COVID-19 Coronavirus Real Time PCR Kit

For Emergency Use Only

INSTRUCTIONS FOR USE

For *in vitro* Diagnostic (IVD) Use

Rx Only

Catalog # JC10223-1NW-50T
Catalog # JC10223-1NW-25T

Jiangsu Bioperfectus Technologies Co, Ltd.
3rd and 4th floors of Building A(G19), 4th floor of Building F(G14), Ground floor of Building G20, Shuaiyu Village, Fuye village, Sixiang town, Taizhou National Medical, Hi-tech Development Zone, 225300 Taizhou, Jiangsu, PEOPLE'S REPUBLIC OF CHINA.
http://en.s-sbio.com/
Tel: +86-523-86201557
Fax: +86-523-86201617

The electronic version of IFU is available at FDA website https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

Contents

1. Intended Use

The COVID-19 Coronavirus Real Time PCR Kit is a real-time fluorescent RT-PCR based assay intended for the qualitative detection of SARS-CoV-2 nucleic acid in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The COVID-19 Coronavirus Real Time PCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The COVID-19 Coronavirus Real Time PCR Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Summary and Explanation

On January 8 2020, the cause of severe unexplained viral pneumonia was identified by sequencing and 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 on the same day, the World Health Organization officially named the disease caused by SARS-CoV-2 as coronavirus disease 2019 (COVID-19). Up until May 2020, about 4.18 million of cases have been confirmed in 211 countries and territories such as the USA and southeast Asia, including severe and death cases.

The COVID-19 Coronavirus Real Time PCR Kit is based on real-time fluorescent reverse transcription PCR technology and is an *in vitro* diagnostic assay for the qualitative detection of nucleic acid from SARS-CoV-2. The kit contains oligonucleotide primers, dual-labeled hydrolysis probes (Taqman®) and quality controls used in real time PCR amplification.

3. Principles of the Procedure

The oligonucleotide primers and probes for specific detection of SARS-CoV-2 are selected from regions of Open Reading Frame 1ab (ORF1ab) and the nucleocapsid gene (N) of the SARS-

CoV-2 genome. The kit includes primers/probes that are specific for the ORF1ab gene (probe labeled with FAM) and N gene (probe labeled with VIC) of SARS-CoV-2. In addition, the kit also contains primers and a probe (labeled with CY5) for the human RNase P gene as an endogenous internal control for specimen integrity, nucleic acid isolation, amplification and detection.

RNA isolated and purified from upper and lower respiratory tract specimens is reverse transcribed to cDNA and amplified in a Real-time PCR instrument using one-step Master Mix. Probes consist of a reporter dye at the 5' end and quenching dye at the 3' end. The fluorescent signals emitted from the reporter dye are absorbed by the quencher. During PCR amplification, probes hybridized to amplified templates are degraded by the Taq DNA polymerase with 5'-3' exonuclease activity, thereby separating the reporter dye and quencher and generating fluorescent signals that increase with each cycle. The PCR instrument automatically draws a real-time amplification curve for each optical channel based on the signal change, and calculates cycle threshold (Ct) values (the point at which fluorescence is detectable above background) that are interpreted by the operator to determine the presence/absence of SARS-CoV-2 RNA.

4. Materials Required (Provided)

Catalog # JC10223-1NW-50T

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

Catalog # JC10223-1NW-25T

Components	Qty	Volume	Test	Ingredients
RT-PCR Buffer	1	188 μL	25T	Tris Hydroxy Methyl Aminomethan, Potassium chloride, Magnesium chloride, Nucleotides mix
RT-PCR Enzyme Mix	1	125 μL	25T	Reverse transcriptase, RNase Inhibitor, Taq DNA polymerase
Reaction Mix	1	100μL	25T	Primers and probes of SARS-CoV-2

				and RNase P
Positive Control	1	500μL	25T	virus-like particles of SARS-CoV-2 and RNase P
Blank Control (RNase-free Water)	1	500μL	25T	RNase-free Water

5. Materials Required but Not Provided

Nucleic acid extraction reagent

Manufacturer	Nucleic Acid Isolation Kit	Cat. No.	Specification
Bioperfectus Technologies	Viral nucleic acid isolation kit	SDK60102 SDK60103	50 T
		SDK60104 SDK60105	32/48/96 T
Qiagen	QIAamp Viral RNA Mini Kit	52904 52906	50/250 T

Instruments and consumables

- Vortex mixer
- Centrifuge
- Pipette (2.5μL, 10μL, 200μL, 1000μL)
- Multi-channel pipette (5-50μL□
- 1.5 mL centrifuge tube stand
- Magnetic grate for 1.5 mL centrifuge tube
- Specimen preservation fluid (2mL/tube)
- Real-time PCR instrument: 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)
- Nucleic Acid Extraction instrument□ SSNP-2000A (32 channels, V1.0)
- 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 (DNase-Free/RNase-Free)
- 0.2 mL PCR plate (Applied Biosystems)
- 0.2 mL PCR tube (Applied Biosystems)

6. Warnings and Precautions

- For *in vitro* diagnostic (IVD) use.

- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner
- Users should be trained in performing real-time PCR.
- Standard precautions should be followed. All patient specimens and positive references should be treated as infectious and processed accordingly.
- Do not eat, drink, smoke, apply makeup or handle contact lenses in the laboratory.
- Specimen processing should be performed according to national biosafety regulations and laboratory procedures.
- Caution is necessary for collection of specimens from suspected cases of COVID-19.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Personal protective equipment including but not limited to gloves, goggles and lab-gown, should be applied when dealing with specimen processing and reagent preparation.
- As PCR technology is highly sensitive to contamination by previous amplification products, incorrect results could be reported if contamination occurs.
- Follow a unidirectional workflow in the PCR laboratory. Working zones in the laboratory should be strictly separated. Work benches should be cleaned immediately after use.
- Check expiry date before use, do not use an expired kit. Please do not replace or interchange reagents from different batches or manufacturers.
- Use pipette tips fitted with filters to reduce contamination.
- Aseptic technique should be used for nucleic acid processing. Good laboratory skill and practice is necessary to minimize the potential for contamination.
- Use qualified instruments and consumables.
- Use clean lab coat and disposable powder-free gloves. Change gloves between samples and whenever contamination is suspected.
- Ensure that PCR reaction tube and plate are properly closed.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on a cold block at all times during preparation and use.
- Thaw and mix reagents properly before reagent preparation.
- Clean work benches, pipette and centrifuge using 10% sodium hypochlorite solution to minimize potential contamination. Use 70% ethanol to remove residue.
- RNA should be prepared and kept on ice to ensure its integrity. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.

- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

7. Reagent Storage, Handling, and Stability

- Store at -20℃±5ºC .
- Always check expiry date before use and do not use expired reagent.
- Keep the Reaction Mix containing the fluorescent probes away from light.
- Properly thaw and mix reagents before use.
- Avoid repeated freeze-thaw.
- Manufacturing date and expiry date: see outer packing box.

8. Specimen Collection, Transportation and Storage

Human nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum specimens can be tested with COVID-19 Coronavirus Real Time PCR Kit. Inappropriate sampling, storage and transportation may lead to incorrect detection results. The following are recommended:

Specimen Collection

- Please refer to https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html for information on collection of appropriate specimens to test for SARS-CoV-2.
- Follow product instructions of instruments and consumables.

Transportation

- Specimen packaging and transportation should follow https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html#specimen.
- Specimens collected from suspected SARS-CoV-2 cases should be preserved at 2-8°C using ice bags or ≤ -70°C on dry ice and sent to qualified laboratories within 24 hours.

