

# FastPlex Triplex SARS-CoV-2 detection kit (RT-PCR)

**Instructions for Use** 

Catalog # 02.01.1038 (96 Tests/kit)

## For in vitro Diagnostic (IVD) Use For Prescription Use Only FDA's review of this EUA is pending

(Reports of results to healthcare providers should note that the test has been validated but FDA's independent review of this validation is pending)



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1. Package Specification 96 tests/kit

### 2. Intended Use

The *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* 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 oropharyngeal swabs from individuals with signs and symptoms of infection who are suspected of COVID-19. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

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.

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 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)* 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)* is being distributed prior to FDA Emergency Use authorization, in accordance with Section IV.C. 2 of the FDA guidance on Policy for <u>Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised).</u>

### 3. Product Overview/Test Principle

The *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* 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 COVID-19.

The oligonucleotide primers and probes for specific detection of SARS-CoV-2 are selected from regions of Open Reading Frame 1ab (ORF1ab) and the nucleocapsid gene (N) of the SARS-CoV-2 genome. The kit includes primers/probes that are specific for the ORF1ab gene (probe labeled



with FAM) and N gene (probe labeled with HEX) 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 one-step Master Mix (SARS-CoV-2 RT-PCR Detection Buffer + SARS-CoV-2 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 (Ct) 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 Kit

Item No.	Components	Composition	Quantities	Reactions/Tube
1	SARS-CoV-2 1-step RT-PCR Detection Mi	Contains primers and probes for ORF1ab (FAM) nucleocapsid (N gene; HEX) and RNase P (RP; Cy5). Formulated at 20X.	672 μl×1	96
2	SASR-CoV-2 1-step RT-PCR Enzyme Mix	Reverse Transcriptase, DNA Polymerase, RNase Inhibitor	96 µl×1	96
3	SARS-CoV-2 negative control	HL-60 cells in Copan VTM	500 μl×1	29
4	SARS-CoV-2 positive control	SARS-CoV-2 Pseudovirus (FNV-2019-nCOV- abEN, Fubio Biological Technology Co., Ltd., Cat. No.: FNV 2001) in a background of HL-60 cells in Copan VTM.	500 μl×1	29

### 5. Reagent Stability and Transportation

The diagnostic kit (in small box) should be stored at -20 °C in the dark and should be transported in a sealed foam box with ice packs. The kit should be stored at -20 °C. Unpacked kits should avoid repeated freeze-thaw cycles.

### 6. Components Required But Not Included within the Test

RNA extraction reagents: QIAamp Viral RNA Mini Kit [Qiagen, (50)-Cat. #52904 or (250)-Cat. #52906].

### **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°C cold blocks
- 10% bleach
- DNAZap<sup>TM</sup> (Ambion, cat. #AM9890)
- Disposable powder-free gloves and surgical gowns

### **Real-Time PCR Instrument(s):**

ABI 7500 Real-Time PCR System

### 7. Warnings and Precautions

This test is being distributed prior to FDA Emergency Use authorization, in accordance with Section IV.C. 2 of the FDA guidance on Policy for <u>Coronavirus Disease-2019 Tests During the</u> <u>Public Health Emergency (Revised)</u>.

Reports of results to healthcare providers should note that the test has been validated but FDA's independent review of this validation is pending.

- Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For in vitro diagnostic use only (IVD).

• This test has not been FDA cleared or approved; this test is use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.

• 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. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-



CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

• 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.

• All used consumables should be disposed of in compliance with local, state and federal regulation.

### 8. Reagent Storage, Handling, and Stability

- Store all reagents at -20°C in the dark when not in use.
- Use the reagents within 30 days once opened.



- Completely thaw the reagents before use; spin briefly before use.
- Do not freeze/thaw cycles for reagents more than 3 times.
- The reagents should be transported in a sealed foam box with ice packs or add dry ice.

