COVID-19 Coronavirus Real Time PCR Kit

IVD **For In Vitro Diagnostic Use Only**

INSTRUCTIONS FOR USE

Version 1.5.6
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Σ **50T** -15℃ -25℃

Cat No. JC10223-1NW-50T

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Contents

1. Intended use

COVID-19 Coronavirus Real Time PCR Kit is an In Vitro Diagnostic (IVD) reagent applying on fluorescent PCR technology and aiming at qualitatively detect Open Reading Frame gene region (ORF1a/b) and viral nucleocapsid region (N) of SARS-CoV-2 RNA from upper and lower respiratory tract specimens.
Upper respiratory tract specimens include throat swab and nasopharyngeal swab. Lower respiratory tract specimens include sputum.
The product is intended for use on populations suspected to have SARS-CoV-2 infection, as an aid in the diagnosis of SARS-CoV-2 infection by trained laboratory personnel on RT-PCR.
Typically, SARS-CoV-2 RNA could be detected from upper and lower respiratory tract specimens if a person is infected. Positive result suggests SARS-CoV-2 infection but bacteria and other virus induced co-infection could not be excluded. SARS-CoV-2 test result is not the only confirmation evidence of suspected cases and all positive results have to be reported to Centers for Disease Control (CDCs) and authorities. Negative result can neither straightforwardly exclude SARS-CoV-2 infection and nor the only decision-making evidence for treatment and patient management. Negative result should be appropriately used by combining clinical observation, medical history and epidemiological information.
The intended use setting of the kit is level 2 laboratories.

2. Background

On January 8 2020, the pathogen of severe unexplained viral pneumonia was identified by sequencing as tentatively named the novel (new) coronavirus 2019 (2019-nCoV). On February 12, 2020, International Committee on Taxonomy of Viruses declared that 2019-nCoV was officially named as SARS-CoV-2, and at the same day, World Health Organization declared the disease caused by SARS-CoV-2 was officially named as coronavirus disease 2019 (COVID-19). To date, there have been more than 10 million confirmed cases of COVID-19 reported to WHO. https://covid19.who.int/
COVID-19 Coronavirus Real Time PCR Kit is an IVD reagent applying fluorescent PCR technology and aiming at qualitatively detect SARS-CoV-2. The kit contains oligonucleotides primer, double-labeled hydrolysis probe (Taqman) and quality controls for PCR reaction.

3. Technical principle

Primer and probe are selected for Open Reading Frame gene region (ORF1a/b) and viral nucleocapsid region (N) after the SARS-CoV-2 genome was decoded. The kit designs primer/probe with specific ORF1a/b gene (labeled by FAM) and N (labeled by VIC) gene for SARS-CoV-2 detection. In addition, the kit also contains human RNase P gene (labeled by CY5) for clinical specimen detection.
RNA separated and purified from upper and lower respiratory tract specimens firstly undergoes reverse transcription to generate cDNA, then amplified in real-time PCR thermal cycler. Probes consist of a reporter dye at 5' and quenching dye at 3'. The fluorescent signals emitted from reporter dye are absorbed by the quencher, so it doesn't emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5'-3' exonuclease activity), separating reporter dye from the quencher, and generating fluorescent signals. The PCR instrument will then automatically draw a real-time amplification curve based on the signal change, and finally realizing the qualitative

detection of SARS-CoV-2 novel coronavirus at the nucleic acid level.

4. Materials provided

Components	Quantity	Volume	Test	Ingredients
RT-PCR Buffer	1	375 μL	50 T	Tris Hydroxy Methyl Aminomethane, Potassium chloride, Magnesium chloride, Nucleotides mix
RT-PCR Enzyme Mix	1	250 μL	50 T	Reverse transcriptase, RNasin, Taq DNA polymerase
Reaction Mix	1	200 μL	50 T	Primers and probes of SARS-CoV-2 and RNase P
Positive Control	1	500 μL	50 T	virus-like particles of SARS-CoV-2 and RNase P
Blank Control (RNase-free Water)	1	500 μL	50 T	RNase-free Water

5. Materials required but not provided

Extraction reagent

Manufacturer	Name	Cat No.	Specification
Bioperfectus Technologies	Viral Nucleic Acid Extraction Kit (Silica-Based Spin Column)	SDK60102	50 T
	Nucleic Acid Extraction Rapid Kit (Magnetic Bead Method)	SDKF60101	32/48/96 T
	Viral Nucleic Acid Extraction Kit (Magnetic Bead Method)	SDK60104	
Qiagen	QIAamp Viral RNA Mini Kit	52904 52906	50/250 T

Instruments and consumables

- Vortex mixer
- Centrifuge
- Calibrated adjustable pipettes (10μL, 100μL, 200μL, 1000μL)
- Calibrated Adjustable Multi-channel pipette (5-50μL)
- 1.5 mL centrifuge tube shelf
- Magnetic grate for 1.5 mL centrifuge tube
- Specimen preservation fluid (Vial transfer medium)
- Real-time PCR thermal cycler: Applied Biosystems 7500 (software

version V2.3 and V2.4), QuantStudio™ 5 (software version V1.4.3 and V1.5.1), Roche LightCycler®480 (software version V1.5.1.62), Bio-Rad CFX96™ (software version V3.1), Bioperfectus STC-48A/96A/96A PLUS (software version V1.0.0)

- Nucleic acid extractor: SSNP-2000B (32 channels), SSNP-3000A (64 channels), SSNP-9600A (96 channels),
- RNase-free Water, molecular grade
- 10% sodium hypochlorite or Pasteurized disinfectant
- Disposable particle-free gloves and operating gown
- Pipette tips with filter
- 1.5 mL centrifuge tube (No DNase/RNase)
- 0.2 mL PCR plate (Applied Biosystems)
- 0.2 mL PCR tube (Applied Biosystems)
- Biological safety cabinet or PCR hood

6.Warning and precautions

- For *in vitro* diagnostic use only.
- Operator should be well trained on real-time PCR techniques.
- All patient specimens should be inactivated at 56℃ for 30 minutes and processed in accordance with laboratory biosafety requirements. For more information, refer to

  https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Nucleic acid extraction of SARS-CoV-2 should be manually carried out in biosafety cabinet or by automatic nucleic acid extraction system.
- Wear personal protective equipment (PPE), including (but not limited to) disposable clean powder-free gloves, mask, goggles.
- Working zones in laboratory should be strictly separated. Use separated and segregated working areas for (i) Reagent preparation, (ii) Specimen preparation and (iii) Amplification. The workflow in the laboratory should proceed in unidirectional manner. The experiment processes shall comply with the Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories.

  https://cdn.intechopen.com/pdfs-wm/23728.pdf
- Work benches should be cleaned immediately after use. Amplicon contamination should be avoided according to

  https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- Clean work benches, pipettes and centrifuge by using 10% sodium hypochlorite and 70% ethanol.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended.

- Use applicable real-time PCR instrument and nucleic acid extraction system to ensure optimal test performance.
- Use reagents before expiry date. DON'T replace or interchange reagents from different batches or manufactures.
- Discard specimens and assay waste according to your local safety regulations.
  http://www.gov.cn/gongbao/content/2003/content_62236.htm

7. Kit storage, processing and reliability

- Store at -20±5°C condition.
- Always check expiry date before use and do not use expired reagent.
- Keep probe away from light.
- Properly unfreeze and mix before reagent preparation.
- Avoid repeatedly freeze-thaw.
- Avoid exposure of the kit to excessive temperature or humidity.
- Manufacturing date and expiry date: see outer packing box.
- Ensure that it is stored on a flat surface.

Indication of instability or deterioration of reagents

When a positive or blank control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested.

8. Specimen type

- Upper respiratory tract specimens: throat swab, nasopharyngeal swab.
- Lower respiratory tract specimens: sputum.

9. Specimen collection, transportation and storage

Inappropriate sampling, storage and transportation may lead incorrect detection results. Following trainings are suggested.

➢ Sampling
  - For sampling, please refer to https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html
  - Follow product instructions of instruments and consumables.
  - Swab specimen should be collected using plastic swab with polypropylene fiber head. Wooden swab with cotton is not allowed. Specimen preserved at virus transport media after collection.
  - Sputum specimen is collected into a 50mL plastic container with viral transfer medium. A digesting buffer (with 1g/L proteinase K in PBS) is added in the specimen tube at a 1:1 ratio (v/v), mixed with shaking, hold for 5 minutes before the nucleic acid extraction. Collect sputum from patient cough and preserve into 50 mL plastic tube with virus transport media.

- Recommended specimen collection kit.

Manufacturer	Product Code	Contents
Yocon Biotech Co., Ltd	Jing 20182400236	1 × viral transfer medium 1 × plastic swab (sterile)
Jiangsu Kangjian Medical Apparatus Co., Ltd	Su 20180169	1 × viral transfer medium
Health Gene Technologies Co., Ltd	Zhe 20190062	1 × viral transfer medium
Jiangsu Bioperfectus Technologies Co., Ltd	Su 20200071	1 × viral transfer medium
Jiangsu Bioperfectus Technologies Co., Ltd.	Su 20200114	1 × viral transfer medium
Jiangsu Bioperfectus Technologies Co., Ltd.	N/A	Physical saline containing 0.9% Sodium chloride

- Transportation
  - Specimen packaging and transportation follows https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/
  - Pack 3 layers according to class A or B infectious articles if external transportation involves.
  - Specimen collected from suspected SARS-CoV-2 cases should be preserved using 2-8°C ice bags or -70°C dry ice and sent to qualified laboratories within 24 hours.
- Storage
  - Specimen preserves at 2-8°C up to 24 hours after received.
  - Specimen preserves at -70°C or colder if extraction is arranged after 24 hours.
  - Extracted RNA preserved at -70°C or colder for 6 months.

10. Specimen transportation

Specimen should be sent to laboratory as soon as possible and using refrigerated preservation if long-distance transportation is inevitable.

- Transport all specimens within 24 hours to qualified laboratories.
- Transport frozen specimen using dry ice and unfrozen specimen using ice bag.
- SARS-CoV-2 viral strain and other infectious biological materials are classified A corresponding to UN2814. Packaging should follow PI602 sub-packaging requirements at International civil aviation organization document Doc9284 “Technical instructions of aviation transport safety of hazardous articles”. Environmental sample is classified B corresponding to UN3373. Packaging should follow PI650 sub-

packaging requirements at International civil aviation organization document Doc9284 “Technical instructions of aviation transport safety of hazardous articles”. Transportation by other ways should refer to above mentioned standards.

- Please inform receiving laboratory before sending specimens.

11. Reagent preparation

1) Preserve kit at -20±5°C after receipt.
2) Attentions: Use reagent at clean environment and preserve reagent at -20±5°C away from direct light. Avoid freeze-thaw cycles for more than 5 times.
3) Properly dissolve and mix before use.
4) Process the RNase-free water and positive reference carefully at specimen processing zone to avoid contamination. Avoid freeze-thaw cycles for more than 5 times.
5) Preserve blank and positive controls at -20±5°C for 12 months.
6) Preserve blank and positive controls at -20±5°C after extraction for 12 months.

12. Reagent setup (in reagent preparation area)

Master Mix and reaction well setting

Note: Setting of reaction wells varies with sample quantity. Each run should contain blank and positive controls.

1) Thaw RT-PCR buffer, enzyme mix and reaction mix from -20±5°C at room temperature in reagent preparation zone.
2) Vortex RT-PCR buffer, enzyme mix and reaction for 5 seconds before use.
3) Invert repeatedly upside down 5 times to mix.
4) Centrifuge at 5000 rpm for 5 seconds.
5) Label the 1.5 mL tube as Master Mix.
6) Double check reaction quantity (N), including sample quantity (n) to be tested, blank and positive control quantity (overdose is accepted). Prepare master mix based on below table.