Storage

- Specimens may be stored at 2-8°C up to 24 hours after receipt.
- Specimens may be stored at -70°C or colder if processing is delayed more than 24 hours.
- Extracted RNA should be stored at -70°C or colder.

9. Reagent Preparation

1) Store kit at -20±5℃ after receipt.
2) Attention: use reagents in a clean environment and store at -20±5℃ without light. Avoid repeated freeze-thaw.
3) Properly thaw and mix reagents before use.
4) Sub-aliquot reagents To reduce the times of multiple freeze-thaw and the likelihood for contamination: aliquot 5 sets RT-PCR Buffer, Enzyme mix, Reaction Mix into labeled centrifuge tubes and store at -20±5℃ without light.

- Sub-pack 75μL/tube RT-PCR Buffer into 5 centrifuge tubes.
- Sub-pack 50μL/tube Enzyme Mix into 5 centrifuge tubes.
- Sub-pack 40μL/tube SARS-CoV-2 Reaction Mix into 5 centrifuge tubes.

- Sub-pack 35μL/tube RNase-free Water into 5 centrifuge tubes.

5) Process the RNase-free water and Positive Control carefully in the specimen processing area to avoid contamination. Avoid repeated freeze-thaw.
6) Store Blank Control and Positive Control at -20±5℃ or≤ -70℃.
7) Store Blank Control and Positive Control at -20±5℃ or≤ -70℃ after extraction.

10. Nucleic Acid Extraction

Instruments preparation

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

Nucleic acid extraction

The performance of the COVID-19 Coronavirus Real Time PCR Kit depends on the quantity of SARS-CoV-2 RNA present in the specimen and the efficiency and purity of nucleic acid extraction. The extraction kits listed below have been experimentally verified and can be used for SARS-CoV-2 RNA extraction.

Bioperfectus Nucleic Acid IsolationKit

SDK60102, Spin column method
SDK60103, Manual magnetic beads method
SDK60104, Auto magnetic beads method (for use with the SSNP 2000A instrument)
SDK60105, Auto magnetic beads method (for use with the SSNP 2000A instrument)

QiagenQIAamp Viral RNA Mini Kit

52904/52906, Spin column method

Extraction should follow the instructions from the respective kit manufacturer.
At least one Blank Control (RNase-free water) and one Positive Control should be processed with each batch of patient samples.

Note: Check below table for control volume used for each extraction

	Bioperfectus Nucleic Acid Isolation kit	Qiagen QIAamp Viral RNA Mini Kit
Blank control (RNase-free water) (μL)	200	140
Positive control (μL)	200	140

11. Assay Set Up

Master Mix and reaction well setting

Note: Set-up of reaction wells varies with the number of specimens. Each run should contain at least one Blank Control and one Positive Control.

1) Thaw RT-PCR Buffer, Enzyme Mix and Reaction Mix from -20±5℃ in the reagent preparation area.
2) Ensure that the RT-PCR Buffer, Enzyme Mix and Reaction Mix are properly thawed/dissolved before

use.

3) Invert tubes 5 times to mix.
4) Centrifuge 5 s.
5) Label a 1.5 mL centrifuge tube.
6) Double check reaction quantity (N), including the number of specimens (n) to be tested, a Blank Control and a Positive Control (allow some overage as indicated below). Prepare master mix based on table below.

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 controls is between 1~14, N=n+1.
- If sample quantity (n) including quality controls >15, N=n+2.

7) Store above master mix in the labeled 1.5 mL centrifuge tube and mix.
8) Centrifuge 5 s.
9) Place PCR reaction tubes, strips or plate into 96-well rack.
10) Pipette 20 µL master mix to each reaction well.
11) Tightly close the PCR reaction wells/plate and transfer to the nucleic acid processing zone.

Add template

1) Wear gloves to avoid contamination.
2) Gently vortex centrifuge tube containing purified RNA 5 s.
3) Centrifuge 5 s to sediment purified RNA.
4) Add 5µL RNA to each PCR well.
5) Close the caps or cover the plate.
6) Continue the steps 3) and 4) and take care to avoid contamination.
7) Close all caps or cover the plate and transfer Positive Control to the processing zone.

Add quality control

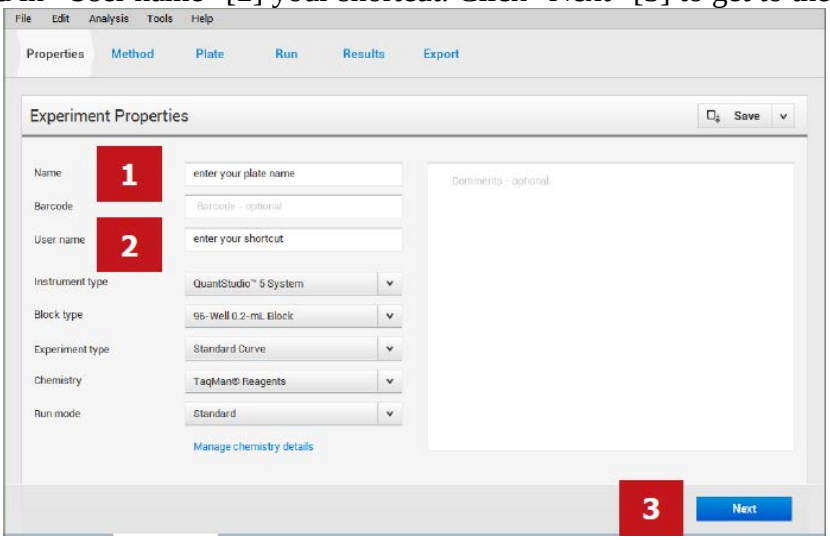
1) Add 5 µL extract from the Blank Control to one reaction well and close the cap or cover the plate.
2) Add 5 µL extract from the Positive Control to one reaction well and close the cap or seal the plate.
   Note: please follow the order from 1 to 8 if using 8-tube strips.
3) Centrifuge the 8-tube strip 10-15 s and then place back to the rack.
   Note: centrifuge 30 s with 1000 rpm if using PCR 96-well plate.

12. Amplification

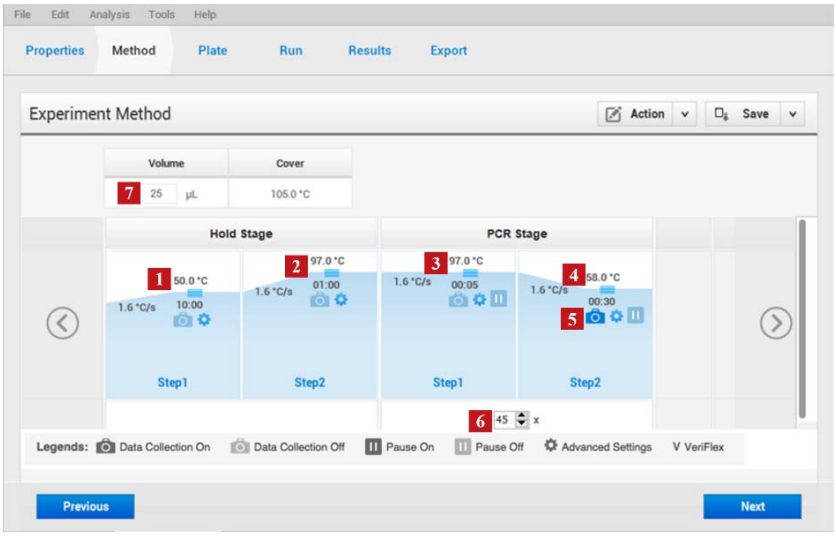
Run the experiment in Applied Biosystems QuantStudio™ 5 Real-time PCR instrument.

