Liferiver®

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Novel Coronavirus (SARS-CoV-2)

Real-Time Multiplex RT-PCR Kit

Instructions For Use

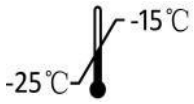
For Emergency Use Only

For *In Vitro Diagnostic* Use Only

REF

Σ 50 **(Catalog # RR-0487-02-50)**

Σ 200 **(Catalog # RR-0487-02-200)**



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Intended Use

The Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is an *in vitro diagnostic* test for the presumptive qualitative detection of nucleic acid from the SARS-CoV-2 in upper and lower respiratory specimens (including nasopharyngeal or oropharyngeal swabs and sputum) collected from individuals as recommended for testing by public health authority guidelines.

Results are for the presumptive identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens during infection. Positive results are indicative of active infection with SARS-CoV-2 but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Positive results should be reported in accordance with local regulations.

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

Testing with Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is intended for use in CLIA certified high-complexity laboratories or similarly qualified non-US laboratoriesby trained laboratory personnel who are proficient in performing real-time RT-PCR assays.

Summary and Explanation

On January 11, 2020, Chinese health authorities preliminarily identified more than 40 human infections with a novel coronavirus in an outbreak of pneumonia under investigation in Wuhan City, Hubei Province, China. The Chinese authorities identified a new type of coronavirus (novel coronavirus, named as SARS-CoV-2), which was isolated on January 7, 2020. By February 25, 2020, SARS-CoV-2 has resulted in more than 80,000 confirmed human infections in a number of countries globally, including close to 2,700 deaths.

The Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used real time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA) and nucleic acid amplification technology. The product contains Super Mix, RT-PCR Enzyme Mix and control material used in rRT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

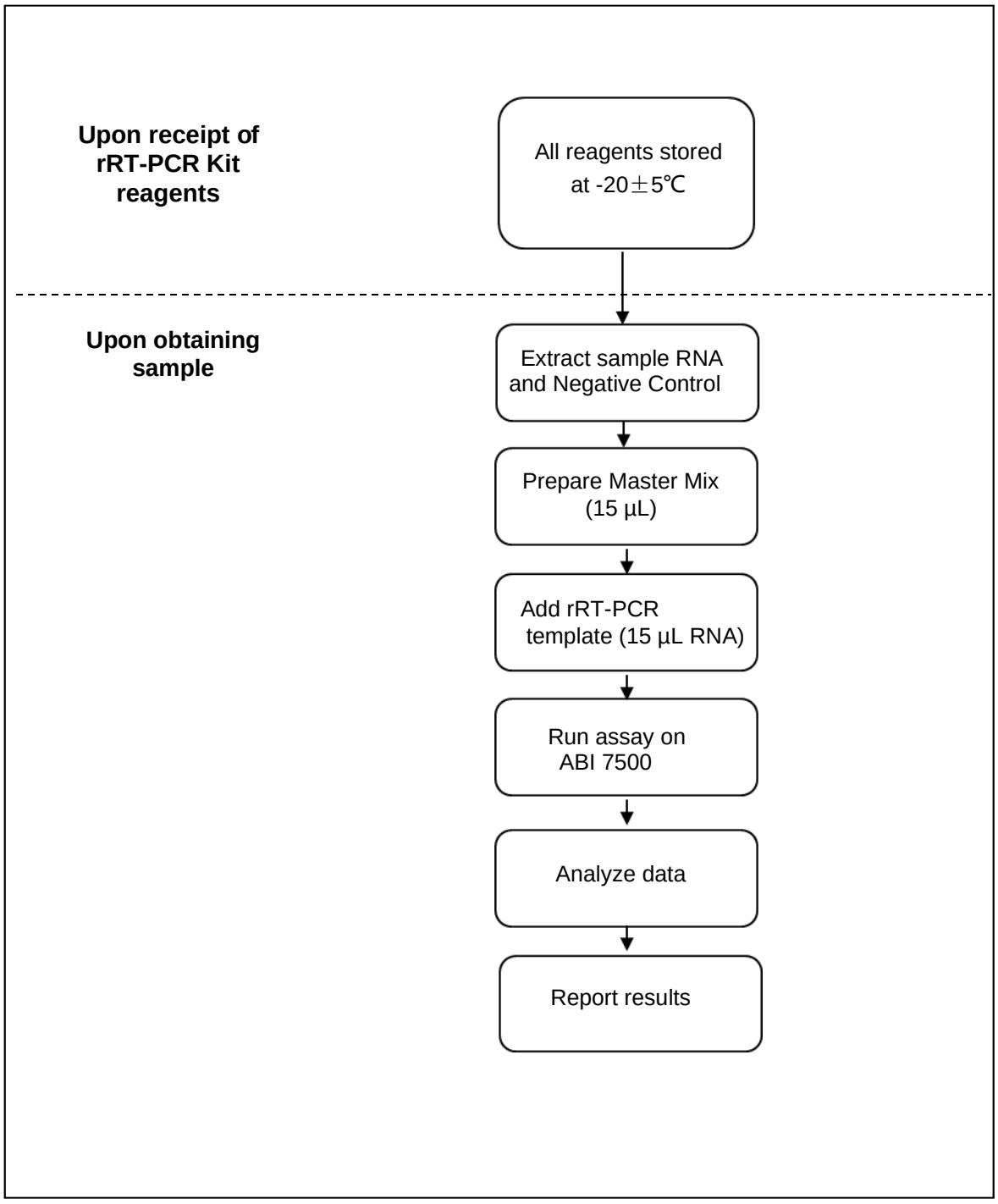
Principles of the Procedure

Three sets of oligonucleotide primers and probes for detection of SARS-CoV-2 were selected respectively from regions of the virus ORF1ab gene, N gene and E gene. An additional primer/probe set to detect the internal control gene processed with the clinical specimens is also included in the kit.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the real-time PCR instrument. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by real-time PCR system.

Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

Summary of Preparation and Testing Process



Materials Required (Provided)

RR-0487-02

Ref.	*Type of Reagent*	*Quantity Sufficient for 50 Rxns*	*Quantity Sufficient for 200 Rxns*
1	Molecular Grade Water	1 vial, 1000 µL	1 vial, 1000 µL
2	Combined Primer/Probe Mix	1 vial, 250 µL	1 vial, 1000 µL
3	One-step RT-qPCR Master Mix	1 vial, 250 µL	1 vial, 1000 µL
4	SARS-CoV-2 Internal Control	1 vial, 60 µL	1 vial, 250µL
5	SARS-CoV-2 Negative Control	1 vial, 400 µL	1 vial, 1000 µL
6	SARS-CoV-2 Positive Control	1 vial, 100 µL	1 vial, 100 µL

Control materials

- SARS-CoV-2 Negative Control is DEPC-water that will serve as an external negative specimen during RNA extraction procedure.
- SARS-CoV-2 Positive Control is a mixture of plasmids containing partial ORF1ab gene, N gene and E gene RNA fragment which are designed to cover the target sequence respectively to react with the real time RT-PCR reagents in this kit to indicate whether the real time RT-PCR worked.
- Internal Control (IC) is a synthetic non-target RNA fragment that will be added into the specimen before RNA extraction procedure to evaluate RNA extraction efficiency and identify possible PCR inhibitors. The RNA fragment in plasmid will be amplified and detected by another set of primer and probe.