Step	Components	Volume
1	RT-PCR Buffer	N × 7.5 µL
2	RT-PCR Enzyme Mix	N × 5.0 µL
3	Reaction Mix	N × 4.0 µL
4	RNase-free water	N × 3.5 µL
	Total	**N × 20.0 µL**

- If sample quantity (n) including quality control is between 1~14, N=n+1.

- If sample quantity (n) including quality control >15, N=n+2.

7) Preserve above master mix into the labeled 1.5 mL centrifuge tube and mix.
8) Centrifuge at 5000 rpm for 5 seconds.
9) Place PCR reaction tube or plate into 96-well shelf.
10) Pipette 20 μL of master mix in each reaction well.
11) Tightly close the PCR reaction well/plate and transfer to the nucleic acid processing zone.

13. Nucleic acid extraction (in specimen preparation area)

Instruments preparation

Clean all work benches, pipettes, centrifuge and other instruments using 5% sodium hypochlorite and 70% ethanol.

Nucleic acid extraction

Performance of COVID-19 Coronavirus Real Time PCR Kit depends on the quantity and purity of nucleic acid extraction. Below listed extraction kits have been validated and could be used for SARS-CoV-2 RNA extraction.

Bioperfectus Viral Nucleic Acid Isolation Kit

SDK60102, spin column
SDKF60101, magnetic beads (auto)
SDK60104, magnetic beads (auto)

Qiagen QIAamp Viral RNA Mini Kit

52904/52906, spin column

Extraction follows instructions from kit manufacturer.
Blank and positive controls shall fully involve nucleic acid extraction process.

Add extraction sample eluate

1) Tenderly vortex mix centrifuge tube containing extraction sample eluate for 5 seconds.
2) Centrifuge at 5000 rpm for 5 seconds to sediment purified RNA.
3) Add 5μL of extraction eluate sample to each well.
4) Close the cap after adding 8 wells.
5) Continue the work to avoid contamination.
6) Wear gloves to avoid contamination.
7) Close all caps and then add extracted quality controls.

Add quality control

1) Add 5 μL blank control to reaction well and close the cap.
2) Add 5 μL positive control to reaction well and close the cap.

Note: please follow the sequence from 1 to 8 if using 8-tube strips.

3) Centrifuge the 8-tube strip at 5000 rpm for 5 s and then place back to the shelf.

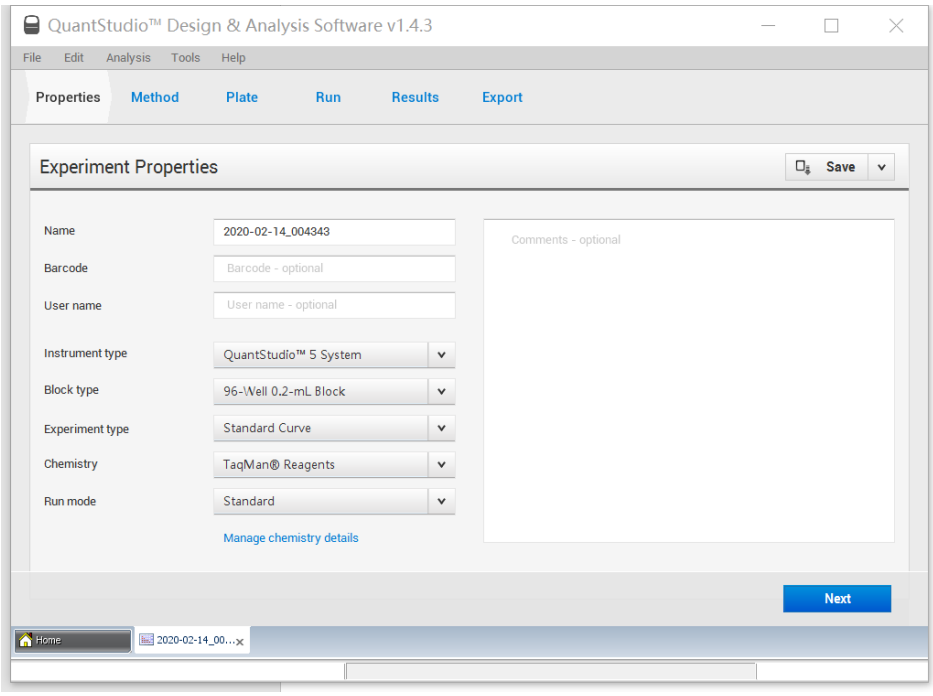
Note: If using a PCR 96-well plate, centrifuge for 30 s at 1000 rpm.

14. Amplification and detection (in amplification area)

Run the experiment in Applied Biosystems QuantStudio™ 5 real-time thermal cycler.

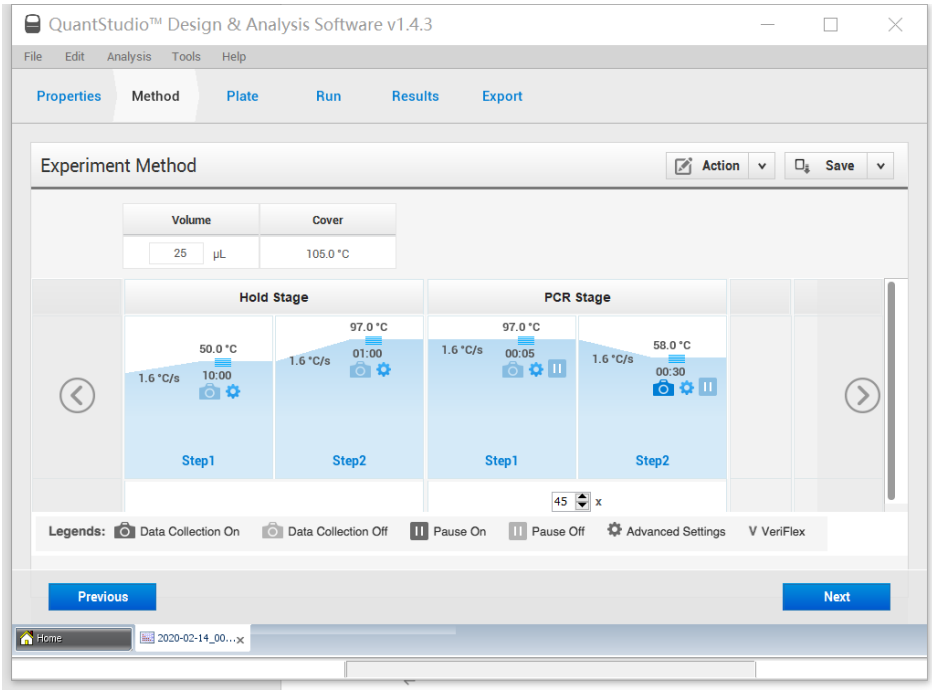
Setup

1) Double click Applied Biosystems QuantStudio™ 5 icon, and run Applied Biosystems QuantStudio™ 5 real-time thermal cycler.
2) Choose “create new files” on the window.
3) Set the following items from the “Properties” menu.
    a. Instrument type:
    b. Block type:
    c. Experiment type:
    d. Chemistry:
    e. Run mode:
4) Click “Next” at the bottom.



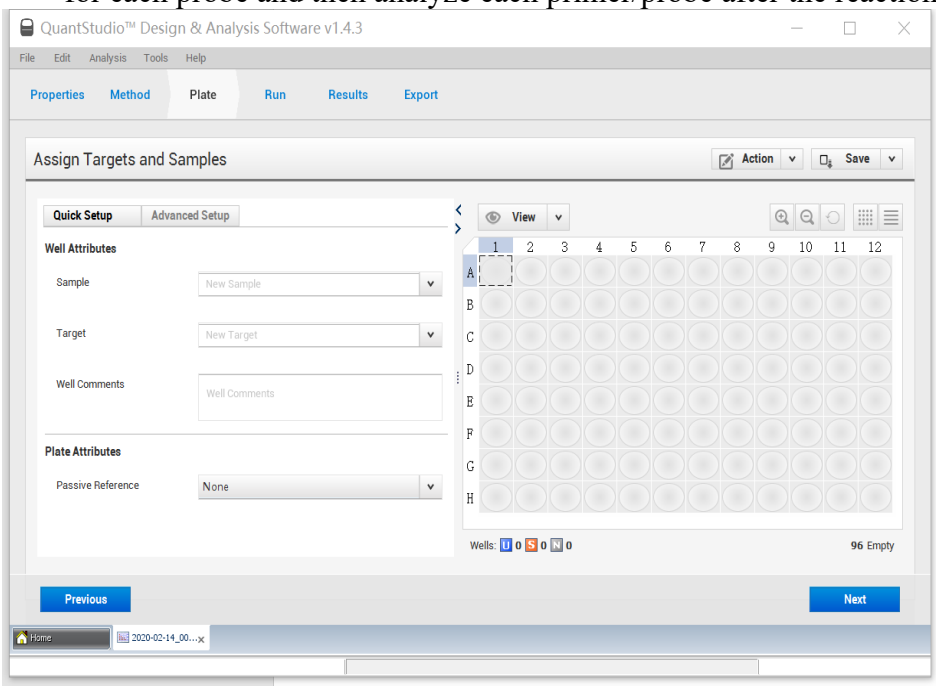
Program setting

1) Choose “Method” in the menu.
2) Modify thermal cycling conditions as follows.
    a Set 50°C, 10 min, 1 cycle at “Hold stage” step 1.
    b Set 97°C, 1 min, 1 cycle at “Hold stage” step 2.
    c Set 97°C, 5s at “PCR stage” step 1.
    d Set 58°C, 30s at “PCR stage” step 2.
    e Fluorescent detection at 58°C, “PCR stage” step 2.
    f Set cycle number 45.
    g Modify “Volume” to 25 µL.
3) Click “Next” at the bottom.



Target setting

1) Choose “Plate” from the menu and then show “Quick setup”.
2) Choose “None” at “Passive reference”.
3) Click “Advanced setup” to set targets. Define luminophore and quencher for each probe and then analyze each primer/probe after the reaction.



4) Create ORF1ab Target including
   a. Name: ORF1ab
   b. Reporter: FAM
   c. Quencher: None

Color: do the following to modify the color of the target

Click color block to show the list.

Click the block to define a color.

Click “apply” to return to “Target” window.

5）Create N Target including

a. Name: N
b. Reporter: VIC
c. Quencher: None

Color: do the following to modify the color of the target

Click color block to show the list.
Click the block to define a color.
Click “apply” to return to “Target” window.

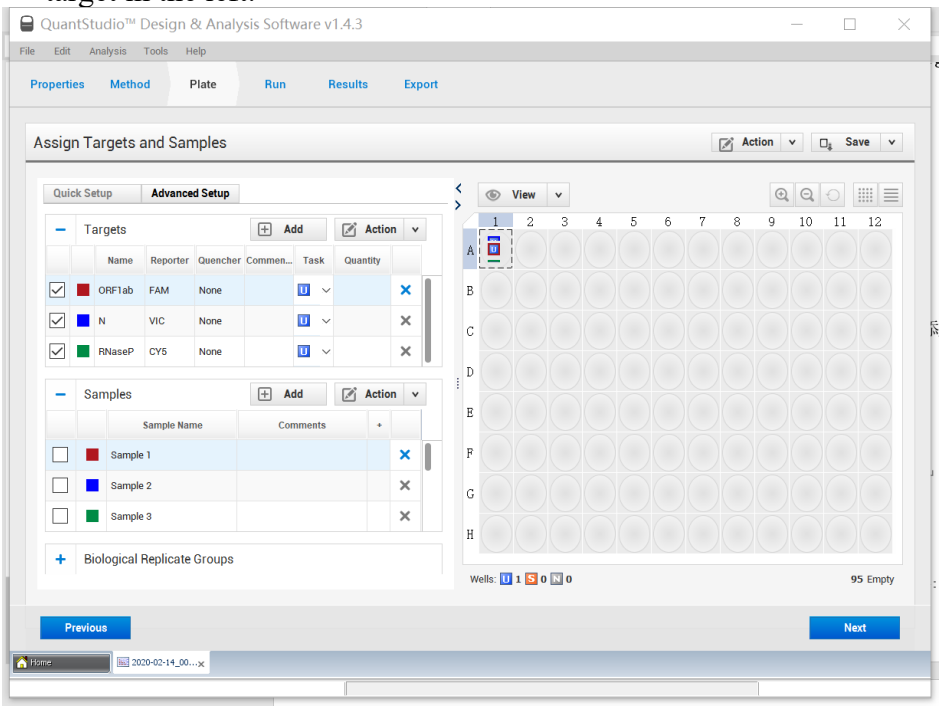
6）Create RNase P Target including

a. Name: RNase P
b. Reporter: CY5
c. Quencher: None

Color: do the following to modify the color of the target

Click color block to show the list.
Click the block to define a color.
Click “apply” to return to “Target” window.