- Settings

1) New experiment: Click on the QuantStudio™ Design and Analysis Software icon to start the software, and choose "Create New Experiment" on the home window.
2) Properties: Enter in "Name" [1] the name of your run file (YYYYMMDD-name) and in "User name" [2] your shortcut. Click "Next" [3] to get to the next page.

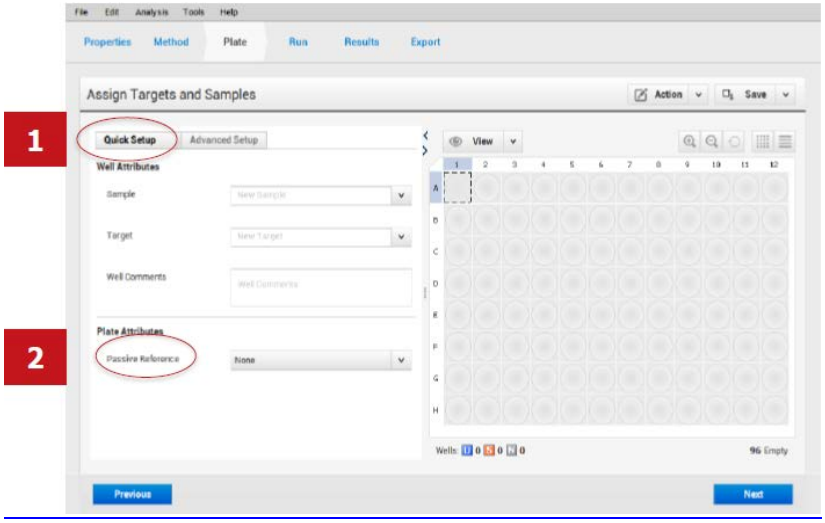


3) Method: Modify thermal cycling conditions as follows.
   a [1]Set 50°C, 10 min, 1 cycle at "Hold stage" step 1.
   b [2]Set 97°C, 1 min, 1 cycle at "Hold stage" step 2.
   c [3]Set 97°C, 5s at "PCR stage" step 1.
   d [4]Set 58°C, 30s at "PCR stage" step 2.
   e [5]Fluorescent detection at 58°C, "PCR stage" step 2.
   f [6]Set cycle number 45.
   g [7]Modify "Volume" to 25 µL.

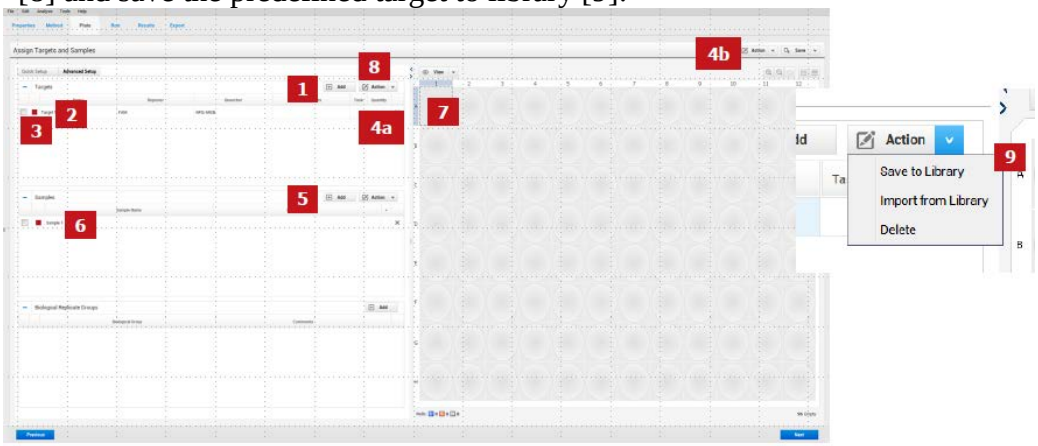


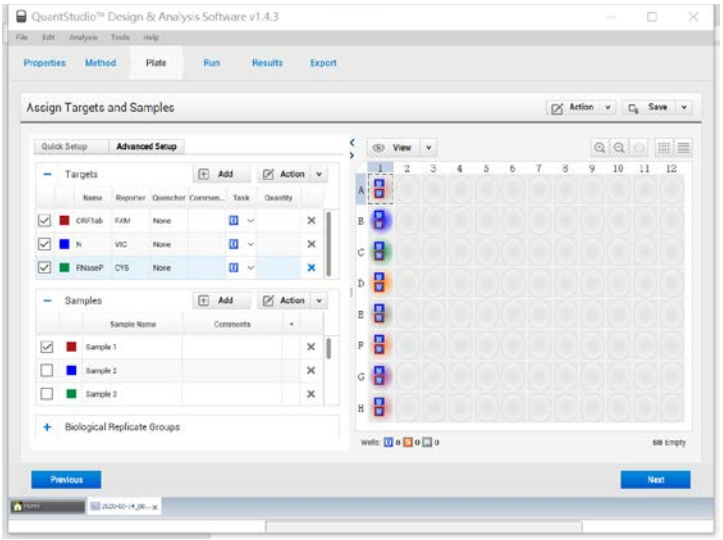
4) Plate:

a. In the "Quick setup" [1] choose " Passive reference-None" [2] in the field "Plate Attribute".

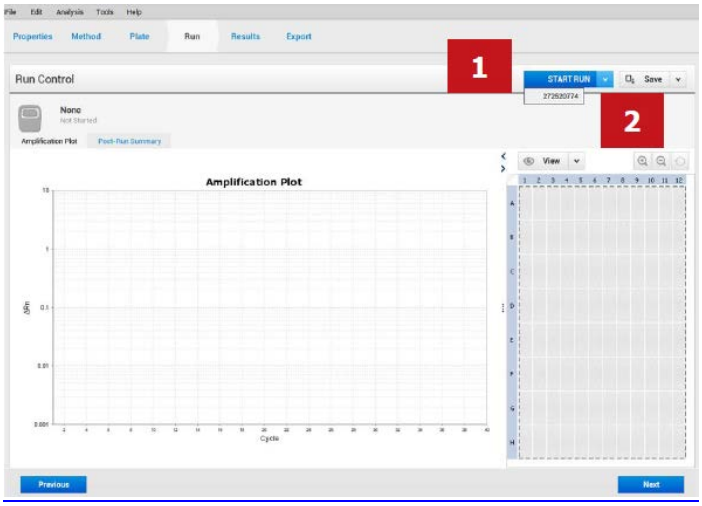


b. To define targets and samples add new target [1] and enter a target name [2]. Choose color for respective target [3]. Select task [4a/b] (unknown, standard, NC) and add the quantity in the empty field next to task if selected standard. Add new sample [5] and enter the sample name [6]. Choose the wells in the plate view [7] to include respective samples and targets. Click on "Action" [8] and save the predefined target to library [9].



