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 Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised).

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 ℃ 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

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℃ cold blocks
- 10% bleach
- DNAZap™ (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 Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised).

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:

I) 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) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html. 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 2-8ºC (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™ 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.

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 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, **EXCEPT** for the elution. **Perform the elution step using an elution volume of 140 µL 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 µl per extraction and 14 µl 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.

Table 1. Preparation of PCR mix

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)	--

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 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.

10) Add 17 µL 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.

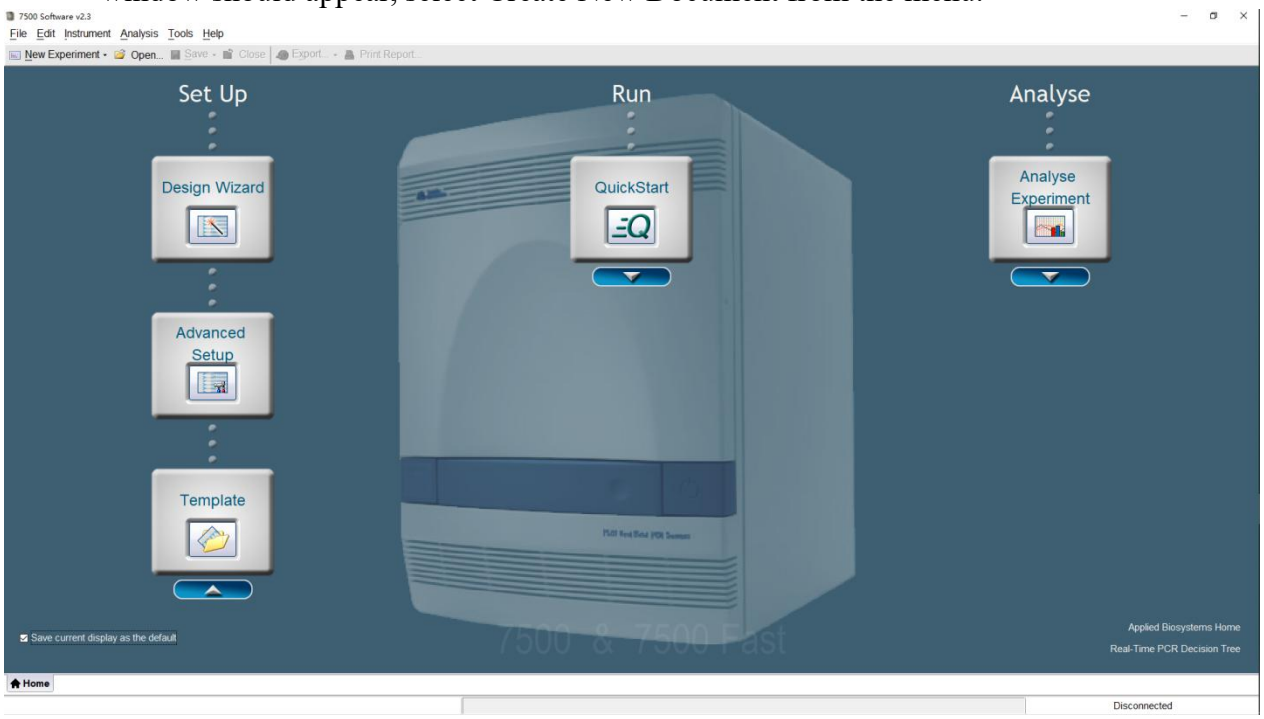
11) Running a PCR amplification on ABI 7500 using 7500 software v2.3:

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.