7）Choose well position from the right “View” window and then check the target in the left.



Sample setting

1） Click “Add” from “Sample” window and set samples.

2）Create Sample 1 including

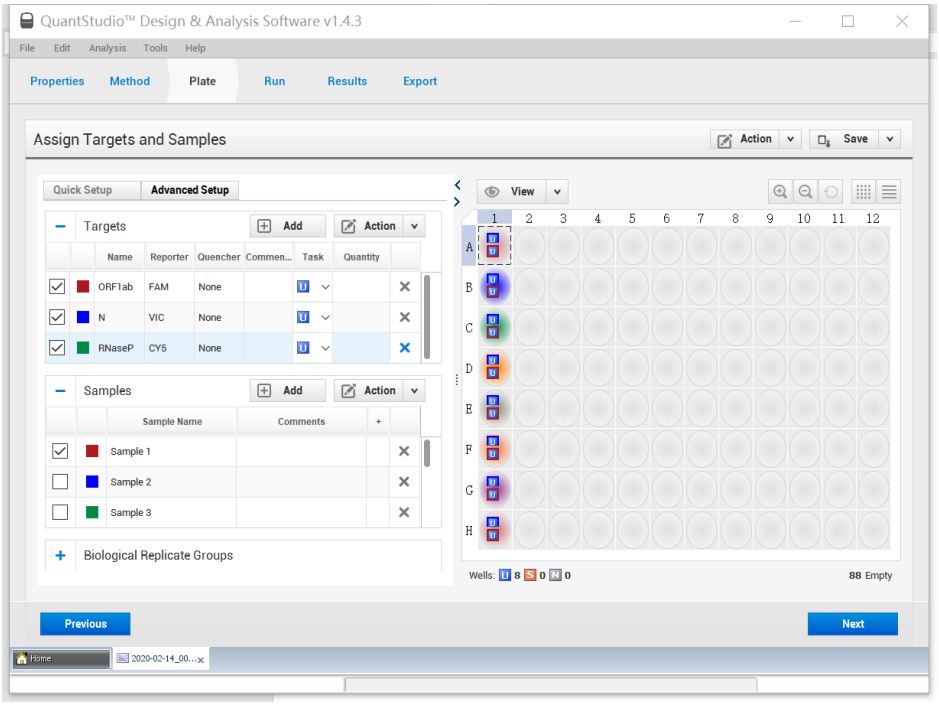
a. Defaulted sample name “Sample 1” and is adjustable.

Color: do the following to modify the color of the sample

Click color block to show the list.
Click the block to define a color.
Click “apply” to return to “Sample” window.

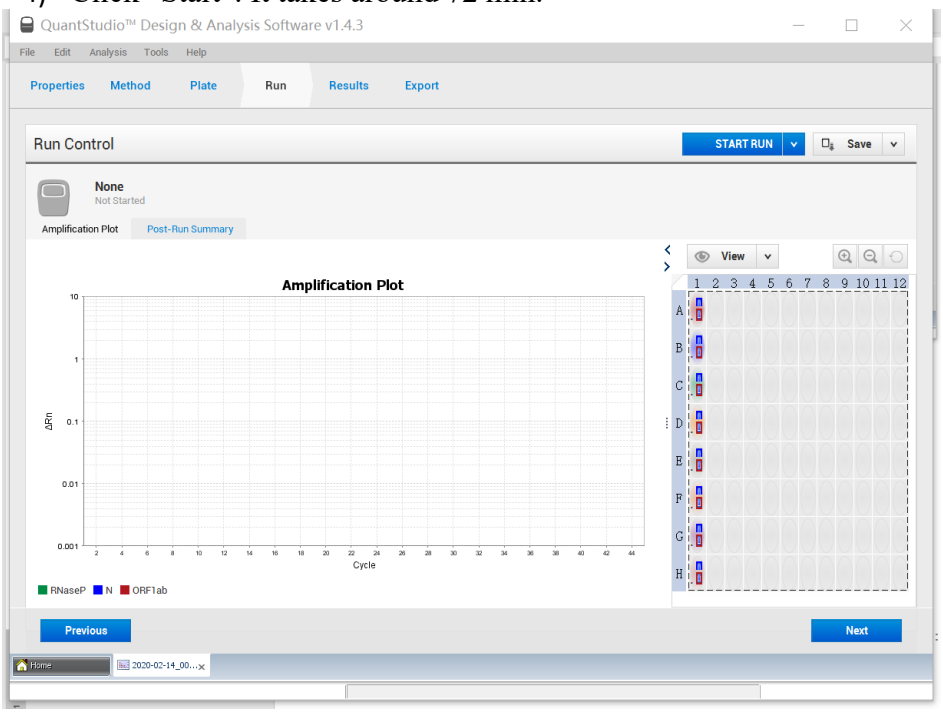
b. Choose well position from the right “View” window and then check the sample in the left.

3）Click “Next”.



Run the program

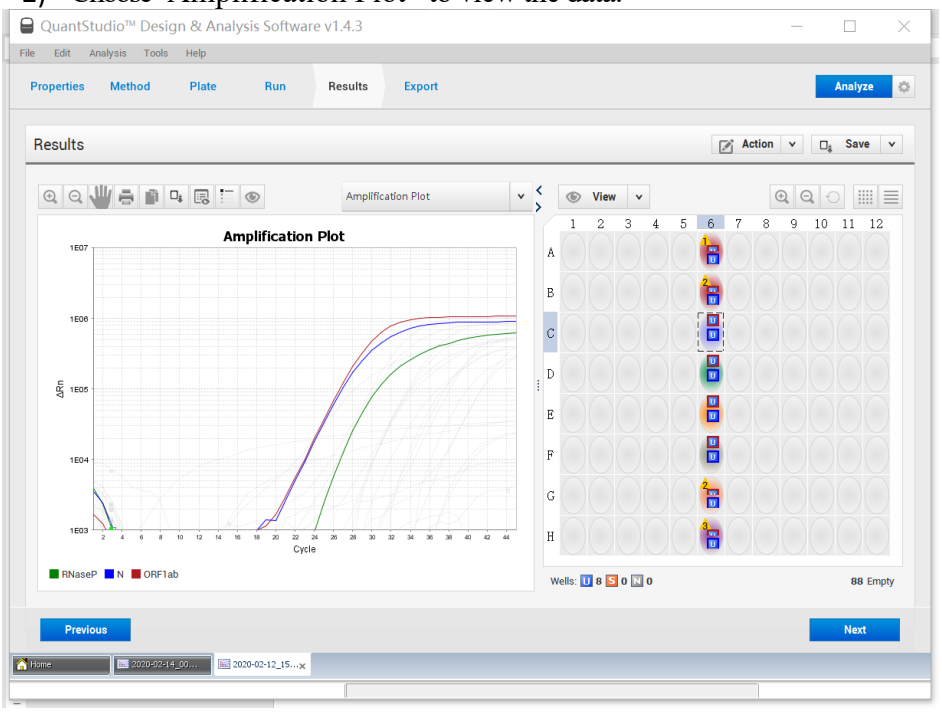
1) Ensure program setting is correct and then mount the PCR plate to the instrument.
2) Click “START RUN” from “Run” menu.
3) File has to be saved before running. Click “File” from main menu and then choose “Save as”. Save in an appropriate folder.
4) Click “Start”. It takes around 72 min.



Analysis

1) After amplification, click “Results”.

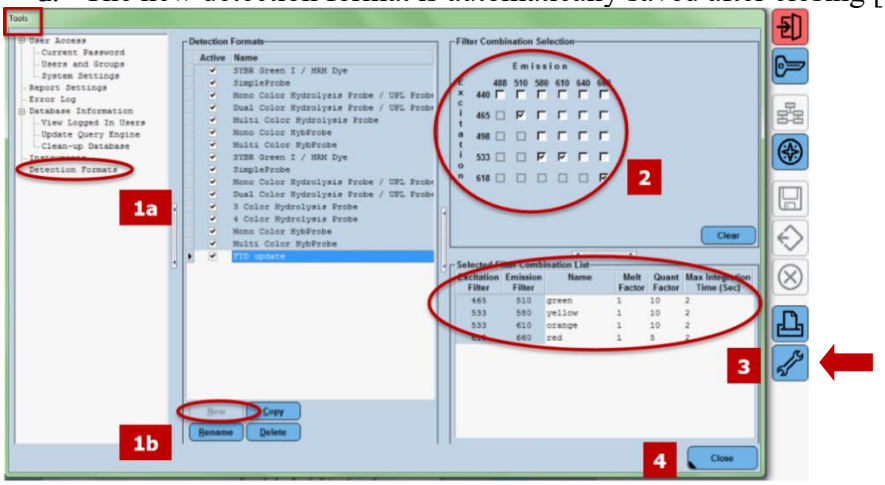
2) Choose "Amplification Plot" to view the data.



Run the experiment in Roche LightCycler® 480 Real-time PCR instrument.

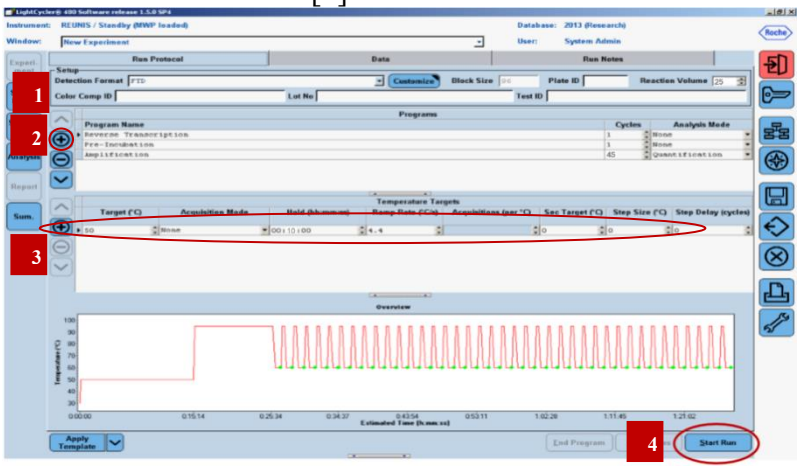
Settings

1) New experiment: Start the LightCycler® 480 software, and choose "New Experiment" on the home window.
2) Tools:
   a. Select "Detection Format" [1a] press "New" [1b].
   b. Choose the right four filter combinations in the "Filter Combination Selection" [2].
   c. Define a "Name" and the correct values for "Quant Factor" and "Max. Integration Time" [3].
   d. The new detection format is automatically saved after closing [4].



3) Create a new experiment:
   a. Change the "Detection Format" [1] to the new created format.
   b. Click on "New Experiment" and press "+" [2] to add further steps of the PCR protocol.

c. Change for each step the “Program Name”, “Cycles”, “Analysis Mode”, “Target”, “Aquisition Mode”, “Hold”, “Ramp Rate” [3].
   a) Set Reverse Transcription at Program Name, 1 at Cycles, None at Analysis Mode, 50 at Target, None at Acquisition Mode, 600 (10min) at Hold, 4.4 at Ramp Rate.
   b) Set Pre-Incubation at Program Name, 1 at Cycles, None at Analysis Mode, 97 at Target, None at Acquisition Mode, 60 (1min) at Hold, 4.4 at Ramp Rate.
   c) Set Amplification at Program Name, 45 at Cycles, Quantification at Analysis Mode
      I) 97 at Target, None at Acquisition Mode, 5 (5s) at Hold, 4.4 at Ramp Rate.
      II) 58 at Target, Single at Acquisition Mode, 30 (30s) at Hold, 4.4 at Ramp Rate.
   d) Modify “Reaction Volume” to 25 µL.
d. Click on the “Start Run” [4] button to start the run.