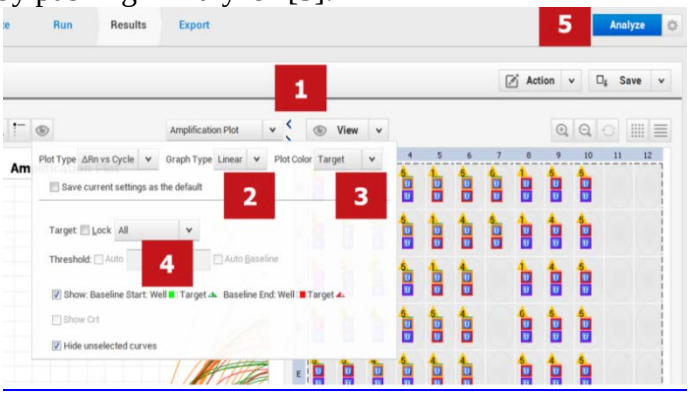


5) Run: To begin the run click the “Start Run” [1] button. All cyclers connected with the computer will be shown as serial numbers [2]. Choose the right serial number and you will be asked to save the file and the run starts. It takes around 72 min.



- **Analysis**: Click on the “Eye” icon and change the settings according to your needs [1]:

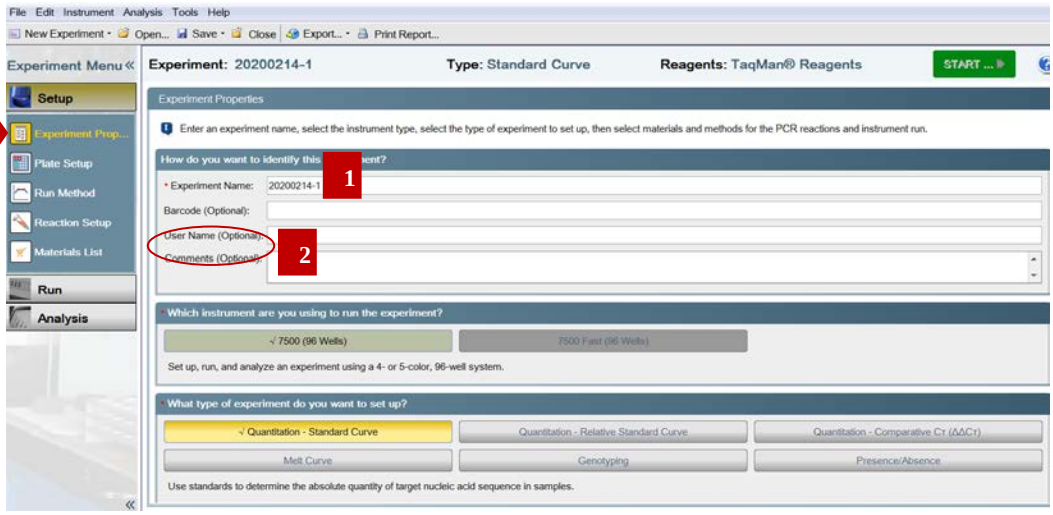
a. Graph from “Logarithmic” to “Linear” type [2].
b. Plot color from “Well” to “Target” [3].
c. Change “Threshold Auto” and “Auto Baseline” [4] to individual adjusted values.
d. Validate your choice by pushing “Analyze” [5].



Run the experiment in Applied Biosystems 7500 Real-time PCR instrument.

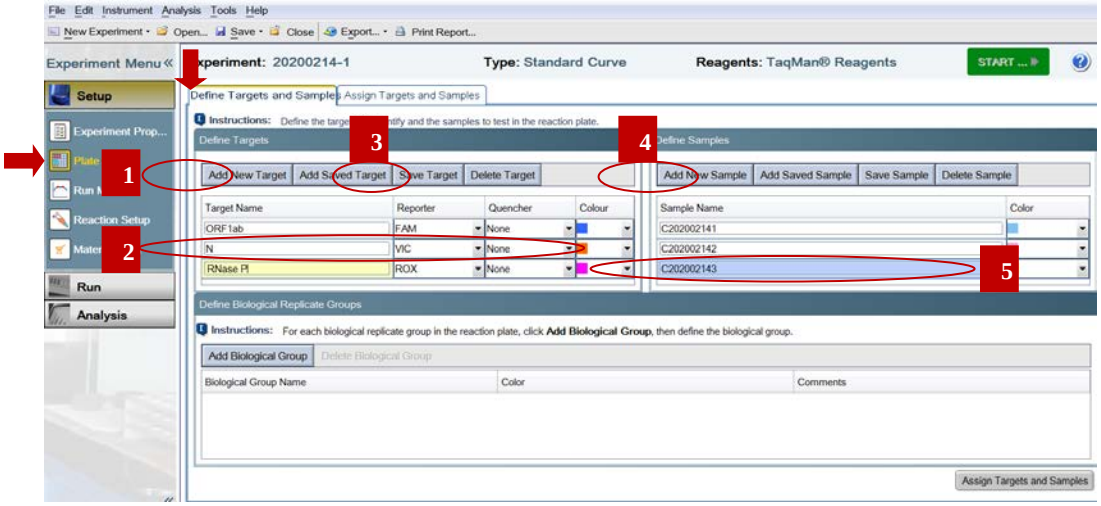
- Settings

1) New experiment: Start the ABI 7500 software, and choose “New Experiment” on the home window.
2) Experiment Properties: You have to enter an Experiment Name [1]. You can enter Barcode, User Name and Comments [2].



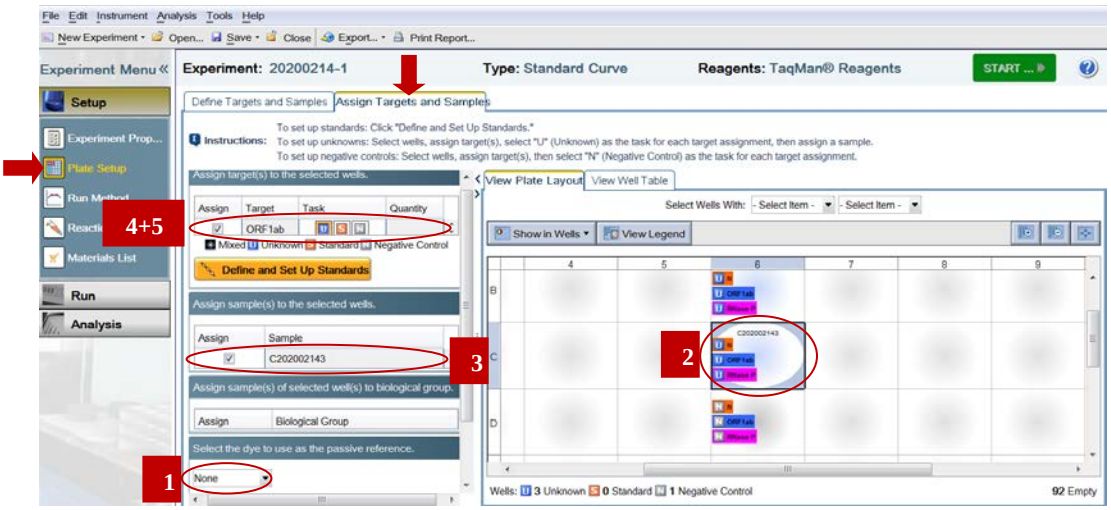
3) Define targets and samples:

a. To define targets click on “Add New Target” [1].
b. Enter a Target Name [2], choose a Reporter, Quencher and the respective colour.
c. Click on “Save Target” to add the new created target to a predefined library [3].
d. To define new samples click on Add New Sample [4] and enter a name [5].



