FastPlex Triplex SARS-COV-2 Detection Kit (RT-PCR, S Gene included)

Instructions for Use

Catalog # 02.01.1022
(96 Tests/kit)

CE IVD

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1. Package Specification

96 tests/kit

2. Intended Use

The ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal/oropharyngeal swabs from individuals with signs and symptoms of infection who are suspected of SARS-CoV-2. This kit meets the provisions of the Directive 98/79/EC of the European Parliament and of the Council on In-Vitro Diagnostic Medical Devices.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

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

The ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene Included)*** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The ***FastPlex Triplex SARS-COV-2 Detection Kit (RT- PCR, S Gene included)*** meets the provisions of the Directive 98/79/EC of the European Parliament and of the Council on In-Vitro Diagnostic Medical Devices.

3. Product Overview/Test Principle

The ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** is a real- time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients with signs and symptoms of infection who are suspected of SARS-CoV-2.

The oligonucleotide primers and probes for specific detection of SARS-CoV-2 are selected from regions of Open Reading Frame 1ab (ORF1ab), the nucleocapsid gene (N) and the spike glycoprotein (S) of the SARS-CoV- 2 genome. The kit includes primers/probes that are specific for the ORF1ab gene (probe labeled with FAM), N gene (probe labeled with HEX) and S gene (Probe labeled with ROX or Cy5.5) of SARS-CoV-2. In addition, the kit also contains primers and a probe (labeled with CY5) for the human RNase P gene as an endogenous internal control for specimen integrity, nucleic acid isolation, amplification and detection.

RNA isolated and purified from upper and lower respiratory tract specimens is reverse transcribed to cDNA and amplified in a Real-time PCR instrument using Master Mix (SARS-CoV-2 Detection Buffer (S Gene included) + Enzyme Mix). Probes consist of a reporter dye at the 5’ end and quenching dye at the 3’ end. The fluorescent signals emitted from the reporter dye are absorbed by the quencher. During

PCR amplification, probes hybridized to amplified templates are degraded by the Taq DNA polymerase with 5'-3' exonuclease activity, thereby separating the reporter dye and quencher and generating fluorescent signals that increase with each cycle. The PCR instrument automatically draws a real-time amplification curve for each optical channel based on the signal change, and calculates cycle threshold (Cq) values (the point at which fluorescence is detectable above background) that are interpreted by the operator to determine the presence/absence of SARS-CoV-2 RNA.

4. Components Included within the Kits

4.1 Rainamp™ collection, Transport and processing kit

Item No.	***Components***	***Composition***	***Quantities***	***Reactions/Tube***
1	Virus Transport Medium with Releasing Agent	Release Reagent	1.2mL×96	96
2	Swab	Swab	96	96

Swab may not be included in the Kit. Rainamp™ Virus Transport Medium with releasing agent is compatible with nasopharyngeal or oropharyngeal swabs from other swab manufacturers. Users can use their preferred swabs.

4.2 SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)

Item No.	***Components***	***Composition***	***Quantities***	***Reactions/Tube***
1	SARS-CoV-2 Detection Buffer (S Gene included)	Primers, Probes, dNTP, $MgCl_2$, PCR buffer	672 µl×1	96
2	Enzyme Mix	Reverse Transcriptase, DNA Polymerase, RNase Inhibitor	96 µl×1	96
3	SARS-CoV-2 Negative Control	Water	500 µl×1	29
4	SARS-CoV-2 Positive Control (S Gene included)	SARS-CoV-2 Pseudovirus (S Gene included) and Human HL-60 cells mix	500 µl×1	29

5. Reagent Stability and Transportation

The Rainamp™ collection, Transport and processing kit should be stored at Room Temperature before use.

The ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** (in small box) should be stored at -20℃ in the dark and should be transported in a sealed foam box with ice packs. The kit should be stored at -20℃. Unpacked kits should avoid repeated freeze-thaw cycles.

6. Components Required But Not Included within the Test

Consumables not supplied:

- 1.5 mL DNase-free and RNase-free Eppendorf tube
- 0.2 mL PCR tube or strip
- Various models of pipettes and pipette tips (10µL, 200µL and 1000µL tips with filters)
- Centrifuge (can reach to 12,000 rpm)
- Microcentrifuge
- Desktop vortex mixer
- 0.9% saline
- -20℃ cold blocks
- 10% bleach
- DNAZap™ (Ambion, cat. #AM9890)
- Disposable powder-free gloves and surgical gowns

Real-Time PCR Instrument(s):
Bio-Rad CFX96 Touch/CFX96 Real-Time PCR System

7. Warnings and Precautions

- This test meets the provisions of the Directive 98/79/EC of the European Parliament and of the Council on In-Vitro Diagnostic Medical Devices.

- For in vitro diagnostic use only (IVD).

- This test is only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

- Handle all specimens as if infectious using safe laboratory procedures.

- Please read the instructions carefully prior to operation.

- Samples must be collected, transported, and stored using the exact procedures and conditions recommended by the swab manufacturer and in this package insert. Improper collection, transport, or storage of specimens may impact the performance of this test.

- False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.

