WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT

Product: COVID-19 Real-Time PCR Kit
EUL Number: EUL-0535-196-00
Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

COVID-19 Real-Time PCR Kit code HBRT-COVID-19, CE-mark regulatory version, manufactured by Chaozhaou Hybribio Biochemistry Ltd, No. 71, Fenghuang 3rd road, Sino-Singapore Guangzhou Knowledge City, Guangzhou, China was listed on 15 June 2020.

Intended use:

According to the claim of intended use from Chaozhaou Hybribio Biochemistry Ltd, "*the COVID-19 Real-time PCR Kit (HBRT-COVID-19) is designed for the qualitative detection of ORFl ab and N genes of SARS-CoV-2 RNA in oropharyngeal swab and nasopharyngeal specimens from patients who meet COVID-19 clinical and/or epidemiological criteria. The product is for aiding the diagnosis of COVID-19 infection.*
Results are for the detection of SARS-CoV-2 RNA that is generally detectable in oropharyngeal swab and nasopharyngeal swab specimen during infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories 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 Real-time PCR Kit (HBRT-COVID-19) for detecting SARS-CoV-2 is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures in level 2 biosafety laboratories."

Specimen type that was validated:

Oropharyngeal swab, Nasopharyngeal swab, sputum, endotracheal aspirate and bronchoalveolar lavage fluid specimens.

Test kit contents:

Component	**24 tests (product code HBRT-COVID-19)**
COVID-1 9 RT-PCR Mix	564 µL×1 vial
Enzyme Mix	36 µL×1 vial
Positive Control	400 µL×1 vial
Negative control	400 mL×1 vial

Items required but not provided:

Specimen collection kits:

Extraction/Purification:

- Thermofisher King Fisher Flex with Prefilled Viral Total NA Kit-Flex (Fisher Scientific, Catalog No.: KFRPF-805H48 4x48).
- Bioer GenePure Pro Nucleic Acid Purification System with MagaBio plus viral DNA/RNA purification kit II (Hangzhou Bioer Technology Co. Ltd. (BIOER), Catalog No. BSC7 l S l E).

Real-Time PCR equipment:

- Applied Biosystemsrn Real time PCR system 7500 with software "7500 Software v2.0.5.
- Bio-Rad CFX96 Real-Time PCR Detection System with software "Bio-Rad CFX Manager 3.1 "/SlAN 96S Real-Time PCR system with software version 8. 2. 2.

General laboratory equipment and consumables

- Vortex mixer.
- Microcentrifuge.
- Micropipettes (2 or l O µl, 200 µl and l 000 µl).
- Multichannel micropipettes (5-50 µl).
- Racks for l .5 ml microcentrifuge tubes.
- Molecular grade water, nuclease-free.
- Disposable powder-free gloves and surgical gowns.

- Aerosol barrier pipette tips.
- 1.5 ml microcentrifuge tubes (DNase/RNase free).
- 96-well 0.2 ml PCR reaction plates (Applied Biosystems).
- 10% bleach (1:10 dilution of commercial 5.25-6% hypochlorite bleach).
- 70% ethanol.

Storage:

Store the kit below -15 °C. Avoid exposing the kit to direct sunlight.

Shelf-life upon manufacture:

9 months.

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Chaozhaou Hybribio Biochemistry Ltd submitted a product dossier for the COVID-19 Real-Time PCR Kit for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347 version 4)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitment for EUL:

As commitments to listing, the manufacturer is required to determine the limit of detection with the WHO international standard when available.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Chaozhaou Hybribio Biochemistry Ltd was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that sufficient information was provided by Chaozhaou Hybribio

Biochemistry Ltd to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_347)*".

Quality management documentation assessment conclusion: acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:
1. Notification to WHO of any planned changes to a EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*WHO guidance on post-market surveillance of in vitro diagnostics" (ISBN 978 92 4 150921 3).*

Chaozhaou Hybribio Biochemistry Ltd is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance "*WHO guidance on post-market surveillance of in vitro diagnostics*".[1]

Scope and duration of procurement eligibility

COVID-19 Real-Time PCR Kit, product code HBRT-COVID-19 manufactured by Chaozhaou Hybribio Biochemistry Ltd is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Chaozhaou Hybribio Biochemistry Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Chaozhaou Hybribio Biochemistry Ltd is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days and any changes to the product.

[1] Available on the web page https://www.who.int/diagnostics_laboratory/postmarket/en/

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

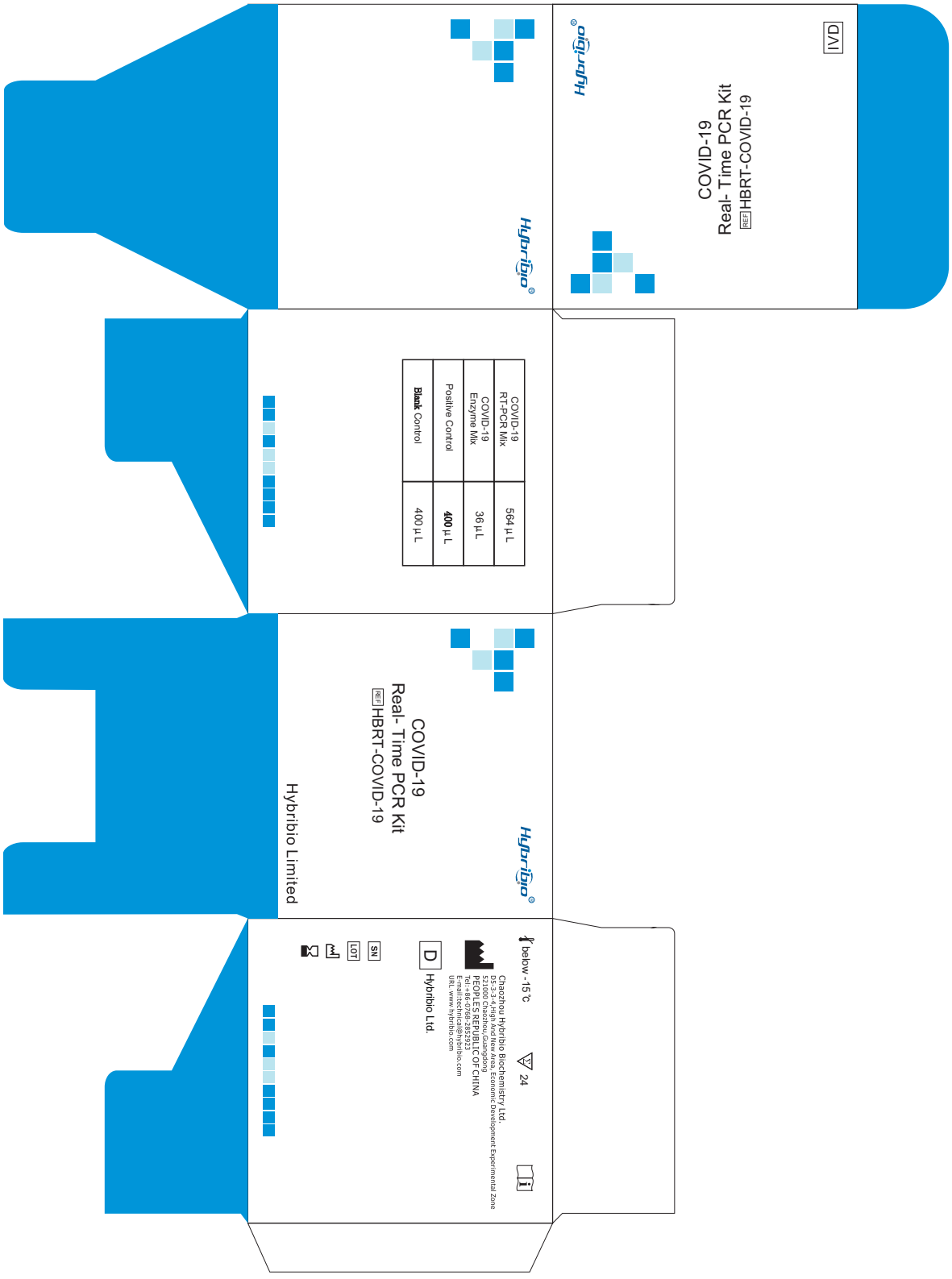
Labelling

1.0 Labels

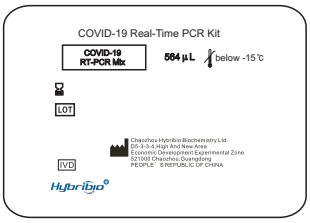
2.0 Instructions for Use (IFU)

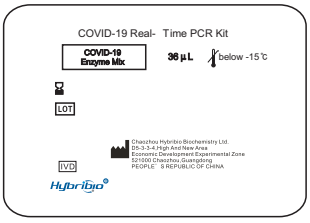
1.0 Product labels

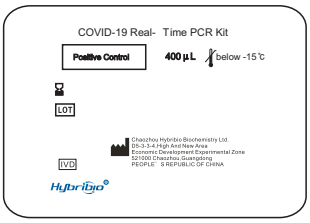
1.1 Outer labels

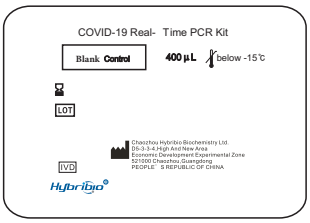


1. 2.Component labels









2.0 Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Hybribio

COVID-19 Real-Time PCR Kit (HBRT-COVID-19) Instructions For Use

2.1

ISO 13485:2016 CE IVD

Table of Contents

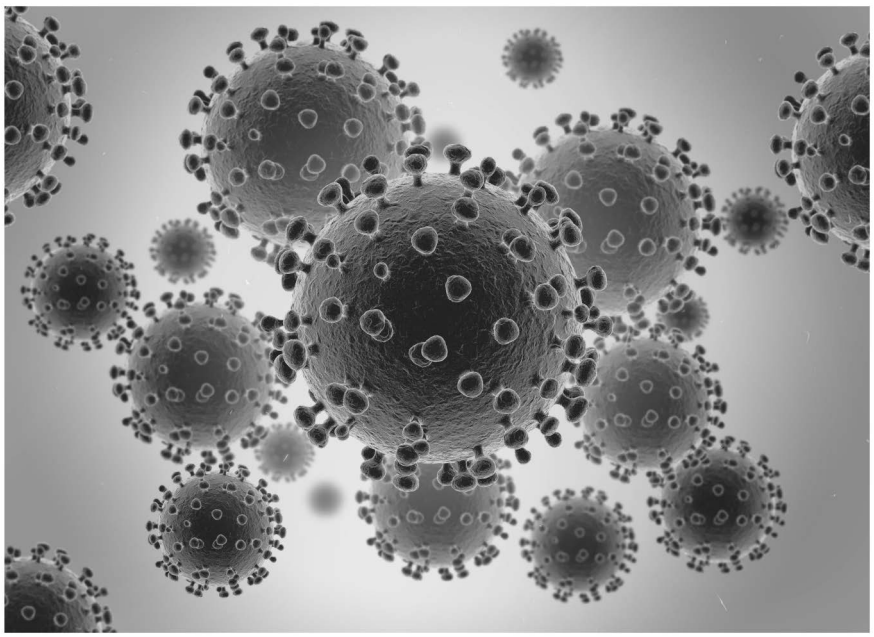
1.Intended Use

The COVID-19 Real-time PCR Kit (HBRT-COVID-19) is designed for the qualitative detection of ORF1ab and N genes of SARS-CoV-2 RNA in oropharyngeal swab and nasopharyngeal specimens from patients who meet COVID-19 clinical and/or epidemiological criteria. The product is for aiding the diagnosis of COVID-19 infection.

