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 \leq -20 °C. 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 °C for up to 72 hours after collection. If any delay in extraction is expected, store specimens at -70°C or lower.
- + Extracted nucleic acids should be stored at -20 $\,^\circ\!\!\mathbb{C}$ or lower.

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 \leq -20 °C, 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.

 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 Onestep 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.
- 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

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/STARIet

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.

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.

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Step	No. of cycles	Temperature	Duration							
1	1	50 ℃	20 min							
2	1	95 ℃	15 min							
3	45	94 ℃	15 sec							
4*	40	58 ℃	30 sec							
5	GOT	O Step 3, 44 more tim	nes							

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

- Click the box next to Sample Volume to directly input 25 $\mu L.$
- Click \mathbf{OK} and save the protocol to open the $\mathbf{Experiment}$ 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)

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

 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.

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Interpretation

Target	IC	E gene	RdRP gene	N gene	Auto-			
Fluorophore	HEX	FAM	CalRed 610	Quasar 670	interpretation	Kesuits		
Case 1	+/-	+	+	+	2019-nCoV Detected	All Target Results are valid. Result for SARS-CoV-2 RNA is Detected.		
Case 2	+/-	+	-	+				
Case 3	+/-	+	+	-	2019-nCoV Detected	All Target Results are valid. Result for SARS-CoV-2 RNA is Detected.		
Case 4	+/-	-	+	+		2019-nCoV Detected	2019-nCoV	1) a sample at concentrations near or below the limit of detection of the test,
Case 5	+/-	-	-	+			 2) a mutation in the corresponding target region, or 3) other factors 	
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,