- Negative results do not preclude infection with SARS-CoV-2 virus and should not be used as the sole basis for treatment or other patient management decision.

- Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. a) 1st Area: Preparation Area—Prepare testing reagent: b) 2nd Area: Sample processing—Process the specimen and controls: c) 3rd Area: Amplification Area—PCR conducted.

- All materials used in one area should remain in that area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.

- All contents in this package are prepared and validated for the intended testing purpose. Do not replace any of the package contents as this will affect the testing performance of the kit.

- Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

- Filter plugged nuclease free pipette tips are required and should be replaced after the addition of each reagent or sample.

- Centrifuge tubes in the assay should be DNase/RNase-free.

8. Reagent Storage, Handling, and Stability

- Store ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** at -20°C in the dark when not in use.

- Use the reagents in ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** within 30 days once opened.

- Completely thaw the ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** before use; spin briefly before use.

- Do not freeze/thaw cycles for ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** more than 3 times.

- The reagents in ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** should be transported in a sealed foam box with ice packs or add dry ice.

9. Controls Materials

Controls – Positive and Negative Controls provided with the test kit include:

- SARS-CoV-2 Positive Control (S Gene include): The SARS-CoV-2 Positive Control consists of a mix of Pseudovirus of SARS-CoV-2 for ORF1ab, N gene, S gene and HL60 cells for internal control RNase P gene. The positive control should be positive for the ORF1ab gene (Cq equals or less than 36), the N gene (Cq equals or less than 36), the S gene (Cq equals or less than 37) and the RNase P gene targets (Cq equals or less than 36). If the results are not positive, the rRT- PCR run is invalid. Please refer to Table 2.

- SARS-CoV-2 Negative Control is RNase free water. SARS-CoV-2 Negative Control is used to detect any reagent or environmental contaminations. The SARS- CoV-2 Negative Control should be

negative for ORF1ab (FAM), N (HEX). S (ROX and Cy5.5) and RNase P (Cy5). If SARS-CoV-2 Negative Control shows any positive results, it indicates contamination of reagents or samples. All sample results need to be invalidated and results must not be reported. It is recommended to decontaminate the PCR lab and use a new box of un-opened reagent before repeating sample testing.

10. Collection, Storage and Shipment of Specimens

- Adequate, appropriate specimen collection, storage, and transport are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results.

- Specimen collection (for Extraction free procedure): **Rinsing mouth clean with water for 20-30 seconds before sample collection to remove any food residue and keep mouth clean. The food residue may inhibit PCR reaction and cause false negative results. To realize direct PCR without RNA extraction, Swab specimens should be collected only using virus preservation medium with releasing agent** in Rainamp™ collection, Transport and processing kit. Place swabs immediately into Virus Preservation Medium with Releasing Agent. If using other sample collection kit, RNA extraction maybe required prior to PCR reaction.

- Specimen Transportation: Specimens must be packaged, shipped, and transported at dry ice, overnight. Specimen Storage: Upon receipt, specimens can be immediately processed or stored at 2-8°C for up to 2 hours after collection. For longer term storage, store specimens at -70°C or lower.

11. Laboratory Procedure

11.1 Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, Bio-Rad CFX96 Touch/CFX96 Real-Time PCR System and other equipment prior to use. The following decontamination agents may be used: 10% bleach, 70% ethanol, or DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.

Warning: Do not use bleach when using specimen collection systems containing guanidinium isothiocyanate as a stabilizer as it may react with bleach to release toxic cyanide gas.

11.2 Preparation of the controls

To avoid contamination, the positive control needs to be prepared in an area separate from the amplification and extraction area.

11.3 Preparation of rRT-PCR Reactions

Thaw enzyme mix on ice. Keep the enzyme mix on ice or cold block all the time during preparation and use, and store it at -20ºC immediately after use.

- Thaw all ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** components at room temperature. Vortex all components and briefly spin to collect all liquid at the bottom of the tube.

• If specimens in Virus Preservation Medium with Releasing Agent were frozen, thaw specimens on ice or a cold block.

• **Vortex specimens in Rainamp™ VTM 2 mins** and centrifuge for 1 minute. After centrifugation, place the tubes in the cold rack or on ice.

• Prepare all PCR mix in an area separate from the sample preparation area.

• Determine the number of reactions (N is the number of reactions including samples, positive control, negative control) that will be included in the test.

• In a 1.5 mL microcentrifuge tubes (DNase/RNase free) prepare the PCR mix by adding detection mix and enzyme mix based on Table 4 below. Mix the PCR mix thoroughly by vortex. The remaining reagent must be stored at -20°C immediately.

Table 1. Preparation of PCR mix

Components	Volume [µL]	Final Concentration
SARS-CoV-2 Detection Mix (S gene included)	7µl x (N+1)	1×
Enzyme Mix	1 µl x (N+1)	1×
Total volume [**µL**]	8 µl x (N+1)	--

• Centrifuge the PCR mix prepared in step 7 for 1 minute to collect contents at the bottom of the tube, and then place the tube in a cold rack.

