In Vitro Diagnostic Medical Device

Allplex™ 2019-nCoV Assay

CE

Cat. No. RP10244Y
RP10243X*

*For use with the 1. Microlab NIMBUS IVD and Microlab STARlet IVD
2. Seegene NIMBUS and Seegene STARlet

NOTICE

- For in vitro diagnostic use only.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- **This test has been validated for the following specimen types: sputum, nasopharyngeal aspirate, throat & nasopharyngeal swab, and bronchoalveolar lavage.**
- This test has not been validated for any other types of specimens.
- **Store RNA samples at ≤ -20℃ until use and keep on ice during use.**
- Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer period of time.
- Workflow in the laboratory should proceed in an 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 equipments 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 all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.
- Please be careful not to contaminate 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 experiment.
- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.
- Store positive materials separated from the kit's reagents.
- 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.
- Expiry date is 8 months from the date of manufacture at ≤ -20℃. Please refer to label for final expiry date.
- Seegene NIMBUS and Seegene STARlet are the same equipment as Microlab NIMBUS IVD and Microlab STARlet IVD, although the manufacturer is different. Since there are no hardware changes on the device, the test results are the same.
- The brand name of "CFX96™ Real-time PCR Detection System-IVD" is changed to "CFX96™ Dx System". Since there are no hardware changes to the systems, it is expected to obtain the same results from both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software-IVD 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.
- This kit is a qualitative in vitro test for the single or multiple detection of 3 types of gene (E gene, RdRP gene, and N gene).

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EC REP

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Intended Use

Allplex™ 2019-nCoV Assay is in vitro diagnostic medical device designed for qualitative detection of novel Corona virus (2019-nCoV) with real-time reverse transcription PCR from sputum, nasopharyngeal aspirate, oropharyngeal(throat) & nasopharyngeal swab, and bronchoalveolar lavage.

Nucleic Acid Extraction

NOTE: 10 µL of RP-V IC must be added to each specimen before nucleic acid extraction.

NOTE: Vortex specimen before use. When the specimen is still viscous, cool down its temperature or add saline solution.

NOTE: Store RNA samples at ≤ -20℃ until use and keep on ice during use.

Microlab NIMBUS IVD / STARlet IVD
Seegene NIMBUS/STARlet

When using STARMag 96 X 4 Universal Cartridge kit

300 µL Specimen
10 µL RP-V IC

Proceed the extraction process using manufacturer's protocol.

SGprep32™

Proceed the extraction process using manufacturer's protocol.

200 µL Specimen
10 µL RP-V IC
10 µL Proteinase K
100 µL Elution

SEEPREP32™

Proceed the extraction process using manufacturer's protocol.

200 µL Specimen
10 µL RP-V IC
10 µL Proteinase K
100 µL Elution

NucliSENS® easyMAG®

Proceed the extraction process using NucliSENS easyMAG generic protocol.

200 µL Specimen
10 µL RP-V IC
50 µL Magnetic silica
100 µL Elution

Ribospin® vRD Kit (GeneAll)

290 µL Specimen
10 µL RP-V IC
500 µL Buffer VL

Pulse-vortexing for 15 sec and then incubate at room temperature for 10 min

Add 700 µL of Buffer RB1 and then vortexing

DO NOT centrifuge after adding Buffer RB1

Apply 750 µL of the lysate onto the column and then centrifuge (15,000 x g (13,000 rpm), 30 sec)

Discard the flow through

Apply the residual lysate onto the column and then centrifuge (15,000 x g (13,000 rpm), 30 sec)

Discard the flow through

Add 500 µL of Buffer RBW and then centrifuge (15,000 x g (13,000 rpm), 30 sec)

Discard the flow through

Add 500 µL of Buffer RNW and then centrifuge (15,000 x g (13,000 rpm), 30 sec)

Discard the flow through

Centrifuge (15,000 x g (13,000 rpm), 1 min) to dry the membrane completely

Place the column in a clean 1.5 mL microcentrifuge tube

Apply 40 µL of Nuclease-free water to the center of the membrane

Incubate at room temperature for 2 min

Centrifuge (15,000 x g (13,000 rpm), 1 min)

QIAamp® DSP Virus Spin Kit

25 µL QIAGEN Protease
190 µL Specimen
10 µL RP-V IC
200 µL Buffer AL

Pulse-vortexing for 15 sec and then incubate at 56℃ for 15 min

Add 250 µL of 100% ethanol

Pulse-vortexing for 15 sec and then incubate at room temperature for 5 min

Apply all of the lysate onto the column and then centrifuge (6,000 x g (8,000 rpm), 1 min)