### 9. Controls Materials

Controls - Positive, Negative (Extraction) and No Template Controls provided with the test kit include:

- SARS-CoV-2 negative control: The SARS-CoV-2 negative control consists of human HL60 cells in VTM (Copan Collection, Transport and Processing Kit UTM 306). It should report negative results for ORF1ab and N genes (Ct > 39), except for RNase P. If any of the ORF1ab or N genes are positive in the negative control, then the RT-PCR run is invalid. Positive and negative are defined based on a cutoff of Ct ≤ 39. Please refer to Table 2. The SARS-CoV-2 negative control is used as an extraction and amplification control and monitors contamination of test reagents with SARS-CoV-2 RNA as well as proper reverse transcription and amplification of the RNase P targets. If the SARS-CoV-2 negative control generates negative results of RNase P (Cy5), which Ct >39, it indicates the failure of RNA extraction. The specimen is required to be re-processed.
- II) SARS-CoV-2 positive control: The SARS-CoV-2 positive control consists of a mix of SARS-CoV-2 pseudovirus and human HL 60 cells in VTM (Copan Collection, Transport and Processing Kit UTM 306). The SARS-CoV-2 pseudovirus contains on one strand all of the following sequences: ORF1ab, E, and N gene sequences in a retroviral vector (lentivirus). The positive control material is provided to the end-user ready-to use. It is prepared by the manufacturer by serially diluting stock concentration of 10^7-10^8 copies/mL of the SARS-CoV-2 pseudovirus in Copan UTM to concentration of 1000 copies/mL. The positive control should be positive for the ORF1ab gene (Ct equals or less than 39), the N gene (Ct equals or less than 39) and the RNase P gene targets (Ct equals or less than 39). If the results are not positive, the rRT- PCR run is invalid. Positive and negative are defined based on a cutoff of Ct ≤ 39. Please refer to Table 2.
- III) NTC control (no template control) is RNase free water added into sterile Copan UTM. NTC control is used to detect any reagent or environmental contaminations. The NTC should be negative for ORF1ab (FAM), N (HEX) and RNase P (Cy5). If NTC 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. CLSI MM13-A may be referenced as an appropriate resource.

• Specimen collection: Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) <u>https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html</u>. Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media or universal transport media. Follow specimen collection devices manufacturer instructions for proper use.

• Specimen Transportation: Specimens must be packaged, shipped, and transported according to Department of Transportation (DoT) and consistent with shipping recommendations from CDC for SARS-Cov-2 specimens. Specimens should be shipped at 2-8°C (on ice packs) or lower (dry ice), overnight.

- Specimen Storage:
- Upon receipt, specimens can be immediately processed or stored at 2-8°C for up to 72 hours after collection. For longer term storage, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or -70°C or lower if stored longer than 4 hours. Avoid repeated freeze-thaw cycles.

### **11. Laboratory Procedure**

### a) Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, ABI 7500 Real Time PCR system and other equipment prior to use. The following decontamination agents may be used: 10% bleach, 70% ethanol, or DNAzap<sup>™</sup> or RNase AWAY<sup>®</sup> 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.

### b) Preparation of the controls

To avoid contamination, the positive control needs to be prepared in an area separate from the amplification and extraction area. The positive control materials in the kit is provided at a concentration of 1000 copies/mL. The positive control material was prepared by serially diluting stock concentration of 107-108 copies/mL using oropharyngeal swab matrix (Copan Collection, Transport and Processing Kit UTM 306) down to concentration of 1000 copies/mL.



### c) RNA isolation

Nucleic acids are isolated and purified from oropharyngeal swab specimens using QIAamp Viral RNA Mini Kit, utilizing 140  $\mu$ L 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  $\mu$ l 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, **EXCEPT** for the elution. **Perform the elution step using an elution volume of 140 \muL AVE buffer.** The extracted RNA can be directly added to the RT-PCR reaction immediately or store at -70°C. Controls are used the same way as the extracted samples (i.e., using 140  $\mu$ l per extraction and 14  $\mu$ l of extracted RNA for the PCR reaction) once per sample run.

