Human adenovirus 7*	ATCC	VR-7	Not Detected
124	*Pooled human nasal wash**	Korean isolate sample		Not Detected

† Specificity tests were repeated 3 times.

※ ATCC: American Type Culture Collection

BEI: BEI Resources
KBPV: Korea Bank for Pathogenic Viruses
KCCM: Korean Culture Center of Microorganisms
KCTC: Korean Collection for Type Cultures
ZMC: ZeptoMetrix Corporation

Competitive Microbial Interference

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is not interfered with the detection of 5 target organisms at a low concentration (3X LoD) by the presence of high concentration (≥10^6 CFU/mL or PFU/mL) of clinically relevant 32 non-target organisms.

No.	Usage	Organism	Source	Isolate No.	Type	Result†
1	Inclusivity	SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated	BEI	NR-52287	RNA virus	SARS-CoV-2 Detected
2	Inclusivity	Respiratory Syncytial Virus Type A (4/2015 Isolate #1)	Zeptometrix	0810481CF	RNA virus	RSV Detected
3	Inclusivity	Respiratory Syncytial Virus Type B (12/2014 Isolate #1)	Zeptometrix	0810450CF	RNA virus	RSV Detected
4	Inclusivity	Influenza B (Brisbane/60/08)	Zeptometrix	0810254CF	RNA virus	Flu B Detected
5	Inclusivity	Influenza A H3N2 (Victoria/361/11)	Zeptometrix	0810240CF	RNA virus	Flu A Detected
6	Exclusivity	Human rhinovirus 14	ATCC	VR-284	RNA virus	Not Detected
7	Exclusivity	Human coronavirus 229E (MRC5)	ATCC	VR-740	RNA virus	Not Detected
8	Exclusivity	Human coronavirus NL63	Zeptometrix	0810228CF	RNA virus	Not Detected
9	Exclusivity	Human parainfluenza virus 1	ATCC	VR-1380	RNA virus	Not Detected
10	Exclusivity	Human parainfluenza virus 2	ATCC	VR-92	RNA virus	Not Detected
11	Exclusivity	Human echovirus 9	ATCC	VR-39	RNA virus	Not Detected
12	Exclusivity	Human coronavirus OC43	KBPV	VR-8	RNA virus	Not Detected
13	Exclusivity	Human coronavirus HKU1	ATCC	VR-3262SD	RNA virus	Not Detected
14	Exclusivity	Human parainfluenza virus 3	ATCC	VR-93	RNA virus	Not Detected
15	Exclusivity	Human adenovirus 1	ATCC	VR-1	DNA virus	Not Detected
16	Exclusivity	Human Metapneumovirus (hMPV) 18	Zeptometrix	0810162CF	RNA virus	Not Detected
17	Exclusivity	Human bocavirus	Twist Bioscience	103004	DNA virus	Not Detected
18	Exclusivity	Human parainfluenza virus 4A	ATCC	VR-1378	RNA virus	Not Detected
19	Exclusivity	*Pseudomonas aeruginosa*	Zeptometrix	801908	Bacteria	Not Detected
20	Exclusivity	*Streptococcus pneumoniae*	KCCM	40410	Bacteria	Not Detected
21	Exclusivity	*Bordetella pertussis*	ATCC	9797	Bacteria	Not Detected
22	Exclusivity	*Chlamydophila pneumoniae*	ATCC	53592	Bacteria	Not Detected
23	Exclusivity	*Klebsiella pneumoniae*	KCCM	40890	Bacteria	Not Detected
24	Exclusivity	*Legionella pneumophila*	KCTC	12009	Bacteria	Not Detected

25	Exclusivity	*Mycoplasma pneumoniae M129*	Zeptometrix	801579	Bacteria	Not Detected
26	Exclusivity	*Moraxella catarrhalis*	ATCC	25238	Bacteria	Not Detected
27	Exclusivity	*Neisseria meningitidis*	KCCM	41562	Bacteria	Not Detected
28	Exclusivity	*Haemophilus influenzae*	KCCM	42099	Bacteria	Not Detected
29	Exclusivity	SARS-coronavirus, Tor2	Zeptometrix	NATSARS-ST	RNA virus	Not Detected
30	Exclusivity	MERS-coronavirus, EMC/2012	Zeptometrix	NATMERS-ST	RNA virus	Not Detected
31	Exclusivity	Enterovirus Type 68 (2014 Isolate)	Zeptometrix	0810300CF	RNA virus	Not Detected
32	Exclusivity	Human coxsackievirus A24	ATCC	VR-583	RNA virus	Not Detected
33	Exclusivity	Candida albicans	KCCM	11282	Fungi	Not Detected
34	Exclusivity	Streptococcus pyogenes	KCCM	11873	Bacteria	Not Detected
35	Exclusivity	Staphylococcus epidermidis	KCCM	40416	Bacteria	Not Detected
36	Exclusivity	Streptococcus salivarius	KCTC	5512	Bacteria	Not Detected
37	Exclusivity	Pooled human nasal wash	LeeBiosolutions	991-13-P	Pooled human donors	Not Detected

† Competitive microbial interference tests were repeated 3 times.