Place the column in a clean 2 mL collection tube

Add 500 µL of Buffer AW1 and then centrifuge (6,000 x g (8,000 rpm), 1 min)

Place the column in a clean 2 mL collection tube

Add 500 µL of Buffer AW2 and then centrifuge (6,000 x g (8,000 rpm), 1 min)

Place the column in a clean 2 mL collection tube

Add 500 µL of 100% ethanol and then centrifuge (6,000 x g (8,000 rpm), 1 min)

Place the column in a clean 2 mL collection tube

Centrifuge at full speed (20,000 x g (14,000 rpm), 3 min) to dry the membrane completely

Place the column in a clean 1.5 mL microcentrifuge tube

Apply 40 µL of Buffer AVE to the center of the membrane

Incubate at room temperature for 2 min

Centrifuge (20,000 x g (14,000 rpm), 1 min)

NOTE: If the RP-V IC is not added during extraction, when analyzing the result, the Negative sample will be invalid. If you want to use without extraction, please contact the manufacturer for PCR control IC.

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Kit stability

- Expiry date is **8 months** from the date of manufacture at ≤ -20℃. Please refer to label for final expiry date.
- This product can be used by **5 days** after opening of the packing and repeatedly frozen and thawed repeat.

Specimen Handling and Storage

- Specimens can be stored at 4 ℃ for up to 72 hours after collection. If any delay in extraction is expected, store specimens at -70℃ or lower.
- Extracted nucleic acids should be stored at -70 ℃ or lower.

Amplification and Detection (CFX96™, Bio-Rad)

1. Preparation for Real-time PCR

NOTE: Centrifuge all reagents stored at ≤ -20℃ after thawing them completely.

NOTE: Positive control amplification and clinical samples require special caution in order to avoid carry-over contamination.

NOTE: PCR setup can be performed on Microlab NIMBUS IVD. Please contact Seegene for NIMBUS method and protocol file.

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

One-step RT-PCR Mastermix for different no. 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 the tube 5 times or quick vortex, and briefly centrifuge.

③ Aliquot **17 µL** of the One-step RT-PCR Mastermix into PCR tubes*.

④ Add **8 µL** of each sample's nucleic acids, 2019-nCoV PC and NC (RNase-free water) into the tube containing aliquot of the One-step RT-PCR Mastermix.

⑤ Close the cap, and briefly centrifuge the PCR tubes.

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.

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.

*** Available PCR Tube**

Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)
Optical Flat 8-Cap Strips (Cat No. TCS0803, Bio-Rad)
Hard-Shell® PCR plates 96-well WHT/WHT (Cat. No. HSP9655, Bio-Rad)

[Analytes]

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

2. Real-time PCR Instrument set up

① Protocol Setup

- In the main menu, select **File** → **New** → **Protocol** to open **Protocol Editor**.
- In **Protocol Editor**, define the thermal profile **as table below**.
- Click the box next to **Sample Volume** to directly input 25 µL.
- Click **OK** and save the protocol to open the **Experiment Setup** window.

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		

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

② Plate Setup

- From **Plate** tab in **Experiment Setup**, click **Create New** to open **Plate Editor** window.
- Click **Select Fluorophores** to indicate the fluorophores (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**) that will be used and click **OK**.
- Select the desired well(s) and then its sample type from the **Sample Type** drop-down menu.
  - **Unknown** : Clinical samples
  - **Negative Control**
  - **Positive Control**
- Click on the appropriate checkboxes (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**) to specify the fluorophores to be detected in the selected wells.
- Type in **Sample Name** and press enter key.
- In **Settings** of the **Plate Editor** main menu, choose **Plate Size (96 wells)** and **Plate Type (BR White).**
- Click **OK** to save the new plate.
- You will be returned to the **Experiment Setup** window.

③ Start Run

- From **Start Run** tab in **Experiment Setup**, click **Close Lid** to close the instrument lid.
- Click **Start Run**.
- Store the run file either in My Documents or in a designated folder. Input the file name, click **SAVE**, and the run will start.

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Data Analysis (CFX96™, Bio-Rad)

1. Pre-setting for Data Analysis

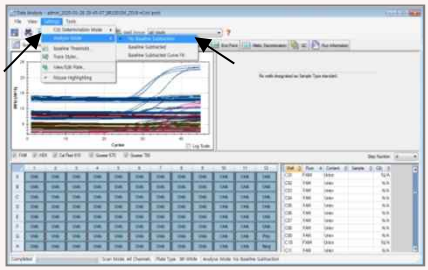
A. Create folders for data export

① Create one folder to save amplification curve detection results.