### d) Preparation of rRT-PCR Reactions

1) Thaw SARS-CoV-2 1-step RT-PCR enzyme mix on ice. Keep the SARS-CoV-2 1-step RT-PCR enzyme mix on ice or cold block all the time during preparation and use, and store it at -20°C immediately after use.

2) Thaw all kit components at room temperature. Vortex all kit components and briefly spin to collect all liquid at the bottom of the tube.

3) If extracted RNA from specimens were frozen, thaw extracted RNA samples on ice or a cold block.

4) Gently vortex RNA extraction sample tubes and centrifuge for 1 minute. After centrifugation, place the RNA extraction sample tubes in the cold rack or on ice.

5) Prepare all PCR mix in an area separate from the RNA extraction.

6) Determine the number of reactions (N is the number of reactions including samples, positive control, negative control and no template control) that will be included in the test.

7) 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.

Components	Volume [µL]	Final Concentration
SARS-CoV-2 1-step RT- PCR Detection Mix	7µl x (N+1)	1×
SARS-CoV-2 1-step RT- PCR Enzyme Mix	1 µl x (N+1)	1×
Total volume [µL]	8 µl x (N+1)	

### Table 1. Preparation of PCR mix



8) 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.

9) Dispense 8  $\mu$ L of the PCR mix into a 200  $\mu$ L 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.

10) Add 17  $\mu$ L of the extracted RNA to the wells pre-filled with PCR mix in the following order: NTC, 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 Applied Biosystems ABI 7500 real-time RT-PCR system and record the exact location of controls and each specimen.

11) Running a PCR amplification on ABI 7500 using 7500 software v2.3:

11.1 . Start ABI 7500 real time PCR system: Turn on the computer connected to the system first, then turn on ABI 7500 real time PCR system.

11.2 Load the instrument: Push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door.

11.3. Set up the experiment run:

11.3.3.1. Double-click ABI 7500 icon (7500 software v2.3) on the desktop. A new window should appear, select Create New Document from the menu.



11.3.3.2. Click **Next Experiment** and a new screen will appear as below.



Revision Date: 07-17-2020

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 7500 Software v2.3

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 Instrument
 Analysis

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 Help

Experiment Menu «	Experiment: Untitled	Type: Standard Curve	Reagents: TaqMan® Reagents	START R »	)				
Setup	Experiment Properties								
Experiment Prope	Enter an experiment name, select the instrument type, select	ect the type of experiment to set up, then select materials and methods for	r the PCR reactions and instrument run.	í	^				
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Run Method	* Experiment Name: Untitled								
Reaction Setup	Barcode (Optional):								
🛒 Materials List	User Name (Optional):								
Run									
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	√ 7500 (96 Wells)	7500 Fast (96 Well	5)						
	Set up, run, and analyze an experiment using a 4- or 5-color, 96-well system.								
	What type of experiment do you want to set up?								
	-/ Quantitation - Standard Curve	Quantitation - Relative Stan	fard Curve Quantitation	Comparative CT (ΔΔCT)					
	Melt Curve	Genotyping	Pre	sence/Absence					
	Use standards to determine the absolute quantity of target nucleic acid sequence in samples.								
	Which reagents do you want to use to detect the target sequence?								
	√ TaqMan® Reagents	SYBR® Green Reag	ents	Other					
	The PCR reactions contain primers designed to amplify the target sequence and a TaqMan® probe designed to detect amplification of the target sequence.								
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11.3.3.3. Enter the Experiment Name and Click 7500 (96 Wells), Quantitation-Standard Curve, TaqMan<sup>®</sup> Reagents, Standard (~2 hours to complete a run) as below.