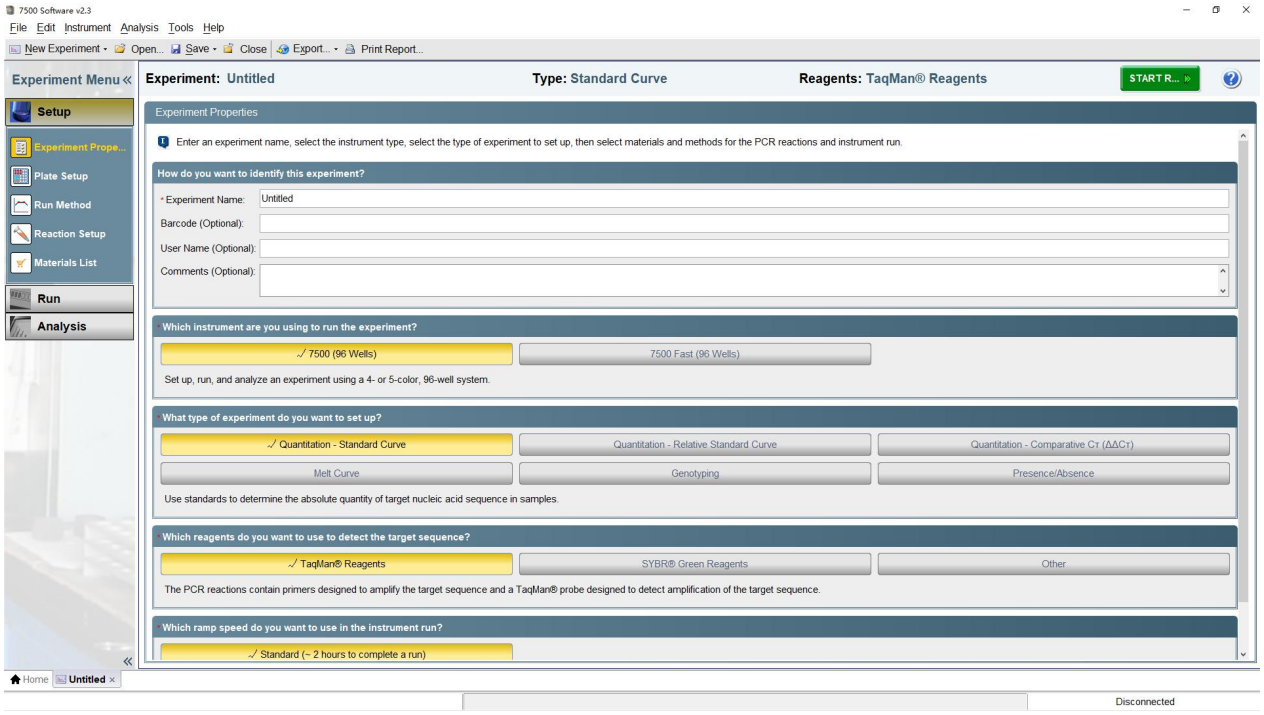
11.2 Load the instrument: Push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door.

11.3. Set up the experiment run:

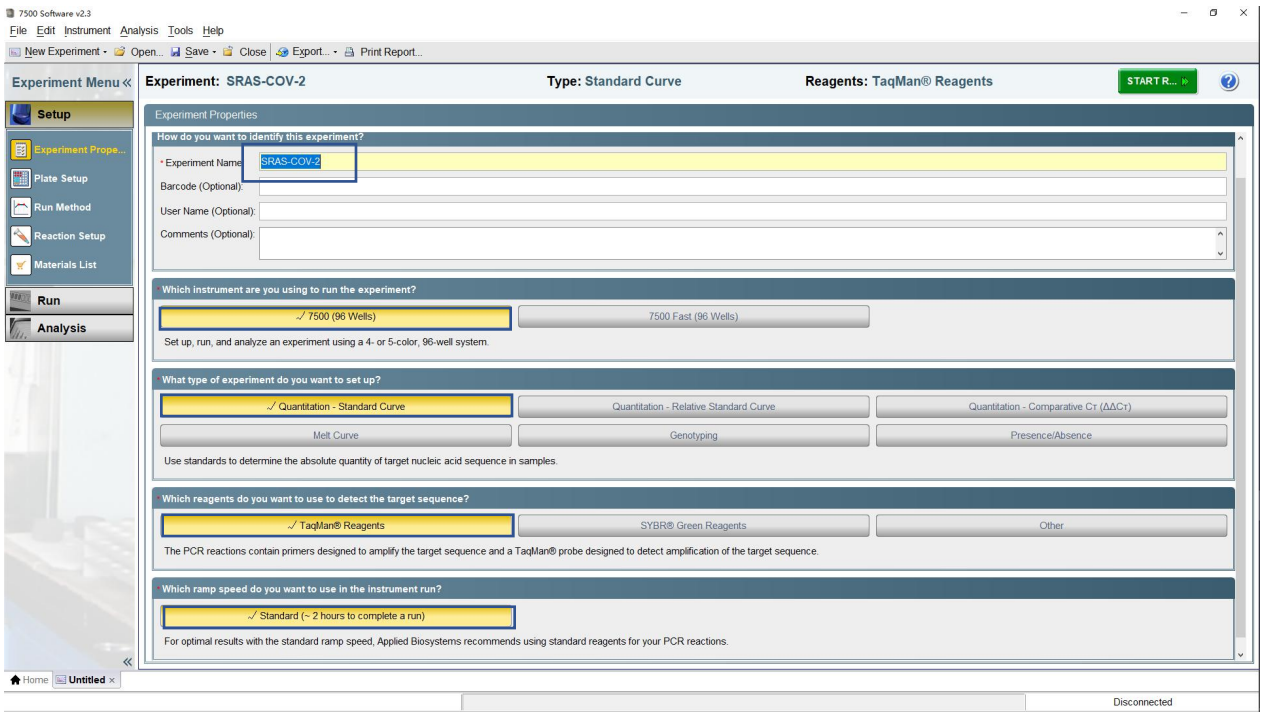
11.3.3.1. Double-click ABI 7500 icon (7500 software v2.3) on the desktop. A new window should appear, select Create New Document from the menu.