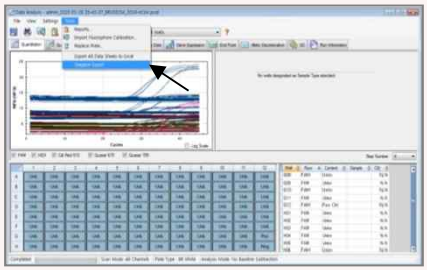
② Folder name may be as desired by user (For 'Seegene Export' function, Folders "QuantStep4" is automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96 Manager™

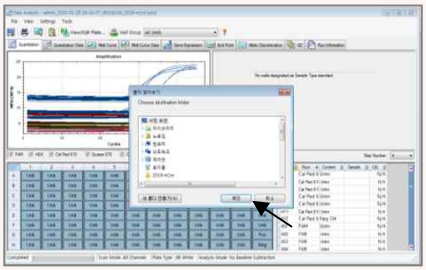
① After the test, Select **No Baseline Subtraction** from Analysis Mode of **Settings** menu.



② Select **Seegene Export** from **Tools** menu.



③ Choose a location to save data and click **OK**.

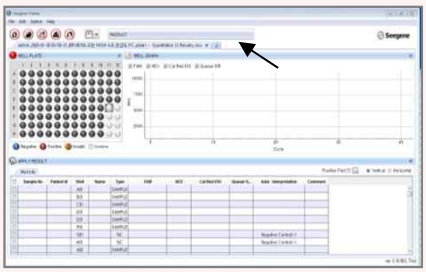


Note : Please, contact Seegene about using 'Seegene Export' or other data file export method on CFX96 Manager.

2. Settings for Data Analysis in Seegene Viewer

① Open **Seegene Viewer** program, and click **Open** to find the saved file in folder "QuantStep4".

② After opening the results file, select the **"Allplex™ 2019-nCoV Assay"** from the **PRODUCT** menu.



③ Check the result for each well.

Data Analysis

[Interpretation]

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
IC (HEX)	+/-	+/-	+/-	+/-	+/-	+	-
E gene (FAM)	+	+/-	-	+/-	+	-	-
RdRP gene (Cal Red 610)	+	-	+	+	-	-	-
N gene (Quasar 670)	+	+	+	-	-	-	-
Result Interpretation	2019-nCoV Detected	Inconclusive Result*			2019-nCoV Not Detected, sarbecovirus Detected	Negative	Invalid

* 1) Recommended to re-test by increasing the sample concentration.
2) Recommended to proceed with sequencing.

[Cut-off]

Ct value	Result
≤ 40	Valid
> 40 or N/A	Invalid

If Ct value of IC is '**> 40**', please conduct a re-test.

Performance Data

1. Specificity

Cross-reactivity of Allplex™ 2019-nCoV Assay was tested using 49 standard materials and organisms as indicated below. Specific targets which were designed for the detection were identified by Allplex™ 2019-nCoV Assay.