4) Assign targets and samples:

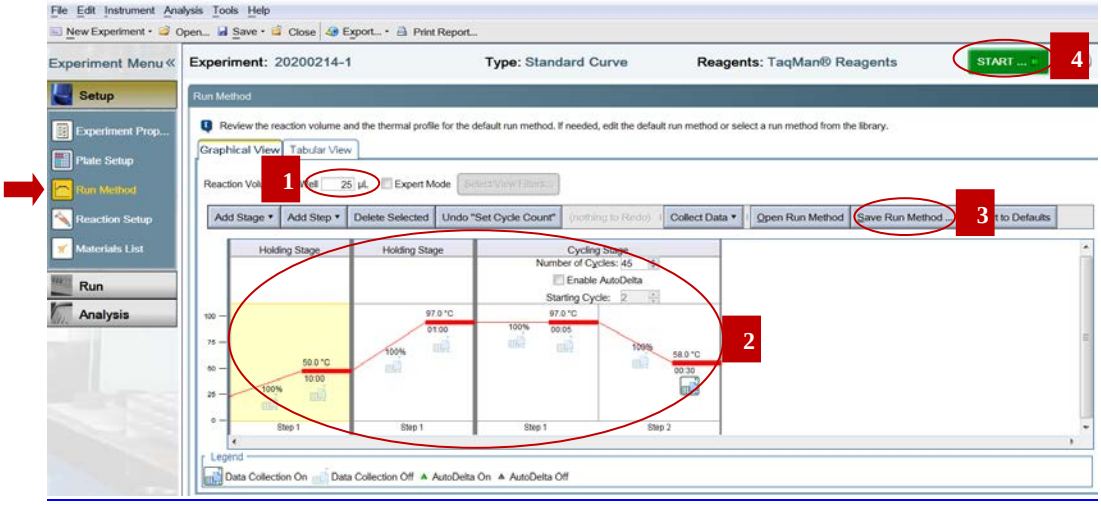
a. Select the passive reference None [1].
b. Choose your well(s) [2].
c. Define the corresponding sample(s) for the respective well(s) [3].
d. Define the corresponding targets for the respective well(s) [4].
e. Select Task: unknown (U), negative control (N), standard (S) [5].



- Run method:
    1) Enter Reaction Volume per Well (25 µL) [1].
    2) Add/modify different stages/temperatures/times [2] based on the reagent.

a) Set 50°C, 10 min, 1 cycle at "Holding Stage" step 1.
b) Set 97°C, 1 min, 1 cycle at "Holding Stage" step 1.
c) Set 97°C, 5s at "Cycling Stage" step 1.
d) Set 58°C, 30s at "Cycling Stage" step 2.
e) Fluorescent detection at 58°C, "Cycling Stage" step 2.
f) Set Number of Cycles 45.

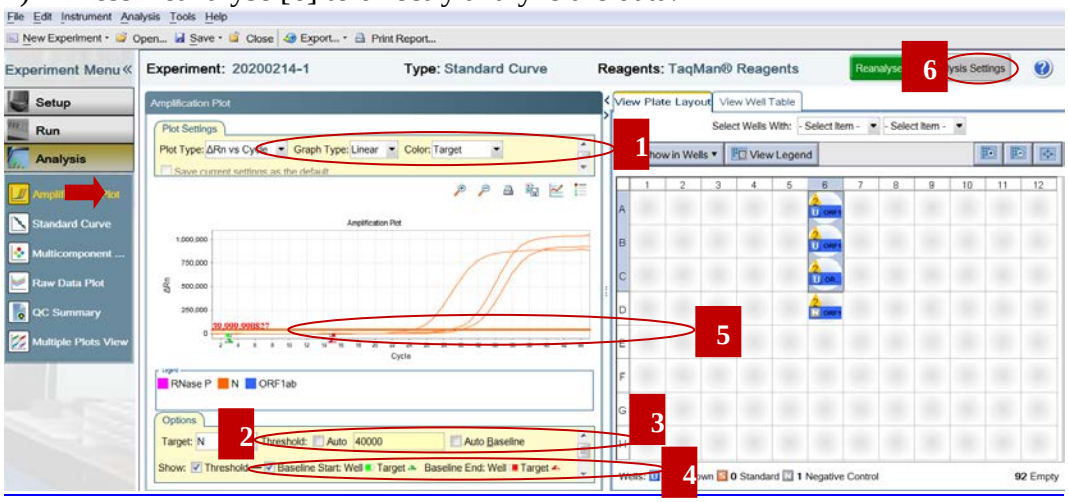
3) Press "Save Run Method" [3] and enter a name. Press Save. For the next real time PCR, you will find your saved run program in Open Run Method.
4) Open the cycler by pushing the tray door and put in the plate.
5) Close the tray door and press "Start" run [4]. After saving your run file in a defined folder the run starts.



- Analysis: On the drop down menu on the left choose Amplification Plot.
    1) Choose your Plot Settings: Rn vs Cycle, Linear and Target [1].
    2) Choose one target [2], then deselect Threshold Auto and Auto Baseline [3].

3) Tick Threshold and Baseline to show the threshold and the baseline start and end cycle [4]. Set the baseline by moving the small triangles (green for the start and red for the end cycle). The threshold can be changed by dragging it in the amplification plot with the mouse [5].

4) Press Reanalyse [6] to directly analyze the data.



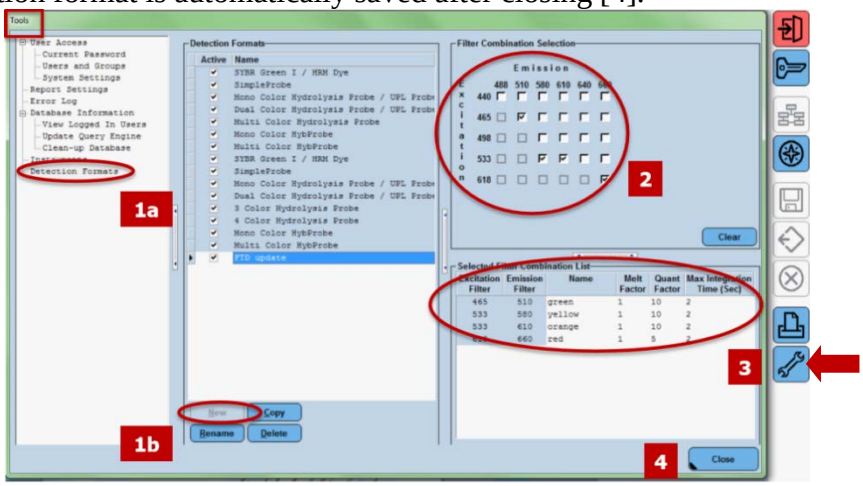
<u>Run the experiment in Roche LightCycler® 480 Real-time PCR instrument.</u>

- 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.

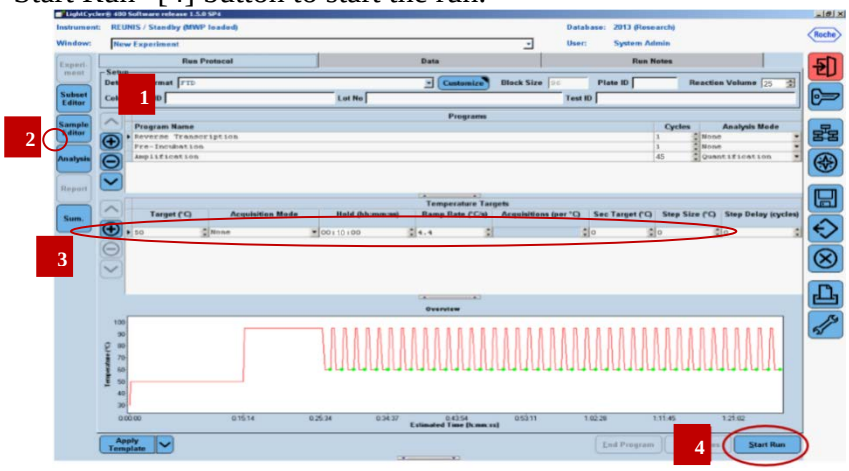
c) Set Amplification at Program Name, 45 at Cycles, Quantification at Analysis Mode

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.