Materials Required (Not Provided)

RNA Extraction Options

Instrument Manufacturer	Extraction Kit	Catalog No.
QIAGEN	QIAamp Viral RNA Mini Kit	52904/52906

Equipment and Consumables Required (Not Provided)

- Vortexmixer (Qinlinbeier; catalog # VORTEX-5) or equivalent
- Microcentrifuge
- Desk top centrifuge with a rotor for 2ml reaction tubes (Eppendorf ; catalog #5415C) or equivalent
- Micropipettes (disposable)
- Multichannel micropipettes (disposable)
- Racks for 1.5 mL microcentrifuge tubes
- 2 x 96-well -20°C cold-blocks
- 7500 Real-time PCR Systems with SDS 2.3 software (Applied Biosystems; catalog #4351104 or #4351105) or 7500 Fast Real-time PCR Systems with SDS 2.3 software (Applied Biosystems; catalog #4351106 or #4351107)
- Molecular grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNA*Zap*™ (Ambion, cat. #AM9890) or equivalent

- RNAseAway™ (Fisher Scientific; cat. #21-236-21) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5 mL microcentrifuge tubes (Axygen; catalog #MCT-150-C) or equivalent
- 8-strip tubes (Axygen; catalog #PCR-0208-C) or equivalent
- 8-strip caps (Axygen; catalog #PCR-2CP-RT-C) or equivalent

Warnings and Precautions

- For *in vitro diagnostic* use (IVD).
- For emergency use only.
- 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-2https://www.cdc.gov/coronavirus/SARS-CoV-2/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Due to the relatively fast molecular revolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
    - Maintain separate areas for assay setup and handling of nucleic acids.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g.,

microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Combined Primer/Probe Mix and One-step RT-qPCR Master Mix must be thawed and maintained on cold-block at all times during preparation and use.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNA*Zap*™" or "RNase AWAY™" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.

- RNA should be maintained on cold-block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Reagent Storage, Handling, and Stability

- Store all reagents at -20±5°C until thawed for use.
- Always check the expiration date prior to use. Do not use expired reagents.
- All reagents must be thawed and kept on a cold-block at all times during preparation and use.
- Repeated thaw-&-freeze for >3 times should be avoided as this may reduce the sensitivity of the assay.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

- Collecting Specimens
  - Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
  - Follow specimen collection device manufacturer's instructions for proper collection methods.
  - Nasopharyngeal (NP) and oropharyngeal (OP) 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. NP and OP specimens should be kept in separate vials.
  - Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.
- Transporting Specimens
- Specimens must be packaged, shipped, and transported according to current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping

regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

- Storing Specimens
  - Specimens can be stored at 2-8°C for up to 72 hours after collection.
  - If a delay in extraction is expected, store specimens at -70°C or lower.
  - Extracted nucleic acid should be stored at -70°C or lower.

General Preparation

Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 5% bleach, 70% ethanol, and *DNAzap*™ or *RNase AWA*™ to minimize the risk of nucleic acid contamination.

Nucleic Acid Extraction

Performance of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kit and procedure has been qualified and validated for recovery and purity of RNA for use with the kit:

QIAamp Virus RNA Mini Kit

Recommendation(s): Utilize 140 µL of specimen and elute with 60 µL of buffer AVE.

It is noted that SARS-CoV-2 Negative Control in this kit should be extracted with the same protocol as for specimens. The Internal Control in this kit should be added into the extraction mixture with 1µl/reaction to monitor the whole process.

Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for sample extraction.

Assay Setup

Reaction Master Mix

Note: Plate set-up configuration can vary with the number of specimens and work day organization. Negative Control and Positive Control must be included in each run.

1) In the reagent setup room clean hood, place Molecular Grade Water, Combined Primer/Probe Mix and One-step RT-qPCR Master Mix on ice or cold-block. Keep cold during preparation and use.
2) Thaw Molecular Grade Water, Combined Primer/Probe Mix and One-step RT-qPCR Master Mix prior to use.
3) Mix Combined Primer/Probe Mix and One-step RT-qPCR Master Mix by inversion 5 times.
4) Centrifuge Molecular Grade Water, Combined Primer/Probe Mix and One-step RT-qPCR Master Mix for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold

rack.

5) Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the Negative Control, Positive Control, and for pipetting error. Use the following guide to determine N:
   - If number of samples (n) including controls equals 1 through 14, then N = n +1
   - If number of samples (n) including controls is 15 or greater, then N = n +2

Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Molecular Grade Water	N x 5 µL
2	Combined Primer/Probe Mix	N x 5 µL
3	One-step RT-qPCR Master Mix	N x 5 µL
	Total Volume	**N x 15 µL**

6) After addition of the reagents, mix reaction mixtures well with vortex mixer.
7) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
8) Set up reaction strip tubes or plates in a 96-well cooler rack.
9) Dispense 15 µL of master mix into each PCR tube.
10) Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.

Template Addition

1) Gently vortex nucleic acid sample including positive and extracted negative control tubes for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
3) After centrifugation, place nucleic acid sample including positive and negative control tubes in the cold rack.
4) Carefully pipette 15 µL of sample including positive and negative control into each well. *Keep other sample wells covered during addition. Change tips after each addition.*
5) Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.
6) Change gloves often and when necessary to avoid contamination.