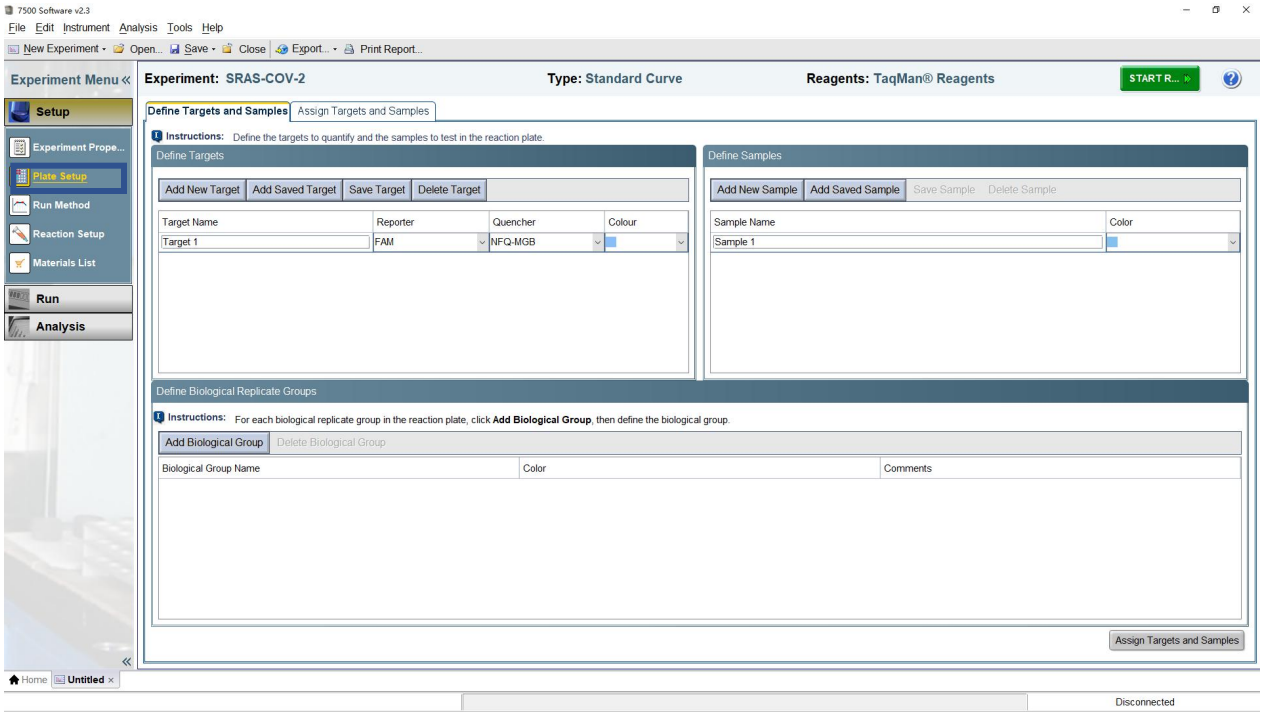
11.3.3.2. Click **Next Experiment** and a new screen will appear as below.



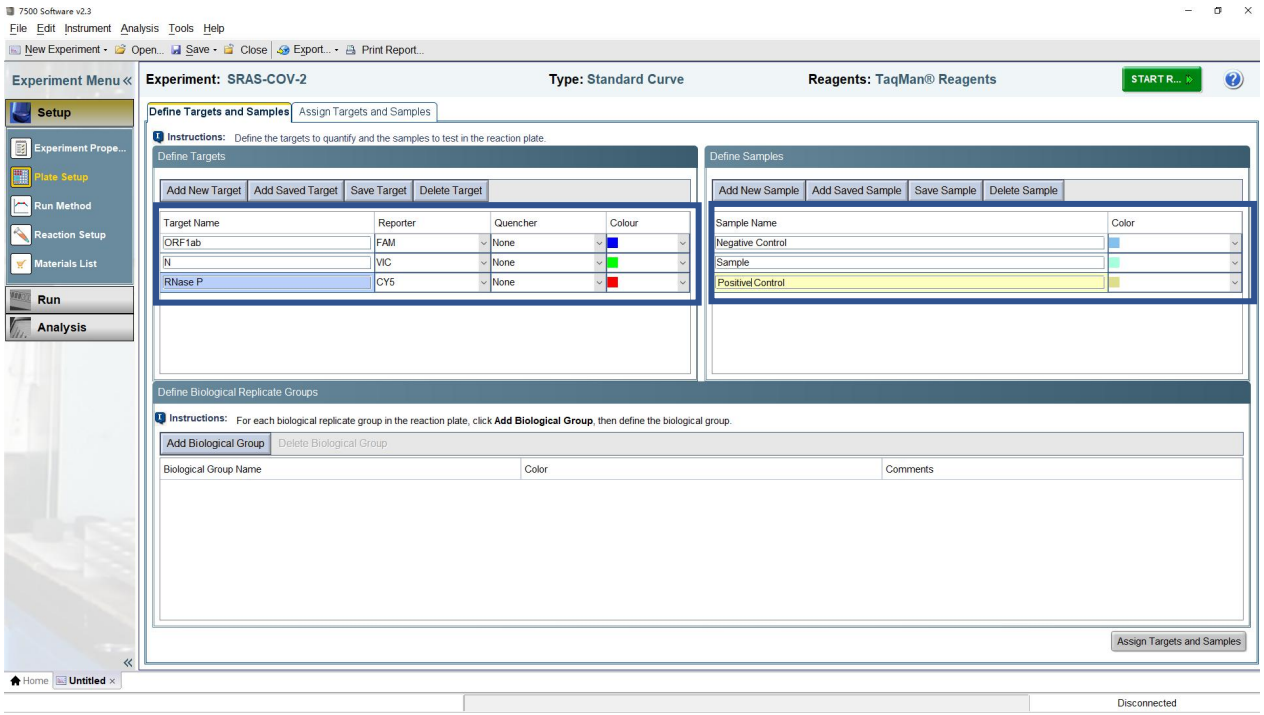
11.3.3.3. Enter the **Experiment Name** and Click **7500 (96 Wells), Quantitation-Standard Curve, TaqMan® Reagents, Standard (~2 hours to complete a run)** as below.