Results are for the detection of SARS-CoV-2 RNA that is generally detectable in oropharyngeal swab and nasopharyngeal swab specimen during infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories 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.

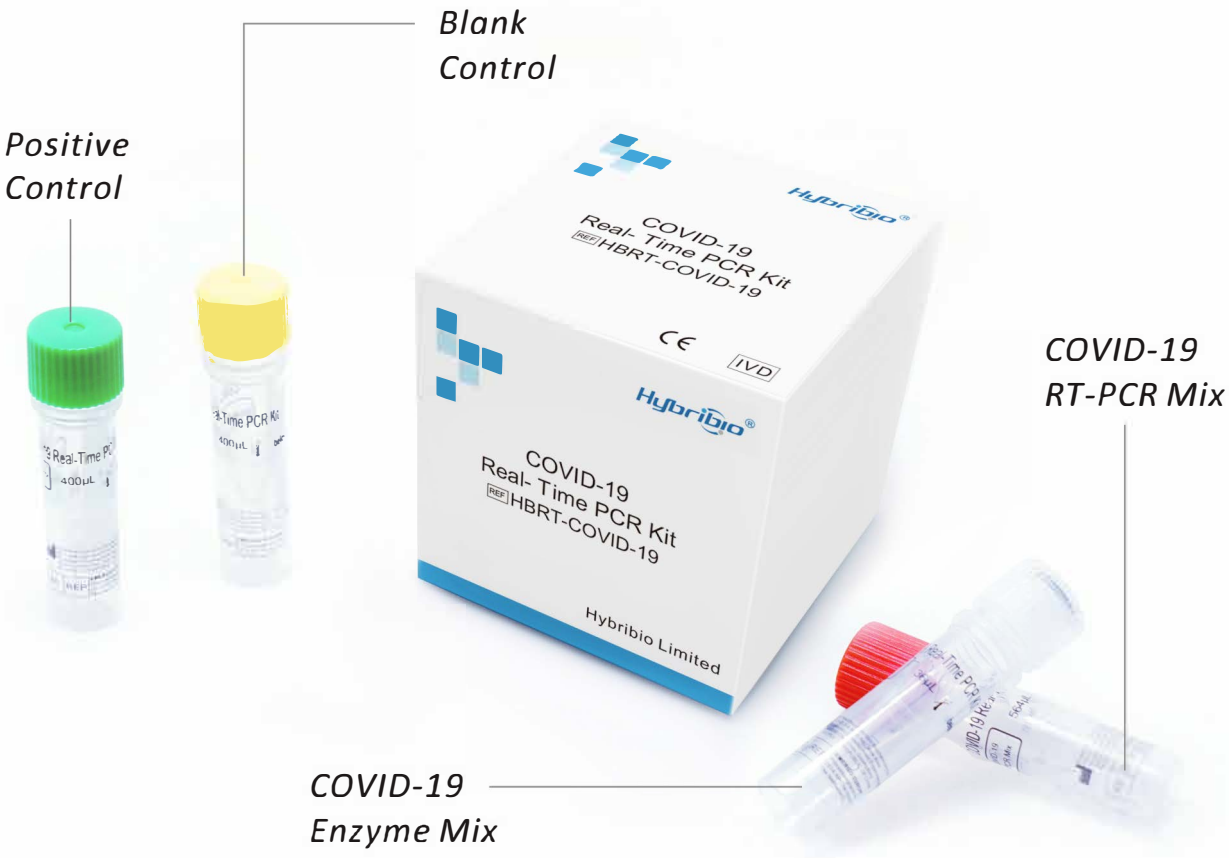
The COVID-19 Real-time PCR Kit (HBRT-COVID-19) for detecting SARS-CoV-2 is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures in level 2 biosafety laboratories



2.Principle of the Test

With use of multiplex real-time PCR technology, this in-vitro diagnostic kit can detect the presence or absence of RNA focusing on 2 targeting genes: ORF1ab and N gene. ORF1ab and N gene signals can be amplified and detected based on the designed Taqman probes of those target genes during the amplification process. B2M RNA gene is also included for each specimen to ensure specimen validity from specimen collection and RNA extraction, and monitor PCR amplification procedure, avoiding false negative results.

3.Kit Contents



The kit contains 24 tests

Kit Components	Specification(tube)	Key Contents
COVID-19 RT-PCR Mix	564 μL	Primer, Probes, dNTP, $MgSO_4$
Enzyme Mix	36 μL	Hot Start DNA Polymerase, Reverse transcriptase
Positive Control	400 μL	COVID-19, B2M
Blank Control	400 μL	Distilled water without RNA enzyme

4.Storage and Period of Validity

Storage and Transportation

The kit should be stored at -15°C or lower. Repeated freeze/thaw should not be more than 5 times to prevent reagent degradation. Both ice-gel / ice-pack / dry ice are required for transportation of the kit.

Period of Validity

9 months period from the manufacturing date stated on the box

5.Material Required But Not Provided

- Applied Biosystems™ Real time PCR system 7500 with software "7500 Software v2.0.5 / Bio-Rad CFX96 Real-Time PCR Detection System with software "Bio-Rad CFX Manager 3.1"/SLAN 96S Real-Time PCR system with software version 8.2.2

- Thermofisher KingFisher Flex with Prefilled Viral Total NA Kit-Flex (Fisher Scientific, Catalog No.: KFRPF-805H48 4x48) / Bioer GenePure Pro Nucleic Acid Purification System with MagaBio plus viral DNA/RNA purification kit II (Hangzhou Bioer Technology Co. Ltd. (BIOER), Catalog No. BSC71S1E)

- Vortex mixer.

- Microcentrifuge.

- Micropipettes (2 or 10 µL, 200 µL and 1000 µL).

- Multichannel micropipettes (5-50 µL).

- Racks for 1.5 mL microcentrifuge tubes.

- Molecular grade water, nuclease-free.

- Disposable powder-free gloves and surgical gowns.

- Aerosol barrier pipette tips.

- 1.5 mL microcentrifuge tubes (DNase/RNase free).

- 96-well 0.2 mL PCR reaction plates (Applied Biosystems).

- 10% bleach (1:10 dilution of commercial 5.25-6% hypochlorite bleach)

- 70% ethanol

6.Warnings and Precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use under Emergency Use Authorization only.

- Positive results are indicative of the presence of SARS-CoV-2 RNA.

- Laboratories are required to report all positive results to the appropriate public health authorities.

- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.1,2 Only personnel proficient in handling infectious materials and perform test procedure.

- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.

- –Laboratories should follow good laboratory practices and comply with all applicable regulatory requirements. Maintain separate areas and dedicated equipment (e.g., pipettes, microcentrifuge) and supplies (e.g., microcentrifuge tubes, pipette tips, gowns and gloves) for assay reagent setup and handling of extracted nucleic acids. Cross-use of equipment from different phases and areas is prohibited.

–Use nuclease-free, sterile disposable aerosol barrier pipette tips for each addition and transfer to avoid cross-contamination in pre-PCR procedures.

–Use nuclease-free, disposable polypropylene tubes for preparing the reaction mixes. Test disposable items should be thoroughly disinfected and inspected in order to avoid contamination or false negative results caused by amplification reaction inhibitor.

–After nucleic acid extraction, immediately take off the 8 sleeve groove tubes from the instrument. The extracting plate should be sealed after use in order to avoid aerosol pollution.

- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.

- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

- Do not eat, drink, or smoke in designated work areas.

- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and the COVID-19 Real-time PCR Kit. Avoid contaminating gloves when handling samples and controls.

- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.

- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

- Make sure the reagents are completely thawed and thoroughly mixed before usage.

- Do not use product after expiration date.

- Only use one Lot No. Kit for one test.

7.Specimen Collection, Storage, and Transfer

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

Collecting the Specimen:

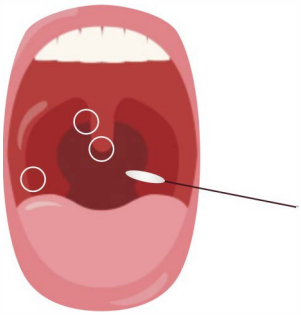
- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV)

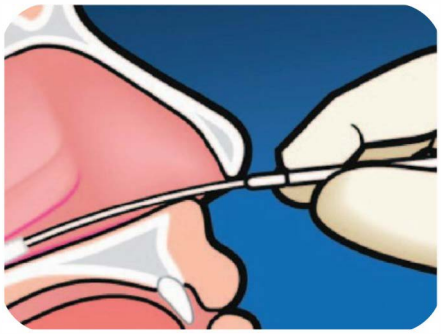
https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

- Follow specimen collection devices manufacturer instructions for proper collection methods.

- Oropharyngeal swab: Use a sterile swab （Model No. 93050, Shenzhen Miraclean Limited.)to wipe the posterior pharynx, avoiding the tongue. Place swabs immediately into labeled sterile tubes containing viral transport medium. Break both applicator sticks off at the score line (flocked swabs) or near the tip, or cut with sterile scissors to permit tightening of the cap. Ship sample immediately on cold packs.

- Nasopharyngeal swab: Insert a sterile swab (Model No. 96000, Shenzhen Miraclean Limited.) into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Place swabs immediately into labeled sterile tubes containing viral transport medium. Break both applicator sticks off at the score line (flocked swabs) or near the tip, or cut with sterile scissors to permit tightening of the cap. Ship sample immediately on cold packs.