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Experiment Menu «	Experiment: SRAS-COV-2	Type: Standard Curve	Reagents: TaqMan® Reagents	START R 👂 🕐
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Experiment Prope Experiment Prope Plate Setup Comment Run Method Reaction Setup Comment Visit	How do you want to identify this experiment?   • Experiment Name Barcode (Optional) User Name (Optional): Comments (Optional):			
Materials List	. Which instrument are you using to run the experiment?			
	~/ 7500 (96 Wells)	7500 Fast (96 Wells)		
Analysis	Set up, run, and analyze an experiment using a 4- or 5-color, 96-well syste	im.		
	What type of experiment do you want to set up?			
	√ Quantitation - Standard Curve	Quantitation - Relative Standard Curve	Quantitation - Comparative Cr	г (ΔΔСт)
	Melt Curve	Genotyping	Presence/Absence	
	Use standards to determine the absolute quantity of target nucleic acid sec	quence in samples.		
	• Which reagents do you want to use to detect the target sequence?			
	√ TaqMan® Reagents	SYBR® Green Reagents	Other	
	The PCR reactions contain primers designed to amplify the target sequence	ce and a TaqMan® probe designed to detect amplification of the target sequ	uence.	
	Which ramp speed do you want to use in the instrument run?			
	✓ Standard (~ 2 hours to complete a run) For optimal results with the standard ramp speed, Applied Biosystems rec	ommends using standard reagents for your PCR reactions.		
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11.3.3.4. Click **Plate Setup**, and a new screen will appear as below:



Doc. Version: V02 Revision Date: 07-17-2020

#### 7500 Software v2.3 File Edit Instrument Analysis Tools Help

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New Experiment - 20 Open... J Save - 21 Close A Export... - A Print Report

Experiment Menu «	Experiment: SRAS-COV-2		Type: Standard Curve	Reagents: TaqMan® Reagents	START R 🔊	?
Setup	Define Targets and Samples Assign Targe	ts and Samples				
Experiment Prope	Instructions: Define the targets to quantify	and the samples to test in the reaction plate				
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### 11.3.3.5. In the **Define Targets and Samples Tab**, add Targets and Sample(s) as below.

Experiment Menu «     Experiment: SRAS-COV-2     Type: Standard Curve     Reagents: TaqMan® Reagents     \$TARTR       Setup     Interview of the targets and Samples (Assign Targets and Samples) <ul> <li>Interview of the targets to quantify and the samples to test in the reaction plate.</li> <li>Define Target (And New Target)</li> <li>Respective Setup</li> <li>Materials List</li> <li>Run</li> <li>N vic vic vice</li> <li>None</li> <li>Vic vice</li> <li>None</li> <li>Vic vice</li> <li>None</li> <li>Vic vice</li> <li>None</li> <li>Vice</li> <li>Vice</li> <li>None</li> <li>Vice</li> <li>None</li> <li>Vice</li> <li>Vice</li></ul>	×
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Add New Target       Add Saved Target       Delete Target       Add Saved Target       Delete Target       Add New Sample       Add Saved Sample       Delete Sample         Image: Name       Reporter       Quencher       Color       Sample       Sample Name       Color         Image: Name       FAM       None       Image: None	
Target Name       Reporter       Quencher       Color       Sample Name       Color       Negative Control       Sample Name       Color         Materials List       N       ViC       None       Image: Color       Sample Name       Color       Color       Sample Name       Color       Sampl	
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Instructions: For each biological replicate group in the reaction plate, click Add Biological Group, then define the biological group.	
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11.3.3.6. In the Assign Targets and Samples Tab, select the well containing the samples and controls in View Plate Layout, and then Select all targets in the Assign target(s) to the selected wells. And choose None in Select the dye to use as the passive reference.



periment Menu «	Experiment: SRAS-COV-2	Type: Standard Curve	Reagents: TaqMan® Reagents	START R 👂
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	Select the dye to use as the passive reference.	i 1 Velis: 🛄 3 Unknown 🔂 0 Standard 🛄 0 Negative Control		93 Em

11.3.3.7. In the same Tab, select the well in View Plate Layout, and assign the sample name in the Assign sample(s) to the selected wells.