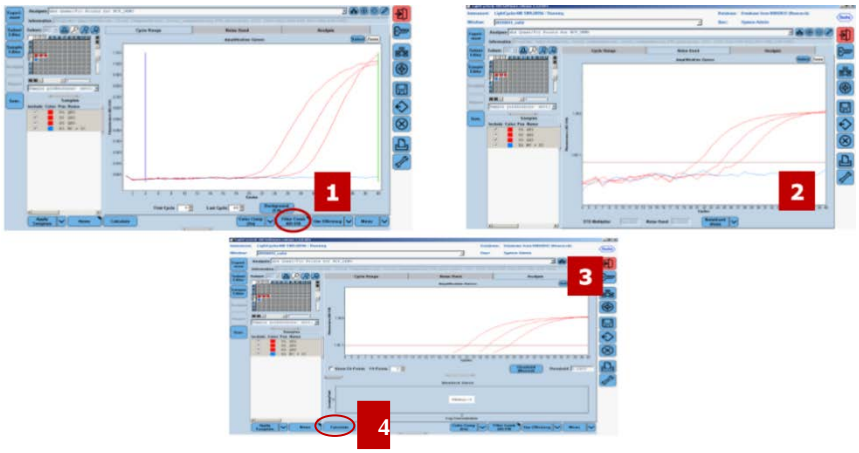
4) Subsets Editor:

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.

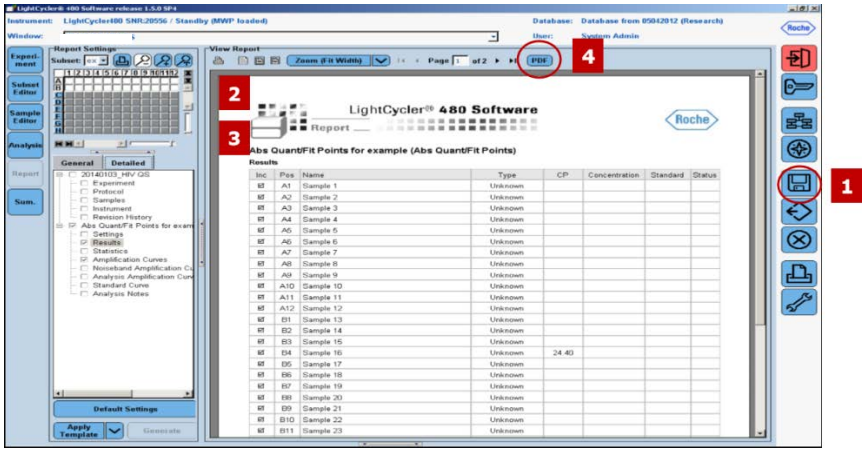
- Analysis: Click on "Analysis" and choose "Abs Quant/Fit Points" under "Create new Analysis" and select the subset of interest and click on " √ " .

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 samples for positives.
4) The report can be saved as a PDF-data.



13. Interpretation of Results

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results must not be interpreted.

1) **Controls - Positive, Negative and Internal**

Prior to evaluating the specimen results, the Positive Control and Negative (Blank) Control must be interpreted using the interpretation table below.

	Threshold cycle (Ct) value		
	FAM	VIC	CY5
Blank Control	UNDET	UNDET	UNDET
Positive Control	Ct≤30	Ct≤30	Ct≤30

FAM channel for ORFlab, VIC channel for N, CY5 channel for the internal control (RNase P).

If the Positive Control and Blank Control do not meet the criteria, the entire run is invalid and results must not be reported. Repeat the entire process (nucleic acid extraction and control preparation, amplification and detection). If the repeat run is still invalid, please contact Technical Support.

All clinical specimens should show amplification in the CY5 channel for the internal control RNase P gene and have Ct≤37. However, if one or both SARS-CoV-2 targets, ORFlab and N, are positive with Ct≤37 and RNase P gene is negative, the result is still valid.

2) **Examination and Interpretation of Patient Specimen Results:**
Assessment of clinical specimen test results must be performed after the positive and blank controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results must not be interpreted.

14. Results Report

Below table shows how to interpret the results of the COVID-19 Coronavirus Real Time PCR Kit.

ORFlab	N	RNase P	Results	Report
+	+	±	Detected SARS-CoV-2	Report SARS-CoV-2 RNA positive
If only one of two targets is positive		±	Detected SARS-CoV-2	Report SARS-CoV-2 RNA positive
gray zone	gray zone	±	Inconclusive results	Report inconclusive (refer to (2) below)
If one of two targets is in gray zone, and the other target gene is negative		±	Inconclusive results	Report inconclusive (refer to (2) below)
-	-	+	SARS-CoV-2 Not detected	Report SARS-CoV-2 RNA 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 inconclusive

Report inconclusive 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 positive 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.

15. Quality Control

- Quality control should be performed according to local regulations, certification requirements or laboratory standard quality control process.
- Quality control is used to monitor the integrity of reagents and result analysis.
- Test positive and blank controls before using each new batch of kits to test patient samples.
- Include at least one Positive Control and one Blank Control with each batch of samples for nucleic acid extraction and purification.
- Amplification and detection of the human RNase P gene should be monitored to ensure specimen quality and the integrity of the extraction and amplification process.

16. Limitations

- All operators, data analysis staff and results reporting staff should be trained and proven to have capabilities of performing the test and interpreting the results. Use of the kit is limited to staff who are trained in performing the test.

- The performance of the COVID-19 Coronavirus Real Time PCR Kit was established using nasopharyngeal swabs, oropharyngeal (throat) swabs and sputum. Anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with the COVID-19 Coronavirus Real Time PCR Kit. Please refer to the CDC Interim Guidelines for Collecting, Handling and Testing Clinical Specimens for COVID-19 for additional information regarding acceptable specimen types for detection of SARS-CoV-2.
- Negative results can neither exclude SARS-CoV-2 infection nor be used as the only decision-making evidence for treatment and patient management. The optimum specimen type for diagnosis of COVID-19 and time of peak virus titer have not been determined.
- Improper specimen collection, transportation and processing may lead to false negative results. Inhibitors or viral load below the limit of detection may also cause false negative results.
- Positive and Negative Predictive Values are dependent on prevalence. False negative results are more likely when the prevalence rate is high and false positive results are more likely when the prevalence rate is medium or low.
- SARS-CoV-2 may be not be detectable or detection may become unpredictable if the target genes of the virus mutate.
- Inhibitors and other interferences may lead to false negative results.
- Detection of viral RNA may not be indicative of infection. Positive results with the COVID-19 Coronavirus Real Time PCR Kit do not rule out bacterial infection or co-infection with other viruses.

- Performance of the kit in monitoring treatment of SARS-CoV-2 infection has not been evaluated.
- Detection of SARS-CoV-2 in blood or blood products using the kit has not been evaluated.

17. Conditions of Authorization for the Laboratory

The Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#2019-ncov.

However, to assist clinical laboratories running the COVID-19 Coronavirus Real Time PCR Kit, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit will use the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit test as outlined in the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit are not permitted.