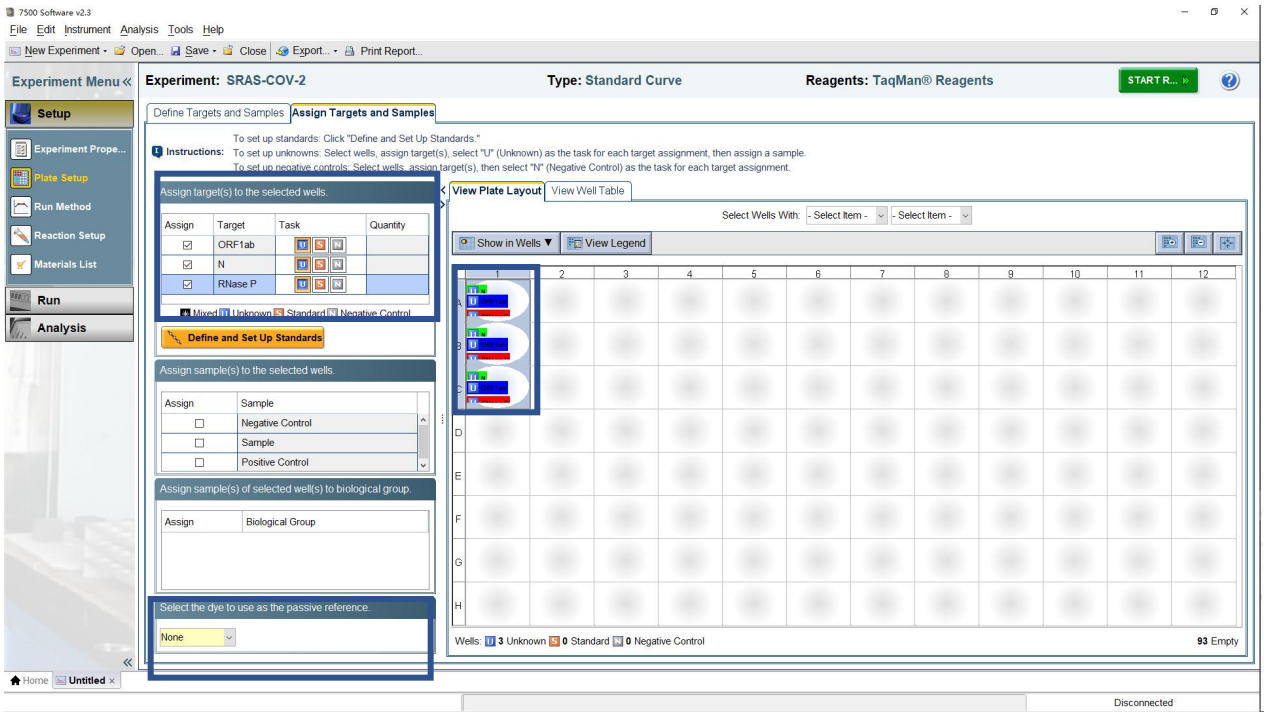
11.3.3.4. Click **Plate Setup**, and a new screen will appear as below:



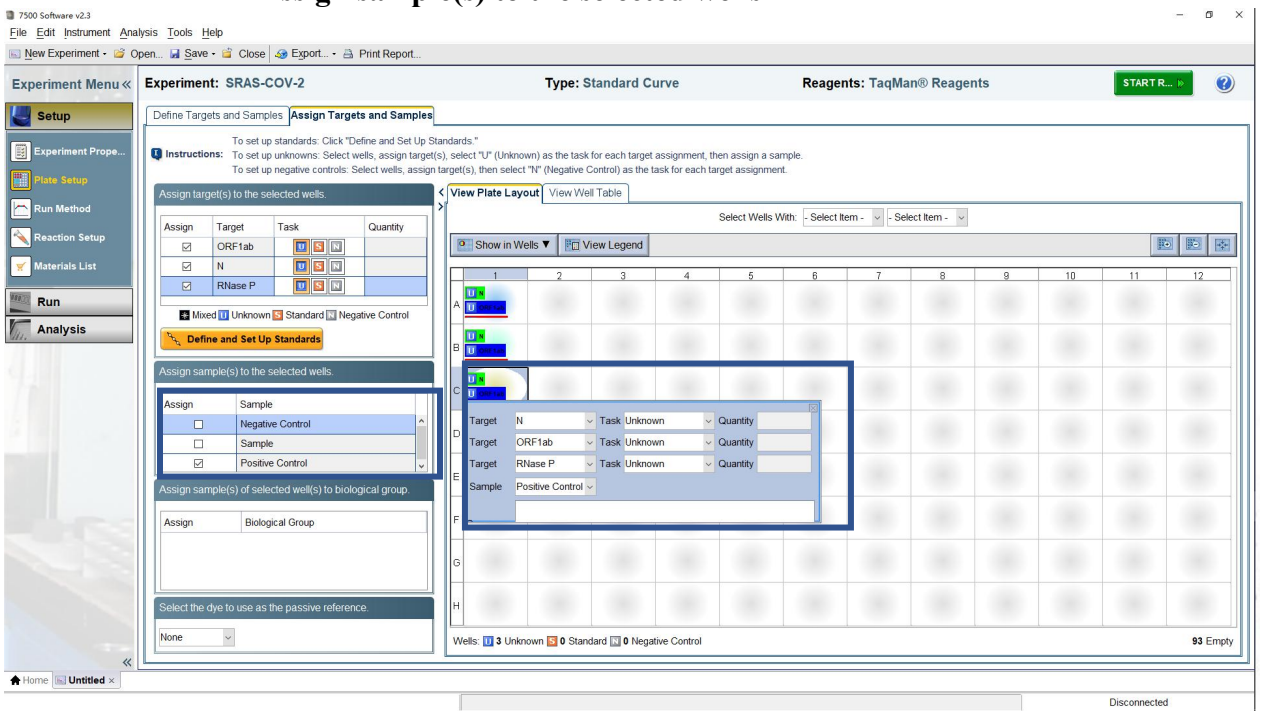
11.3.3.5. In the **Define Targets and Samples Tab**, add Targets and Sample(s) as below.



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**.



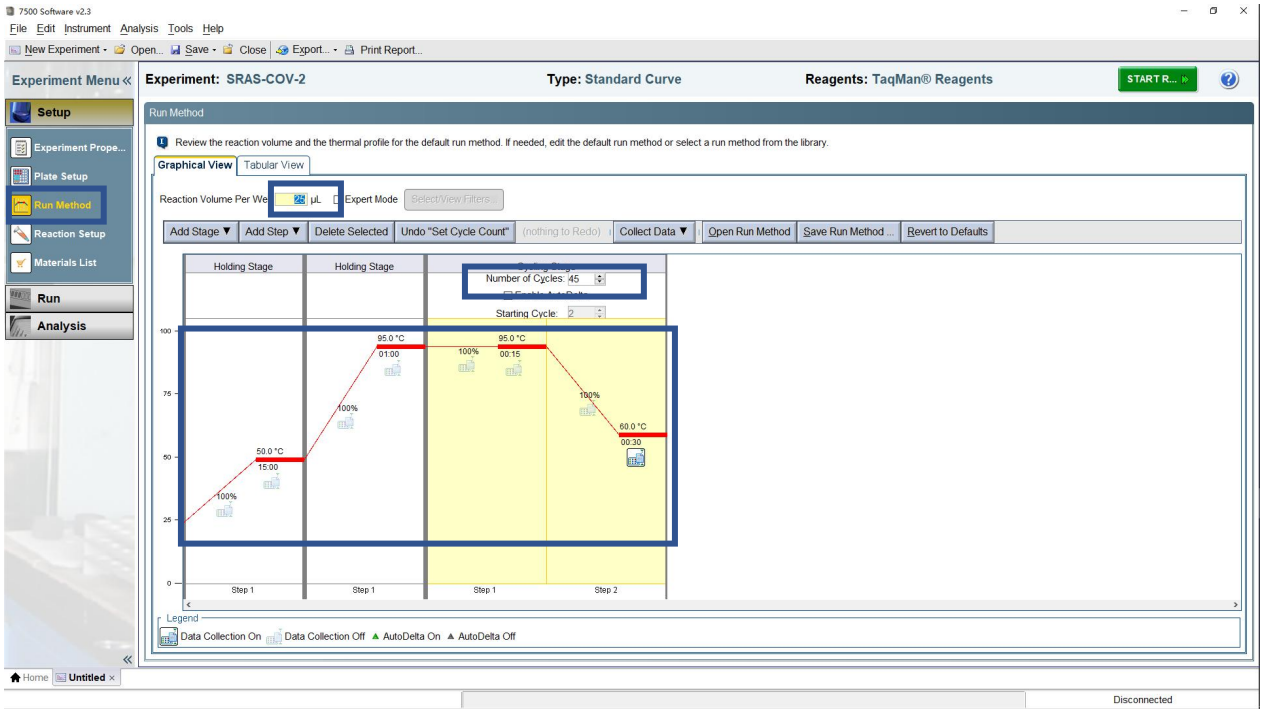
11.3.3.7. In the **same** Tab, select the well **in View Plate Layout**, and assign the sample name in the **Assign sample(s) to the selected wells**.