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	Negative Control	Target N V Task Unknown V Quantity		1000
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	Positive Control V	Target RNase P v Task Unknown v Quantity		1000
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11.3.3.8. Click **Run Method**, set the parameters as follows:

- Stage 1: 50°C for 15 min, 1 cycle;
- Stage 2: 95°C for 1 min, 1 cycle;



- Stage 3: 95°C for 15 sec, 60°C for 30 sec, 45 cycles.
- Sample Volume:25 μL
- Data Collection at Stage 3, Step 2 (60.0 @ 0:30)



11.3.3.9. Click **Run**, click **Save** to save the document and click **START RUN** to run the evaluation.





### e) Data Analysis

See below for step-by-step operation of ABI 7500 using 7500 software v2.3 for Data analysis.

1) After the run is completed, click **Analysis**. Click **Amplification Plot** tab and view and adjust the raw data.

- Click Analysis Settings, check the baseline Start Cycle and End Cycle. In the The Start Cycle window should be "3-15." The End cycle window should be 5-20. Users can adjust the values according to the actual situation. Click Apply Analysis Settings.
- In the Plot Settings window, Delta Rn vs Cycle should be selected in Plot Type, Liner should be selected in Graph Type, and Target should be selected in Color.
- In the **Option** tab, select one target in **Target** and adjust the threshold just above the curve from NTC (noise).
- Lastly, be sure to click "Re-analyse" icon to update the analysis.

periment Menu «	Experiment: 2020052	5-RX		Type: Standa	rd Curve	Reagents: TaqMan® Reager	ts	Reanalyse	llysis Settings	۲
Setup Run Analysis Implification Flot Standard Curve Multicomponent PL. Raw Data Flot QC Summary Multiple Plots View	Arrelification Plot Plot Specific Rays Cycle Rows current settings  700.000 e0.000 e0.	Analysis Settings for CT.Settings Elec Review the de a target, selec Default CT Settin Default CT Settin Threshold. AUTC Select a Target N ORF1ab RNase P	20200523-IXX Settings ( <u>A</u> dvanced fault settings for analysis of an analysis are used to calculate the the target from the table gs are used to calculate the the target from the table of a set used to calculate the the target from the table set of the table of the table the target from the table of the table of target from the table of the table of target from the table of the table of target from the table of target from the table of target from the table of target from the table of target from table of target from the table of target from table of target from the table of target from table of target from table of target from table of target from target from target from table of target from target from table of target from target from table of target from table of target from target from table of target from target	Settings of targets in this experimer , deselect "Use Default Set E CT for targets without cus UTO Baseline End Cycle: Baseline Start AUTO AUTO AUTO	It. To edit the default setting tings," then change the setti tom settings. To edit the de AUTO Edit Default Sett Baseline End AUTO AUTO AUTO AUTO	Atomatic Baseline Start Cycle: 33 End Cycle	x	Select Item - V		
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Il 7500 Software v2.3 <u>File Edit</u> Instrument <u>A</u>nalysis <u>T</u>ools <u>H</u>elp

New Experiment - Open... Save - Close & Export... - Print Report.



### 2) Click View Well Table tab to display the cycle threshold (Ct) 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 Ct cutoff value of this kit is set as 39 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.

1) Positive and Negative Controls

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

SARS-CoV-2 Positive Control			SARS-CoV-2 Negative Control			<b>D V</b>	
ORF1ab (FAM)	N (HEX)	IC (CY5)	ORF1ab (FAM)	N (HEX)	IC (CY5)	Results	Actions
+	+	+	-	-	-	Valid	Continue to result interpretation
Any one of them shows negative			Not considered				rRT-PCR failed, re-run
Not considered Any one of them shows positive				Invalid	Extraction, rRT-PCR contaminated, re-run		
esult of (-): Ct value >39 or Undetermined							

Table 2	Positive	and Negative	<b>Control Inter</b>	nretation
Table 2.	I USILIVE	and negative	Control Inter	pretation.

Result of (+): Ct value  $\leq 39$ 

If there is contamination for the re-run, please perform decontamination procedures.

### 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 Ct cutoff value of this kit is set as 39 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.