NOTE: *If using 8-tube strips, label the TAB of each strip to indicate sample position.* ***DO NOT LABEL THE TOPS OF THE REACTION TUBES!***

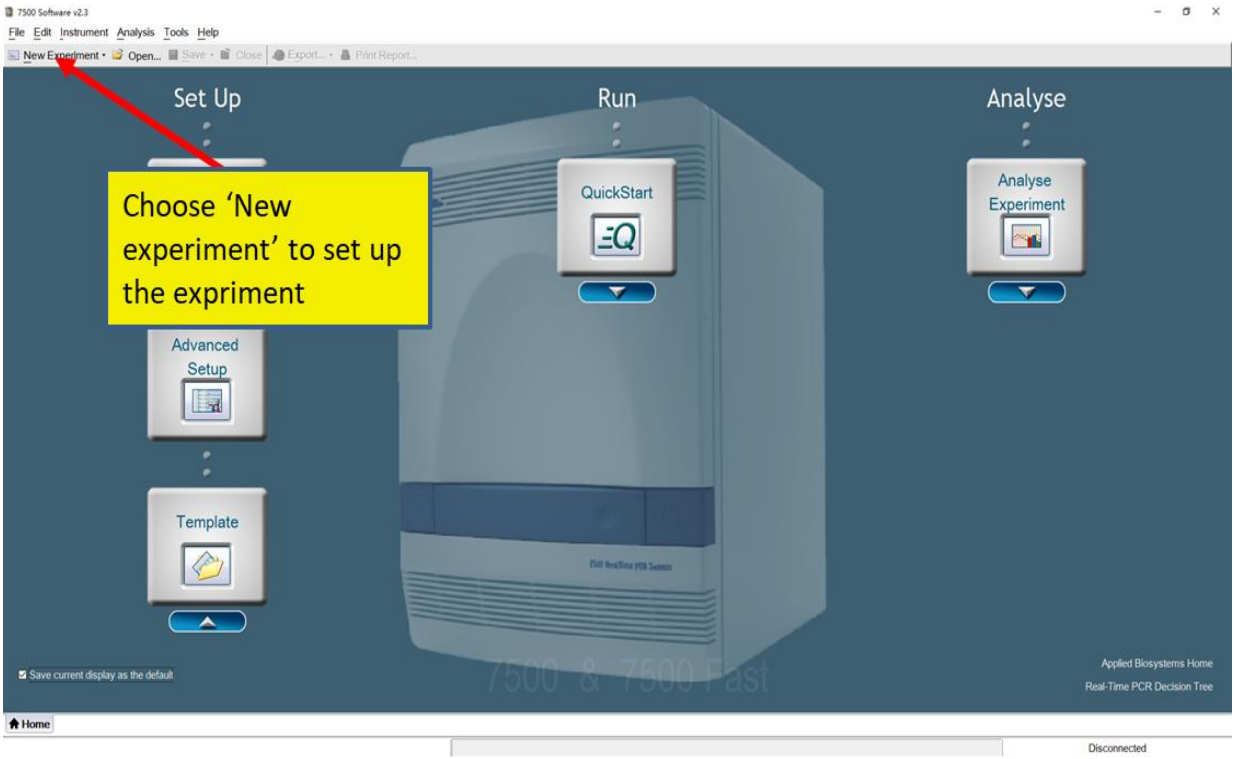
7) Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack.

NOTE*: If using 96-well plates, centrifuge plates for 30 seconds at 500 x g, 4°C*.

Create and Run an Experiment on Applied Biosystems 7500 or 7500 Fast Real-time PCR Instrument

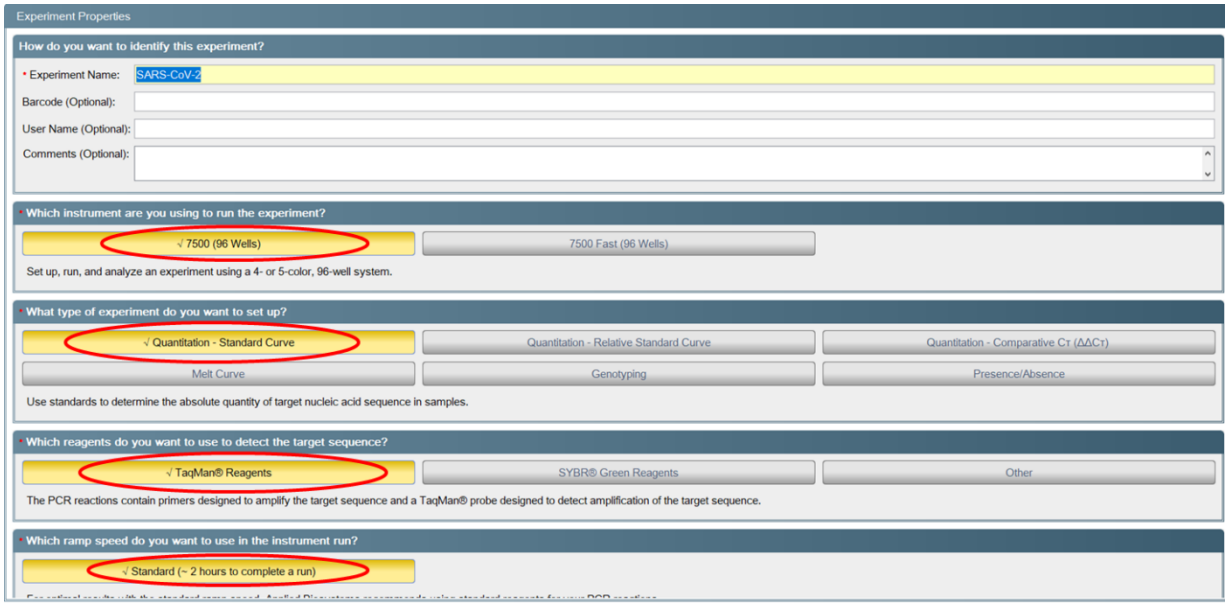
1) Launch Applied Biosystems 7500 or 7500 Fast Real-time PCR Instrument by double clicking on the 7500 Software v2.3 icon on the desktop.
2) A new window should appear, click **Log in as Guest** to log in anonymously.
3) Choose the **New Experiment** to start an experiment (see **Figure 1**)