Transporting Specimens:

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. All specimens must be transported with ice cool / ice-gel box /dry ice and securely sealed and handled.

Storing Specimens:

- Specimens can be stored at 2-8°C for up to 48 hours after collection.
- Specimens can be stored at -70°C or lower for up to 6 months after collection.
- Extracted RNA can be stored at -15°C to – 20°C for 20 days, and should be stored at -70°C or lower for up to 6 months.

8.Test Procedures

8.1 RNA Extraction

Performance of the COVID-19 Real-time PCR Kit (HBRT-COVID-19) is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction system have been qualified and validated for recovery and purity of RNA for use with the panel: Thermofisher KingFisher Flex and Bioer GenePure Pro Nucleic Acid Purification System

For other extraction system or manual extraction method, please consult with the technical support of Hybribio before using the test kit.

Thermofisher KingFisher Flex

Bioer GenePure Pro Nucleic Acid Purification System

The test procedure is described in detail in the Thermofisher KingFisher Flex and Bioer GenePure Pro Nucleic Acid Purification System– User Guide. Below information summarizes the procedure on Thermofisher KingFisher Flex .

1. Samples and reagents, including magnetic particles, are dispensed into the plates according to the corresponding instructions. The protocol that is selected by the user via the keyboard and display has already been preloaded into the onboard software.

2. Go to the Factory protocols/User protocols menu, Select the DNA/RNA row by using the cursor keys and press START OR use BindIt Software to run the desired protocol via the PC.

3. Open the sliding door if the see-through lid is in place.

4. Load the plates in the order that the protocol requests. Place the A1 well of the plate so that it is in the upper right corner. The first A1 row is consequently always in the inner circle. Once you have loaded the requested plates into the plate stations, press START. The tip comb always has to be placed manually onto a KingFisher plate. The instrument also functions with either one plate or up to eight plates depending on the amount of steps. Only one tip comb is placed onto a KingFisher plate (= tip-plate) per run. Confirm the plate loading by pressing START.

The loading position, that is, plate station 4, is labeled. The eight plate stations and the A1 positions of the eight plate stations are clearly marked on the turntable. When the instrument is in its basic position, plate station 1 is under the KingFisher Flex head. After the protocol has been run, note that the turntable may stop in a different position than the basic position.

5. The tip comb is automatically locked onto the tip comb holder from the tip-plate.

6. When the turntable moves, the shield plate moves over the plate underneath forming a protective cover.

7. Close the sliding door. The see-through lid protects the instrument against environmental contamination.

8. After the run, remove the plate(s) according to the protocol request. Confirm each plate removal by pressing the START key. Note that the plate containing your samples is removed first.

9. Press the STOP key after completing the run.

Following extraction, the RNA should be used immediately processed or stored at -70°C or lower for use later.

8.2 PCR Amplification

Reagent preparation:

1. Take out COVID-19 RT-PCR Mix and COVID-19 Enzyme Mix from -15°C or lower. Thaw thoroughly at ambient temperature. Mix contents well before use. Centrifuge at 8000r.p.m for 10 seconds.

2. Calculation of PCR Mix and Taq Polymerase volume mixture is as the table below:

No. of tests	PCR Mix	Taq Polymerase
1 test	23.5 μL	1.5 μL
10 tests	235 μL	15 μL

3. The mixture of PCR Mix with Taq Polymerase is 25 μL for each PCR reaction.

4. Adding 5 μL extracted RNA sample into each PCR reaction, and spin down (pulse/short).

5. The total volume of each PCR reaction system is 30 μL.

6. One positive and one blank control are required for every run of test regardless of the quantity of samples.

Real-Time RT-PCR:

PCR programs Setup Fluorescence detecting channels

Detector Name	Target genes	Reporter Dye	Quencher
FAM	ORF1ab	FAM	none
HEX	N	HEX/JOE	none
Cy5	B2M	Cy5	none

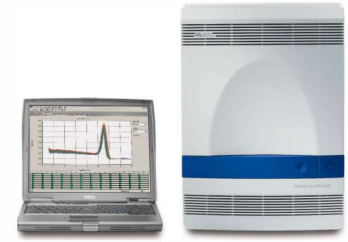
Programs setting on Real-Time PCR

Program	Number of cycles	Temperature	Constant time	Sampling mode
1	1	55°C	15min	none
2	1	95°C	30sec	none
3	45	95°C	10 sec	none
		60°C	35 sec	Signal Taken
4	1	38°C	30 sec	none

Baseline and threshold value setting

Please consult instructions of companies for detail setting procedure.
For threshold selection: the threshold should be adjusted above the amplification line of Blank Control.

The following commercially available PCR Amplification system have been qualified and validated for PCR amplification for use with the kit: Applied Biosystems™ Real time PCR system 7500 with software v2.0.5. / Bio-Rad CFX96 Real-Time PCR Detection System with software / SLAN 96S Real-Time PCR System with software.



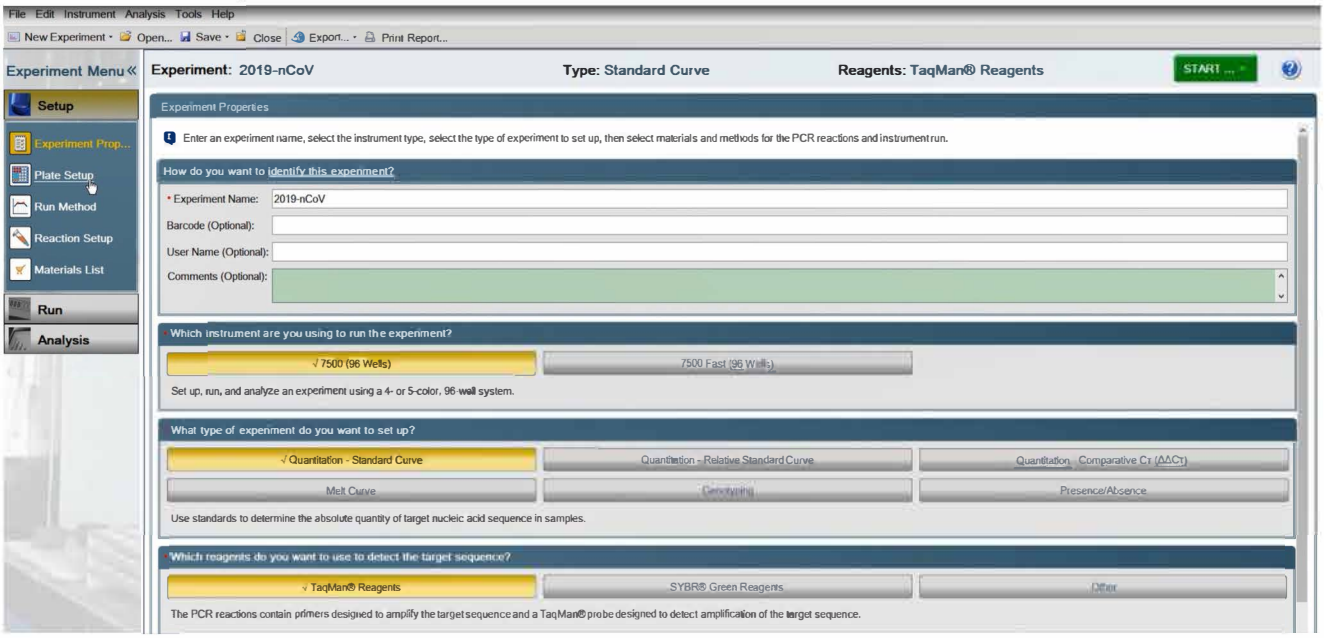
ABI7500

Bio-Rad CFX96

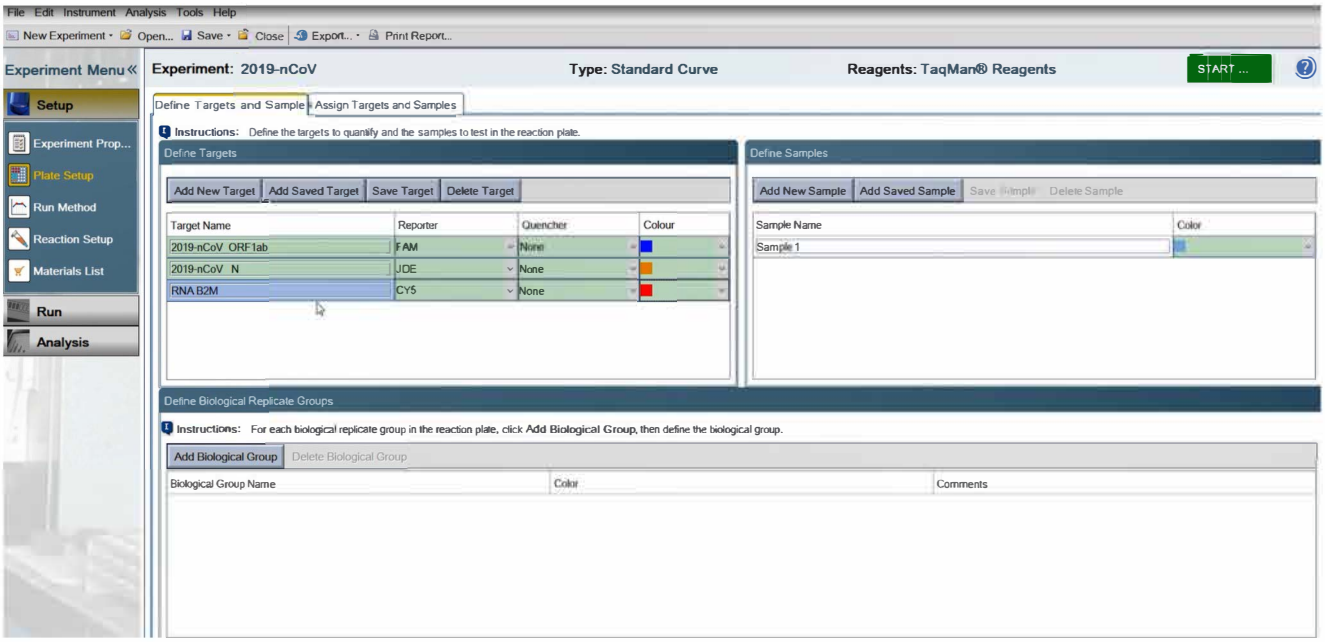
SLAN-96S

See below for step-by-step operation of ABI 7500 using 7500 software v2.0.5:

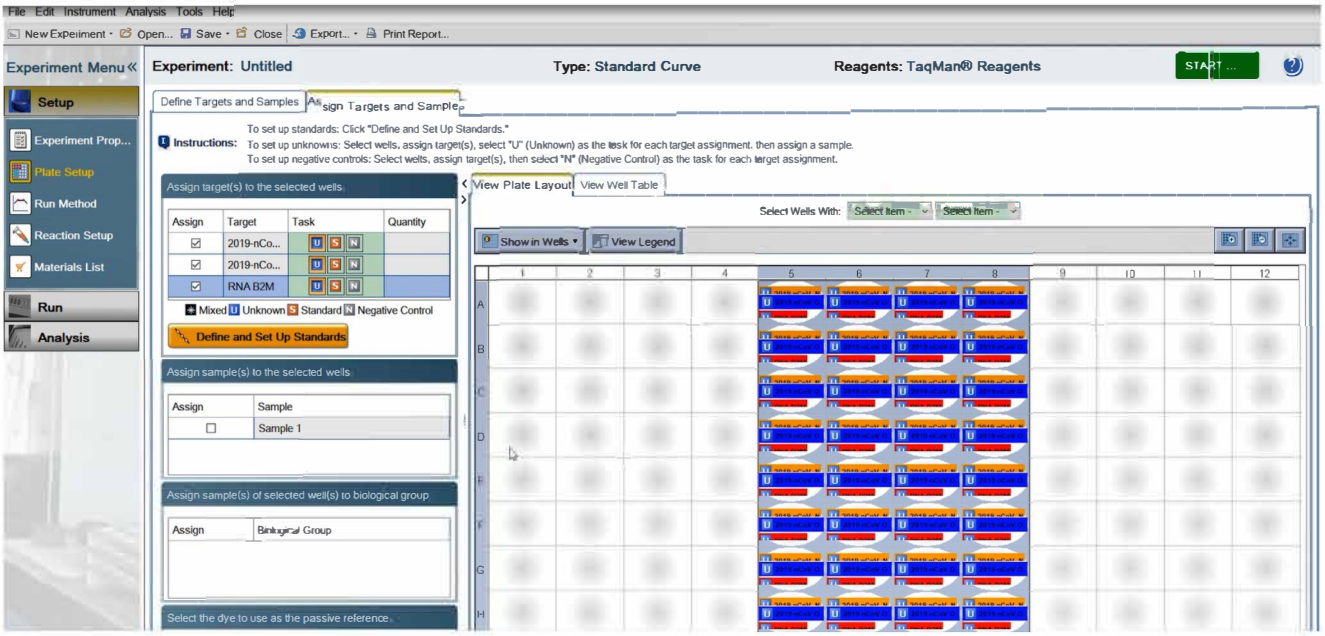
1. Open the software, input the program name in Experiment Name: 2019-nCoV.



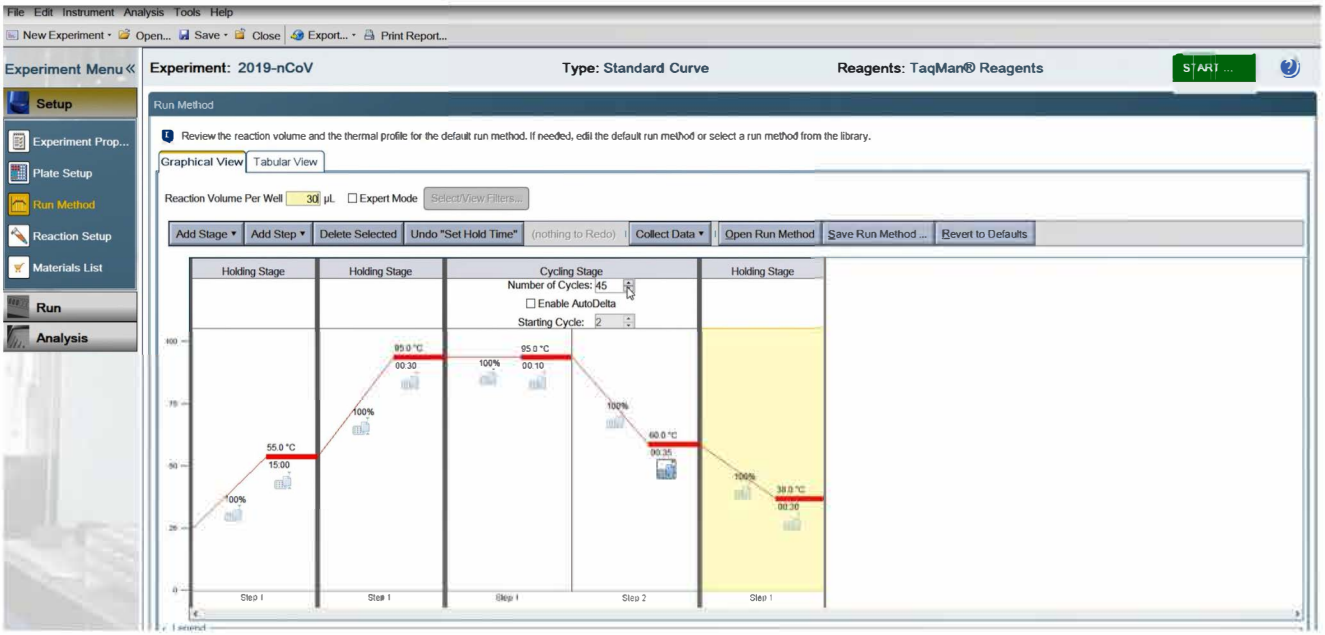
2. Click "Plate Setup", click "Add New Target" twice,and select three channels in total, "FAM", "JOE" and "CY5" respectively under "Reporter", input "2019-nCoV ORF1ab", "2019-nCoV N", "RNA B2M" corresponding to target name, and select "None" under "Quencher".



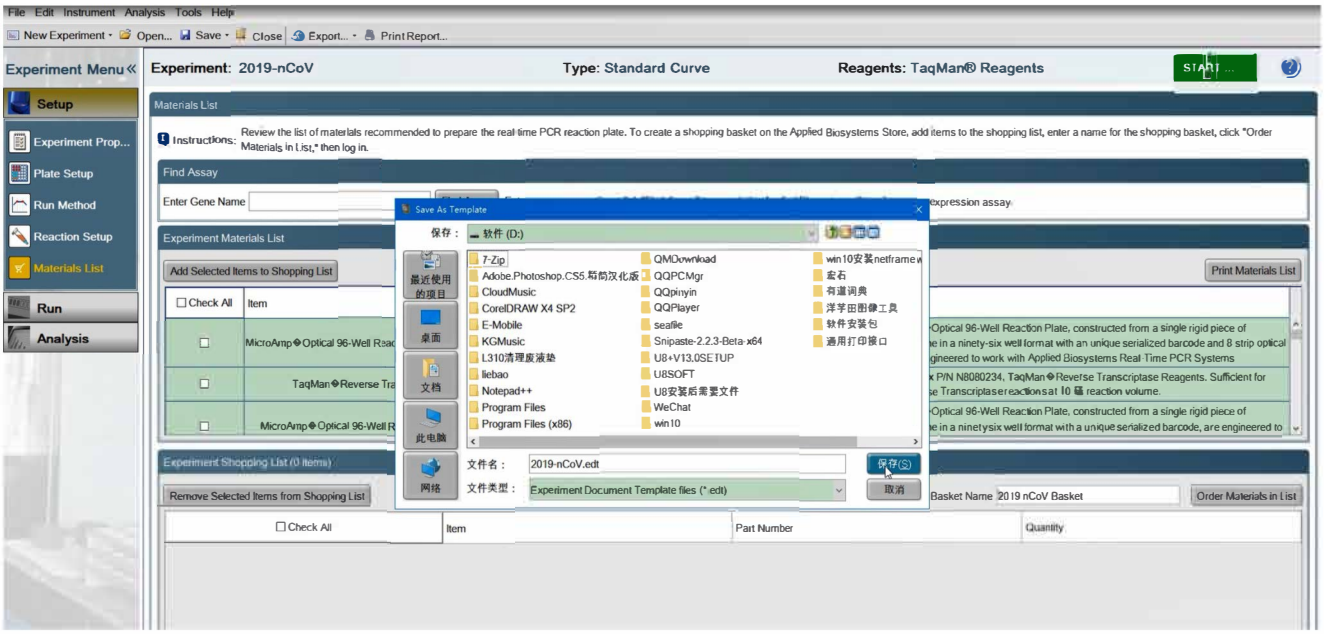
3. Click "Assign Targets and Samples", and select None under "View Plate Layout", select the corresponding hole position, select the sample placement position, then select the target on the left and click the checkmark in the box. Select dye to use as the passive reference at the bottom left of the interface, and select "Rox" as "None"



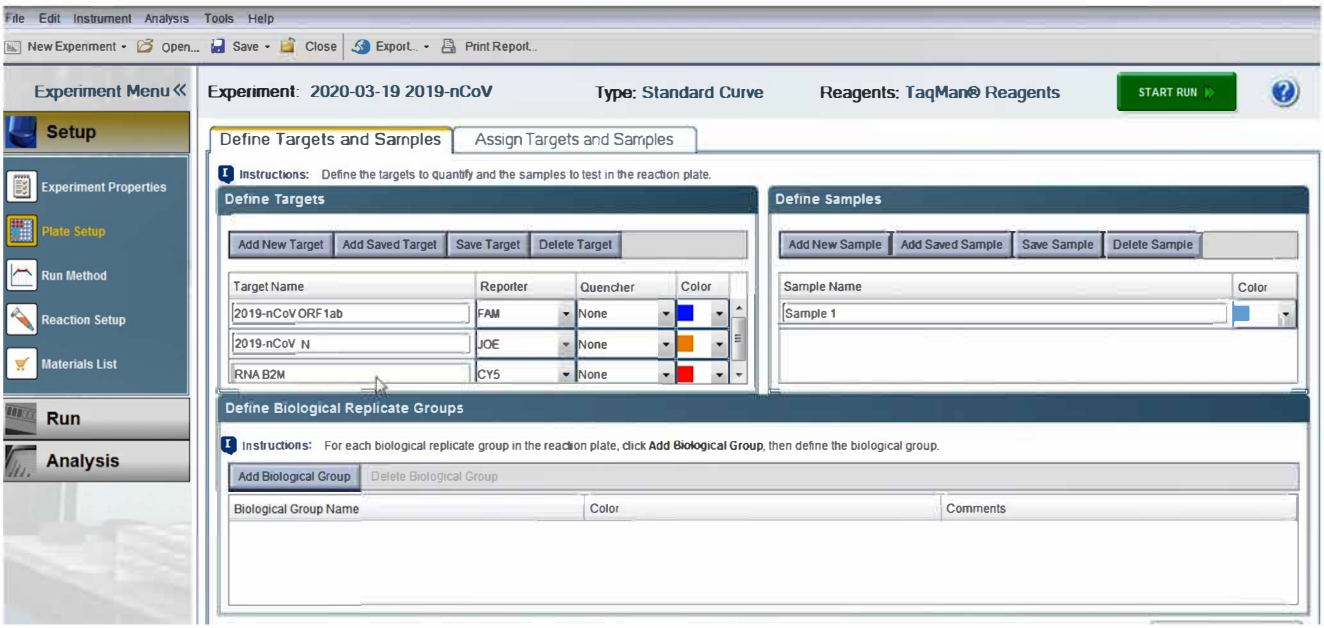
4. Click "Run Method", set the program according to the product manual, and pay attention to setting the lighting position, system and cycle number.