	1		
ORF1ab (FAM)	N (HEX)	IC (CY5)	Results
+	+		
+	-	Not considered	SARS-CoV-2 Positive
-	+		
-	-	+	SARS-CoV-2 Negative
-	-	-	Invalid

 Table 3. Interpretation of Results based on Controls.

Result of (-): Ct value >39 or Undetermined

Result of (+): Ct value  $\leq$  39

Invalid Result: There is no typical S-shape amplification curve or Ct > 39 or No Ct detected for ORF1ab gene (FAM), N gene (ROX) and internal control (CY5), indicating that the specimen concentration is too low, 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.



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)* was established using oropharyngeal swabs. Nasal swabs, nasopharyngeal, mid-turbinate nasal swabs, and 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.

The ORF1ab and N gene primer/probes may detect SARS-coronavirus based on in silico analysis.

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples,	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.
positive control	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use.
control reaction	PCR tube not properly sealed	Ensure plates are sealed correctly.
If the fluorescent	Components degraded	Use a new batch.
signal does not display the sigmoidal	Poor quality of RNA samples carrying interferences	Repeat the test with the neat extracted RNA and 1:10 dilution of the extracted RNA.
characteristic	PCR equipment failure	Repeat the test or contact the equipment supplier

### 14. Troubleshooting



#### **15. Performance Characteristics**

### a) Limit of Detection (LoD) - Analytical Sensitivity

To determine the tentative LoD, negative clinical matrix was prepared by pooling oropharyngeal swabs specimens collected from person negative for SARS-CoV-2. The negative matrix was used to serially dilute quantified whole viral genomic SARS-CoV-2 RNA extracted from cells from a COVID-19 positive patient (NTHL-20200212) from 11.43 to 1.14 x10<sup>5</sup> copies/mL in replicates of three. Replicates were individually processed using the QIAamp Viral RNA Mini Kit according to the FastPlex Triplex SARS-CoV-2 detection kit (RT-PCR) instructions for use. The tentative LoD was determined to be 285.7 copies/mL.

The tentative LoD was confirmed by testing 20 additional replicates of clinical specimens diluted by negative clinical matrix at the preliminary LoD concentration of 285.7 copies/mL. The 20 replicates were prepared and extracted following the same procedure above. The LoD was confirmed to be 285.7 copies/mL as > 95% (20/20) of the replicates were positive. Data in shown in Table 4.

RNA Input (copies/reaction)	Clinical Sample Input (copies/mL)	ORF1ab Ct	N Ct	RNase P Ct	Interpretation	#of Positive Samples	Detection Rate
		34.27	34.23	25.39	Positive		
		34.95	34.55	25.43	Positive		
		33.39	35.11	25.41	Positive		
		34.43	34.83	25.54	Positive		100%
		33.97	34.48	25.38	Positive		
		34.69	35.23	25.42	Positive		
	285.70	33.94	35.35	25.41	Positive		
		34.44	34.40	25.35	Positive	20/20	
4.86		36.57	34.93	25.49	Positive		
		34.34	34.27	25.33	Positive		
		34.16	34.94	25.42	Positive	20/20	
		33.62	36.55	25.34	Positive		
		34.96	35.74	25.22	Positive		
		35.03	34.54	25.39	Positive		
		34.11	35.83	25.28	Positive		
		33.39	35.91	25.34	Positive	-	
		39.03	34.33	25.38	Positive		
		34.65	33.85	25.18	Positive		
		34.05	34.15	25.27	Positive		
		35.67	34.77	25.26	Positive		

### Table 4. Confirmatory LoD Study Results

b) Inclusivity (analytical sensitivity):



Of 1114 published SARS-COV-2 sequences, the following was observed for the ORF1ab target:

- •The ORF1ab forward primer shows 100% alignment with 1114 published sequences
- •The ORF1ab reverse primer shows 100% alignment in 1113 of 1114 sequences; the sequence that did not yield 100% homology had a single mismatch that based on the Tm of the annealing temperature is not expected to impact binding.
- •The ORF1ab probe shows 100% alignment to 1111 of 1114 sequences. Three sequences with single nucleotide mismatches with the ORF1ab probe were observed. Based on the Tm of the annealing temperature it is not expected that these mismatches would impact binding of the probe to these sequences.
- Of 1114 published SARS-COV-2 sequences, the following was observed for the N target:
  - •The N-gene forward primer shows 100% alignment with 1020 out of 1114 published sequences For 33 sequences a single bp mismatches was observed in positions 1, 7, 16 or 19 of the primer. For 63 sequences 3 bp mismatches were observed, which are all in one stretch right at the 5' end of the sequence. For one sequence 4 bp were mismatched, of which one was a single nucleotide mismatch and the other was a 3-bp mismatch at the 5' end of the sequence.
  - •The N-gene reverse primer shows 100% alignment with 1113 out of 1114 published sequences
  - •The N assay probe shows 100% alignment to 1114 of 1114 sequences

### c) Cross-reactivity (Analytical Specificity):

Cross-reactivity of the *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* was evaluated using both *in silico* analysis and wet testing against normal and pathogenic organisms found in the respiratory tract.

### I) In silico analysis:

*In silico* analysis for possible cross-reactivity with the organisms listed in **Table 5** was performed. With the exception of SARS-coronavirus, no organisms, including other related coronaviruses, were amplified by either the ORF1ab or N target PCR primers and probes. The results of the *in silico* analysis suggest that the *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* is specific for SARS-CoV-2.

SARS-coronavirus, a closely related human SARS virus exhibited > 90% homology to the forward primer, probe of ORF1ab target, forward and reverse primer of N target and – based on the *in silico* analysis - would be expected to result in cross-reactivity with the *FastPlex Triplex SARS-CoV-2 Detection Kit (RT- PCR).* However, cross-reactivity was not observed in the wet testing (see Table 5 below). The impact of such cross-reactivity is further mitigated through the fact that SARS-CoV virus is not currently circulating.



### Table 5. Cross-Reactivity In Silico Study (Reference Sequences of Organisms)

Pathogen	GenBank Acc#	Pathogen	GenBank Acc#		
Human coronavirus 229E	AF304460.1	A7 Respiratory syncytial virus	U39661.1		
Human coronavirus OC43	NC_006213.1	Rhinovirus	NC_009996.1		
Human coronavirus HKU1	NC_006577.2	Chlamydia pneumoniae	NC_000922.1		
Human coronavirus NL63	NC_005831.2	Haemophilus influenzae	NC_000907.1		
SARS-coronavirus	NC_004718.3	Legionella pneumophila	NC_002942.5		
MERS-coronavirus	NC_019843.3	Mycobacterium tuberculosis	NC_018143.2		
Adenovirus (e.g. C1 Ad. 71)	KF268207	Streptococcus pneumoniae	NC_003098.1		
Human Metapneumovirus (hMPV)	MG431250.1	Streptococcus pyogenes	NC_002737.2		
Parainfluenza virus 1	NC_003461.1	Bordetella pertussis	NC_002929.2		
Parainfluenza virus 2	NC_003443.1	Mycoplasma pneumoniae	NC_000912.1		
Parainfluenza virus 3	KF687319	Pneumocystis jirovecii (PJP)	NW_017264775. 1		
Parainfluenza virus 4	KF483663.1	Candida albicans	NC_032089.1		
Influenza A	KT388699.1	Pseudomonas aeruginosa	NC_002516.2		
Influenza B	AF101982.1	Staphylococcus epidermis	NC_004461.1		
Enterovirus (e.g. EV68)	NC_038308.1	Streptococcus salivarius	CP013216.1		

### II) Wet-Testing:

Wet testing against normal and pathogenic organisms of the respiratory tract, sourced from China National Institute of Food and Drug control and Fubio Biological Technology Co., was performed to confirm the results of the *in silico* analysis. The organism identified in **Table 6** below were tested with 3 lots of *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* at the organism concentrations indicated (1 replicate per lot). Each replicate was tested with a different reagent lot. All results were negative with the ORF1ab and N genes (no cross-reactivity).