C. Authorized laboratories that receive the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Jiangsu Bioperfectus Technologies Company, Ltd. (via telephone: +86-523-86201557; web address: http://en.s-sbio.com/) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

F. All laboratory personnel using the test must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

G. Jiangsu Bioperfectus Technologies Company, Ltd., authorized distributors, and authorized laboratories using the Jiangsu Bioperfectus Technologies COVID-19 Coronavirus Real Time PCR Kit test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

18. Performance Characteristics

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. Nucleic acid extraction was performed using the SSNP-2000A automated specimen processor and SDK60105 extraction kit and RT-PCR was performed using the QuantStudio™ 5 Real-time PCR instrument. For each specimen type, the LoD was estimated to be 3.5×10^2copies/mL (Table 1).

Table 1. The result of three clinical specimens

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

Samples in shaded cells all had Ct values between 37 and 40 for ***both*** the N gene and ORF1ab (grey zone).
For the LoD Study, samples with only one target that exhibited amplification and which had a Ct value in the range

of 37 to 40 were recorded as negative for SARS-CoV-2.

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 using the SSNP-2000A automated specimen processor and SDK60105 extraction kit, 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 3.5×10^2copies/mL (Table 2).

Table 2. Validation of the LoD

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

Confirmation of LoD Using Alternative PCR Instrument Systems

To demonstrate the compatibility of the COVID-19 Coronavirus Real Time PCR Kit with alternative PCR instruments, a study was performed using clinical samples that were diluted in the appropriate matrix to achieve target levels of 0.5×, 1×, 1.5×, 2× and 3× LoD, as determined by quantitative RT-PCR. Five replicates at each level were tested on each PCR instrument after processing using the SSNP-2000A automated specimen processor and SDK60105 extraction kit. For each specimen type and instrument system, 5/5 replicates (100%) were reported positive at ≥ 1×LoD, whereas at 0.5× LoD, ≤ 4/5 replicates (≤ 80%) produced positive results. The LoD of the COVID-19 Coronavirus Real Time PCR Kit is therefore considered comparable when used on the ABI 7500, QuantStudio™ 5 and Roche LightCycler® 480 Real-time PCR instruments.

Confirmation of the LoD using Alternative Nucleic acid extraction methods

To verify the compatibility of the COVID-19 Coronavirus Real Time PCR Kit with alternative nucleic acid extraction methods, 32 clinical/contrived specimens were used that included 16 negative specimens, 8 SARS-CoV-2 negative specimens that were spiked with pseudo-virus particles and 8 specimens containing SARS-CoV-2 nucleic acid added in negative matrix (including 4 nasopharyngeal swabs, 2 throat swabs and 2 sputum specimens). The specimens were extracted using five extraction kits (No. SDK60102, SDK60103, SDK60104, SDK60105 from Bioperfectus and No. 52904 from Qiagen). RT-PCR was performed using a QuantStudio™ 5 Real-time PCR instrument. Results showed 100% positive and negative agreement and a Kappa value 1 > 0.75 (Table 3).

Twenty replicates of another 8 contrived specimens containing viral nucleic acid at 1×LoD were also tested with each extraction kit. The detection rate of all of the specimens was more than 95% (Table 4). The results support use of the specified Bioperfectus and Qiagen extraction reagents

interchangeably.

Table 3. 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 SDK60103	100%（16/16）	100%（16/16）	100%（32/32）	1
Bioperfectus SDK60104	100%（16/16）	100%（16/16）	100%（32/32）	1
Bioperfectus SDK60105	100%（16/16）	100%（16/16）	100%（32/32）	1
Qiagen 52904	100%（16/16）	100%（16/16）	100%（32/32）	1

Table 4. The detection rate of specimens of 1×LoD

Extraction kit	nasopharyngeal swab	throat swab	sputum
Bioperfectus SDK60102	100%（80/80）	100%（40/40）	100%（40/40）
Bioperfectus SDK60103	100%（80/80）	100%（40/40）	100%（40/40）
Bioperfectus SDK60104	100%（80/80）	100%（40/40）	100%（40/40）
Bioperfectus SDK60105	98.75%（79/80）	100%（40/40）	100%（40/40）
Qiagen 52904	100%（80/80）	100%（40/40）	100%（40/40）

Inclusivity analytical sensitivity

In silico analysis was conducted to evaluate the extent of homology between the COVID-19 Coronavirus Real Time PCR Kit and all SARS-CoV-2 sequences available in NCBI and in GISAID databases. A total of 15486 sequences (NCBI (n=2160); GISAID (n=13326)) collected from Africa, America, Europe, Oceania and Asia that were available through May 11 2020, were examined using BLAST to identify the extent of predicted assay inclusivity. Overall, >99% of available N gene and ORF1ab sequences exhibited 100% homology with the COVID-19 Coronavirus Real Time PCR Kit primers and probes. For those sequences with <100% homology, there is a single mismatch located in the middle of one primer that is not expected to affect test performance. Based on in silico analysis, the COVID-19 Coronavirus Real Time PCR Kit is predicted to detect all currently available SARS-CoV-2 sequences.

Table 5. The result of the in inclusivity of the primers and probes

a. Sequences from GISAID (n=13326)

SARS-CoV-2 Target	Number of Sequences	Number of Mismatches		
		Forward Primer	Probe	Reverse Primer
ORFlab Gene	13325 (99.99)	0	0	0
	1 (0.01)	0	0	1
N Gene	13290 (99.73)	0	0	0
	33 (0.25)	1	0	0
	3 (0.02)	0	0	1

b. Sequences from NCBI (n=2160)

SARS-CoV-2 Target	Number of Sequences	Number of Mismatches		
		Forward Primer	**Probe**	**Reverse Primer**
N Gene	2154 (99.72)	0	0	0
	5 (0.23)	1	0	0
	1 (0.05)	0	0	1

Cross-reactivity

In silico exclusivity analysis was conducted to examine the possible cross-reactions between all the organisms (shown in Table 6) and the COVID-19 Coronavirus Real Time PCR Kit primers and probes. SARS-CoV (including bat-like SARS coronavirus) is the only organism identified as potentially cross-reactive by in silico analysis. While the primer binding sites are well conserved between sequenced isolates of SARS-CoV, bat-like SARS-CoV and SARS-CoV-2, the N gene and ORF1ab detection probe binding regions share only 72% and 74% homology respectively. Based only on sequence analysis, the possibility that the COVID-19 Coronavirus Real Time PCR Kit may cross-react with SARS-CoV cannot be ruled out. However, SARS-CoV has not been detected in the human population since 2004. In addition, the COVID-19 Coronavirus Real Time PCR Kit was challenged with nucleic acids isolated from SARS-CoV and other human coronaviruses to confirm that the kit did not cross-react.

The microorganisms listed below were added to SARS-CoV-2 negative sputum at 10^6 cfu/mL for bacteria and yeast, and 10^5 pfu/mL for viruses. The SARS-CoV-2 target for use in the study was obtained from known positive clinical specimens and was quantified by RT-PCR. Each microorganism was tested in the presence and absence of SARS-CoV-2 RNA at 3× LoD using the SSNP-2000A automated specimen processor and SDK60105 extraction kit and QuantStudio ™ 5 Real-time PCR instrument. No cross-reactivity or interference was observed. The results are summarized in table below.