Figure 1. Home Window



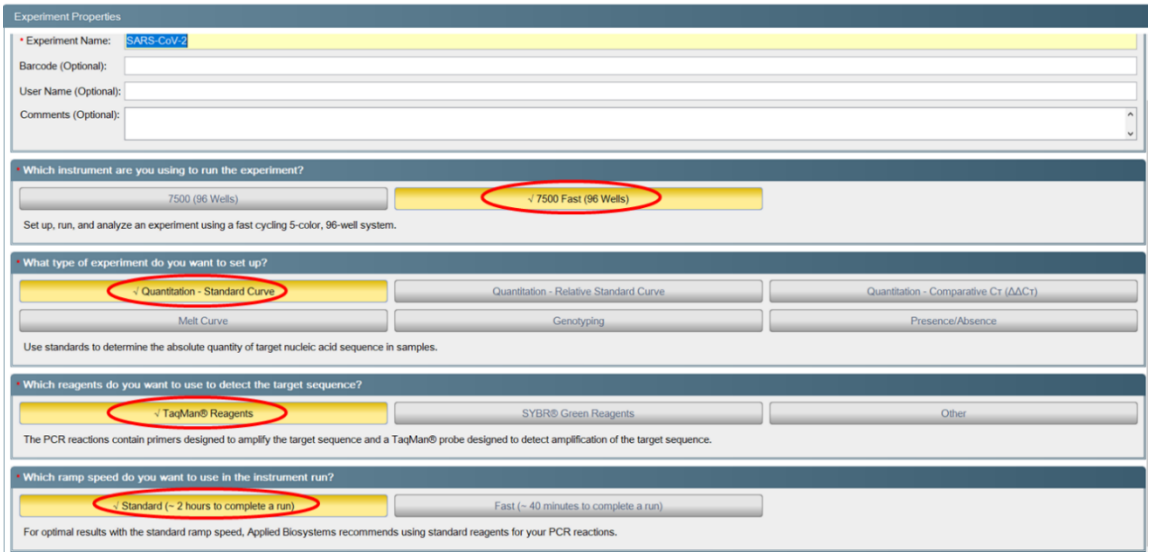
4) Set up the **Experiment Properties**. Fill in or select:
   - For 7500 Real-time PCR Systems (see **Figure 2**)
     - **a.** Experiment name: ***your own customized choice***
     - **b.** Barcode (optional): *leave blank or your choice*
     - **c.** User Name (optional): ***leave blank or your name***
     - **d.** Comments (optional): ***leave blank or your choice***
     - **e.** Which instrument are you using to run the experiment: **7500 (96-wells)**
     - **f.** What type of experiment do you want to set up: **Quantitation-Standard Curve**
     - **g.** Which reagents do you want to use to detect the target sequence: **TaqMan® Reagents**
     - **h.** Which ramp speed do you want to use in the instrument run: **Standard (~2 hours to complete a run)**

Figure 2. Experiment Properties Window (For 7500 Real-time PCR Systems)



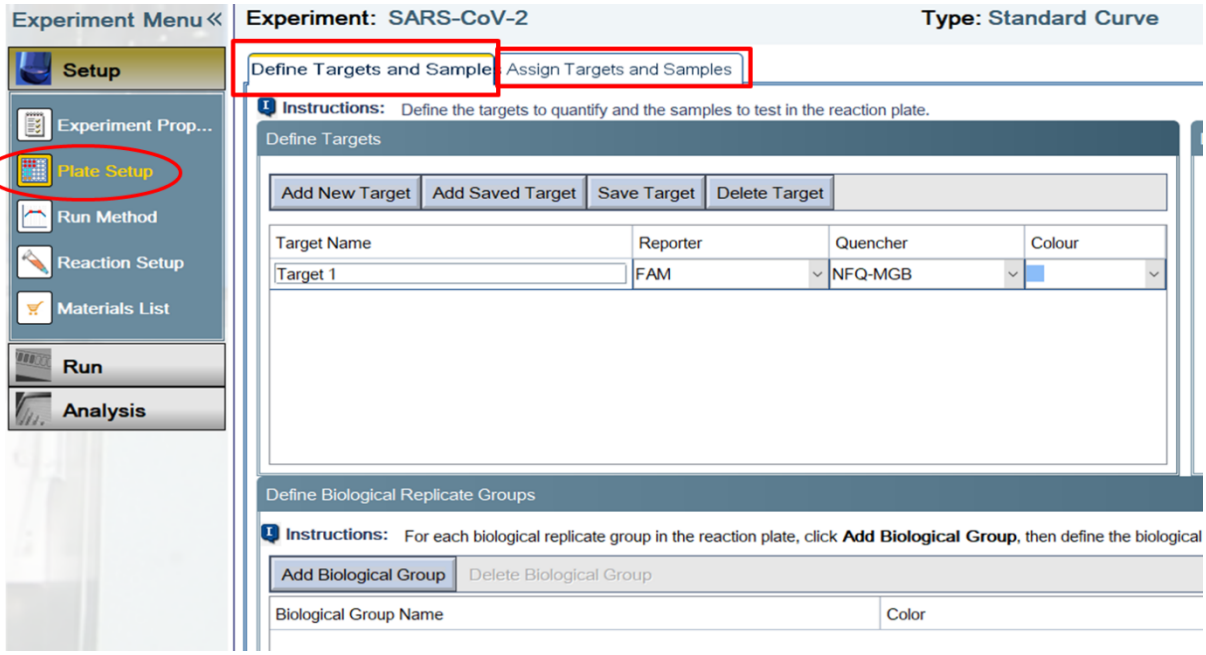
- For 7500 Fast Real-time PCR Systems (see **Figure 3**)
  - **a.** Experiment name: ***your own customized choice***
  - **b.** Barcode (optional): *leave blank or your choice*
  - **c.** User Name (optional): ***leave blank or your name***
  - **d.** Comments (optional): ***leave blank or your choice***
  - **e.** Which instrument are you using to run the experiment: **7500 Fast (96-wells)**
  - **f.** What type of experiment do you want to set up: **Quantitation-Standard Curve**
  - **g.** Which reagents do you want to use to detect the target sequence: **TaqMan® Reagents**
  - **h.** Which ramp speed do you want to use in the instrument run: **Standard (~2 hours to complete a run)**

Figure 3. Experiment Properties Window (For 7500 Fast Real-time PCR Systems)



5) After making selections click **Plate Setup** at the left side of the window. Then the **Define Targets and Samples** and **Assign Targets and Samples** will appear as below (see **Figure 4**).

Figure 4. Plate Setup



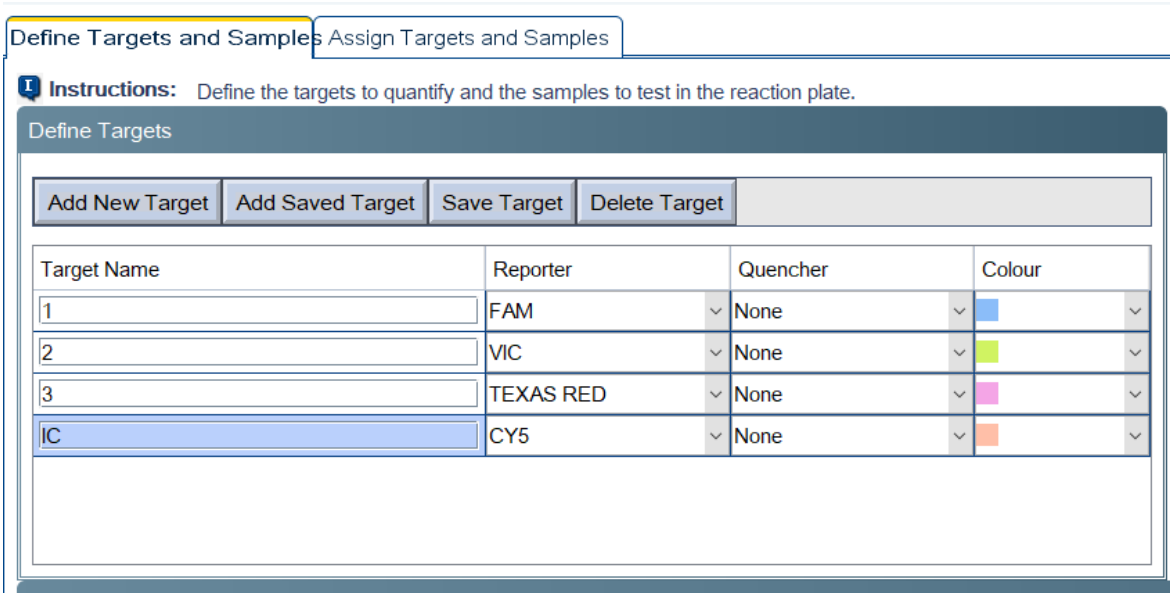
6) Define targets (see **Figure 5**). Fill in or select:
   - **a.** Target Name: ***1***
   - **b.** Reporter: **FAM**
   - **c.** Quencher: **None**
   - ***d.*** Color: *to change the color of the detector indicator, do the following:*
     ⇒Click on the color square to reveal the color chart
     ⇒Select a color by clicking on one of the squares
   - **e.** When necessary to add a new target or delete an exited target, click **Add New Target** or **Delete Target**.