• Dispense 8 µL of the PCR mix into a 200 µL centrifuge tube. Be sure not to introduce any foam or bubbles into the tubes when aliquoting PCR reaction Mix. Cover the wells and transfer to the sample processing area.

• Add RNA Template:

1） For Extraction-free

Add 17 µL of the **specimens in Rainamp™ VTM** to the wells pre-filled with PCR mix in the following order: SARS-CoV-2 Negative Control, specimen(s), and SARS-CoV-2 Positive Control. Be sure to deposit samples with the pipette directly into the reaction mix in PCR tubes. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into Bio-Rad CFX96 Touch/CFX96 Real-Time PCR System and record the exact location of controls and each specimen.

2） For Extraction

Extraction Performed Procedure
Manual RNA isolation:

Nucleic acids are isolated and purified from Nasopharyngeal swab specimens using QIAamp Viral RNA Mini Kit, utilizing 140 µL of sample. In the extraction steps all controls, the SARS-

CoV-2 positive control, the SARS-CoV-2 negative control (contains RNase P only) and the no template control (water/VTM), are included with 140 µL control material instead of sample and are processed in an identical manner to the sample. Please follow the sample processing steps of the Manufacturer's instructions for use. 50 µL elution volume is used. The extracted RNA can be directly added to the rRT-PCR reaction immediately or store at -70℃. Controls are used the same way as the extracted samples (i.e., using 140 µL per extraction, 50 µL elution volume and 17 µL of extracted RNA for the PCR reaction) once per sample run. Manual RNA extraction can also be done using other suppliers' spin column-based RNA extraction kits according to manufacturers' instruction.

Automatic RNA isolation:

Nucleic acids are isolated and purified from Nasopharyngeal swab specimens can be extracted using automatic extractor (magnetic beads based), *e.g.,* QIAcube, Kingfisher, NPA-96 or other automatic RNA extractors according manufacturer's instructions.

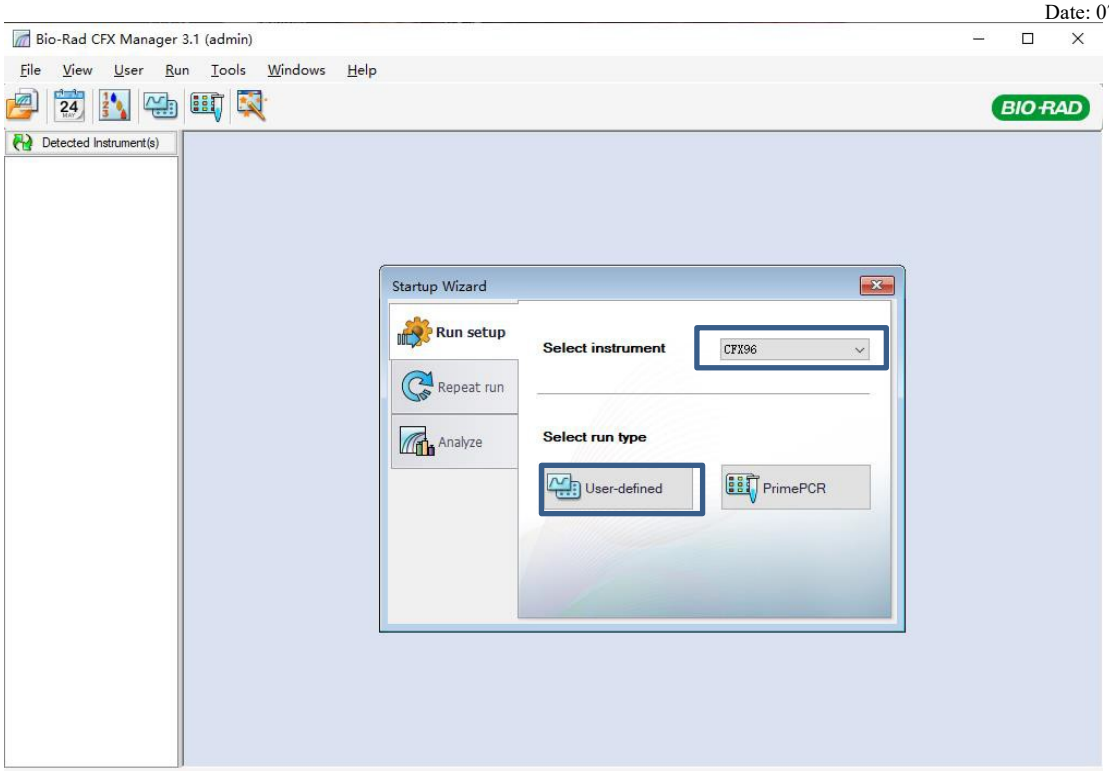
Add 17 µL **Extracted RNA** to the wells pre-filled with PCR mix in the following order: SARS-CoV-2 Negative Control, specimen(s), and SARS-CoV-2 Positive Control. Be sure to deposit samples with the pipette directly into the reaction mix in PCR tubes. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into Bio-Rad CFX96 Touch/CFX96 Real-Time PCR System and record the exact location of controls and each specimen.