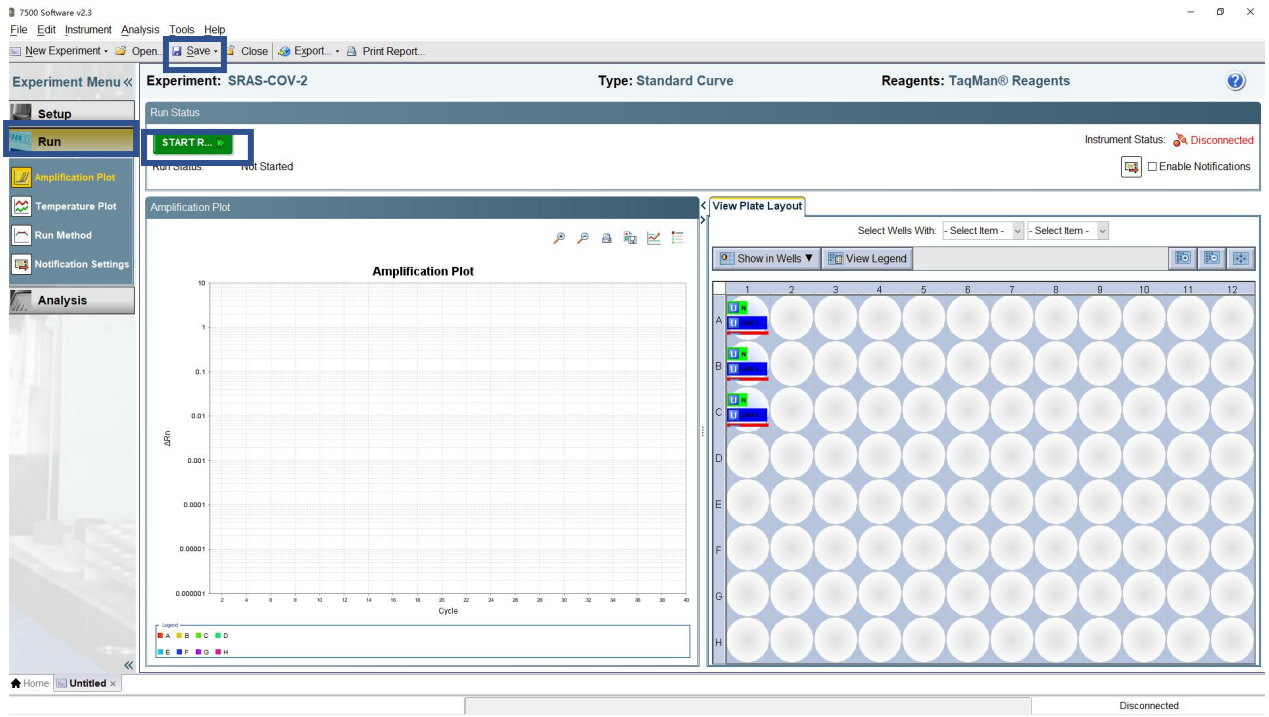
11.3.3.8. Click **Run Method**, set the parameters as follows:

- 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.