7) Repeat Step 6 for each target in **Define Targets** window.

Name	Reporter Dye	Quencher Dye	Corresponding Gene
1	FAM	None	ORF1ab
2	VIC	None	N
3	TEXAS RED	None	E
IC	CY5	None	IC

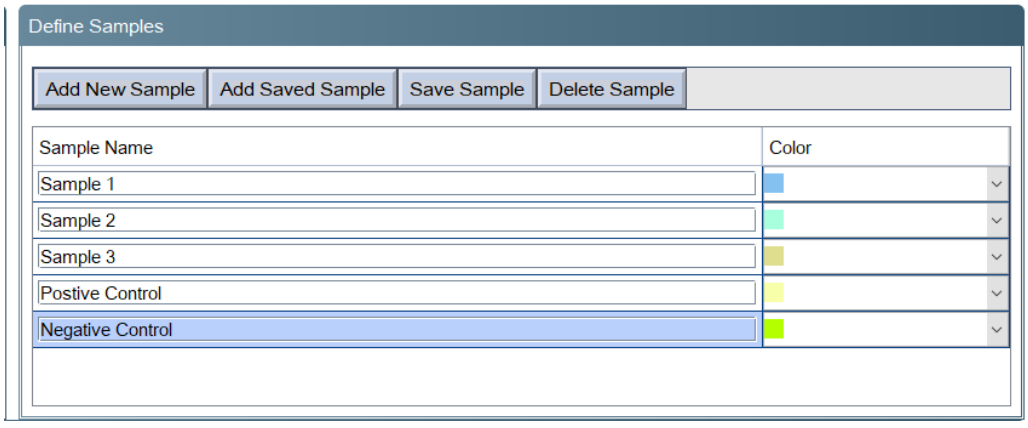
'Target 1' represents 'ORF1ab gene', 'Target 2' represents 'N gene', and 'Target 3' represents 'E gene'.

Figure 5. Define Targets



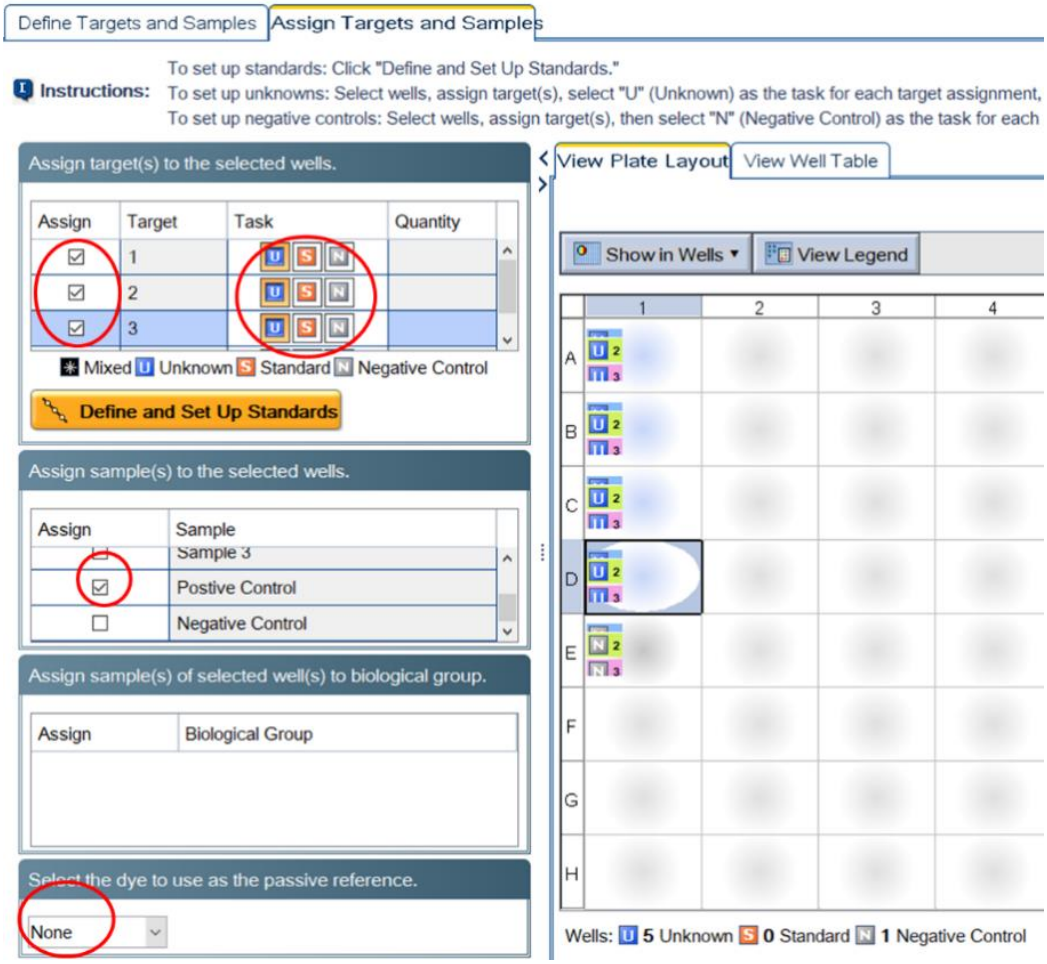
8) Define Samples (see **Figure 6**). Fill in or select:
   - **a.** Sample Name: ***Your Choice***
   - **b.** Color: *to change the color of the detector indicator do the following:*
     ⇒Click on the color square to reveal the color chart
     ⇒Select a color by clicking on one of the squares
   - **c.** When necessary to add a new sample or delete an exited sample, click **Add New Sample** or **Delete Sample**.