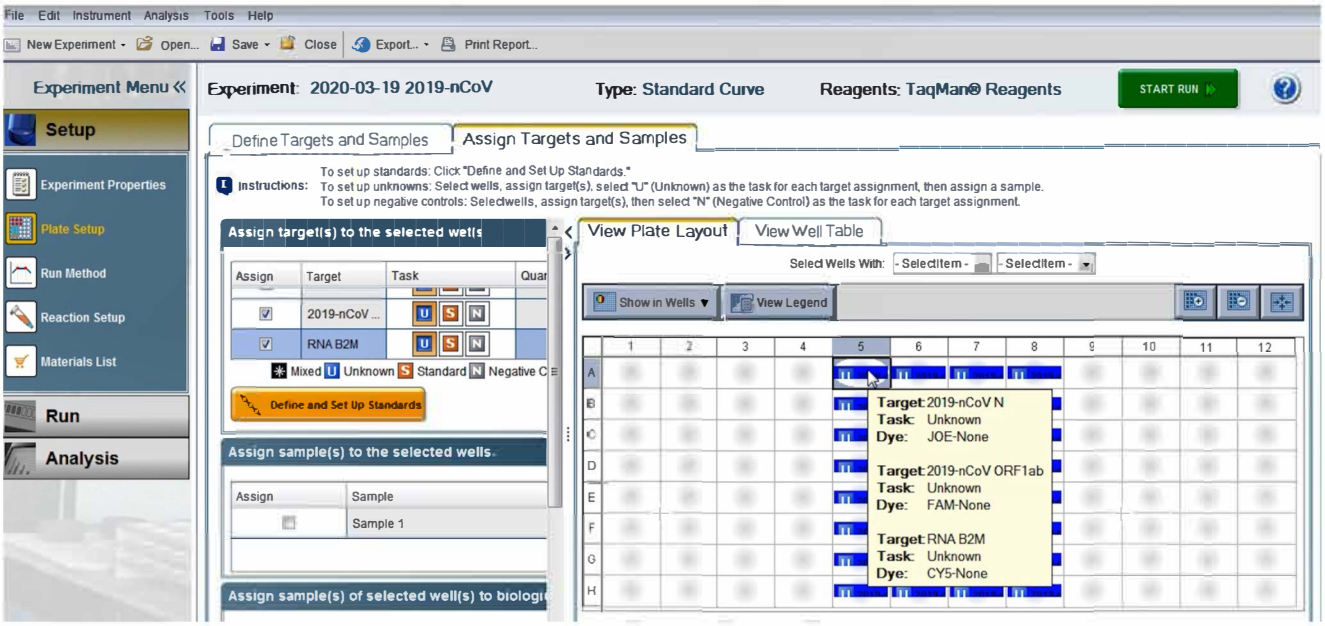
5. When finished, click Save as template under Save (be sure to keep the end of. edt), Then close this page.



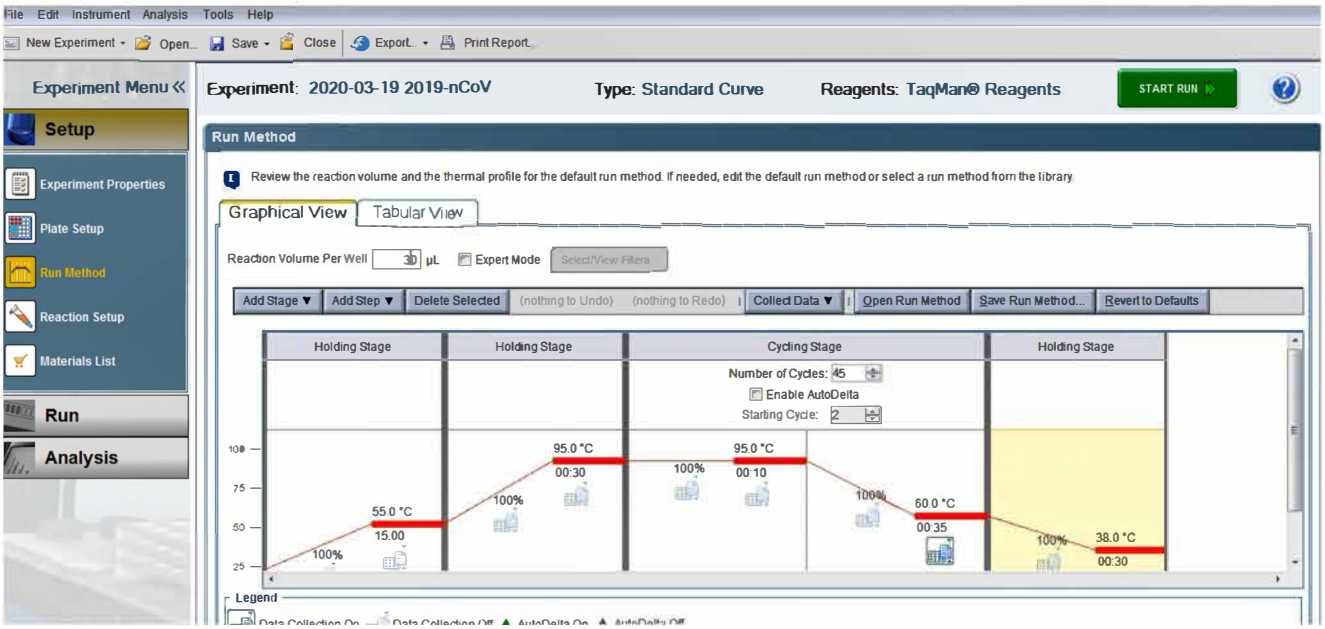
6. Then click from template under Experiment Name, select the previously saved 2019 nCoV program, and confirm that there is no error in the channel under Define Targets and Samples interface.



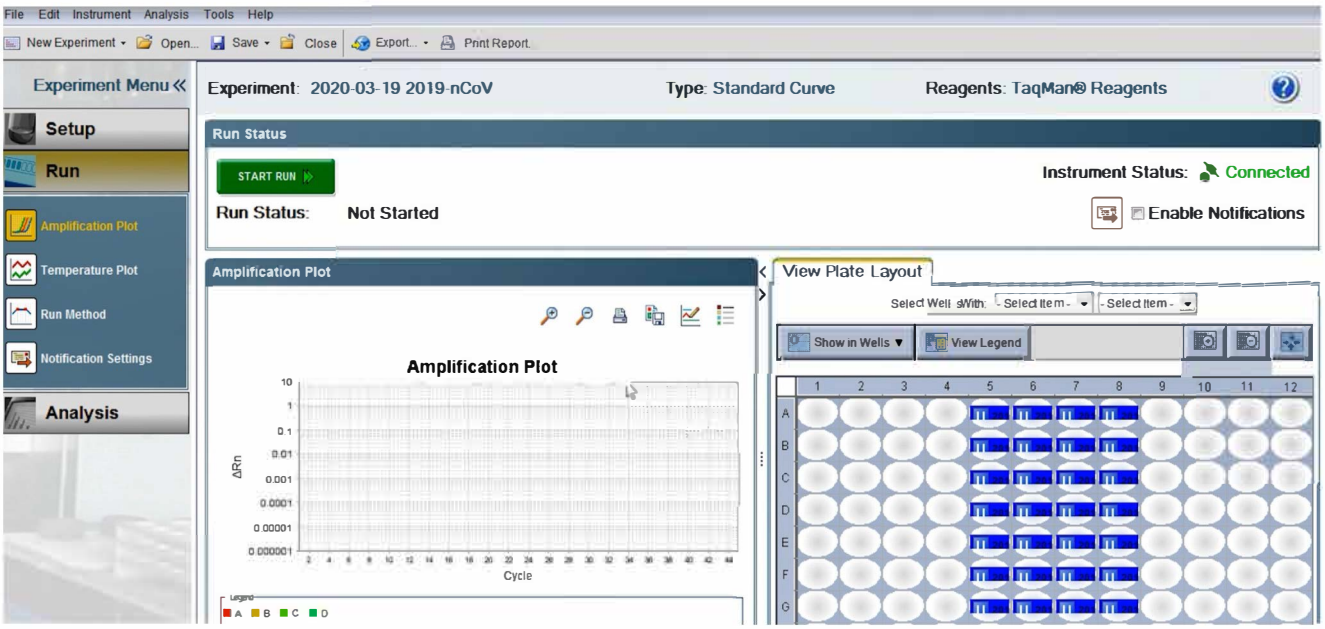
7. Click Assign Targets and Samples , select the corresponding hole position under View Plate Layout, select the sample placement position, and then select the target on the left and click the hook in the box.



8. Confirm that select dye to use as the passive reference is "None". Confirm that the procedure under run method is consistent with the instruction.