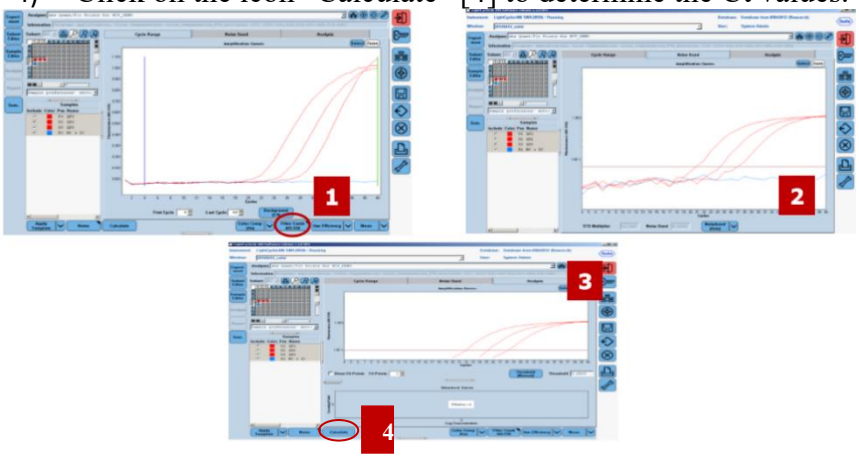
4) Subsets Editor:
   a. Click on the window “Subset Editor”.
   b. To enter new subsets, click on the icon “+”.
   c. Add the name of the subset.
   d. Select the correct wells.
   e. Click on the icon “Apply”.
5) Sample Editor: Click on the window “Sample Editor”, and select the subset created previously.

Analysis

Click on “Analysis” and choose “Abs Quant/Fit Points” under “Create new Analysis” and select the subset of interest and click on “√” .

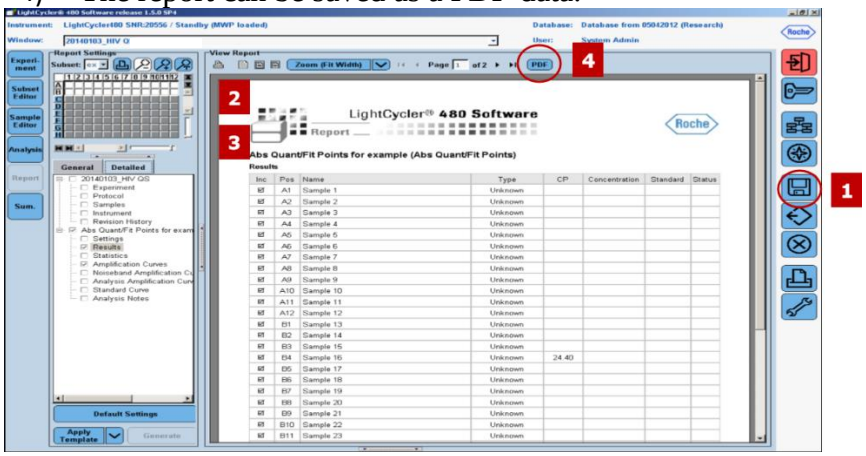
1) Click on the icon “Filter Comb” [1], to choose the correct channel according to the color which is to be analyzed. Correctly place the baseline by moving the vertical line above the negative control. The threshold is determined visually in the window “Cycle Range”.
2) The setup of the threshold will be done in the window “Noise Band” [2]. Correctly place the threshold accordingly by moving the threshold line with Start value 3 ~ 15 and End value 5 ~ 20.
3) In the window “Analysis” [3], the threshold can be placed with more precision.

4) Click on the icon “Calculate” [4] to determine the Ct values.



Report

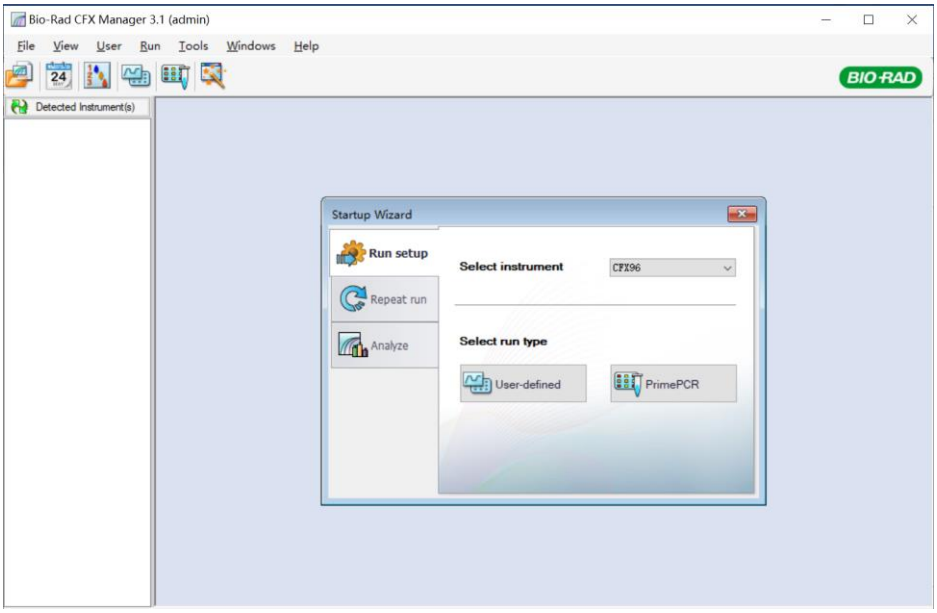
1) Save the data and generate a report.
2) Check the Ct values of the positive, internal and negative control.
3) If all controls meet the specified ranges, check the clinical specimens for positives.
4) The report can be saved as a PDF-data.



Run the experiment in Bio-Rad CFX96™ real-time thermal cycler.

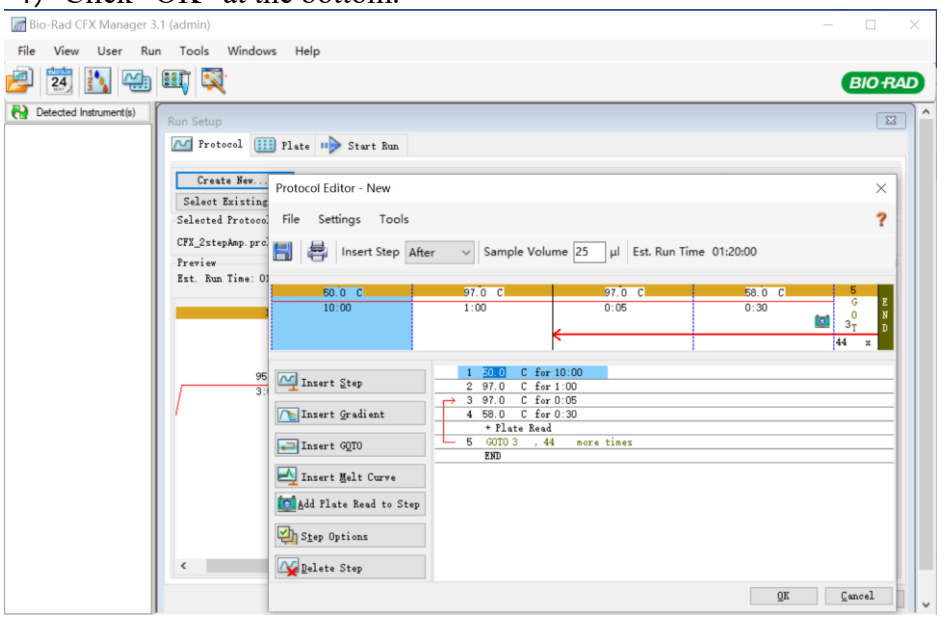
Setup

1) Double click Bio-Rad CFX96 Manager icon, and run Bio-Rad CFX96™ real-time thermal cycler.
2) Choose “run setup” on the window. Select run type as “User-defined”.



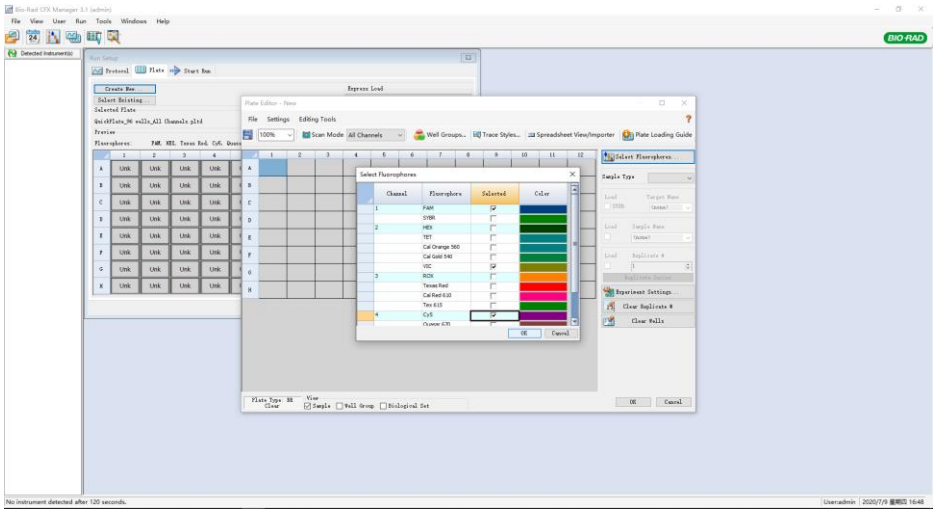
Program setting

1) Choose “Create new protocol” in the menu.
2) Modify thermal cycling conditions as follows.
   a Set 50°C, 10 min as step 1.
   b Set 97°C, 1 min as step 2.
   c Set 97°C, 5s as step 3.
   d Set 58°C, 30s as step 4, and set “Add Plate Read to Step”.
   e And then set “GOTO 3, 44 more times”.
   f Modify “Sample Volume” to 25 µL.
3) Press “Save” and enter a name for the program.
4) Click “OK” at the bottom.

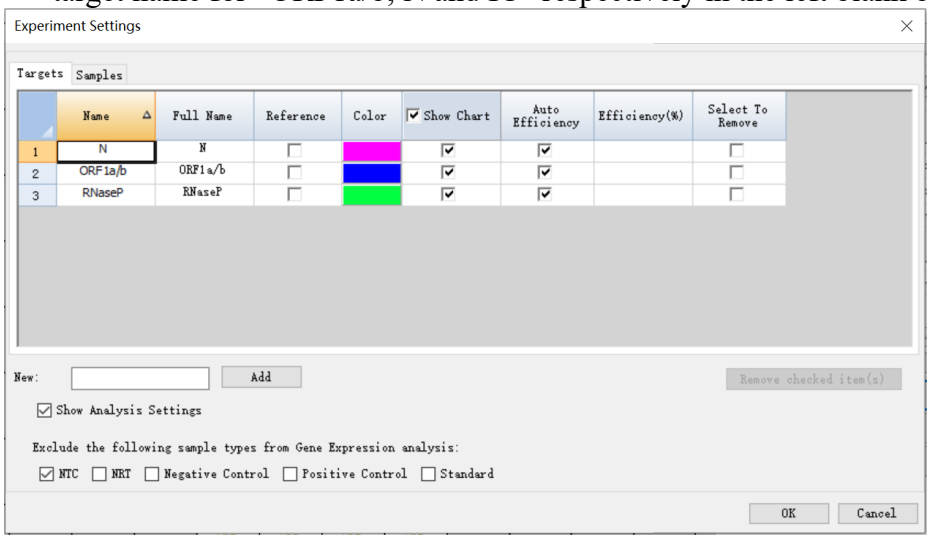


Target and sample setting

1) Click “Next” and then “Create new plate”.
2) Click “Select Fluorophores” to select FAM, VIC and CY5, and change the color accoding to your needs.