Figure 6. Define Samples



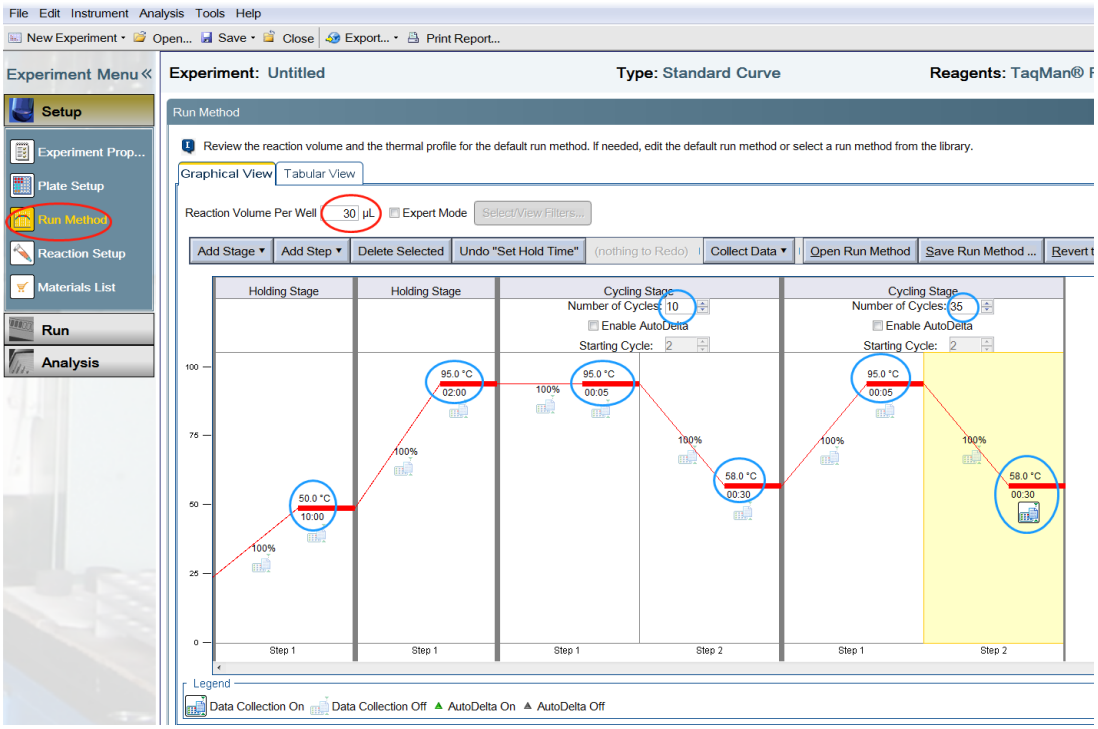
9) Click **Assign Targets and Samples** (see **Figure 7**) to layout samples.
   - **a.** Assign target(s): Select wells and assign the four targets including 'ORF1ab/N/E/IC'. Then specify the reaction well under **Task** tab (S means a standard while U represents an unknown sample and N is a negative control).
   - **b.** Assign sample(s): Select wells and assign sample.
   - **c.** Select the dye: **None**

Figure 7. Assign Targets and Samples



10) After finishing **Plate Setup**, proceed to **Run Method** (see **Figure 8**) in the **Experiment Menu**.
   - ***a.*** Reaction Volume Per Well: ***30***
   - **b.** In First Holding Stage, set to **10 min** at **50°C**.
   - **c.** In Second Holding Stage, set to **2 min** at **95°C**.
   - **d.** In First Cycling Stage, Step 1 set to**5 sec** at **95°C.**
   - **e.** In First Cycling Stage, Step 2 set to **30 sec** at **58°C**.
   - **f.** In First Cycling Stage, Numbers of Cycles should be set to **10.**
   - **g.** The icon under the time in Step 2 of the First Cycling Stage should not be highlighted to indicate data without collection.
   - **h.** In Second Cycling Stage, Step 1 set to **5 sec** at **95°C.**
   - **i.** In Second Cycling Stage, Step 2 set to **30 sec** at **58°C**.
   - **j.** In Second Cycling Stage, Numbers of Cycles should be set to **35.**
   - **k.** The icon under the time in Step 2 of Second Cycling Stage should be highlighted to indicate data collection.

Figure 8. Define Run Method

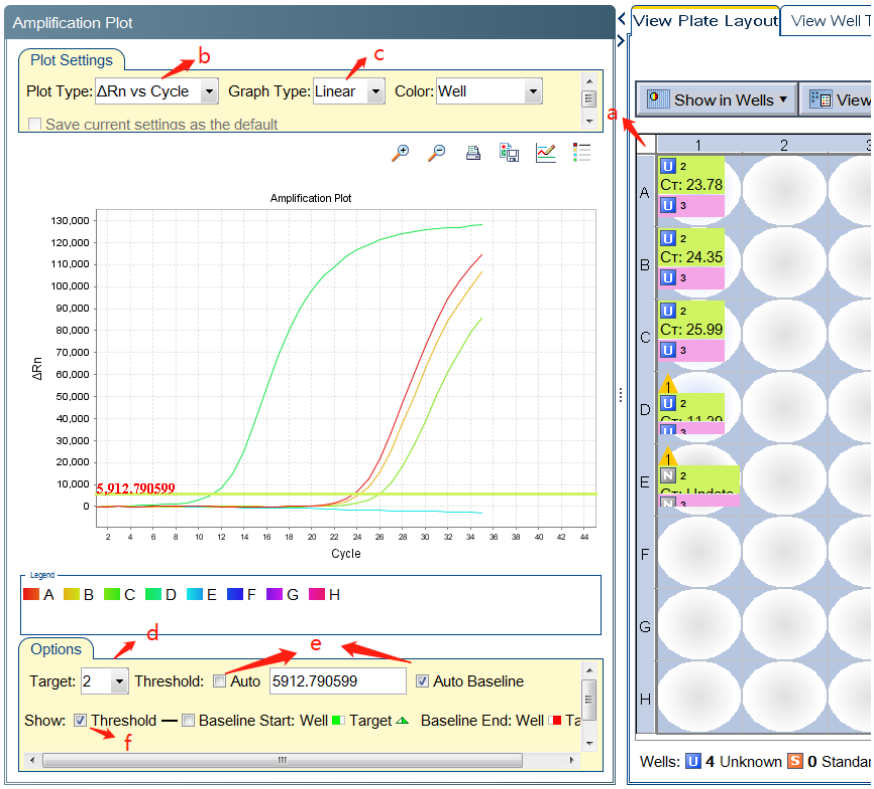


11) Before proceeding, the run file must be saved; from the main menu, select **File,** then **Save As**. Save in appropriate run folder designation.
12) Turn on ABI 7500 or 7500 Fast Real-time PCR Instrument.
13) Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.
14) Once the run file is saved, click **Start** button. *Note: The run should take approximately 1 hour and 20 minutes to complete.*

