For use only under Emergency Use Authorization

Allplex™ 2019-nCoV Assay

Cat. No. RP10250X

For U.S. ONLY

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 nucleic acid from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate and sputum specimens from individuals with signs and symptoms of infection who are suspected of COVID-19 by their health care provider. Testing is limited to U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Kit stability

- Expiry date is **8 months** from the date of manufacture at ≤ -20℃. Please refer to product label for final expiry date.
- This product can be used for **30 days** after opening the vials.
- This product can be used for maximum 7 repeats of freezing and thawing.

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 -20 ℃ or lower.

Nucleic Acid Extraction

NOTE: Vortex specimen before use. If the specimen is still viscous, let it cool down or add saline solution.
NOTE: Refer to volume of internal control (IC) in Seegene Launcher program.

Seegene NIMBUS/STARlet

Extraction reagent : STARMag™ 96 X 4 Universal Cartridge kit*

Required (or Minimum) specimen volume : 300 µL
Elution volume : 100 µL

Proceed the extraction step following 'Tutorial' of Seegene Launcher program.
RP-V IC tube must be loaded on extraction equipment before nucleic acid extraction.

Amplification and Detection (CFX96 Touch™, Bio-Rad)

NOTE: If the RP-V IC is not added during extraction, the negative sample is interpreted as 'Invalid'.

1. Preparation for Real-time PCR

NOTE: After completely thawing all reagents stored at ≤ -20℃, centrifugation must be performed.
NOTE: Positive control and clinical samples require special caution in order to avoid carry-over contamination.
NOTE: PCR setup can be performed automatically via Seegene NIMBUS or STARlet. The following is a guide for manual users.

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

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 an 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 PCR.

NOTE: Be sure to centrifuge the PCR tube before running PCR reaction in order to set 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)
Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)*
PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)*
* The above mentioned heat seal and plate sealer must be used in combination.

[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**.

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.

- Click the box next to **Sample Volume** to directly input 25 µL.
- Click **OK** and save the protocol to open the **Experiment Setup** window.

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.

** Required, but not provided (Cat. No. 744300.4.UC384)*

04/2020 V1.0

Data Analysis (CFX96™ Touch, Bio-Rad)

1. Pre-setting for Data Analysis

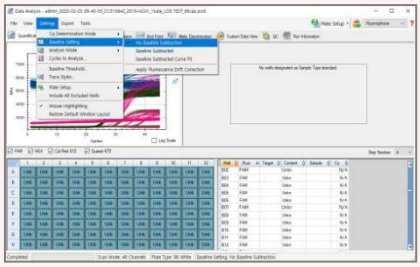
A. Create folders for data export

① Create a folder to save amplification curve detection results.

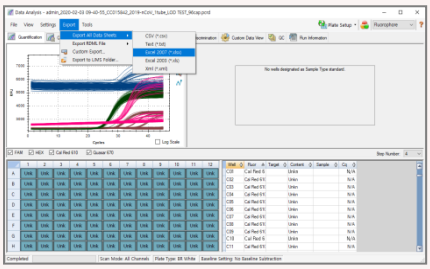
② The location and name of the folder is specified by user, but in case of using 'Seegene Export' function, folder named "QuantStep4" is created automatically in selected location.

B-1. Pre-settings for Data Analysis in CFX Manager™ Software V3.1 of CFX96™ Touch

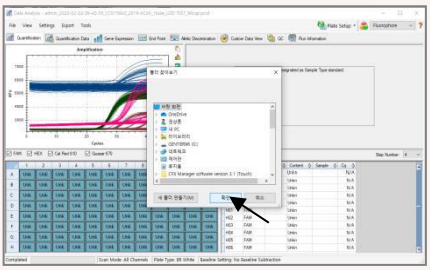
① After the PCR reaction, select **No Baseline Subtraction** from **Baseline Setting** of **Settings** menu.



② Select **Excel 2007** from **export All Data Sheets from Export** menu.

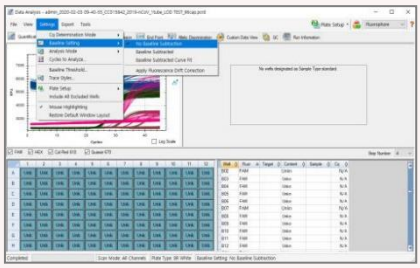


③ Choose a location to save data and click **OK**.

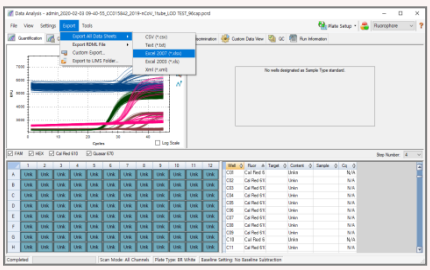


B-2. Pre-settings for Data Analysis in CFX Maestro™ Software of CFX96™ Touch

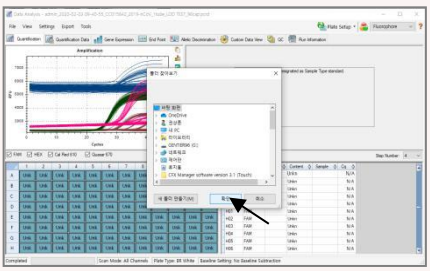
① After the PCR reaction, select **No Baseline Subtraction** from **Baseline Setting** of **Settings** menu.



② Select **Excel 2007** from **export All Data Sheets from Export** menu.



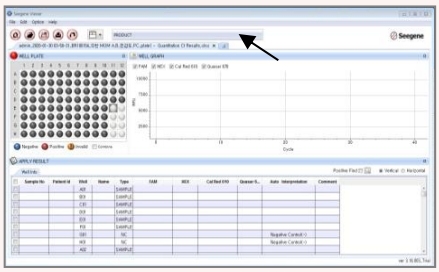
③ Choose a location to save data and click **OK**.



2. Settings for Data Analysis in Seegene Viewer

① Open **Seegene Viewer** program and click **Open,** select the exported data.

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



③ Check the result for each well.

Interpretation

Target	IC	E gene	RdRP gene	N gene	Auto-interpretation	Results
Fluorophore	HEX	FAM	CalRed 610	Quasar 670		
Case 1	+/-	+	+	+	2019-nCoV Detected	All Target Results are valid. Result for SARS-CoV-2 RNA is Detected.
Case 2	+/-	+	-	+	2019-nCoV Detected	All Target Results are valid. Result for SARS-CoV-2 RNA is Detected. Negative target results are suggestive of 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	All Target Results are valid. Result for Sarbecovirus RNA is detected. Result for SARS-CoV RNA is Presumptive Positive. Negative target results are suggestive of 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. Repeat test with more nucleic acids (up to 13 µL) instead of RNase-free Water. For sample with the same result on a repeated test, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Case 8	+	-	-	-	Negative	All Target Results are valid. Result for 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.

[Cut-off]

For all targets,

Ct value	Result
≤ 40	Detected
> 40 or N/A	Not detected

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