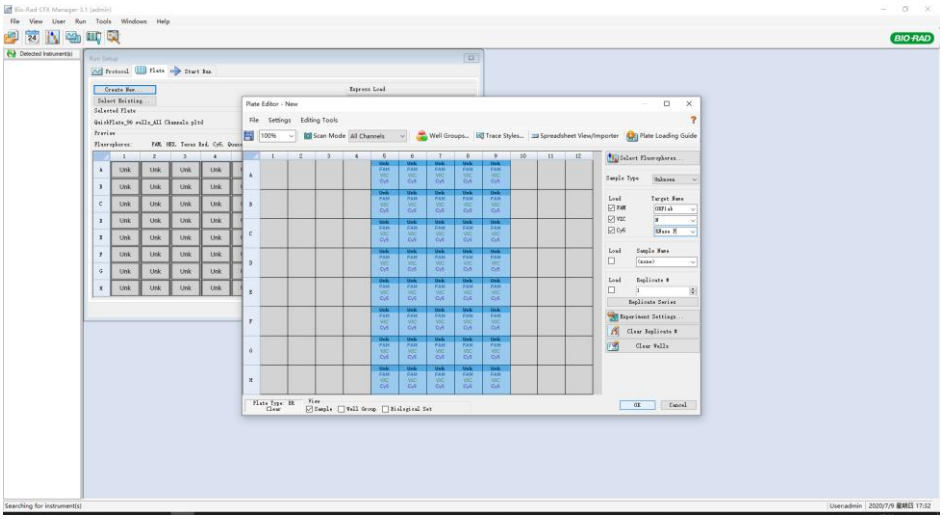
3) Choose well position from the left window and then select sample type as “Unknown”.
4) Click “Experiment setting”, and tick “Show Analysis Setting”.Add the target name for “ORF1a/b, N and IC” respectively in the left blank box.



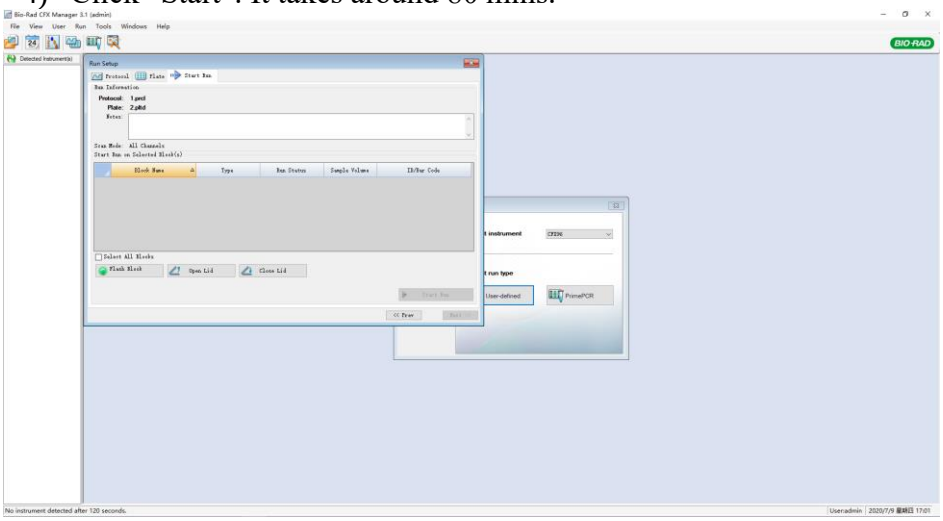
5) Click “OK” and turn to the Plate Edtor window.
6) Choose the target on the right. Give the target name for each reporter. ORF1a/b for FAM, N for VIC and RNase P for CY5.
7) Name for the detection samples.
8) Click “OK” and then click “Next”.

Run the program

1) Click “Open Lid”, place the PCR plate to the instrument and then click “Close Lid”.
2) Click “Start Run”.
3) File has to be saved before running. Click “File” from main menu and then choose “Save as”. Save in an appropriate folder.

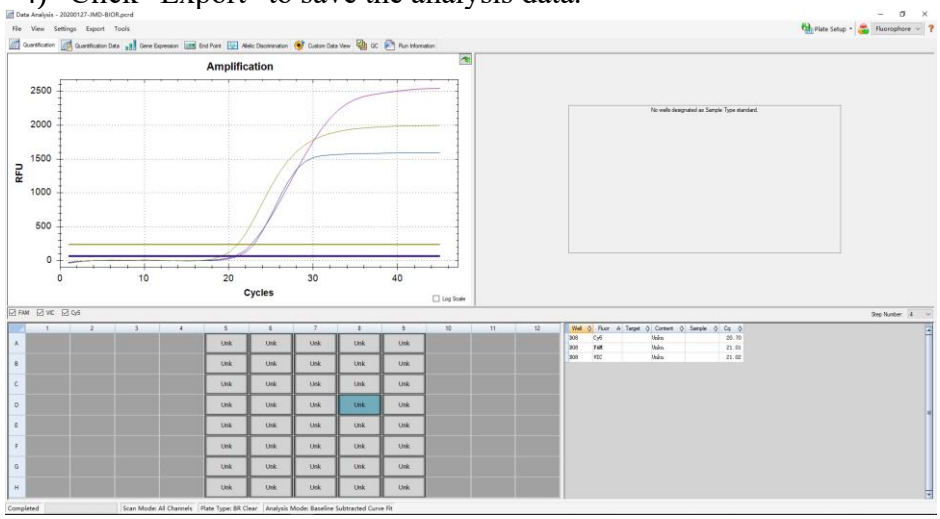


4) Click “Start”. It takes around 80 mins.



Analysis

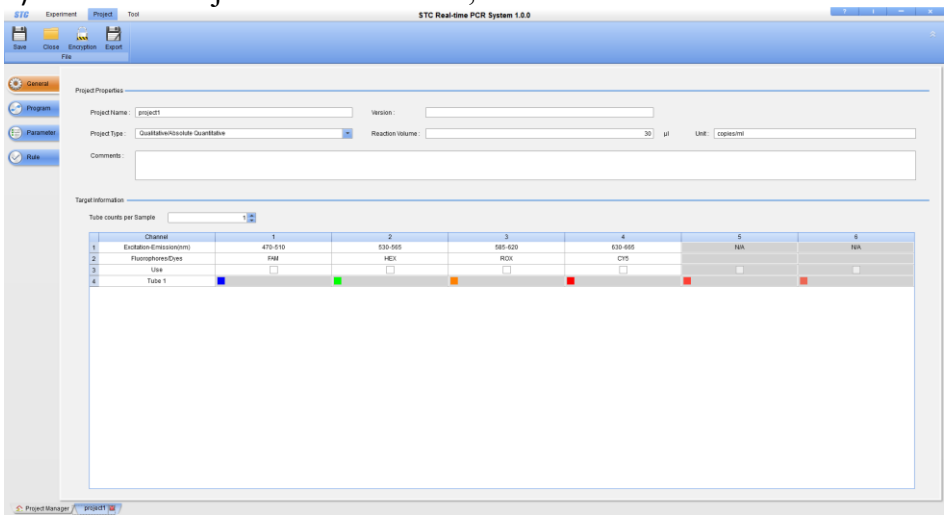
1) After amplification, View results on the Quantification window.
2) Click the well to see the amplification plot.
3) Tick one target, and click “Settings” to set the baseline and threshold line.
4) Click “Export” to save the analysis data.



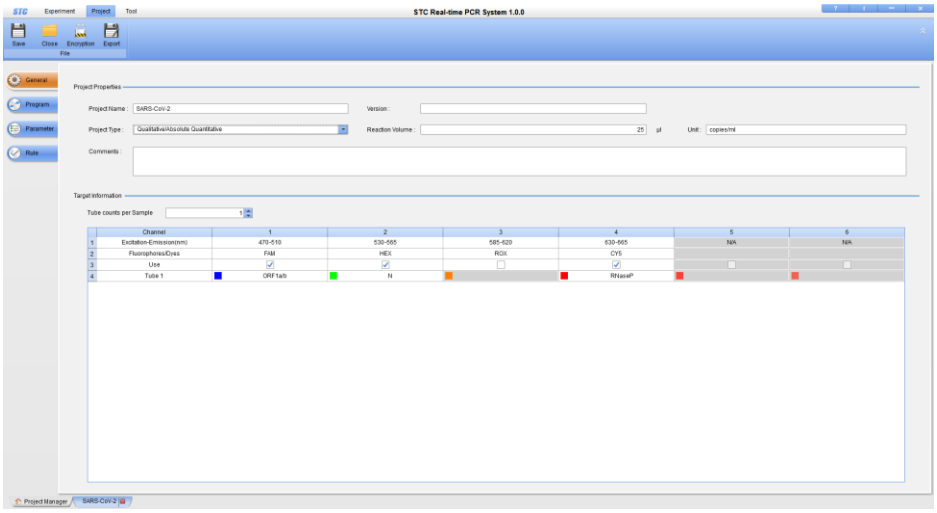
Run the experiment in Bioperfectus STC-48A/96A/96A PLUS real-time thermal cycler.

Program setting

1) Double click Bioperfectus STC 1.0.0 icon, and run STC real-time thermal cycler.
2) Choose “Project” on the window, and then click “Create”.

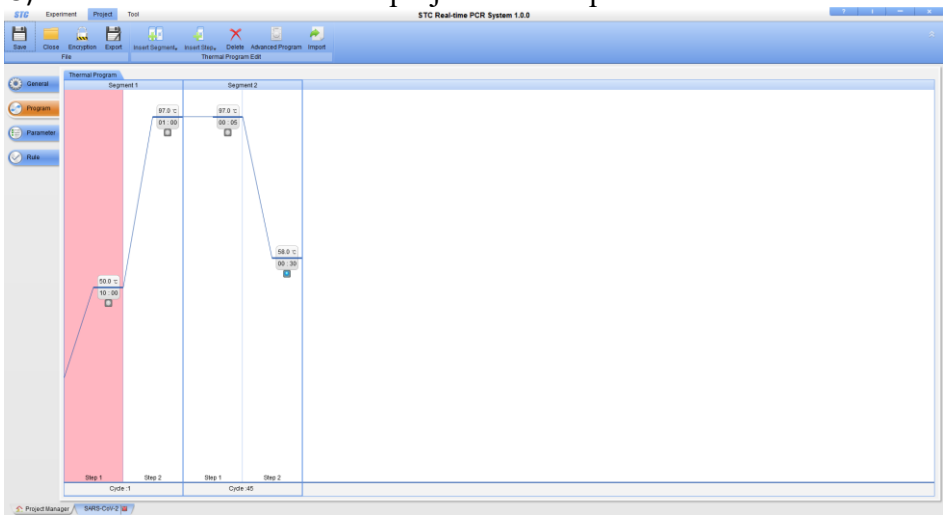


3) Click “General” on the left to set the following items.
    a. Project name： SARS-CoV-2
    b. Project type： Qualitative/Absolute Quantitative
    c. Reaction Volume： 25μL
    d. Target information：
    Set “Tube counts per Sample” 1;
    Tick FAM, HEX and CY5 channel, named as “ORF1a/b, N, and RNaseP”.