- Running a PCR amplification on Bio-Rad CFX96 Real-Time PCR System using Bio-Rad CFX Manager 3.1:

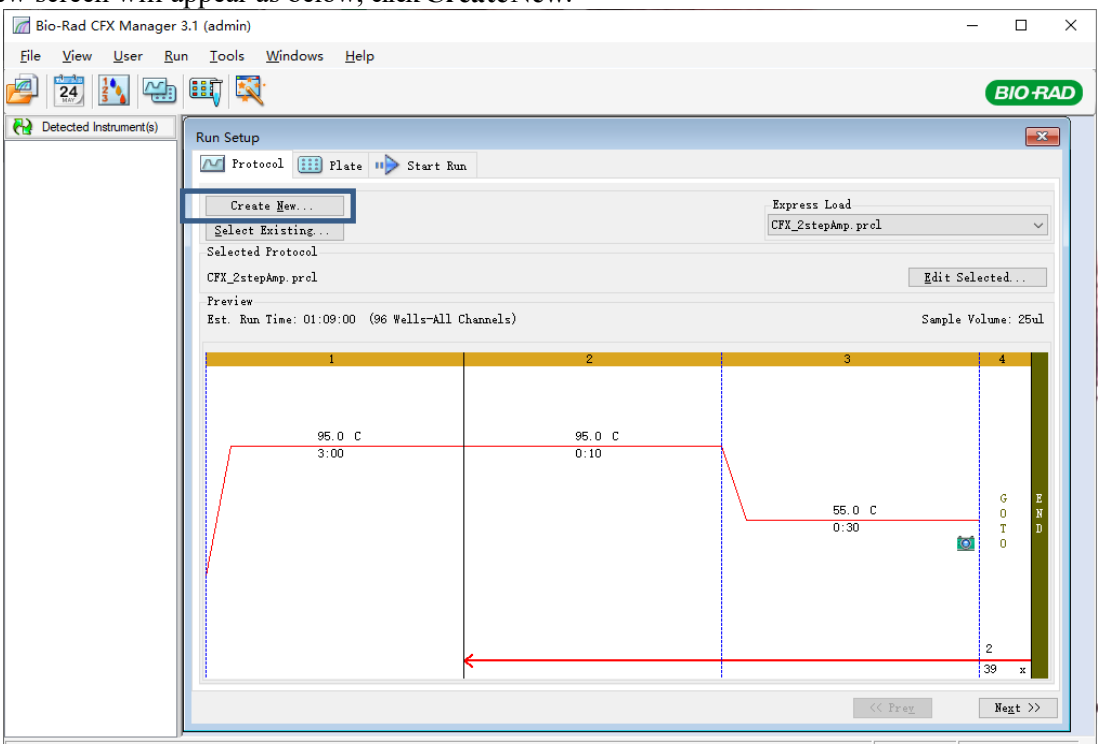
1）Start Bio-Rad CFX96 Real-Time PCR System: Turn on the computer connected to the system first, then turn on Bio-Rad CFX96 Real-Time PCR System.

2）Load the instrument: Open the lid, lift the lid lever completely until the reaction module stays open without assistance. Place the plate in the block and close the lid. Make sure increase the lid force by a half turn after the lid touches the plate.

3）Set up the experiment run:
Double-click Bio-Rad CFX Manager icon on the desktop. A new window should appear, select instrument as **CFX96** and click **User-defined**.