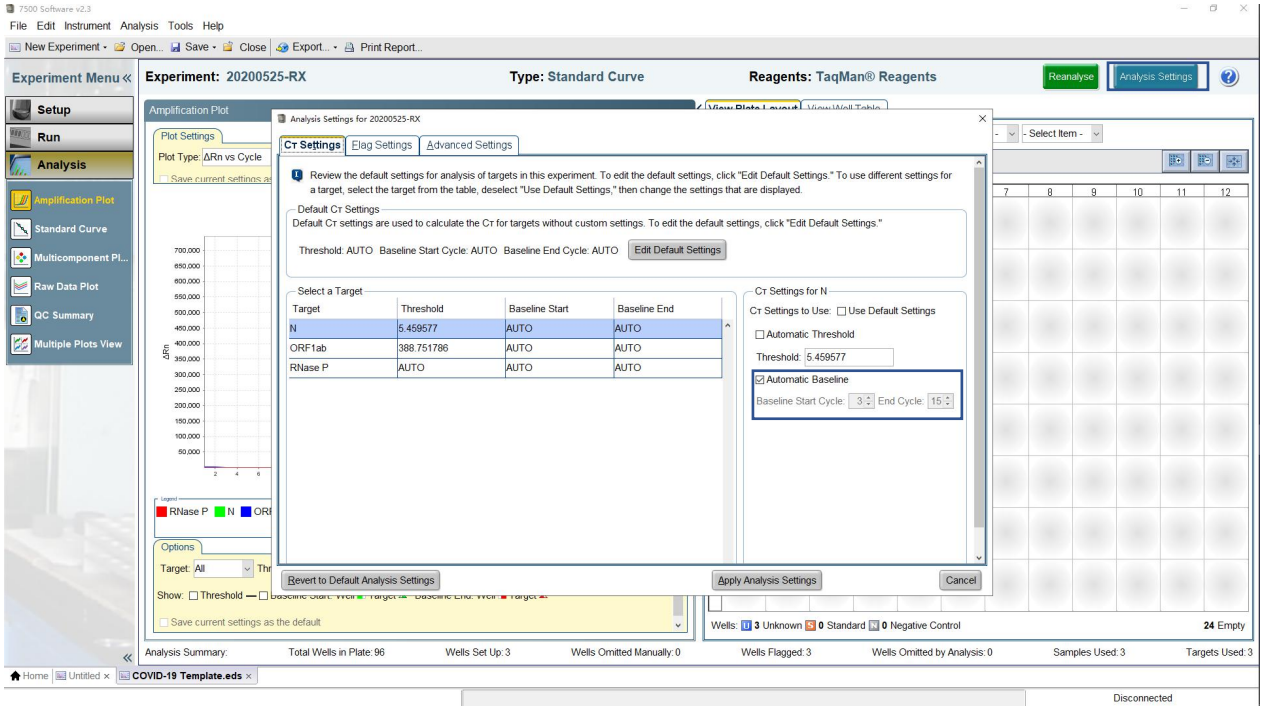


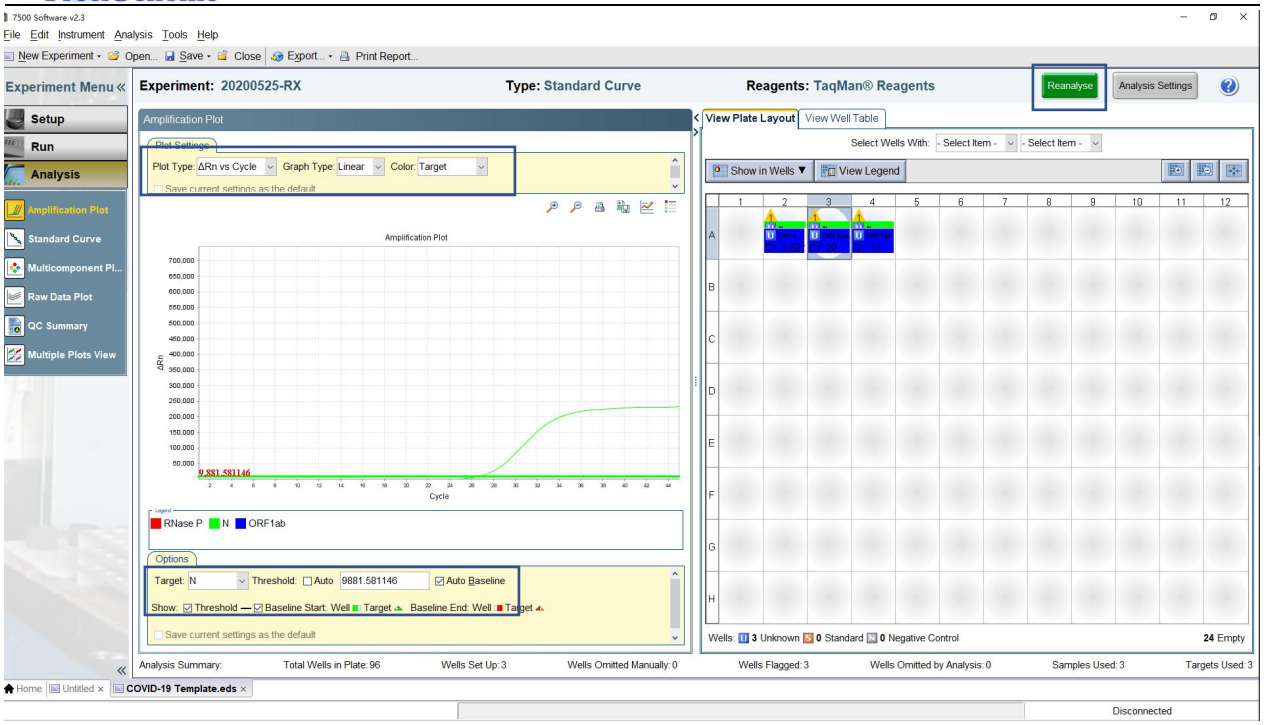
e) Data Analysis

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

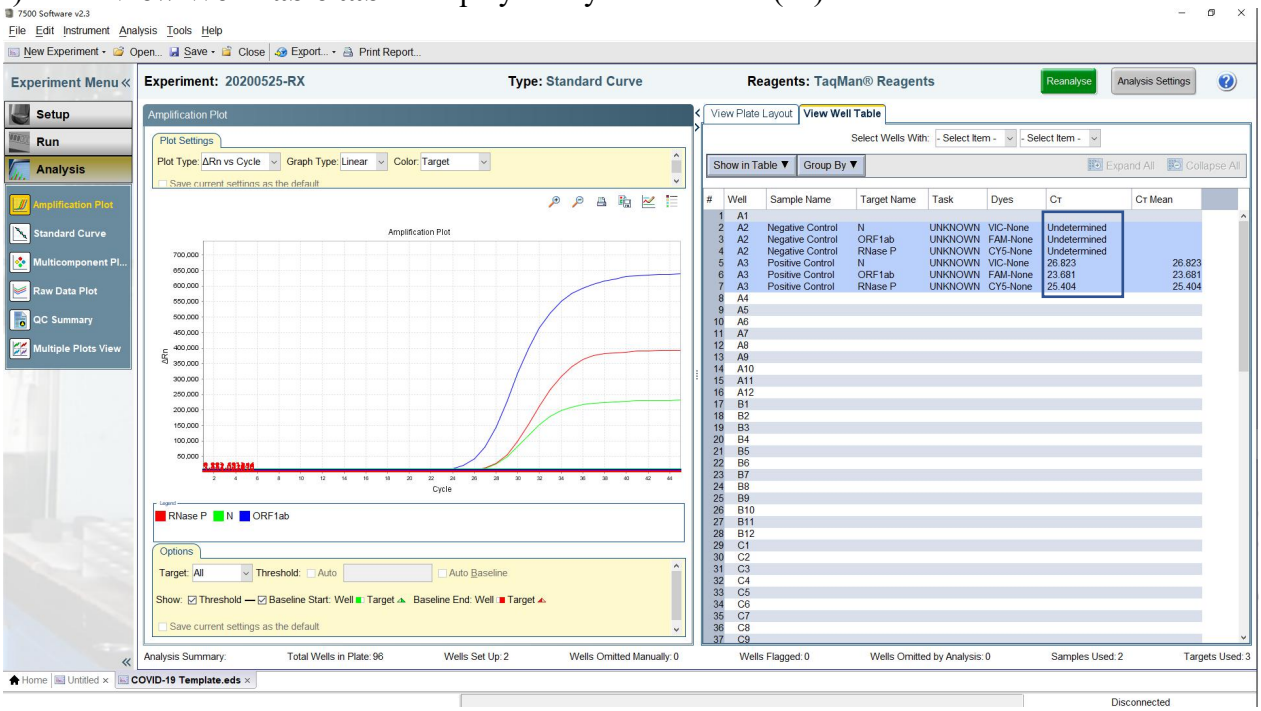
1) After the run is completed, click **Analysis**. Click **Amplification Plot** tab and view and adjust the raw data.

- 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.





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.

Table 2. Positive and Negative Control Interpretation.

SARS-CoV-2 Positive Control			SARS-CoV-2 Negative Control			Results	Actions
ORF1ab (FAM)	N (HEX)	IC (CY5)	ORF1ab (FAM)	N (HEX)	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, rRT-PCR contaminated, re-run
Result of (-): Ct value >39 or Undetermined Result of (+): Ct value ≤ 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.

Table 3. Interpretation of Results based on Controls.

ORF1ab (FAM)	N (HEX)	IC (CY5)	Results
+	+	Not considered	SARS-CoV-2 Positive
+	-		
-	+		
-	-	+	SARS-CoV-2 Negative
-	-	-	Invalid
Result of (-): Ct value >39 or Undetermined Result of (+): Ct value ≤ 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.			

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）*** 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.

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 RNA samples carrying interferences	Repeat the test with the neat extracted RNA and 1:10 dilution of the extracted RNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

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 x10^5 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.

Table 4. Confirmatory LoD Study Results