NO.	Organism	Source	Isolate No.	Result
1	human coronavirus HKU1	Korean isolate		Not Detected
2	human coronavirus OC43	Korean isolate		Not Detected
3	human coronavirus NL63	Korean isolate		Not Detected
4	human coronavirus 229E	ATCC	VR-740	Not Detected
5	influenza A virus (H1N1)	ATCC	VR-95 (H1N1)	Not Detected
6	Influenza A virus (H3N2)	ATCC	VR-547	Not Detected
7	influenza B virus	ATCC	VR-523	Not Detected
8	Human Rhinovirus 1	KBPV	VR-81	Not Detected
9	Rhinovirus 21	KBPV	VR-40	Not Detected
10	Human rhinovirus type 90	ATCC	VR-1291	Not Detected
11	Human rhinovirus type 16	ATCC	VR-283	Not Detected
12	Human rhinovirus type 42	ATCC	VR-338	Not Detected
13	Human rhinovirus type 8	ATCC	VR-488	Not Detected
14	Human rhinovirus type 14	ATCC	VR-284	Not Detected
15	Human enterovirus type 68	ATCC	VR-1826	Not Detected
16	Human enterovirus type 70	ATCC	VR-836	Not Detected
17	Human enterovirus type 71	ATCC	VR-784	Not Detected
18	human respiratory syncytial virus A	ATCC	VR-26	Not Detected
19	human respiratory syncytial virus B	ATCC	VR-955	Not Detected
20	Parainfluenza 1 virus	ATCC	VR-1380	Not Detected
21	Human parainfluenza virus 2	ATCC	VR-92	Not Detected
22	Human parainfluenza virus 3	ATCC	VR-93	Not Detected
23	human parainfluenza 4 virus 4a	ATCC	VR-1378	Not Detected
24	Human parainfluenza virus 4b	ATCC	VR-1377	Not Detected
25	Human Metapneumovirus (MPV)	KBPV	VR-87	Not Detected
26	Human adenovirus 1	ATCC	VR-1	Not Detected
27	Human adenovirus 11	KBPV	VR-63	Not Detected
28	Human adenovirus 18	ATCC	VR-1095	Not Detected
29	Human adenovirus 23	ATCC	VR-1101	Not Detected
30	Human adenovirus 3	ATCC	VR-3	Not Detected
31	Human adenovirus 4	ATCC	VR-1572	Not Detected
32	Human adenovirus 8	ATCC	VR-1368	Not Detected
33	Human adenovirus type 31	ATCC	VR-1109	Not Detected
34	Human adenovirus type 40	ATCC	VR-931	Not Detected
35	Human adenovirus type 5	KBPV	VR-61	Not Detected
36	Human adenovirus type 35	ATCC	VR-718	Not Detected
37	Human Bocavirus (HBoV)	Korean isolate		Not Detected
38	Legionella pneumophila Serotype 2	ATCC	33154	Not Detected
39	Legionella pneumophila subsp. fraseri Serotype 4	ATCC	33156	Not Detected
40	Legionella pneumophila Serotype 7	ATCC	33823	Not Detected
41	Legionella pneumophila Serotype 10	ATCC	43283	Not Detected
42	Legionella pneumophila Serotype 11	ATCC	43130	Not Detected
43	Legionella pneumophila Serotype 12	ATCC	43290	Not Detected
44	Legionella pneumophila Serotype 13	ATCC	43736	Not Detected
45	Legionella pneumophila Serotype 14	ATCC	43703	Not Detected
46	Legionella pneumophila subsp. fraseri Serotype 15	ATCC	35251	Not Detected
47	Mycoplasma pneumoniae	ATCC	15293	Not Detected
48	Mycoplasma pneumoniae	ATCC	29342	Not Detected
49	Mycoplasma pneumoniae M129-B7	ATCC	29342	Not Detected

2. Sensitivity

In order to determine the sensitivity of Allplex™ 2019-nCoV Assay, a standard serial dilution of In vitro transcription RNA was set up from $2X10^3$ to 10 RNA copies/reaction and was analyzed with Allplex™ 2019-nCoV Assay. Detection limit of Allplex™ 2019-nCoV Assay was **100** RNA copies/reaction.

TROUBLESHOOTINGS

Allplex™ 2019-nCoV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis and export the data again. There is no need to repeat the test in this case.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage conditions and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Nucleic acid extraction failure	No signal including IC may indicate loss of nucleic acid during extraction. Make sure that you use recommended extraction method. If due to inhibitors, re-extract the original specimen or dilute the specimen (1/3~1/10) in saline solution and repeat the test from extraction step.
No Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not IC, then IC amplification may have been inhibited by high titer of target pathogen. In order to confirm IC signal, dilute the specimen (1/3~1/10) in saline solution and repeat the test from extraction step.
	Presence of RT-PCR inhibitor	Please dilute the specimen (1/3~1/10) in saline solution and repeat the test from extraction step.
Putative false positive or target signals observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with a new set of reagents.

TROUBLESHOOTINGS

Allplex™ 2019-nCoV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative false negative or no signal observed in Positive Control	Error in specimen collection	Please check the specimen collection method, and re-collect specimen.
	Incorrect storage of the specimen	Please re-collect the specimen and repeat the entire procedure. Ensure that the specimen is stored as recommended.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract nucleic acid.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid in to the correct PCR tubes and carefully repeat the test if necessary.
	Presence of inhibitor	Please dilute the specimen (1/3~1/10) in saline solution and repeat the test from extraction step.
	Incorrect PCR mixture	Confirm that all components are added to the RT-PCR mixture (Sensitivity is compromised with pre-composed premix). All reagents must be homogenized and spun down before use.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Centrifuge the PCR tube before run.

KEY TO SYMBOLS

Symbol	Explanation
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use-by date
	Upper limit of temperature
ENZYME	Enzyme Mix
PREMIX	Oligonucleotide mix for amplification and detection
BUFFER	Buffer
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