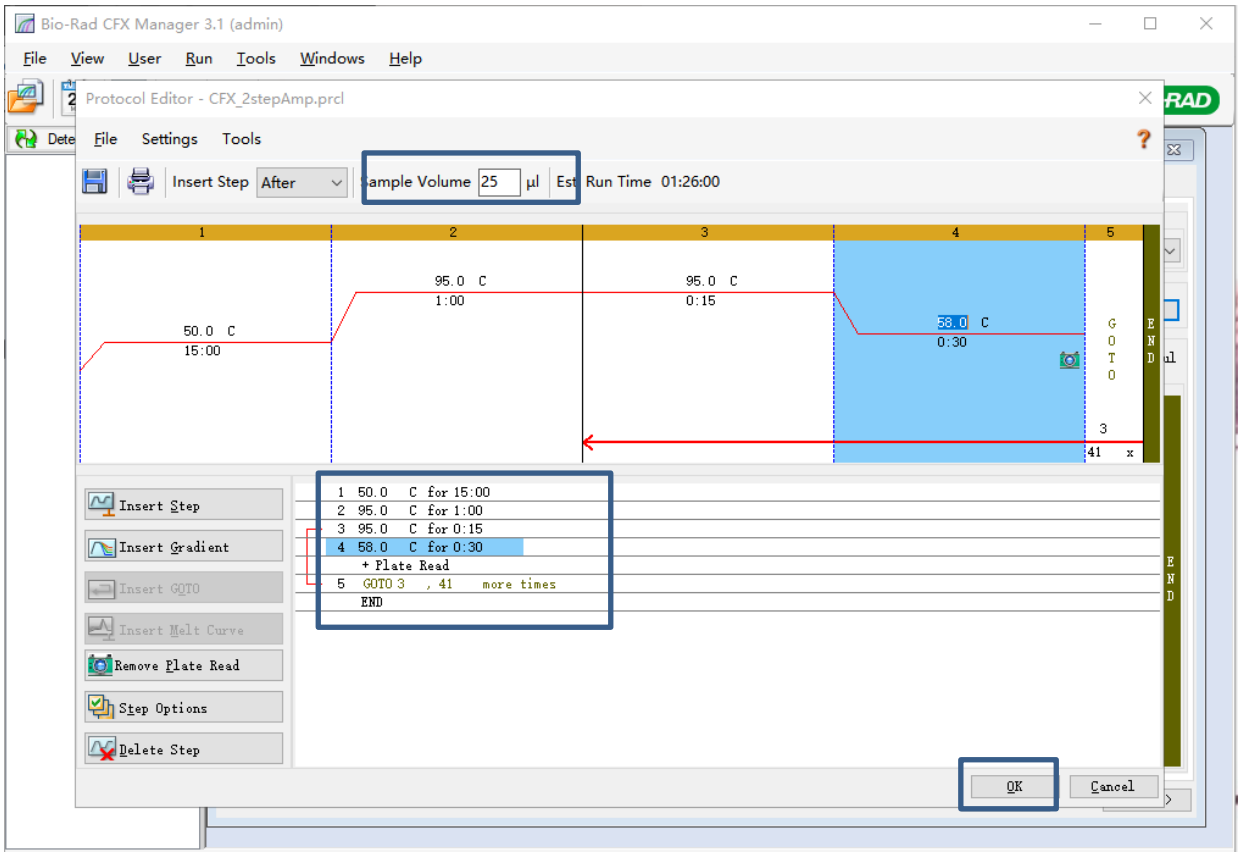


A new screen will appear as below, click **Create New**.

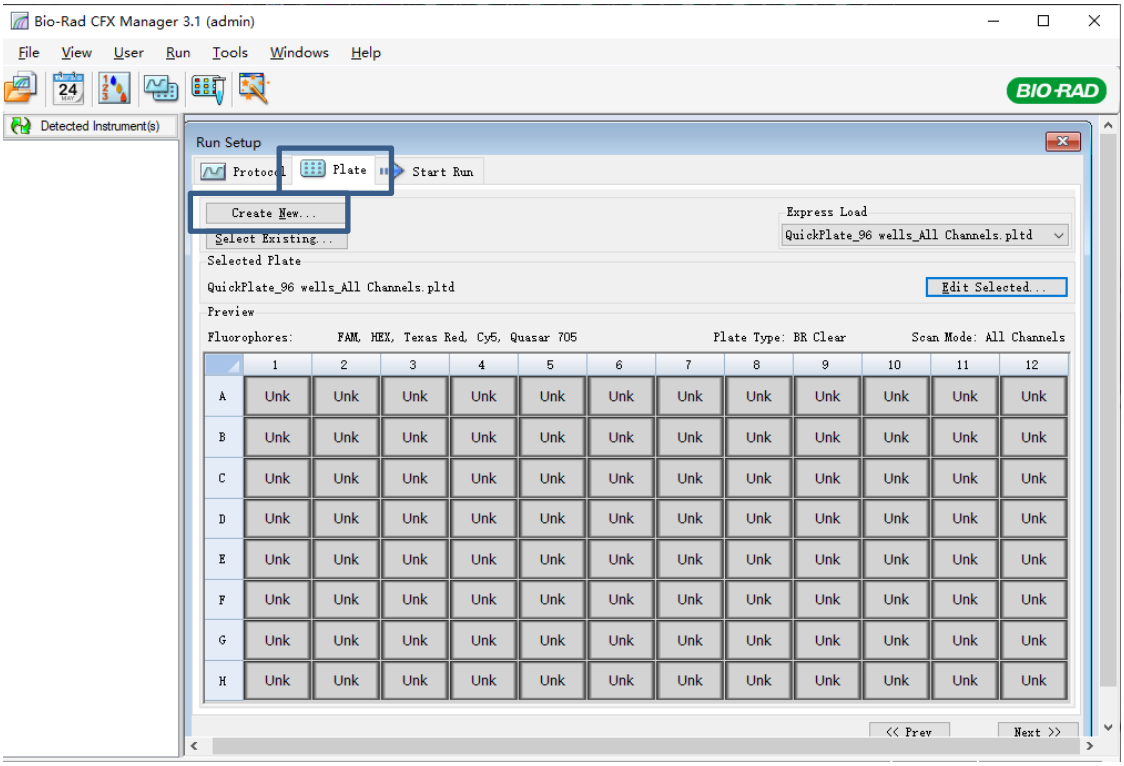


Set the parameters as follows in **Protocol Editor**, then click **OK** to confirm.