RNA Input (copies/reaction)	Clinical Sample Input (copies/mL)	ORF1ab Ct	N Ct	RNase P Ct	Interpretation	#of Positive Samples	Detection Rate
4.86	285.70	34.27	34.23	25.39	Positive	20/20	100%
		34.95	34.55	25.43	Positive		
		33.39	35.11	25.41	Positive		
		34.43	34.83	25.54	Positive		
		33.97	34.48	25.38	Positive		
		34.69	35.23	25.42	Positive		
		33.94	35.35	25.41	Positive		
		34.44	34.40	25.35	Positive		
		36.57	34.93	25.49	Positive		
		34.34	34.27	25.33	Positive		
		34.16	34.94	25.42	Positive		
		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		

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).

Table 6: Wet testing of organisms in VTM (Copan Collection, Transport and Processing Kit UTM 306) for cross reactivity with the *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*

Pathogen	Source/Sample Type	Concentration	Fastplex Triplex SARS-CoV- 2 Detection Kit (RT-PCR)											
			LOT1 Ct				LOT2 Ct				LOT3 Ct			
			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/INS132/2009(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/1514A07305892N/2013(H3N2 (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/1/2014 (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/Guangdong/1/1996(H5N1))	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) Clinical Evaluation:

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 x 100% = 97.9%, (95% CI: 88.7%-99.9%)
NPA = 67/70 x 100%= 95.7%, (95% CI: 88.0%- 99.1%)

16. Symbols

Symbols	Meanings	Symbols	Meanings
	In Vitro Diagnostic Medical Device		Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
	Lot Number		Reference Number
	Number of Tests		Any warnings and/or precautions to take

17. Contact Information and Product Support

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