9. Click the Amplification Plot under Run, and click the green button "START RUN".



8.3 Data Analysis

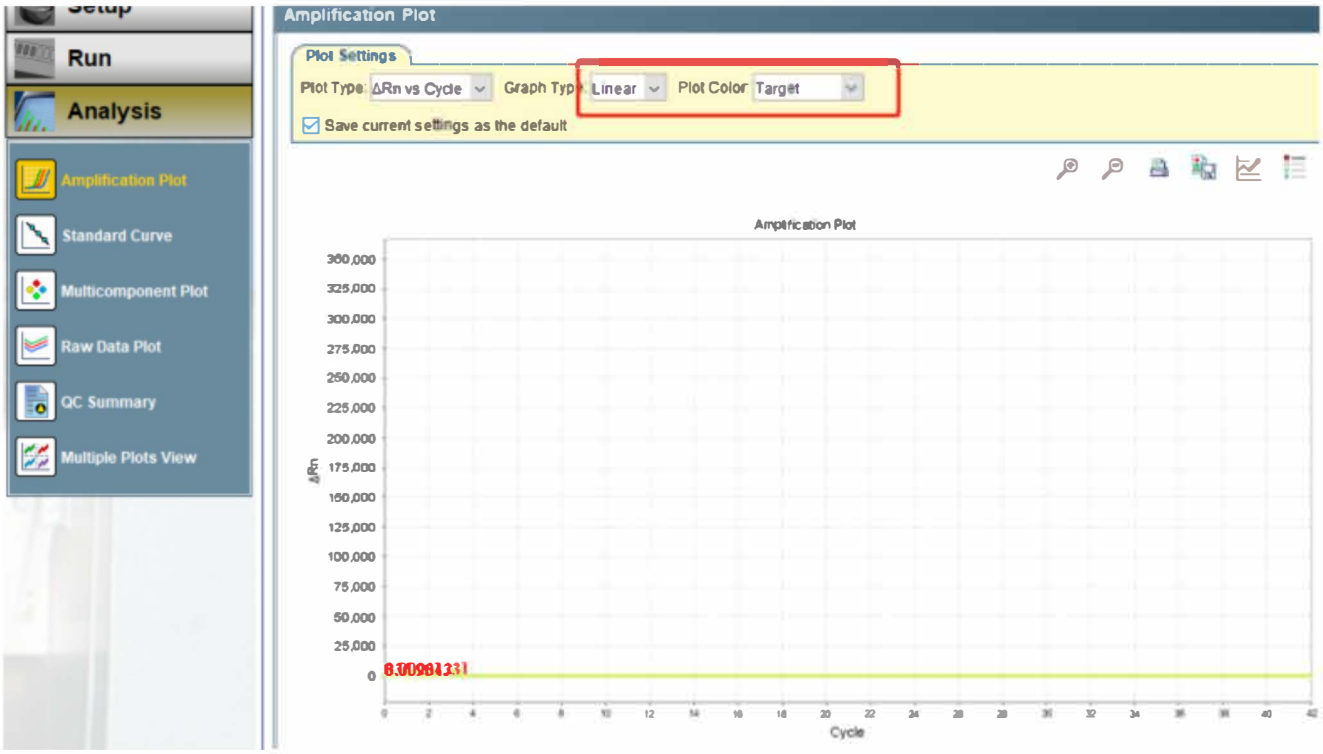
See below for step-by-step operation of ABI 7500 using 7500 software v2.0.5 for Data analysis:

1. Click Analysis. In the Amplification Plot screen under Plot Settings tab:

a. In the Plot Type drop-down list, select ΔRn vs Cycle (default).

b. In the Graph Type drop-down list, select Linear.

c. In the Plot Color drop-down list, select Target as showed in the figure below.



2. Set the baseline starting point at cycle 3 and ending at cycle 15.

3. Manually set thresholds:

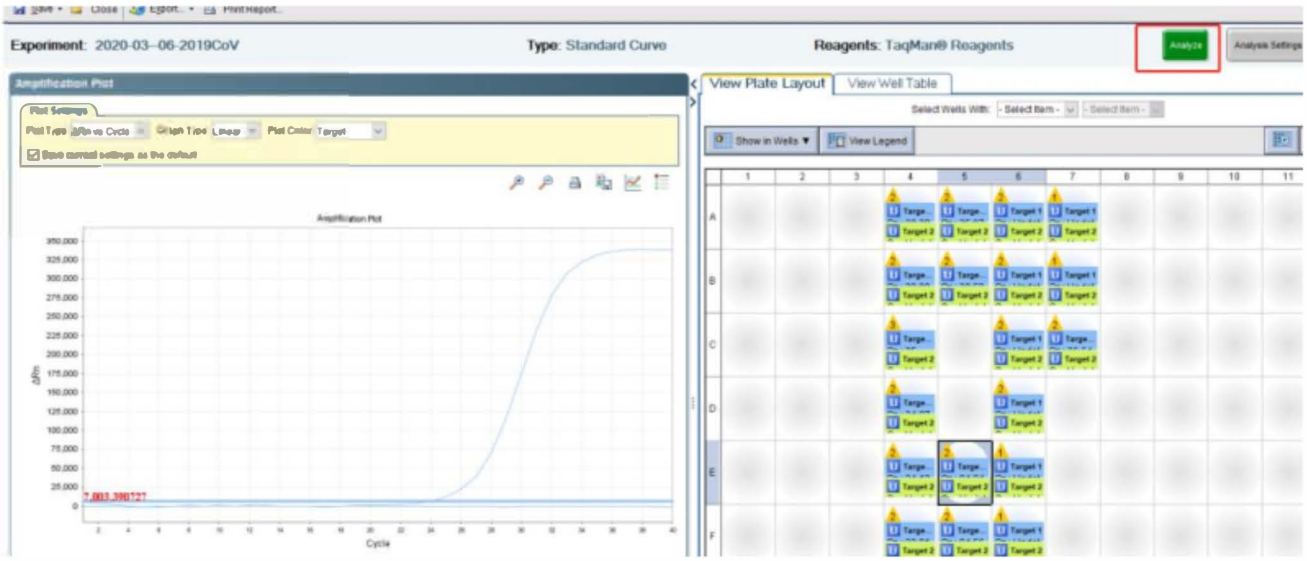
a. In the Target drop-down list, select Target 1(ORF 1ab).

b. Uncheck Auto to □ Auto as shown in the figure below.

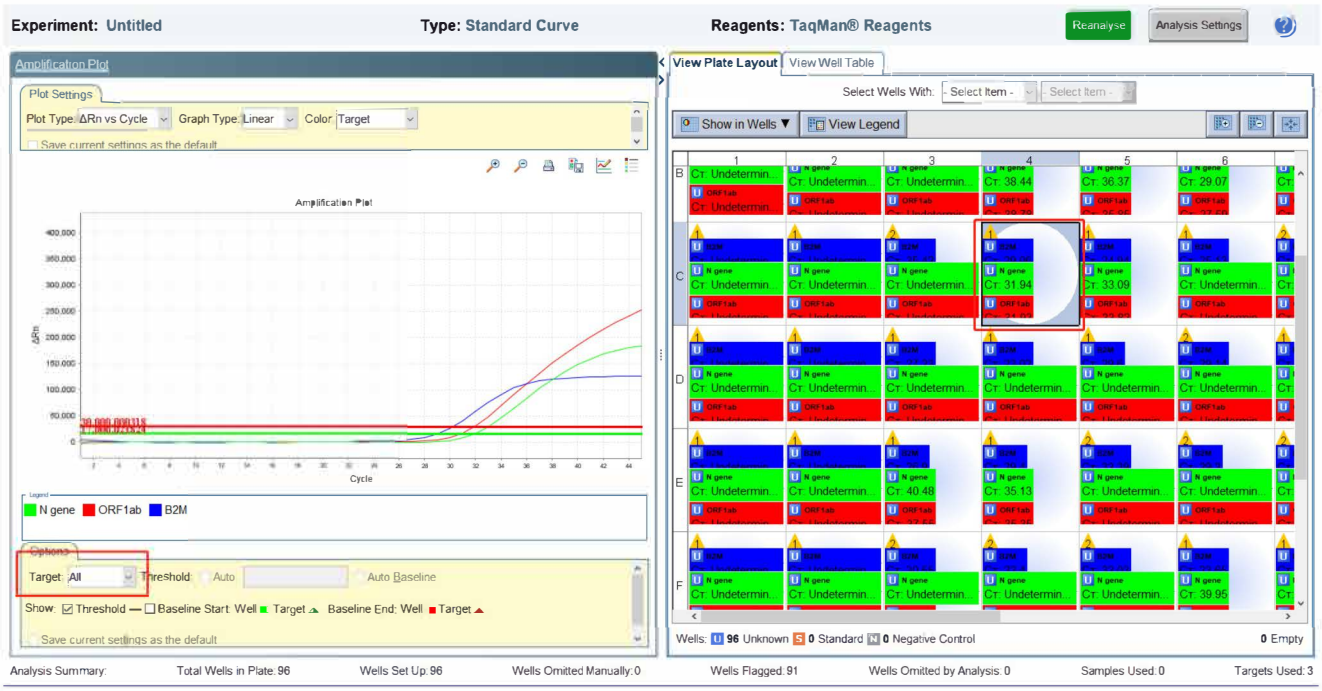
c. Adjust the threshold just above the curve from NTC (noise).

d. Repeat the steps for Target 2(N gene) and Target 3(B2M).

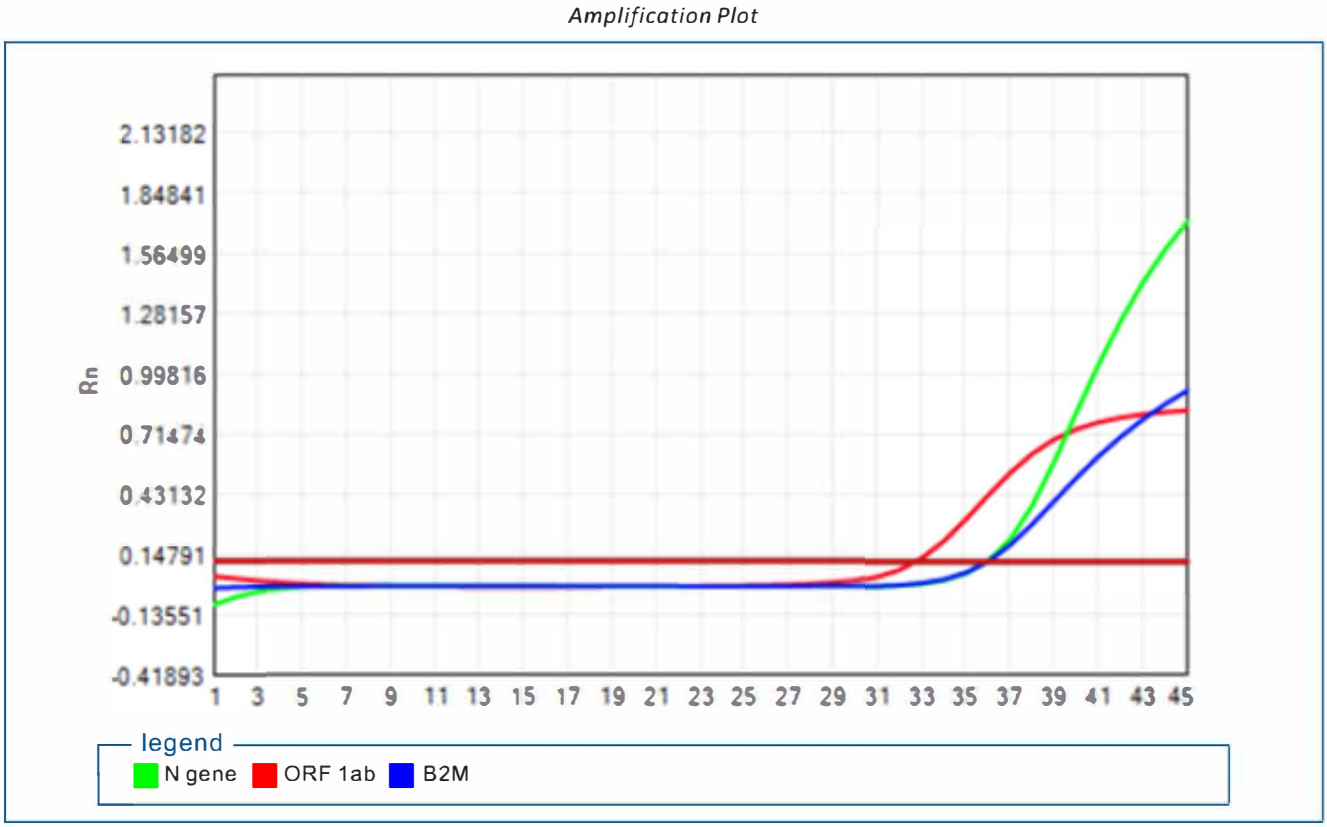
4. Click Analyze. The software analyzes the data with the settings.