Reproducibility

The reproducibility test was prepared including High Negative (0.1 X LoD), Low positive (1XLoD) and Moderate positive (3XLoD) samples. At each testing site, the kit was tested for five days, two runs per day by two different experimenters and triplicate of each target. The positive rates were observed for each target for reproducibility study: 100.0% for Moderate positive samples, ≥95% for Low positive samples. The reproducibility of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was evaluated between runs, sites and product lots. Positive rates for all concentrations and CV values met criteria of less than 10 (<10).
The results were satisfied with the Criteria set above, thus confirming the reproducible performances of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay.

Interfering substances

There were not effects on the results by adding the substances: non-specific detections or inhibitions on target amplification. Based on the results, 7 interfering substances had no effect on Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay results.

No.	Interfering Substances	Source	Test Concentration
1	Mucin (bovine submaxillary gland, type I-S)	Sigma-Aldrich (Cat.No.M3895)	60 μg/ml
2	Mupirocin (Antibiotic, nasal ointment)	Sigma-Aldrich (Cat.No.1448901)	6.6 mg/ml
3	Oxymetazoline (Afrin Nasal Spray)	Sigma-Aldrich (Cat.No.O2378)	15% (v/v)
4	Blood	Human	2% (v/v)
5	Tobramycin (Antibacterial, systemic)	Sigma-Aldrich (Cat.No.T4014)	4.0 μg/mL
6	Zanamivir (Anti-viral drug-Relenza)	Sigma-Aldrich (Cat.No.SML0492)	3.3 mg/mL
7	Oseltamivir (Anti-viral drug-Tamiflu)	Sigma-Aldrich (Cat.No.1479304)	25 mg/mL

Clinical Evaluation

A total of 361 nasopharyngeal swab sample were included in this clinical performance. Clinical performance of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was evaluated through the comparison with reference products which are CE-IVD approved before. The result has shown higher than 95% of agreement in clinical samples. Therefore, it is confirmed that the quality of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is valid. The performance is summarized in table.

SARS-CoV-2		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2/ FluA/FluB/RSV Assay	Positive	48	0	48
	Negative	1*	76	77
	Total	49	76	125

- PPA (Positive Percent Agreement): 97.96% (95% CI: 89.15% to 99.95%)
- NPA (Negative Percent Agreement): 100.00% (95% CI: 95.26% to 100.00%)
- OPA (Overall Percent Agreement): 99.20% (95% CI: 95.62% to 99.98%)
- Kappa value: 0.983 (95% CI: 0.950 to 1.000)

* Sample confirmed true positive by sequencing

Influenza A virus (Flu A)		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2/ FluA/FluB/RSV Assay	Positive	63	0	63
	Negative	1*	172	173
	Total	64	172	236

- PPA (Positive Percent Agreement): 98.44% (95% CI: 91.60% to 99.96%)
- NPA (Negative Percent Agreement): 100.00% (95% CI: 97.88% to 100.00%)
- OPA (Overall Percent Agreement): 99.58% (95% CI: 97.66% to 99.99%)
- Kappa value: 0.989 (95% CI: 0.968 to 1.000)

* Sample confirmed true positive by sequencing

Influenza B virus (Flu B)		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2/ FluA/FluB/RSV Assay	Positive	29	0	29
	Negative	0	207	207
	Total	29	207	236

- PPA (Positive Percent Agreement): 100.00% (95% CI: 88.06% to 100.00%)
- NPA (Negative Percent Agreement): 100.00% (95% CI: 98.23% to 100.00%)
- OPA (Overall Percent Agreement): 100.00% (95% CI: 98.45% to 100.00%)
- Kappa value: 1.000 (95% CI: 1.000 to 1.000)

Respiratory syncytial virus (RSV)		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2/ FluA/FluB/RSV Assay	Positive	63	8*	71
	Negative	0	165	165
	Total	63	173	236

- PPA (Positive Percent Agreement): 100.00% (95% CI: 94.31% to 100.00%)
- NPA (Negative Percent Agreement): 95.38% (95% CI: 91.09% to 97.98%)
- OPA (Overall Percent Agreement): 96.61% (95% CI: 93.43% to 98.53%)
- Kappa value: 0.917 (95% CI: 0.860 to 0.973)

* 4 Samples confirmed true positive by sequencing

■ CHAPTER 12: 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
PREMIX	Enzyme mix
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
UDI	Unique Device Identifier
rxns	Reaction barcode for automated extraction system

■ CHAPTER 13: 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 CANADA located at 240 Richmond Street West Toronto ON M5V1V6 Canada.

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