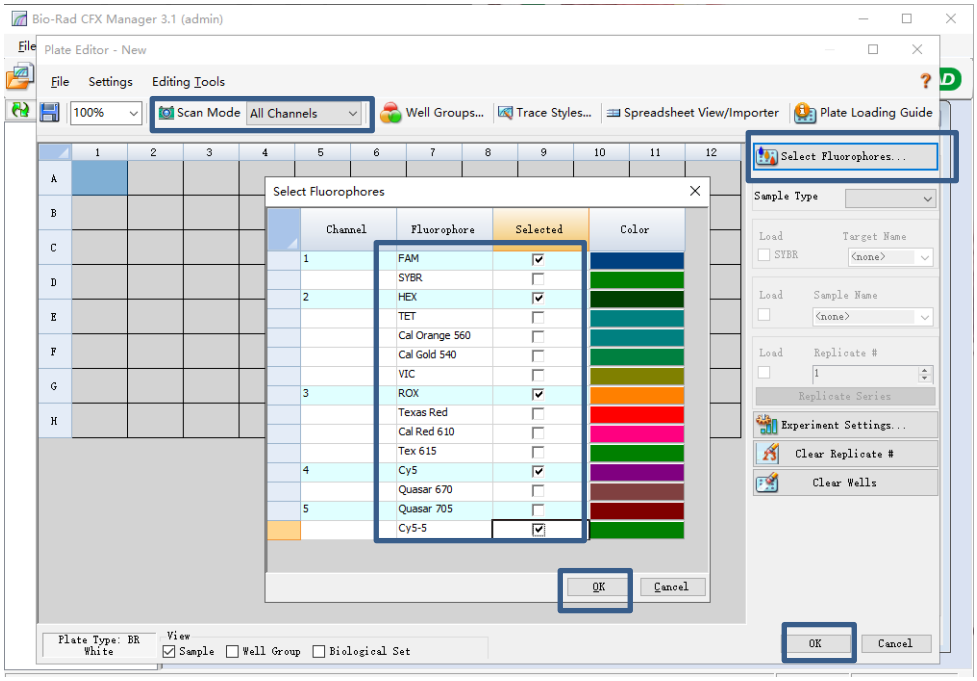
- Stage 1: 50℃ for 15 min, 1 cycle;
- Stage 2: 95℃ f or 1 min, 1 cycle;
- Stage 3: 95℃ for 15 sec, **58℃** for 30 sec, 42 cycles.
- Sample Volume: **25 µL**
- Data Collection at Stage 3, Step 2 (58.0℃ @ 0:30)



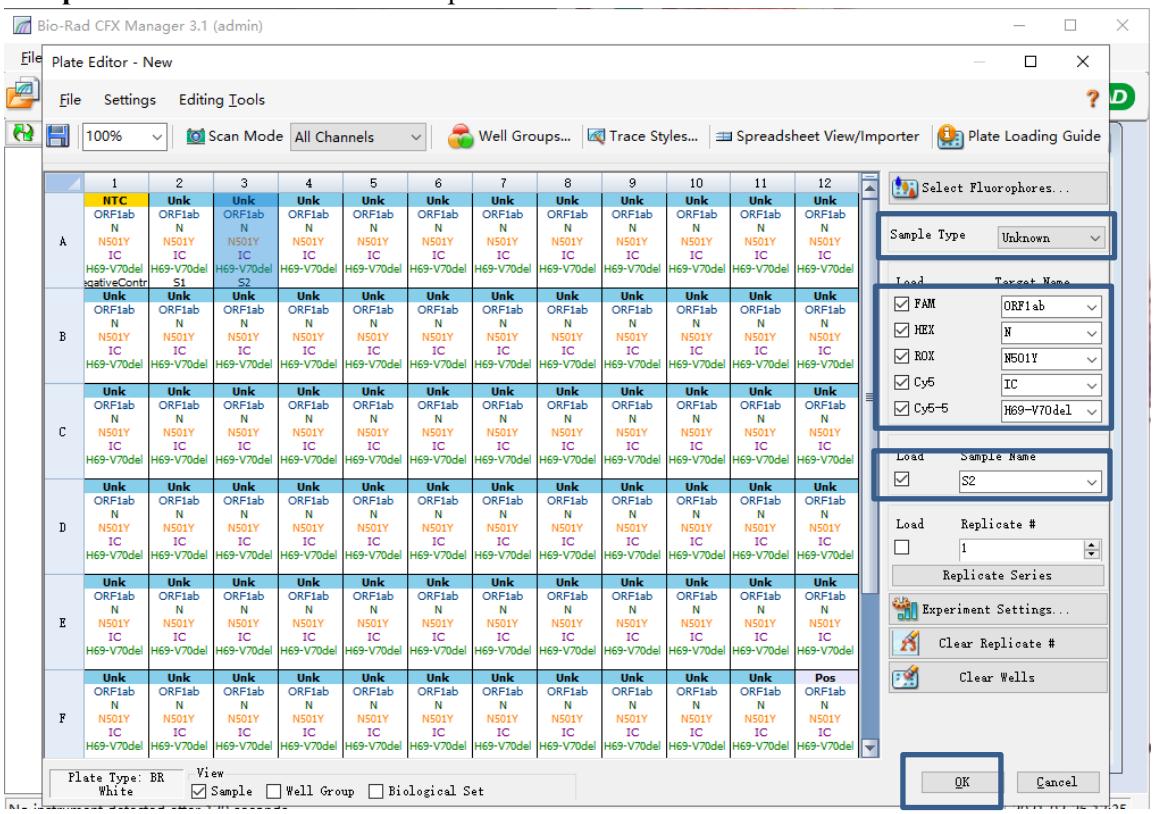
Click **Plate** Tab and a new screen will appear as below:



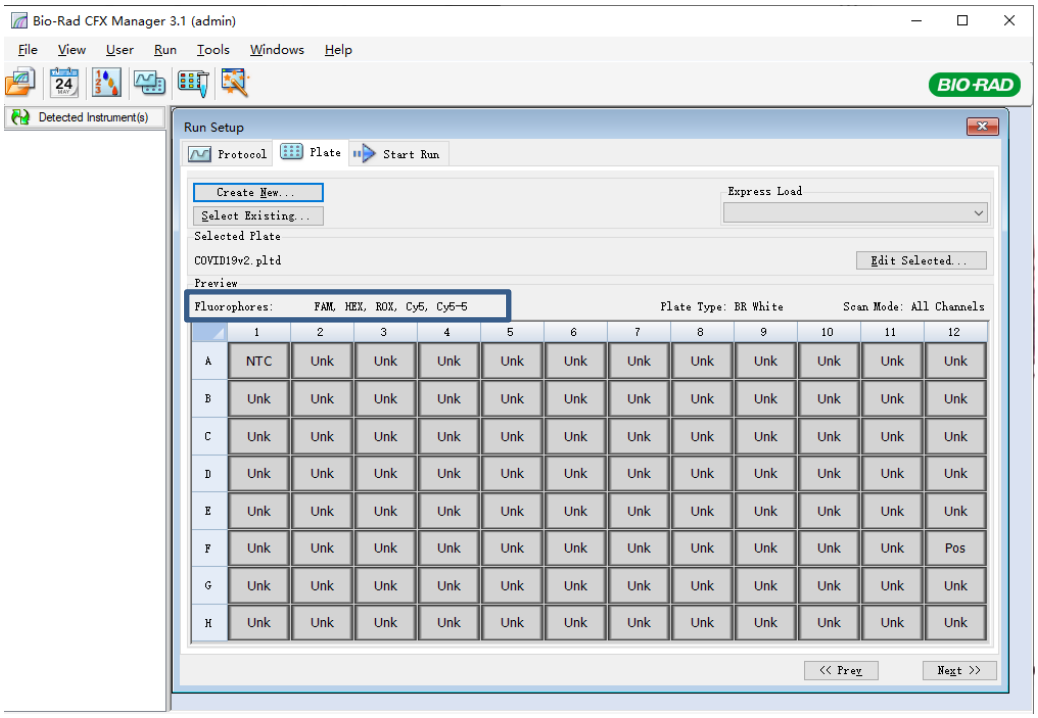
Click **Create New**, and a new screen will appear. Click **All Channels** in **Scan Mode**, and then click **Select Fluorophores**, and in the window select FAM, HEX, ROX, Cy5 and Cy5.5 as below. Then click **OK** toconfirm.



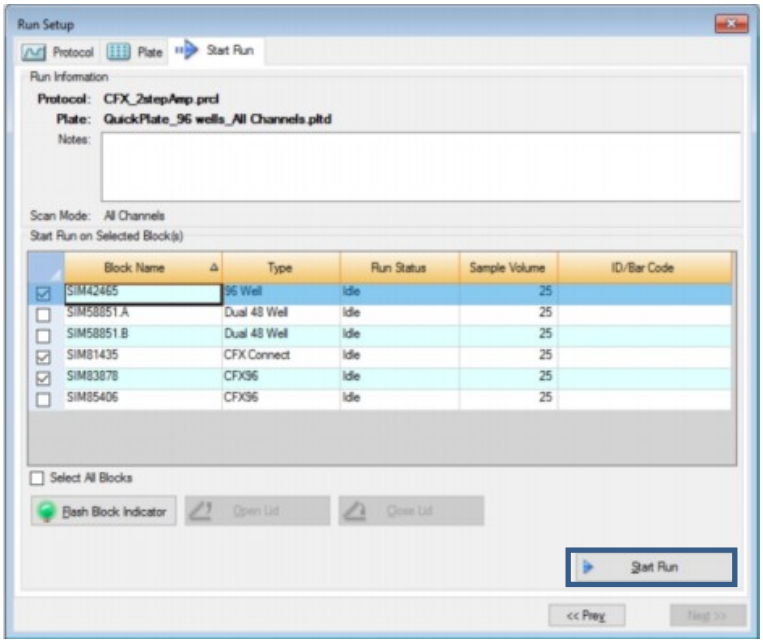
Select sample type in **Sample Type**, Negative Control select **NTC**, Positive Control select **Positive Control** and specimen(s) select **Unknown**. Rename **Target Name** as ORF1ab (FAM), N (HEX), N501Y (ROX), IC (Cy5) and Cy5.5 (H69-V70del). Then input sample name in **Sample Name**. Click OK to save the plate.



Run Setup window appear, and double check the **Fluorophores.**



Click **Next** to select the block to **Start Run**.