		So	C	Fastplex Triplex SARS-CoV- 2 Detection Kit (RT-PCR				.)							
		urce	once	LOT1 Ct			LOT2 Ct					LOT	3 Ct		
	Pathogen	e/Sample ype	ntration	ORF1ab FAM	N HEX	RNase P Cy5	Interpretation	ORF1ab FAN	N HEX	RNase P Cy5	Interpretation	ORF1ab FAM	N HEX	RNase P Cy5	Interpretation
· · · · · · · · · · · · · · · · · · ·	Influenza A virus (A/Aalborg/INS1 32/2009(H1N1 (HA-NA)	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	Influenza A virus (A/Addis Ababa/1514A073 05892N/2013(H3 N2 (HA-NA)	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	Influenza A virus (A/Anhui/1- DEWH730/2013( H7N9 (HA-NA))	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	Influenza B virus (B/Yamagata/222 /2002 (HA-NA)	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	Influenza B virus (B/Victoria/1/201 4 (HA-M1))	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	Influenza A virus (A/goose/Guangd ong/1/1996(H5N 1))	Pseudo-virus	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	FNV-SARS- ORF1a-N	Pseudo-virus	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
	FNV-MERS- abEN	Pseudo-virus	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative



### 1) Endogenous Interference Substances Studies:

Since this test uses the QIAamp Viral RNA Mini Kit, a well-established extraction method, an endogenous interference study for the test was not performed.

### 2) <u>Clinical Evaluation:</u>

### a. Clinical Sample Testing

The performance of *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* with oropharyngeal swab clinical samples was evaluated using 117 clinical specimens. Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were stored frozen at -80°C until use. All clinical samples were randomized, blinded and tested with *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)* and the FDA EUA authorized comparator (Sansure BioTech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (EUA authorized on 05-04-2020). The positive or negative is determined based on the manufacturers' instructions.

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARSCoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, midturbinate swabs, nasal washes and nasal aspirates from individuals who are suspected of COVID19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Nucleic acid extraction was performed with the QIAamp Viral RNA Mini Kit. A total of 117 specimens were available for analysis when using the QIAamp Viral RNA Mini Kit. All 117 specimens were tested with *FastPlex Triplex SARS-CoV-2 Detection kit (RT-PCR)* and with Sansure BioTech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) on ABI 7500 Real Time System. The positive percent agreement (PPA) was 97.9%, 95% CI [88.9%-99.9%] and the negative percent agreement (NPA) was 95.7%, 95% CI [88.0%-99.1%] as shown in Table 7.

 Table 7. Clinical Evaluation of the FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR).

	Comparator Test Positive	Comparator Test Negative	Total	
FastPlex Triplex SARS-CoV-2 Positive	46	3	48	
FastPlex Triplex SARS-CoV-2 Negative	1	67	69	
Total	47	70	117	

 $PPA = 46/47 \times 100\% = 97.9\%, (95\% \text{ CI: } 88.7\% - 99.9\%)$ 

NPA = 67/70 x 100% = 95.7%, (95% CI: 88.0% - 99.1%)



### 16. Symbols

Symbols	Meanings	Symbols	Meanings
IVD	In Vitro Diagnostic Medical Device	М	Date of Manufacture
$\square$	Use By	i	Consult Instructions for Use
X	Temperature Limitation	••••	Manufacturer
LOT	Lot Number	REF	Reference Number
Σ	Number of Tests	$\triangle$	Any warnings and/or precautions to take

### **17. Contact Information and Product Support**

Company Name: PreciGenome LLC Commercial Entity Address: 2176 Ringwood Ave, San Jose, CA, 95131 Tel, Fax: 408-708-4602 Email: info@precigenome.com Website: https://www.precigenome.com/coronavirus-covid19-pcr-assay