Data Analysis

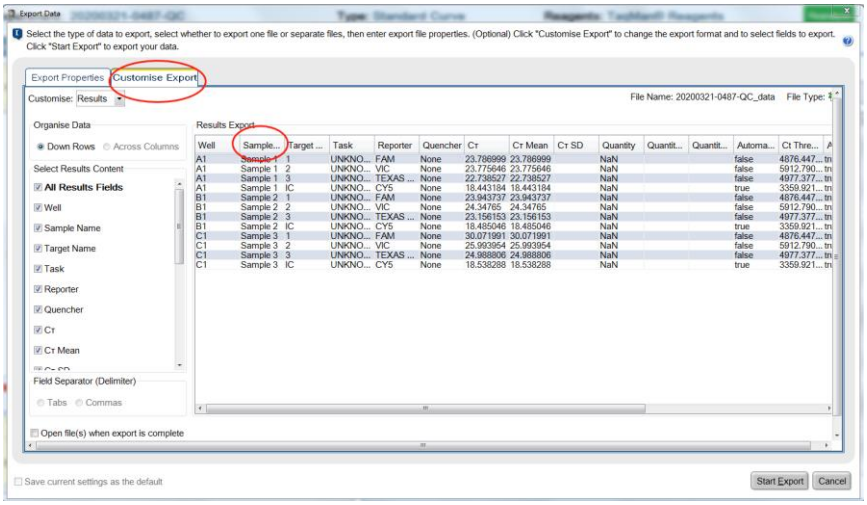
1) Once the run has completed, select the **Analysis** tab at the upper left corner of the software.
2) Select the Amplification Plot tab to view the raw data (see **Figure 9**).
3) Start by highlighting all samples from the run; to do this, click on the upper left hand box **(a)** of the sample wells (see **Figure 9**). All the growth curves should appear on the graph.
4) On the top of the window **(b),** the **Plot Type** drop down selection should be set to **Δ Rn vs Cycle**. The **Graph Type** drop down selection should be set to **Linear (c)**.
5) Select **1** from **(d)** the Target drop down menu, using the downward arrow.
    a. Please note that each target is analyzed individually to reflect different performance profiles of target.
6) Cancel the check of **Auto** in **Threshold (e)**.
    b. Do not cancel the check of **Auto Baseline**.
7) Add the check of **Threshold** in **Show (f)**.
8) Using the mouse, click and drag the blue threshold line **(g)** until it lies within the exponential phase of the fluorescence curves.

Figure 9. Amplification Plot Window



9) Click the **Reanalyse**button in the upper right corner of the window.
10) Repeat Steps 5-9 to analyze results generated for each set of markers (i.e. 1, 2, 3, etc).
11) Save analysis file by selecting **File**, then **Save As** from the main menu.
12) After completing analysis for each of the markers, click the **Export** tab, then the **Export Data** screen (see **Figure 10**) will appear. Select **Customise Export** to display the Ct values (see **Figure 10**).
13) To filter report by sample name in ascending or descending order, simply click on **Sample Name** in the table.

Figure 10. View Well Table



Interpretation of Results and Reporting

Expected Performance of Controls Included in Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit

Control Name	Detection Target			
	ORF1ab	N	E	IC
Positive Control	⩽25Ct	⩽25Ct	⩽25Ct	None detected
Negative Control	None detected	None detected	None detected	Standard 'S' amplification curve

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

SARS-CoV-2 Real-Time Multiplex RT-PCR Results Interpretation Guide

The table below lists the expected results for the SARS-CoV-2 Real-Time Multiplex RT-PCR Kit. If results are obtained that do not follow these guidelines, re-extract and re-test the sample. If repeat testing yields similar results, contact Liferiver for consultation.

Ct Value				Result Interpretation[a]
ORF1ab	N	E	IC	
+	+	+	/	All target results are valid. SARS-CoV-2 RNA is detected.
+	-	+	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab and E are both positive andTarget N is negative, suggesting 1) a sample at concentrations near or below LoDof the test, 2) a mutation in Target N, target region, or 3) other factors.
+	+	-	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab and N results are both positive and Target E is negative, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target E, target region, or 3) other factors.
-	+	+	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab result is negative and Target N and E are both positive, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target ORF1ab, target region, or 3) other factors.
+	-	-	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab result is positive and Target N and E are both negative, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target N and Target E, target region, or 3) other factors.
-	+	-	/	All target results are valid. SARS-CoV-2 RNA is detected. Target N result is positive and Target ORF1ab and E are both negative, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target ORF1ab and Target E, target region, or 3) other factors.
-	-	-	+	All target results are valid. SARS-CoV-2 RNA is not detected[b].
-	-	-	-	All target results are invalid. Sample should be retested; if the result is still invalid, a new specimen should be obtained.
-	-	+	/	All target results are valid. SARS-CoV-2 RNA is presumptive positive. Sample should be retested. For sample with a repeated presumptive positive result, additional confirmatory test may be performed if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans for epidemiological purposes or patient management.

'+' represents a positive detection signal, which is defined as Ct≤31;
'-' represents a negative detection signal, which is defined as Ct>31;
'/' represents no requirement. Detection of Internal Control is not required if result positive in any of the other three detection channels.

Note:

[a]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

[b]Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus.

Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good Laboratory Practice (cGLP) recommends a positive extraction control in each nucleic acid isolation batch.
- The internal control must be extracted and processed with each specimen at the same time to monitor the process of extraction.
- Always include negative control and positive control in each amplification and detection run.

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Performance of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit has only been established in upper and lower respiratory specimens (including nasopharyngeal or oropharyngeal swabs and sputum).
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the test result is obtained that Target ORF1ab and N are both negative while Target E is positive only, the sample should be retested. For sample with repeated presumptive positive result, additional confirmatory test may be performed if it is necessary to differentiate between SARS-CoV-2 and SARS-

CoV-1 or other Sarbecovirus currently unknown to infect humans for epidemiological purposes or patient management.

- If the virus mutates in the rRT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of viral RNA that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.

Performance Characteristics

Limit of Detection (LoD):

The LoD is defined as the lowest amount of analyte in a sample that is detected with a 95% probability, and it is determined by probit analysis.

For this purpose, a dilution series consisting of 5 different dilution levels was used, starting with 0.02 $TCID_{50}$/ml cultured virus(SARS-CoV-2/ZJU-02/Human/2020, inactivated) spiked into SARS-CoV-2 negative NPS or sputum samples.