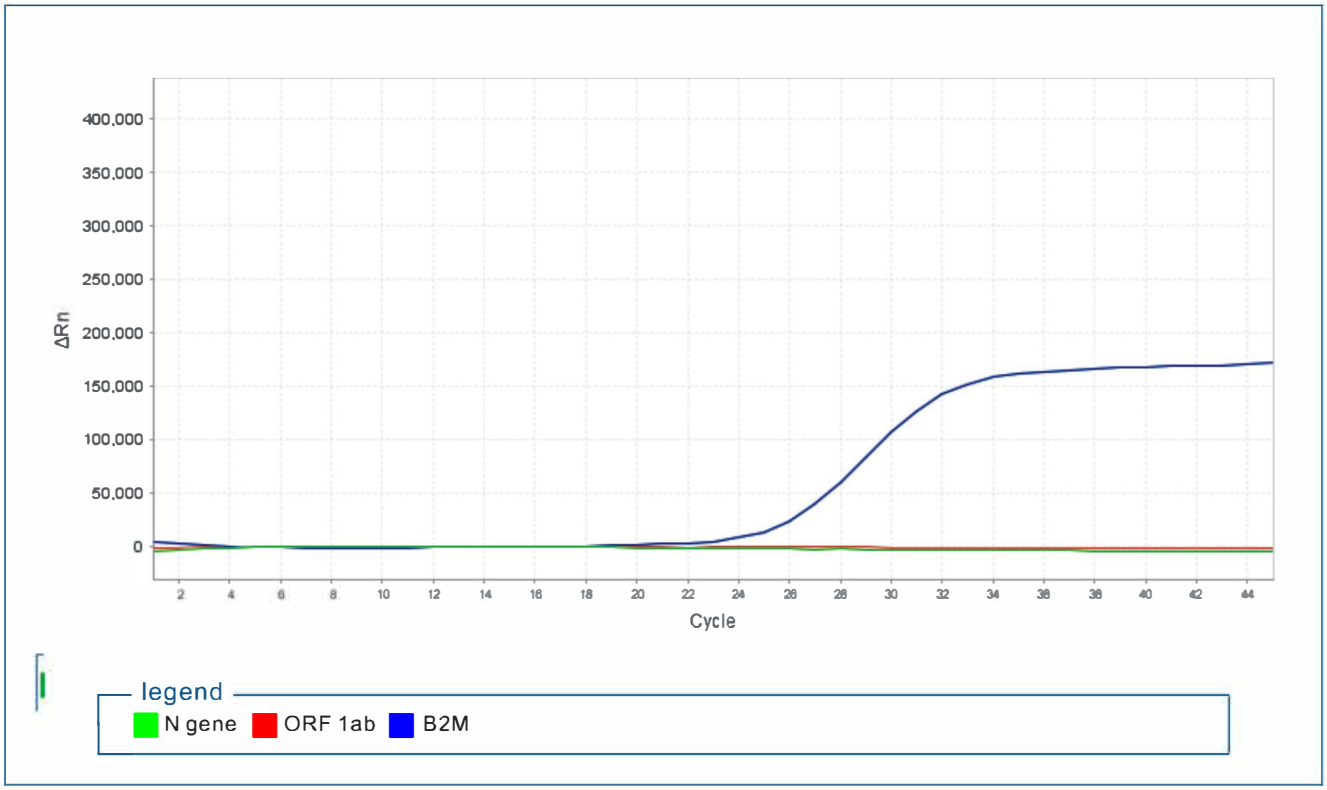
5. To review a Ct value of a sample, click the well containing the sample as shown in the figure below. In the Target drop down, select the target for review.



6. Example of a positive sample amplification curve



7.Example of a nagative sample amplification curve



9.Results

9.1 Quality Control and Validity of Results

Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. Quality control procedures are intended to monitor reagent and assay performance.

- One positive control and one blank control are processed with each batch.

- Always include a blank control and a positive control in each amplification and detection run. If below two situations are achieved, this test is deemed to be valid.

The Ct value in any fluorescent detection channel of Blank Control should be Undet.

The Ct value in any fluorescent detection channel of positive control should be ⩽34.

9.2 Interpretation of Results

Examination and Interpretation of Controls – Positive, Blank and Internal:

The controls for the COVID-19 Real-time PCR Kit (HBRT-COVID-19) for detecting SARS-CoV-2 are evaluated using the nucleic acid amplification curve and Ct values generated by the RT-PCR system software. The Ct cut-off values were determined using the receiver operator characteristic curves of the tested clinical samples. The Ct value in any fluorescent detection channel of blank control should be Undet, and there should be no sigmoidal amplification curve.The Ct Value of any fluorescent detection channel for a valid positive control should not be higher than 34 and there should be sigmoidal amplification curve for each channel (FAM, HEX/JOE, and Cy5).

All clinical samples should exhibit fluorescence growth curves in the Cy5 channel that cross the threshold line within 40 cycles (Ct⩽ 40), thus indicating the presence of the human B2M gene. Experimental analysis found that the Ct values for valid clinical specimen either negative or positive should not be no higher than 40. Thus, the Ct value in the Cy5 channel for a valid internal control should not be no higher than 40, and there should be a sigmoidal amplification curve.

Below table is a brief summary of expected Performance of Controls Included in the COVID-19 Real-time PCR Kit (HBRT-COVID-19).

Control Type	Used to monitor	FAM	HEX/JOE	Cy5	Expected Ct Values
Positive	Substantial reagent failure including primer and probe integrity	+	+	+	⩽34
Blank	Reagent and/or environmental contamination	-	-	-	None detected
Internal (Included in PCR Mix)	Failure in specimen collection, lysis and extraction, and PCR amplification procedure	-	-	+	⩽40

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

Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and blank control have been examined and determined to be valid and acceptable.

A specimen is positive for SARS-CoV-2 if there is a sigmoidal amplification curve in the FAM and HEX/JOE channel, the Ct value is not higher than 40.

A specimen is negative for SARS-CoV-2 if there is no sigmoidal amplification curve in the FAM and HEX/JOE channel, there is a Ct value of "0" or "no data available", and there is a sigmoidal amplification curve in the CY5 channel with Ct value is not higher than 40.

An exemplary interpretation of the test results using COVID-19 Real-time PCR Kit for detecting SARS-CoV-2 is provided in below Table.

FAM	HEX/JOE	Cy5	Result Interpretation	Report	Actions
+	+	+/-	SARS-CoV-2 detected	Presumptive positive SARS-CoV-2	Report results to CDC and sender. Contact CDC immediately for instructions for transfer of the specimen to CDC for additional testing and further guidance.
If only one of the two targets is positive		+/-	Inconclusive Result	Inconclusive	Repeat extraction and rRT-PCR. If the repeated result remains inconclusive, contact CDC immediately for instructions for transfer of the specimen to CDC for additional testing and further guidance.
-	-	+	SARS-CoV-2 not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.
-	-	-	Invalid Result	Invalid	Repeat extraction and RT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

Note:

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

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

9.3 Procedural Limitations

Reliable results depend on proper sample collection, storage and handling procedures.

- This test is intended to be used for the detection of SARS-CoV-2 RNA in oropharyngeal swab and nasopharyngeal swab specimen. Other specimen types (such as: Sputum, Anal swab, Stool, blood etc.) need to be further validated.

- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g.,presence of symptoms), and/or stage of infection.

- False negative or invalid results may occur due to interference. False-negative results may arise from:

  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use

- False-positive results may arise from:

  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling

- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

- A positive result indicates the detection of nucleic acid from the relevant virus.

- Nucleic acid may persist even after the virus is no longer viable.

- Laboratories are required to report all positive results to the appropriate public health authorities.

10.Conditions of Authorization for the Laboratory

Clinical laboratories using the COVID-19 Real-Time PCR Kit for detecting SARS-CoV-2, the relevant Conditions of authorization are listed below:

A. Authorized laboratories using COVID-19 Real-Time PCR Kit will include with result reports of this product, 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 COVID-19 Real-Time PCR Kit will use COVID-19 Real-Time PCR Kit as outlined in the 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 use this product are not permitted.

C. Authorized laboratories that receive COVID-19 Real-Time PCR Kit will notify the relevant public health authorities of their intent to run this product prior to initiating testing.

D. Authorized laboratories using COVID-19 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 COVID-19 Real-Time PCR Kit and report to Hybribio (isw@hybribio. cn) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of this product of which they become aware.

F. All laboratory personnel using COVID-19 Real-Time PCR Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use this product in accordance with the authorized labeling.

11.Performance Characteristics

11.1 Analytical Performance

Limit of Detection (LoD):

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive.

A preliminary LoD was determined by testing 5-fold serial dilutions of SARS-CoV-2 pseudovirus spiked into pooled negative samples. The approximate LoD was further fine-tuned by testing 2-fold dilutions of clinical samples 20 replicates extracted by each extraction method. Both of the oropharyngeal swab and nasopharyngeal swab were tested.