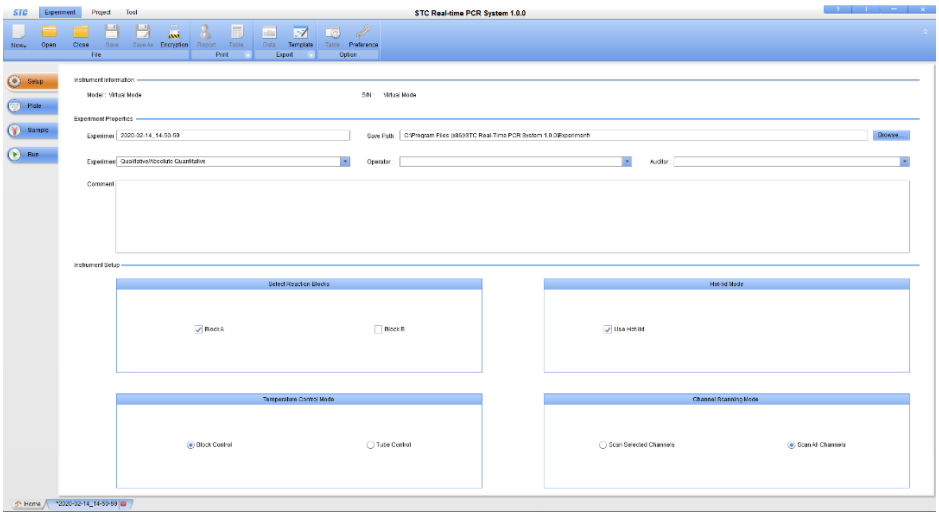
4) Click “Program” on the left to set amplification conditions.
    a. Set 50°C, 10 min, 1 cycle at “Segment 1” step 1.
    b. Set 97°C, 1 min, 1 cycle at “Segment 1” step 2.
    c. Set 97°C, 5s at “Segment 2” step 1.
    d. Set 58°C, 30s at “Segment 2” step 2.
    e. Fluorescent detection at 58°C, “Segment 2” step 2.
    f. Set cycle number 45.

5) Click “Save” to save the project as a template.

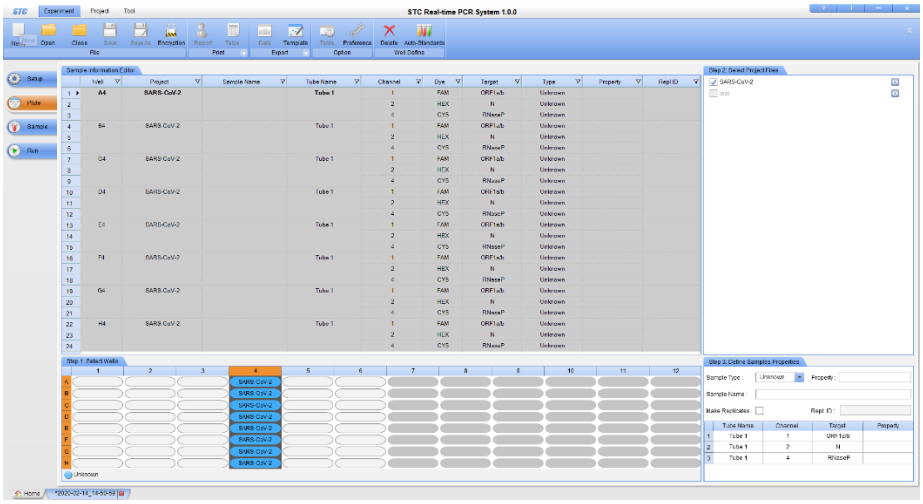


Experiment

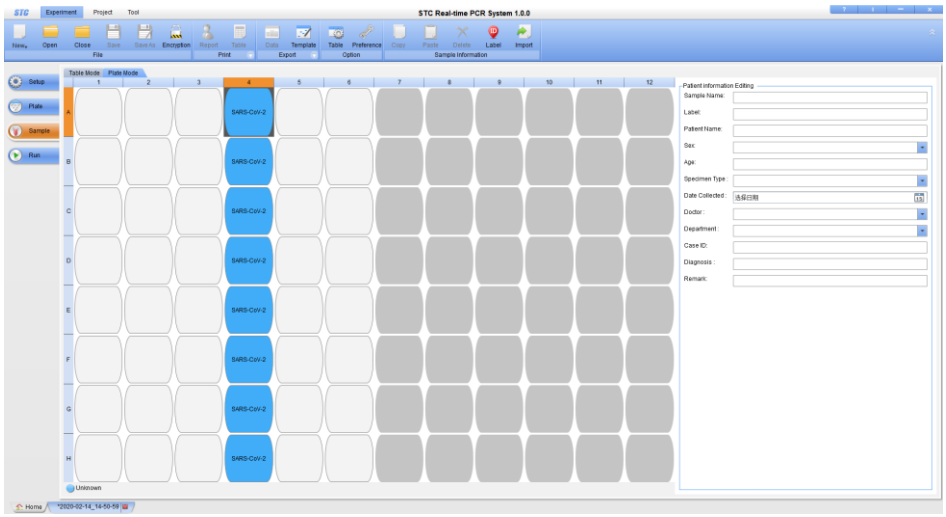
Choose “Experiment” in the menu and click “Experiment Wizard” to create a new experiment.



1) Set the following items in the “Setup” menu.
   b. Experiment Name:
   c. Experiment Type: Qualitative/Absolute Quantitative
   d. Save Path:
   e. Select Reaction Blocks:
   f. Temperature Control Mode: Block Control
   g. Channel Scanning Mode:
2) Click “Plate”.
3) Click wells and the project template “SARS-CoV-2”.

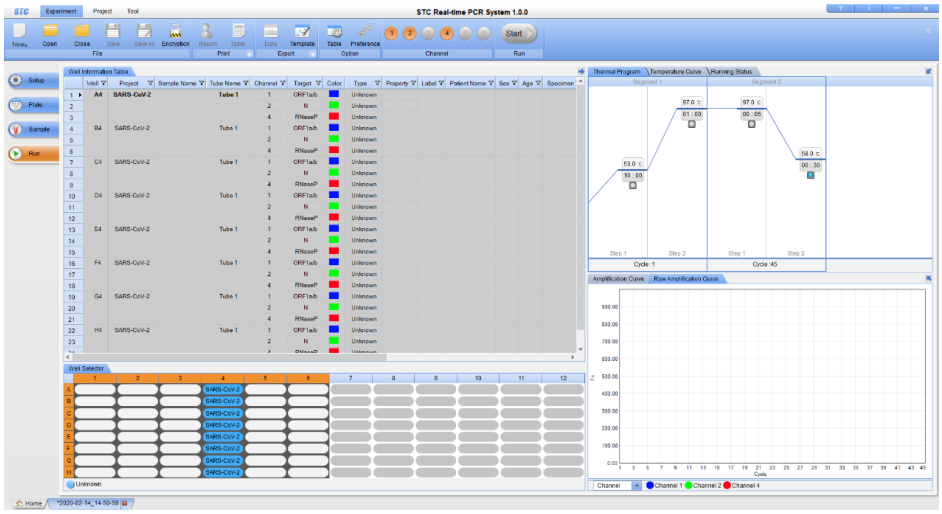


4) Click “Sample” to mark the detected specimens.



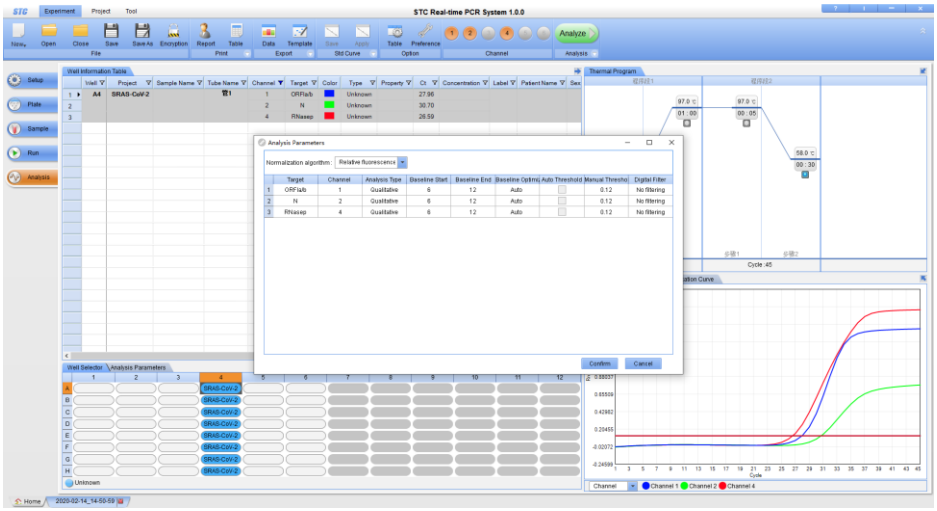
Run the program

1) Ensure program setting is correct and then mount the PCR plate to the instrument.
2) Click “Run” menu.
3) File has to be saved before running. Click “File” from main menu and then choose “Save as”. Save in an appropriate folder.
4) Click “Start”. It takes around 72 min.

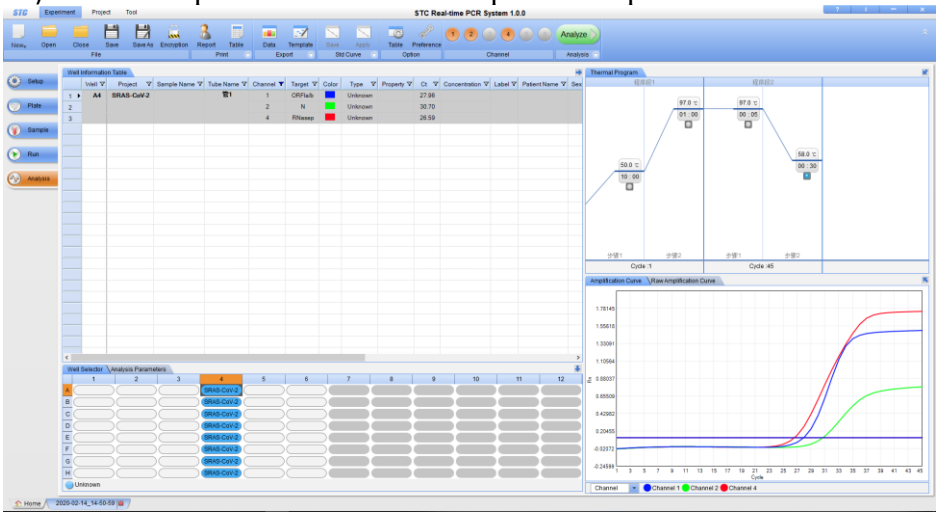


Analysis

1) After the run is completed, View amplification curve in the same window.
2) Under the “Analysis”, click “Analysis Parameters”, set “Baseline Stat”, “Baseline End”, and “Threshold” values, then click “Confirm” to read the result.



3) Click “Export” in the menu to export the experimental results.



15.Quality control

- Quality control should be enforced according to local regulations, certificate requirements or laboratory standard quality control process.
- Quality control is used to monitor reagent and results analysis.
- Check positive and blank controls before using each new batch of kits.
- Add positive control to each nucleic acid extraction and purification.
- Each nucleic acid testing should include positive and blank controls. Human RNase P gene should be monitored to guarantee specimen quality and extraction process.
- Expected performance of COVID-19 Coronavirus Real Time PCR Kit quality control follow.

Quality controls	Name	Used for	ORF1ab	N	RNsae P	Expected Ct
Positive control	PTC	Failure of kit or experiment	+	+	+	Ct ⩽ 30
Blank control	NTC	Reagent/environment contamination	-	-	-	Not detected

Internal control RNase P gene monitors sampling and extraction processes and potential contamination of extraction process.
If any of above controls shows incorrect Ct value, it means that the testing is incorrect or reagent/instrument has problems. Repeating the test is necessary.