Each dilution of each specimen type was extracted in 20 replicates using the QIAGEN QIAamp Virus RNA Mini Kit and tested with Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit on Applied Biosystems 7500 Real-time PCR Instrument.

A probit regression with SPSS Software was performed and the 95% LoD value was determined. The results for NPS and sputum samples are shown in Table 1-8.

Table 1. Positive rate of NPS (Target: ORF1ab)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.02	20	20	100%
0.01	20	19	95%
0.005	20	13	65%
0.0025	20	9	45%
0.00125	20	8	40%

Table 2. Positive rate of NPS (Target: N)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.02	20	20	100%
0.01	20	20	100%
0.005	20	19	95%
0.0025	20	16	80%
0.00125	20	8	40%

Table 3. Positive rate of NPS (Target: E)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.02	20	20	100%
0.01	20	19	95%
0.005	20	18	90%
0.0025	20	17	85%
0.00125	20	6	30%

Table 4. LoD of NPS by Probit

	Target	95% LoD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	ORF1ab	0.014	0.009	0.040
	N	0.005	0.003	0.010
	E	0.007	0.005	0.015

Table 5. Positive rate of sputum (Target: ORF1ab)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.02	20	20	100%
0.01	20	18	90%
0.005	20	18	90%
0.0025	20	10	50%
0.00125	20	7	35%

Table 6. Positive rate of sputum (Target: N)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.02	20	20	100%
0.01	20	20	100%
0.005	20	18	90%
0.0025	20	15	75%
0.00125	20	13	65%

Table 7. Positive rate of sputum (Target: E)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate(%)
0.02	20	20	100%
0.01	20	20	100%
0.005	20	19	95%
0.0025	20	16	80%
0.00125	20	12	60%

Table 8. LoD of sputum by Probit

	Target	95% LOD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	ORF1ab	0.010	0.007	0.026
	N	0.006	0.004	0.025
	E	0.005	0.003	0.015

Note: Variants VUI 202012/01 and 20C/501Y.V2,the two mutant strains of SARS-CoV-2, can be successfully detected by Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit, with no change of specificity and accuracy.
Analytical sensitivity: The positive rate of the kit is 100% when testing VUI 202012/01 and 20C/501Y.V2 with a concentration of 0.02 $TCID_{50}$/mL.
Precision: The CV of Ct value is < 5% when testing VUI 202012/01 and 20C/501Y.V2 with a concentration of 0.04 $TCID_{50}$/mL.

Analytical Specificity (Cross-reactivity)

The analytical specificity was evaluated by testing the cross-reactivity of a panel of different pathogens consisting of 17 viruses, 1 fungi, 2 chlamydia and mycoplasma, 1 protozoa, 8 bacteria and 1 pooled human nasal wash. The organisms selected were clinically relevant organisms (colonizing the respiratory tract or causing respiratory symptoms), common skin flora or laboratory contaminants, or microorganisms for which much of the population may have been infected. Each organism was tested with Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit.

For SARS-coronavirus, the results of target ORF1ab and N were negative, and the result of target E was positive.

For the rest of 29 pathogens, there were no false positive or invalid results tested (Table 9).

Table 9. Analytical specificity

Pathogen	ORF1ab	N	E
Human coronavirus 229E	-	-	-
Human coronavirus OC43	-	-	-
Human coronavirus HKU1	-	-	-
Human coronavirus NL63	-	-	-
MERS-coronavirus	-	-	-

Adenovirus	-	-	-
Human Metapneumovirus (hMPV)	-	-	-
Parainfluenza virus , type 1	-	-	-
Parainfluenza virus, type 2	-	-	-
Parainfluenza virus , type 3	-	-	-
Parainfluenza virus , type 4	-	-	-
Influenza A	-	-	-
Influenza B	-	-	-
Enterovirus71	-	-	-
Respiratory syncytial virus	-	-	-
Rhinovirus	-	-	-
Chlamydia pneumoniae	-	-	-
Haemophilus influenzae	-	-	-
Legionella pneumophila	-	-	-
Mycobacterium tuberculosis	-	-	-
Streptococcus pneumoniae	-	-	-
Streptococcus pyogenes	-	-	-
Bordetella pertussis	-	-	-
Mycoplasma pneumoniae	-	-	-
Pneumocystis jirovecii (PJP)	-	-	-
Pooled human nasal wash	-	-	-
Candida albicans	-	-	-
Pseudomonas aeruginosa	-	-	-
Staphylococcus epidermis	-	-	-

Note: "–" means negative for target ORF1ab, N and E.

Diagnostic Evaluation

For the clinicalperformance of the *Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit*, a method of comparison study was conducted to assess its performance. The comparator reagent used in this clinical study is BGI's diagnostic reagent, *Real-Time Fluorescent RT-PCR kit for Detecting SARS-CoV-2*, which has been authorized by the US Food and Drug Administration for emergency use on March 26, 2020. In total, 51 confirmed cases and 49 excluded cases were selected as the clinical samples in this study. In terms of specimen type, there were 32 cases of nasopharyngeal swab (15 positives while 17 negatives), 33 cases of oropharyngeal swab (15 positives while 18 negatives) and 35 cases of sputum (17 positives while 18 negatives). The 100 selected specimens were tested with evaluation reagent ("Liferiver Assay") and comparator reagent ("BGI Assay") simultaneously; the positive percent agreement and the negative percent agreement of each specimen type were all 100% between LiferiverAssay and BGI Assay.

Table 10. The compared results of Liferiver Assay and BGI Assay(specimen count)

Specimen count		BGI Assay		Total
		Positive	Negative	
Liferiver Assay	Positive	47	0	47
	Negative	0	53	53
Total		47	53	100
Positive percent agreement:47/47=100%(95%CI:90.59%~100.00%) **Negative percent agreement:53/53=100%(95%CI:91.58%~100.00%)**				

Note: A total of 100 specimens were collected for clinical evaluation from 51 clinically confirmed patients and 49 clinically excluded patients. Four specimens from clinically confirmed patients, attributable to specimen degradation, were tested negative with both Liferiver Assay and BGI Assay. In that, the sensitivity of both Liferiver Assay and BGI Assay is 92% when compared with clinical diagnostic results.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

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4. National Health Commission of the People’s Republic of China, “Technical Guidance on Laboratory Test of Novel Coronavirus Infected Pneumonia (current edition).’’

5. National Health Commission of the People’s Republic of China, “The Surveillance Protocol for Novel Coronavirus Infected Pneumonia Cases (current edition).”

6. Centers for Disease Control and Prevention (CDC), “Instructions for Use of CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. ”

Contact Information, Ordering, and Product Support

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