Table 6. The results of cross-reactivity and microbial interference testing

Microorganism	Result(No.Positive/ No.Tested)		Final Result (cross-reactivity)
	Negative samples (with interfering pathogen)	3× LoD (with interfering pathogen)	
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 substances

The potential for interference with the COVID-19 Coronavirus Real Time PCR Kit by substances that may be found in respiratory specimens was evaluated by testing each substance listed in Table 7 in SARS-CoV-2 positive and negative sputum. The positive specimens were diluted in SARS-CoV-2 negative sputum to achieve a concentration of 3×LoD. RNase-free water was used as a control. Three batches of reagents were included in the study and the specimens were tested using a QuantStudio™ 5 PCR instrument after processing using the SSNP-2000A automated specimen processor and SDK60105 extraction kit. Results showed that these substances did not interfere with the detection of negative and weak positive specimens by the COVID-19 Coronavirus Real Time PCR Kit.

Table 7. The result of interference substance

Group	Interference with substances	Concentration	Group	Interference with substances	Concentration
1	Fresh blood	5%	15	Histamine hydrochloride	100mg/L
2	Nasal secretions	5%	16	α- interferon	100 units/ mL
3	Mucus	5%	17	Zana Miv	5mg/L
4	Mucin	2g/dL	18	Limbavirin	0.2g/L
5	Phenylephrine	5%	19	Oseltamivir	100mg/L
6	Oxymetazoline	10mg/L	20	Paramibe	100mg/L
7	Sodium chloride	1%	21	Lopinavir/Litonavir	200mg/100mg/L
8	Perchloromethasone	100mg/L	22	Mopiro	0.2%
9	Dexamethasone	100mg/L	23	Arbidol	0.2g/L
10	Flunisolide	100mg/L	24	Levofloxacin	0.4g/L
11	Triamcinolone acetonide	100mg/L	25	Azithromycin	1g/L
12	Budesonide	100mg/L	26	Ceftriaxone	1g/L
13	Mometasone	100mg/L	27	Meropenem	50mg/mL
14	Fluticasone	100mg/L	28	Tobramycin	10mg/L

Clinical trial

I) Positive agreement:

The ability of the Bioperfectus COVID-19 Real Time PCR to detect SARS-CoV-2 positive samples was evaluated further using 30 simulated nasopharyngeal swabs, sputum samples and throat swabs containing pseudo-virus at concentrations of 1× to 5× LoD, as well as with 20 SARS-CoV-2 positive clinical specimens of each type that had been characterized using another NMPA-authorized assay. The COVID-19 Real Time PCR kit exhibited 100% agreement with the expected results for each specimen type.

Table 8. Positive agreement (simulated specimens)

Specimen type	Number of specimens	Positive Agreement
Nasopharyngeal swab	30	100% (88.65-100%)
Sputum	30	l00% (88.65-100%)
Throat swab	30	l00% (88.65-100%)

Table 9. Positive agreement (clinical specimens)

Specimen type	Number of specimens	Positive Agreement
Nasopharyngeal swab	20	100% (83.89-100%)
Sputum	20	l00% (83.89-100%)
Throat swab	20	l00% (83.89-100%)

II) Negative agreement:

The ability of the Bioperfectus COVID-19 Real Time PCR kit to report SARS-CoV-2 negative samples correctly was evaluated using 100 nasopharyngeal swab specimens, 30 sputum

specimens and 100 throat swabs that were confirmed as SARS-CoV-2 negative by clinical diagnosis and nucleic acid testing using another NMPA-authorized assay. The COVID-19 Real Time PCR kit exhibited 100% agreement with the expected results for each specimen type.
The 95% confidence interval for negative agreement with nasopharyngeal swabs and throat swabs was 96.30%-100%, while the value for sputum was lower, i.e. 88.65%-100%, due to lower specimen number.

Table 10. Negative agreement

Specimen type	Number of specimens	Negative Agreement
Nasopharyngeal swab	100	100% [96.30-100]
Sputum	30	100% [88.65-100]
Throat swab	100	100% [96.30-100]

III) Agreement with clinical diagnosis and comparator RT-PCR methods:

In total 970 clinical specimens were involved in this clinical study, including 420 SARS-CoV-2 positive and 550 SARS-CoV-2 negative, as determined using a comparator molecular diagnostic assay for the detection of SARS-CoV-2 RNA. The specimen types tested included sputum, throat swabs and nasopharyngeal swabs. Overall, the Bioperfectus COVID-19 Real Time PCR kit exhibited 100% positive agreement and 95.1% negative agreement in comparison to another China National Medical Products Administration (NMPA)-certified molecular diagnostic assay used to characterize the specimens (Kappa value 0.9437). There were 27 specimens that were reported as positive by the Bioperfectus COVID-19 Real Time PCR kit and negative by the comparator, including 22 clinical confirmed cases, 1 case with incomplete clinical history and 4 clinical excluded cases, but these 4 cases were confirmed as weak positive by digital PCR.

Table 11. Agreement between the Bioperfectus COVID-19 Real Time PCR kit and Comparator kit, stratified by specimen type

Nasopharyngeal Swabs			
Bioperfectus kit	Comparator kit		Total
	Positive	Negative	
Positive	185	14	199
Negative	0	127	127
Total	185	141	326
Positive Agreement	100% (185/185); 98.0-100%		
Negative Agreement	90.1% (127/141); 84.0-94.0%		

Throat Swabs			
Bioperfectus kit	Comparator kit		Total
	Positive	Negative	
Positive	153	4	157
Negative	0	276	276

Total	153	280	433
Positive Agreement	100% (153/153); 97.6-100%		
Negative Agreement	98.6% (276/280); 96.4-99.4%		

Sputum			
Bioperfectus kit	Comparator kit		Total
	Positive	Negative	
Positive	82	9	91
Negative	0	120	120
Total	82	129	211
Positive Agreement	100% (82/82); 95.5-100%		
Negative Agreement	93.0% (120/129); 87.3-96.3%		

All Specimen Types Combined			
Bioperfectus kit	Comparator kit		Total
	Positive	Negative	
Positive	420	27	447
Negative	0	523	523
Total	420	550	970
Positive Agreement	100% (420/420); 99.1-100%		
Negative Agreement	95.1% (523/550); 93.0-96.6%		

Compared to clinical diagnosis of COVID-19 (symptoms consistent with COVID-19 and a positive test result with an NMPA-certified molecular assay), the Bioperfectus COVID-19 Real Time PCR kit exhibited positive and negative agreement of 94.92% and 98.72%, respectively, with Kappa value 0.9378 (Table 12).

Table 12. Agreement between the Bioperfectus COVID-19 Real Time PCR kit and clinical diagnosis

Bioperfectus kit	Clinical diagnosis [1]		Total
	Confirmed [2]	Excluded [3]	
Positive	411	6	417
Negative	22	464	486
Total	433	470	903

Positive Agreement	94.9% (411/433); 92.4-96.6%
Negative Agreement	98.7% (464/470); 97.2-99.4%

[1] Signs and symptoms of COVID-19 or epidemiological history of contact with a known case
[2] Clinical diagnosis confirmed by a positive test result with an NMPA-certified molecular assay
[3] Negative test result with an NMPA-certified molecular assay

19. References

1) Diagnosis and Treatment Protocol for COVID-19 (Trial Version 7). http://en.nhc.gov.cn/2020-03/29/c_78469.htm
2) Laboratory Guidelines for Detection and Diagnosis of the Novel Coronavirus (2019-nCoV) Infection. https://iris.paho.org/bitstream/handle/10665.2/51895/ncov-lab-recommendations-en.pdf?sequence=1&isAllowed=y
3) Laboratory testing for 2019 novel coronavirus in suspected human cases. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117
4) Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected

20. Symbols

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

21. Contact Information and Product Support

For more information about Bioperfectus Technologies Co, Ltd., please visit our website at: http://en.s-sbio.com/ or contact at E-mail: trade@s-sbio.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@s-sbio.com.