11.4 Data Analysis

See below for step-by-step operation of Bio-Rad CFX96 Real-Time PCR System using Bio-Rad CFX Manager for Data analysis.

11.5 Click B**rowse** to select the amplification file.

11.6 Click **Quantification** Tab to adjust the cycle threshold (Cq) values.

11.7 Click **Quantification Data** Tab to display the cycle threshold (Cq) values.

12. Interpretation of Results

All test controls should be examined prior to interpretation of results. If the controls are not valid, the results cannot be interpreted. The Cq cutoff value of this kit is set as 36 for ORF1ab (FAM), N (HEX) and IC (Cy5), and 37 for N501Y (ROX) and H69-V70Del (Cy5.5). And the end user is required to review fluorescent curves before final interpretation. All the positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples.

12.1 Positive and Negative Controls

The positive control and negative control for each run are interpreted as described in Table 2 below.

Table 2. Positive and Negative Control Interpretation.

SARS-CoV-2 Positive Control					SARS-CoV-2 Negative Control					Result	Actions
ORF1ab (FAM)	N (HEX)	N501Y (ROX)	H69-V70Del (Cy5.5)	IC (CY5)	ORF1ab (FAM)	N (HEX)	N501Y (ROX)	H69-V70del (Cy5.5)	IC (CY5)		
+	+	+	+	+	-	-	-	-	-	Valid	Continue to result interpretation
Any one of them shows negative					Not considered					Invalid	rRT-PCR failed, re-run

Not considered	Any one of them shows positive		Extraction or rRT-PCR contaminated, re-run
Result of (-): Cq value >36 or Undetermined for ORF1ab (FAM), N (HEX) and IC (Cy5), and Cq value >37 or Undetermined for N501Y (ROX) and H69-V70Del (Cy5.5). Result of (+): Cq value ≤ 36 for ORF1ab (FAM), N (HEX) and IC (Cy5), and Cq value≤ 37 for N501Y (ROX) and H69-V70Del (Cy5.5) If there is contamination for the re-run, please perform decontamination procedures.			

12.2 Examination and Interpretation of Specimen Results

Assessment of specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the results cannot be interpreted. **Table 3** below describes the results interpretation concerning the use of the controls provided with the test. The Cq cutoff value of this kit is set as 36 for ORF1ab (FAM), N (HEX) and IC (Cy5), and 37 for N501Y (ROX) and H69-V70Del (Cy5.5). And the end user is required to review fluorescent curves before final interpretation. **All positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples as below.**

Table 3. Interpretation of Results based on Controls.

ORF1ab (FAM)	N(HEX)	H69-V70del (Cy5.5)	N501Y(ROX)	IC(Cy5)	Results
+	+	+	+	+/-	UK B.1.1.7 Positive Generate report
+	-				
-	+				
+	+	-	+	+/-	SARS-CoV-2 Positive Mutation Positive Sequencing or 2nd RT-PCR test is required to identify mutation type
+	-				
-	+				
+	+	-	-	+/-	SARS-CoV-2 Positive Mutation Negative Generate report
+	-				
-	+				
-	-	-	-	+	SARS-CoV-2 Negative Generate report
Result of (-): Cq value >36 or Undetermined for ORF1ab (FAM), N (HEX) and IC (Cy5), and Cq value >37 or Undetermined for N501Y (ROX) and H69-V70Del (Cy5.5). Result of (+): Cq value ⩽ 36 for ORF1ab (FAM), N (HEX) and IC (Cy5), and Cq value⩽ 37 for N501Y (ROX) and H69-V70Del (Cy5.5) Invalid Result: There is no typical S-shape amplification curve or Cq value >36 or Undetermined for ORF1ab (FAM), N (HEX) and IC (Cy5), and Cq value >37 or Undetermined for N501Y (ROX) and H69-V70Del (Cy5.5), indicating that the specimen concentration is below detection limit, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.					

13. Limitations

False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.

Mutation in the target sequence of SARS-CoV-2 or change in the sequence due to virus evolution may lead to false negative results. Improper reagent storage may lead to false negative results.

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

The performance of the ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR, S Gene included)*** was established using oropharyngeal swabs, nasal swabs, nasopharyngeal, and mid-turbinate nasal swabs. Bronchoalveolar lavage fluid specimens are also considered acceptable specimen types for use with the kit. but performance has not been established.

Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

14. Troubleshooting

Problems	**Possible Causes**	**Action**
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use.
	PCR tube not properly sealed	Ensure plates are sealed correctly.
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of specimen carrying interferences	Repeat the test with fresh sample. **Make sure no food residue or visible impurities in the VTM.**
	PCR equipment failure	Repeat the test or contact the equipment supplier

15. Symbols

IVD	The product is used in vitro, please don't swallow it.	EC REP	European union authorization representative
	Validity		Refer to instruction book
	Warning, please refer to the instruction in the annex		Manufacturer
	Product temperature scope	REF	Catalogue number
LOT	Batch number	Σ	Contains sufficient for <*n*> tests
	Avoid overexposure to sun		Date of manufacture
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

16. Contact Information and Product Support

PreciGenome LLC

Commercial Entity

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EC REP

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