RNase P（CY5 channel, control of sampling and extraction）

- All clinical specimens should show fluorescent amplification plot with internal control RNase P gene and Ct ⩽ 37, and therefore indicate the existing of human RNase P gene. If RNase P gene is not detected, it is possible that
  - Nucleic acid extraction from clinical specimen was not successful and then leads RNA missing and/or RNA degradation.
  - Insufficient human cell collected due to poor extraction or loss of integrity
  - Improperly operation or instruments.
  - Failure of reagent or instruments.
- If positive RNase P gene is not detected, the explanation could be
  - If both SARS-CoV-2 targets ORF1ab and N are positive, and RNase P gene is negative for the patient sample, the result is valid. Some specimens do not have RNase P amplification plot because of rare cells contained. Negative RNase P cannot exclude the existing of SARS-CoV-2 in these specimens.
  - If all of SARS-CoV-2 targets ORF1ab and N and RNase P gene are negative for the patient sample, the result should be invalid. If there's excess specimens, repeat the test. If all three genes are negative in repeating test, report the test invalid. It is

suggested that the sampling is invalid and should re-collect specimen.

SARS-CoV-2 targets（ORF1ab, FAM channel; N, VIC channel）

- When all positive control and blank control work properly, if SARS-CoV-2 targets (ORF1ab, N) Ct > 40, and RNase P gene Ct ≤ 37, report the specimen negative.
- When all positive control and blank control work properly, if any SARS-CoV-2 targets (ORF1ab, N) Ct ≤ 37, although RNase P gene is not positive, SARS-CoV-2 positive result is still valid and report positive.
- When all positive control and blank control work properly, if SARS-CoV-2 targets (ORF1ab, N) Ct > 40, and RNase P gene Ct > 37, the result is invalid. Repeat extraction, amplification and detection. If all of SARS-CoV-2 targets (ORF1ab, N) and RNase P gene are negative, the result is invalid. Repeat sampling from patients.
- When all positive control and blank control work properly, if any of SARS-CoV-2 targets (ORF1ab, N) 37 < Ct ≤ 40, the result is inconclusive. Consider repeat extraction, amplification and detection and repeat sampling from patient if necessary.

16. Results interpretation and reporting

Below table lists expected results of COVID-19 Coronavirus Real Time PCR Kit.

If these criteria are not satisfied, repeat the test.

ORFlab	N	RNase P	Results	Report
+	+	±	Detected SARS-CoV-2	Report SARS-CoV-2 positive
If only one of two targets is positive		±	Detected SARS-CoV-2	Report SARS-CoV-2 positive
gray zone	gray zone	±	Inconclusive results	Report inconclusive
If one of two targets is in gray zone, and the other target gene is negative		±	Inconclusive results	Report inconclusive
-	-	+	Undetected SARS-CoV-2	Report SARS-CoV-2 negative
-	-	-	Invalid	Invalid

Note: for SARS-CoV-2 targets: Ct value ⩽37 is considered positive(+) ; Ct value > 40 is considered negative(-) ; 37 < Ct ⩽40 is considered diagnostic gray zone.

For RNase P: Ct value ⩽37 is considered positive (+); Ct value > 37 is considered negative (-).

(1) Report positive

Any of which is satisfied:

1) ORFlab and N gene are both positive.
2) One of two target genes is positive.

(2) Report suspicious

Report suspicious when:

1) One target gene is in the diagnostic gray zone and the other target gene is negative.
2) Both target genes are in the gray zone.

For inconclusive results, repeating nucleic acid extraction is recommended, followed by amplification of the extracted RNA. If results for both targets (N and ORF1ab) are positive or in the gray zone, or one of the two targets is positive, then report the specimen as positive, otherwise report as negative.

Alternatively, when inconclusive is reported, the following actions can also be considered: 1) test the specimen with an alternative method 2) repeat sample collection or collect an alternative specimen type from the patient and repeat the test with the COVID-19 Coronavirus Real Time PCR Kit.

(3) Report negative

Report " Negative for SARS-CoV-2 RNA" when neither of the target genes has a Ct value ⩽37 but RNase P has a Ct value ⩽37. Negative results may be due to the absence of viral RNA or to the presence of viral RNA below the limit of detection of the assay. Repeat RNA extraction or collect an alternative specimen type from the patient and repeat the test when clinically indicated.

17.Limitations

- All operator, data analysis staff and results reporting staff should be trained and proven to have competence for doing test and explaining results independently. User of the kit is limited to staff who has successfully passed the training.
- The kit only works for throat swab, nasopharyngeal swab, sputum, other upper and lower respiratory tract specimens (nasal swab, respiratory tract extract, bronchial perfusate and bronchoalveolar lavage fluid) were not validated.
- Negative results can neither straightforwardly exclude SARS-CoV-2 infection, nor the only decision-making evidence for treatment and patient management. Optimized specimen type and time of peek virus value induced by SARS-CoV-2 infection has not been finalized, and therefore multiple specimen collection (type and time) is necessary.
- Improper specimen collection, transportation and processing may lead to false negative results. Inhibitors or insufficient viral load may also lead false negative results.

- Predictive positive and negative rate is based on prevalence rate. Prevalence thus impacts the positive predictive value (PPV) and negative predictive value (NPV) of tests. As the prevalence increases, the PPR also increases but the NPV decreases. Similarly, as the prevalence decreases the PPV decreases while the NPV increases. https://www.ncbi.nlm.nih.gov/books/NBK430867/
- SARS-CoV-2 may be undetectable if target genes of the virus mutate.
- Inhibitors and other interferences may lead false negative results.
- Epidemiological or clinical features of SARS-CoV-2 induced infection have not been fully understood and therefore the performance of the kit might be affected. For example, optimized specimen type and sampling time of which containing best viral RNA are still unknown.
- Detected viral RNA may not straightforwardly show infection, or SARS-CoV-2 is not the root cause of clinical symptoms.
- Performance has not been validated for treatment monitoring.
- Detection of SARS-CoV-2 with specimen type of blood or blood products using the kit has not been validated.
- Detection using the kit cannot exclude other bacteria or pathogen induced diseases.

18.Performance characteristics

Analytical performance

Limit of detection (LoD)

Fifteen SARS-CoV-2 positive clinical specimens that included equal numbers of nasopharyngeal swabs, throat swabs and sputum, were serially diluted to 3.5×10^4 copies/mL, 3.5×10^3 copies/mL, 3.5×10^2 copies/mL, 3.5×10^1 copies/mL, and 3.5×10^0 copies/mL, respectively in SARS-CoV-2 negative clinical matrix. Quantitative RT-PCR was used to estimate the concentration of virus in each specimen prior to dilution. Each specimen dilution was tested 20 times and the level at which ⩾95% of results were positive was used to estimate the limit of detection (LoD). Three lots of COVID-19 Coronavirus Real Time PCR Kits were included in the study. For each specimen type, the LoD was estimated to be 3.5×10^2 copies/mL.

LoD Determination of COVID-19 Coronavirus Real Time PCR Kit on QuantStudio™ 5

Specimens	Concentration (copies /mL)	Number of positive(n)			Total number(n)	Positive detection rate (%)
		Batch 1	Batch 2	Batch 3		
nasopharyngeal swab	3.5×10^4	100	100	100	300	100.0
	3.5×10^3	100	100	100	300	100.0
	3.5×10^2	99	100	100	300	99.7
	3.5×10^1	28	28	22	300	26.0
	3.5×10^0	6	10	6	300	7.3
throat swab	3.5×10^4	100	100	100	300	100.0
	3.5×10^3	100	100	100	300	100.0
	3.5×10^2	100	100	100	300	100.0
	3.5×10^1	27	37	32	300	32.0
	3.5×10^0	11	11	9	300	10.3

sputum	3.5×10^4	100	100	100	300	100.0
	3.5×10^3	100	100	100	300	100.0
	3.5×10^2	100	100	99	300	99. 7
	3.5×10^1	30	34	33	300	32.3
	3.5×10^0	8	9	12	300	9. 7

To confirm the LoD, 15 other clinical specimens that included nasopharyngeal swabs, throat swabs and sputum, were gradient diluted to 3.5×10^2 copies/ml. Three lots of Real-time PCR reagents were used to test each diluted specimen 20 times, followed by amplification using the QuantStudio™ 5 Real-time PCR instrument. For each specimen type, ≥95% of results were reported as positive with each lot of reagents and the LoD was therefore confirmed as 3.5×10^2 copies/mL.

LoD Verification of COVID-19 Coronavirus Real Time PCR Kit on QuantStudio™ 5

Specimens	Number of positive (n)			Total number (n)	Positive detection rate (%)
	Lot 1	Lot 2	Lot 3		
nasopharyngeal swab	99	99	100	300	99.3
throat swab	100	100	100	300	100.0
sputum	100	100	100	300	100.0

Cross-reactivity

No cross-reactivity of the COVID-19 Coronavirus Real Time PCR Kit with the selected microorganisms was observed. The results are summarized in table below.

Results of cross-reactivity

Microorganism	Result (Positive/ Total)		cross-reactivity
	Negative samples	3 x LoD	
Human coronavirus 229E	0/3	3/3	NO
Human coronavirus OC43	0/3	3/3	NO
Human coronavirus HKU1	0/3	3/3	NO
Human coronavirus NL63	0/3	3/3	NO
SARS-coronavirus	0/3	3/3	NO
MERS-coronavirus	0/3	3/3	NO
Adenovirus (Ad. 71)	0/3	3/3	NO
Human Metapneumovirus (HMPV)	0/3	3/3	NO
Parainfluenza virus 1	0/3	3/3	NO
Parainfluenza virus 2	0/3	3/3	NO
Parainfluenza virus 3	0/3	3/3	NO
Influenza A (H1N1)	0/3	3/3	NO
Influenza A (H3N2)	0/3	3/3	NO
Influenza B	0/3	3/3	NO
Enterovirus Type 71	0/3	3/3	NO
Respiratory syncytial virus	0/3	3/3	NO
Rhinovirus	0/3	3/3	NO

Chlamydia pneumoniae	0/3	3/3	NO
Haemophilus influenzae	0/3	3/3	NO
Legionella pneumophila	0/3	3/3	NO
Mycobacterium tuberculosis	0/3	3/3	NO
Streptococcus pneumoniae	0/3	3/3	NO
Streptococcus pyogenes	0/3	3/3	NO
Bordetella pertussis	0/3	3/3	NO
Mycoplasma pneumoniae	0/3	3/3	NO
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract	0/3	3/3	NO
Candida albicans	0/3	3/3	NO
Pseudomonas aeruginosa	0/3	3/3	NO
Staphylococcus epidermis	0/3	3/3	NO
Staphylococcus salivarius	0/3	3/3	NO

Interfering substance

Apply interfering substances to weak positive SARS-CoV-2 and negative specimen with same concentration. Apply RNase-free water with same volume as reference. One batch real-time PCR kits were selected and the test was carried out using QuantStudio™ 5 real-time PCR thermal cycler. The following endogenous interfering substances including fresh human blood, nasal secretions, mucus and mucin showed no interference to COVID-19 Coronavirus Real Time PCR Kit detection. The following exogenous interfering substances showed no interference to COVID-19 Coronavirus Real Time PCR Kit detection: oxymetazoline, nasal skin steroids (Beclomethasone, Dexamethasone, flunisolide, Triamcinolone, Budesonide, Mometasone, fluticasone), allergy symptom relief drug histamine hydrochloride, antiviral drug (α-interferon, Zanamivir, Ribavirin, Oseltamivir, Peramivir, Lopinavir/ritonavir, Aspirin), antibacterial agents (Mupirocin), general antibacterial drug (Tobramycin), Nasal gel, Throat lozenges, Lidocaine.

Results using interfering substances

Interference with substances	Concentration	Interference with substances	Concentration
Fresh blood	5%	α- interferon	100 units/ mL
Nasal secretions	5%	Zanamivir	5mg/L
Mucus	5%	Ribavirin	0.2g/L
Mucin	2g/dL	Oseltamivir	100mg/L
Oxymetazoline	10mg/L	Peramivir	100mg/L
Beclomethasone	100mg/L	Lopinavir/ ritonavir	200mg/100mg/L
Dexamethasone	100mg/L	Mupirocin	0.2%
Flunisolide	100mg/L	Tobramycin	10mg/L
Triamcinolone	100mg/L	Nasal gel	5%
Budesonide	100mg/L	Throat lozenges	5mg/mL
Mometasone	100mg/L	Lidocaine	5%

Fluticasone	100mg/L	Aspirin	50mg/L
Histamine	100mg/L	/	/

Precision(repeatability and reproducibility)

For repeatability, the test specimens were clinical positive throat swabs, nasopharyngeal swabs and sputum diluted to both 3×LoD and 6×LoD concentrations. One negative specimen of each of the three specimen types was also included. One identical batch of testing reagents was used for testing the specimens, each of which was tested replicates of 10, and the CV of Ct values was calculated. The specimens with both 3×LoD and 6×LoD concentrations were detected as positive in all the 10 replicates, and the CV of the Ct value among those 10 replicates all less than 5% (CV<5%). Additionally, negative specimens were detected as negative, and the CV of the Ct value for the internal control among the 10 replicates was less than 5%. In a word, this kit can meet the requirement of repeatability.

Repeatability analysis

Specimen type	Concentration	CV (%)		
		ORF1ab	N	Internal control
throat swab	6×LoD	0.91	0.83	1.95
	3×LoD	1.07	0.90	2.01
	negative	Undet.	Undet.	1.49
nasopharyngeal swab	6×LoD	0.73	0.75	1.63
	3×LoD	0.91	0.98	1.39
	negative	Undet.	Undet.	1.76
sputum	6×LoD	0.93	0.97	1.54
	3×LoD	0.76	0.74	1.86
	negative	Undet.	Undet.	2.26

For reproducibility, the precision of reference J1 and J2 was carried out by 2 operators for 20 consecutive days using 3 batches of kits. In addition, the clinical SARS-CoV-2 positive specimens (10×LoD) of throat swab, nasopharyngeal swab and sputum specimens and negative specimens of those three types, referring to the principle of 3 × 5 × 5 in EP05-A3 standard, were tested by the same operator using three batches of reagent kit for 5 consecutive days with 5 repeats per day. Results showed that inter/inner batch, inter/inner day and inter/inner operator were all <5%. Therefore, it is concluded that the precision of COVID-19 Coronavirus Real Time PCR Kit is satisfactory.

Reproducibility of the precision of reference J1 and J2

Precision（CV，%）	J1		J2	
	ORF1ab	N	ORF1ab	N
Inter-lot	1.48%	1.77%	1.28%	1.28%
Inner-lot	1.36%	1.45%	1.44%	1.09%
Inter-day	1.64%	1.20%	1.65%	1.19%
Inner-day	1.29%	1.48%	1.38%	1.06%
Inter-operator	1.48%	1.19%	1.52%	0.37%
Inner-operator	0.95%	1.02%	1.00%	0.76%

Reproducibility for clinical specimens

Specimen type	Channel	Mean	RUN reproducibility	Within-batch reproducibility	Intra-batch reproducibility

			SD	CV	SD	CV	SD	CV
nasopharyngeal swab	FAM	33.10	0.35	1.06%	0.54	1.64%	0.56	1.69%
	VIC	32.48	0.35	1.08%	0.52	1.60%	0.52	1.60%
throat swab	FAM	33.00	0.42	1.27%	0.66	2.00%	0.69	2.10%
	VIC	32.75	0.44	1.35%	0.64	1.96%	0.65	2.00%
sputum	FAM	32.97	0.47	1.42%	0.61	1.85%	0.65	1.97%
	VIC	32.72	0.44	1.36%	0.62	1.89%	0.63	1.94%

Nucleic acid extraction

32 clinical/simulated specimens were selected, of which 8 were virus-like particle-positive simulated specimens and 8 were viral nucleic acid simulated specimens with 4 cases of Nasopharyngeal swab, 2 cases of Throat swab, 2 cases of Sputum, and 16 negative specimens. The specimens were extracted using four extraction kits (No. SDK60102, SDKF60101, SDK60104, from Bioperfectus and No. 52904 from Qiagen) on a QuantStudio™ 5 fluorescent quantitative PCR instrument. Results showed that 100% positive percent agreement, 100% negative percent agreement, 100% overall percent agreement and Kappa=1 greater than 0.75.

Nucleic acid extraction kit

Extraction kit	Positive detection rate	Negative detection rate	Overall detection rate	Kappa value
Bioperfectus SDK60102	100%（16/16）	100%（16/16）	100%（32/32）	1
Bioperfectus SDKF60101	100%（16/16）	100%（16/16）	100%（32/32）	1
Bioperfectus SDK60104	100%（16/16）	100%（16/16）	100%（32/32）	1
Qiagen 52904	100%（16/16）	100%（16/16）	100%（32/32）	1

Compatible instruments

Three batches of kits were used for the evaluation performance (LoD, precision and accuracy) of 5 different real time PCR thermal cyclers (ABI7500, QuantStudio™ 5, Roche LightCycler®480, Bio-RadCFX96™, Bioperfectus STC-96A PLUS). LoD reference L1 was performed to run 20 replicates of 350copies/mL. Evaluation of precision adopted 5x5x5 method, i.e. one operator using same method and same batch of kits to run the test in 5 different thermal cyclers for 5 consecutive days and repeat 5 times each. Specimens included precision reference J1 and J2. For the accuracy studies, positive references (P1-P12) and positive references (N1-N10) were test respectively on 5 instruments. The detection rate of P1-P12 and N1-N10 by 5 instruments were analyzed. Results showed that LoD was greater than 95%, CV values of instruments were less than 5%, and percent agreement of positive and negative reference were 100%. It was concluded that COVID-19 Coronavirus Real Time PCR Kit is compatible to the different thermal cyclers (ABI7500, QuantStudio™ 5, Roche LightCycler®480, Bio-Rad CFX96™, Bioperfectus STC-96A PLUS).

Evaluation of LoD on 5 different real time PCR thermal cyclers

Batch	Consistency of LoD

	ABI 7500	QuantStudio™ 5	Roche LightCycler®480	Bio-Rad CFX96™	Bioperfectus STC-96A PLUS
Batch 1	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）
Batch 2	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）
Batch 3	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）

Evaluation of precision on 5 different real time PCR thermal cyclers

Refenrence	Channel	Mean	Inner run		Inner instrument		Inter instrument	
			SD	CV	SD	CV	SD	CV
J1	FAM	21.31	0.34	1.61%	0.42	1.97%	0.55	2.56%
	VIC	21.31	0.40	1.86%	0.42	1.96%	0.54	2.52%
J2	FAM	33.89	0.62	1.84%	0.75	2.20%	0.86	2.53%
	VIC	33.80	0.65	1.92%	0.72	2.13%	0.80	2.37%

Evaluation of accuracy on 5 different real time PCR thermal cyclers

Batch	Consistency of positive and negative reference				
	ABI 7500	QuantStudio™ 5	Roche LightCycler®480	Bio-Rad CFX96™	Bioperfectus STC-96A PLUS
Batch 1	100%(10/10)	100%（10/10）	100%（10/10）	100%（10/10）	100%（10/10）
	100%(12/12)	100%（12/12）	100%（12/12）	100%（12/12）	100%（12/12）
Batch 2	100%(10/10)	100%（10/10）	100%（10/10）	100%（10/10）	100%（10/10）
	100%(12/12)	100%（12/12）	100%（12/12）	100%（12/12）	100%（12/12）
Batch 3	100%(10/10)	100%（10/10）	100%（10/10）	100%（10/10）	100%（10/10）
	100%(12/12)	100%（12/12）	100%（12/12）	100%（12/12）	100%（12/12）

Clinical Evaluation

I) Clinical sensitivity

Sensitivity of Bioperfectus COVID-19 Coronavirus Real Time PCR Kit was clinically evaluated using 50 nasopharyngeal swabs, 50 sputum and 50 throat swabs specimens. The 50 specimens consisted of 20 clinical specimens and 30 simulated specimens with positive virus RNA dissolved in negative specimens confirmed by clinical diagnosis and nucleic acid testing using another NMPA-authorized assay. 30 nasopharyngeal swab specimens, 30 sputum specimens, and 30 throat swab specimens were adopted to evaluate the sensitivity from LoD concentration to 5 times LoD concentration.

All these specimens showed 100% clinical sensitivity. 95% confidence interval of nasopharyngeal swab, sputum and throat swab was 92.86%-100%. Sensitivity of all specimens was consistent with acceptable criteria.

Clinical sensitivity

Specimen type	Population	Positive results	Sensitivity
Nasopharyngeal swab	50	50（100%）	100% [92.86-100]

Sputum	50	50（100%）	100% [92.86-100]
Throat swab	50	50（100%）	100% [92.86-100]

II) Clinical specificity

Specificity of Bioperfectus COVID-19 Coronavirus Real Time PCR Kit was clinically evaluated using 100 nasopharyngeal swab, 30 sputum and 100 throat swab specimens. These specimens were confirmed negative by clinical diagnosis and nucleic acid testing. Moreover, all these specimens were from clinical suspicious cases by referring to "Diagnostic and therapeutic schemes of COVID-19 induced pneumonia".

All these specimens showed 100% clinical specificity. 95% confidence interval of nasopharyngeal swab and throat swab was 96.30%-100%, while the value of sputum was lower, i.e. 88.65%-100%, due to fewer specimens tested. Specificity of all specimens was consistent with acceptable criteria.

Clinical specificity

Specimen type	Population	Positive results	Specificity
Nasopharyngeal swab	100	0（0%）	100% [96.30-100]
Sputum	30	0（0%）	100% [88.65-100]
Throat swab	100	0（0%）	100% [96.30-100]

III) Clinical study

In total 970 clinical specimens were tested in this clinical study, including 420 SARS-CoV-2 positive and 550 SARS-CoV-2 negative, as determined using comparator molecular diagnostic assays for the detection of SARS-CoV-2 RNA. The specimen types tested included sputum, throat swabs and nasopharyngeal swabs specimens. Overall, the Bioperfectus COVID-19 Coronavirus Real Time PCR Kit exhibited 100% positive percent agreement and 95.1% negative percent agreement in comparison to the other molecular diagnostic assays used to characterize the specimens (Kappa value 0.9437).

Consistency between the Bioperfectus COVID-19 Coronavirus Real Time PCR Kit and Contrast Kit

Bioperfectus kit	Contrast kit		Total
	Positive	Negative	
Positive	420	27	447
Negative	0	523	523
Total	420	550	970

There were 27 specimens that were reported as positive by the Bioperfectus COVID-19 Coronavirus Real Time PCR Kit and negative by the comparator, including 22 clinically confirmed cases(symptoms consistent with COVID-19 and a positive test result with an NMPA-certified molecular assay) , 4 weak positive cases confirmed by digital PCR, 1 case with incomplete clinical history that was counted as negative.

Consistency between the Bioperfectus COVID-19 Coronavirus Real Time PCR

Kit and reference method

Bioperfectus kit	Reference method		Total
	Positive	Negative	
Positive	446	1	447
Negative	0	523	523
Total	446	524	970

Note: The results of reference method were based on the clinical symptoms, other NMPA-certified molecular assay and digital PCR.

Compared to reference method, the positive percent agreement of Bioperfectus COVID-19 Coronavirus Real Time PCR Kit is 100% (95% CI: 99.15%-100%) and negative percent agreement is 99.8% (95% CI: 98.93%-99.87%), and the overall percent agreement is 99.9% (95% CI: 99.42%-99.98%).

19.Appendix

Index of Symbols

IVD	In vitro diagnostic medical device
Σ	Contains sufficient for <n> tests
	Consult instructions for use
	Temperature limit
REF	Catalogue number
	Date of manufacture
	Use-by date
LOT	Batch code
	Manufacturer
EC REP	Authorized representative in the European Community

20.Contact and Support

For more information about Jiangsu Bioperfectus Technologies Co, Ltd., please visit our website at: http://www.bioperfectus.com or contact at E-mail: info@bioperfectus.com.

For detailed programming instructions regarding the use of the Bioperfectus Technologies Real Time PCR Kits on specific Real Time PCR instruments please contact our Technical Support at E-mail:support@bioperfectus.com.