As shown in Table 1, the concentration level with observed hit rates greater than or equal to 95% were 5×10^2 copies/μL for ORF1ab gene (Target 1) and 1×10^2 copies/ml for N gene (Target 2).

Table 1 Preliminary LoD results

concentration (copies/μL)	total valid results	Hit rate (%)		Mean Ct	
		Target1 (ORF1ab gene)	Target 2 (N gene)	Target1 (ORF1ab gene)	Target 2 (N gene)
2.5×10^3	5	100	100	35.37	32.24
5×10^2	5	100	100	37.23	34.41
1×10^2	5	60	100	38.58	35.46

As shown in Table 2-3, the 95% hit rates were 5×10^2 copies/ml for ORF1ab gene (Target 1) of both oropharyngeal swab specimens and nasopharyngeal swab specimens. And the hit rates were all 100% for N gene (target2) at test concentration. Therefore, LoD of HBRT-COVID-19 was determined to be 5×10^2 copies/ml for oropharyngeal swab and nasopharyngeal swab.

Table 2 LoD validation of oropharyngeal swab sample (OPS)

oropharyngeal swab sample(OPS)		Extracted by Thermofisher KingFisher Flex System		Extracted by Bioer GenePure Pro Nucleic Acid Purification System	
concentration (copies/μL)	total valid results	Hit rate (%)		Hit rate (%)	
		Target1 (ORF1ab)	Target 2 (N)	Target1 (ORF1ab)	Target 2 (N)
1×10^3	20	100	100	100	100
5×10^2	20	≥95	100	≥95	100
2.5×10^2	20	<80	<95	<80	<95

Table 3 LoD validation of nasopharyngeal swab sample (NPS)

nasopharyngeal swab sample(NPS)		Extracted by Thermofisher KingFisher Flex System		Extracted by Bioer GenePure Pro Nucleic Acid Purification System	
concentration (copies/μL)	total valid results	Hit rate (%)		Hit rate (%)	
		Target1 (ORF1ab)	Target 2 (N)	Target1 (ORF1ab)	Target 2 (N)
1×10^3	20	100	100	100	100
5×10^2	20	≥95	100	≥95	100
2.5×10^2	20	<80	<95	<80	<95

Reactivity/inclusivity:

In silico analysis concluded that HBRT-COVID-19 kit will detect all analyzed SARS-CoV-2 sequences in NCBI databases (n=100), and had 100% match for target1 (ORF1ab) and target 2 (N).

Cross-reactivity:

In silico analysis

The in silicon analysis for possible cross-reactions with all the organism listed in Table 4 was conducted by mapping primers in HBRT-COVID-19 individually to the sequence download from NCBI databases. If any two of the primers were mapped to a sequence on opposite strands with short distance apart, potential application were flagged. Analysis results were shown in Table 4.

Table 4 *In silico analysis for SARS-CoV-2*

Strain	In silico analysis for % identity to Target 1(ORF1ab)	In silico analysis for % identity to Target 2 (N)
SARS coronavirus	9.9%	81.8%
Human coronavirus 229E	58.7%	55.3%
Human coronavirus OC43	51.1%	56.9%
Human coronavirus HKU1	47.1%	53.5%
Human coronavirus NL63	56.7%	52.3%
MERS coronavirus	No alignment was found	No alignment was found
Adenovirus	No alignment was found	No alignment was found
Human Metapneumovirus (hMPV)	No alignment was found	No alignment was found
Parainfluenza virus type1	No alignment was found	No alignment was found

Strain	In silico analysisi for % identity to Target 1(ORF1ab)	In silico analysisi for % identity to Target 2 (N)
Parainfluenza virus type2	No alignment was found	No alignment was found
Parainfluenza virus type3	No alignment was found	No alignment was found
arainfluenza virus type4	No alignment was found	No alignment was found
Influenza A(H1N1)	No alignment was found	No alignment was found
Influenza B	No alignment was found	No alignment was found
EV	No alignment was found	No alignment was found
RSV	No alignment was found	No alignment was found
RV	No alignment was found	No alignment was found
Chlamydia pneumoniae	No alignment was found	No alignment was found
Haemophilus influenzae	No alignment was found	No alignment was found
Legionella pneumophila	No alignment was found	No alignment was found
MTB Mycobacterium bovis subsp. Bovis	No alignment was found	No alignment was found
Streptococcus pneumoniae	No alignment was found	No alignment was found
Streptococcus pyrogenes	No alignment was found	No alignment was found
Bordetella pertussis	No alignment was found	No alignment was found
Mycoplasma pneumoniae	No alignment was found	No alignment was found
Pneumocystis jirovecii	No alignment was found	No alignment was found
Influenza C	No alignment was found	No alignment was found
Parechovirus	No alignment was found	No alignment was found
Candida albicans	No alignment was found	No alignment was found
Corynebacterium diphtheriae	No alignment was found	No alignment was found
Legionella non-pneumophila	No alignment was found	No alignment was found
Bacillus anthracosis(Anthrax)	No alignment was found	No alignment was found

Strain	In silico analysisi for % identity to Target 1(ORF1ab)	In silico analysisi for % identity to Target 2 (N)
Moraxella cararrhails	No alignment was found	No alignment was found
Neisseria elongate and meningitides	No alignment was found	No alignment was found
Pseudomonas aeruginosa	No alignment was found	No alignment was found
Staphylococcus epidermis	No alignment was found	No alignment was found
Staphylococcus salivarius	No alignment was found	No alignment was found
Letospirosis	No alignment was found	No alignment was found
Chlamydia psittaci	No alignment was found	No alignment was found
Coxilla burneti(Q-Fever)	No alignment was found	No alignment was found
Streptococcus aureus	No alignment was found	No alignment was found

Cross reactivity testing

Cross-reactivity of HBRT-COVID-19 was evaluated by testing a panel of multiple unique sub-species of microorganisms. High titer stocks of the potentially cross-reacting microorganisms or corresponding extracts were spiked into negative simulated clinical matrix to a concentration level of 1.0×10^{7} CFU/mL for bacterial and fungal isolates, or 1.0×10^{6} copies/mL for virus. All microbial samples were tested in triplicate.

The BLAST searches did not identify any cross-reactivity with the exception of SARS coronavirus, which is in the same subgenus (Sarbecovirus) as SARS-CoV-2(identical sites>80%). Therefore, the region of low homologous was chosen for probes design in the kit to ensure the analysis specificity. In the cross reactivity test, none of the organisms tested interfered with HBRT-COVID-19 performance by generating false positive results, including SARS coronavirus.

Sample type equivalency:

Equivalence between nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) sample types was evaluated using SARS-CoV-2 VLPs spiked into paired negative samples (individual samples, not pooled) to prepare contrived low positive (approximately 2x Target 1 LoD) and moderate positive (approximately 6x Target 1 LoD) samples for each sample type. A total of 20 low positive paired samples, 10 moderate positive paired samples, and 10 negative paired samples were tested.

As shown in Table 5, all low positive and moderate positive paired samples were positive in both sample matrices. All negative paired samples were negative in both sample types. The observed Ct values for contrived positive samples were comparable in both sample types.

Table 5 Nasopharyngeal vs oropharyngeal sample type comparison

Specimen type	Sample Concentration	N	Target 1(ORF1ab)		Target 2(N)	
			% Positive	Mean Ct (95% CI)	% Positive	Mean Ct (95% CI)
NPS	Low positive	20	100	36.50 (36.22-36.78)	100	33.52 (33.32-33.72)
OPS			100	36.35 (36.07-36.64)	100	33.42 (33.10-33.74)
NPS	Moderate positive	10	100	35.80 (35.39-36.21)	100	33.40 (32.73-34.07)
OPS			100	35.37 (35.02-35.72)	100	32.55 (32.33-32.77)
NPS	Negative	10	0	n/a	0	n/a
OPS			0	n/a	0	n/a

11.2 Clinical Performance

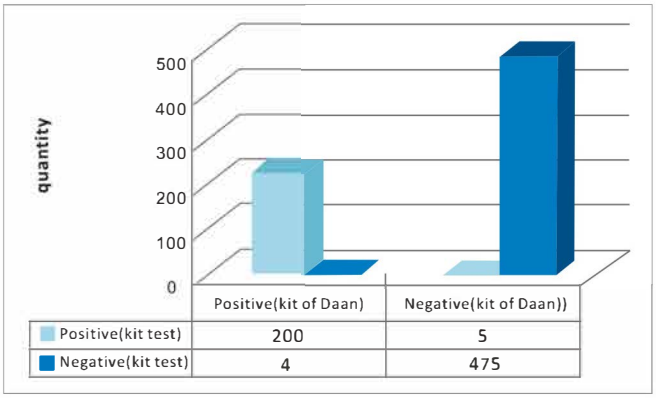
Retrospective Clinical Trail:

This study was conducted with 684 clinical specimens,total 510 cases collected by three hospitals.

- Consistency with comparator: In this clinical study, a commercial kit was used as a comparator to compare consistency, below graph shows the result:

Table 1 Clinical evaluation with 684 specimens

Specimen type	Number	Hybribio		Comparator	
		Positive	Negative	Positive	Negative
Oropharyngeal swab	684	205	479	204	480
Clinical Performance		**Agreements**		**95% CI**	
Positive coincidence rate		98.04%		95.07%-99.24%	
Negative coincidence rate		98.96%		97.59%-99.55%	
Total coincidence rate		98.68%		97.52%-99.31%	
Kappa=0.969		P <0.05			



- Clinical sensitivity and specificity:

Clinical diagnostic criteria (patient status determination):

Criterion 1. Fourteen days prior to the onset of illness, the patient (i) traveled to or resided in

Wuhan, (ii) had contact with a patient with a fever and respiratory symptoms, or (iii) was exposed to a cluster of COVID-19 patients.

Criterion 2. Clinical presentation indicates that (i) the patient has a fever, (ii) the patient's chest images shows multiple mottling, consolidation, or ground glass opacities, or (iii) the patient shows leukopenia or lymphopenia.

Criterion 3. Laboratory test of sputum, oropharyngeal swabs, or lower respiratory specimens for SARS-Cov-2 returns positive. Laboratory detection of SARS-CoV-2 virus includes RT-PCR detection and viral sequencing showing high homology with known SARS-CoV-2 sequence.

*Clinical status of a patient is determined as positive if all three criteria above are met.

Summary of the result:

According to the statistics, the clinical sensitivity was 99.41 %(95% CI: 96.71%-99.90%), the clinical specificity was 99.71 %(95% CI: 98.36%-99.95%). See table below for details.

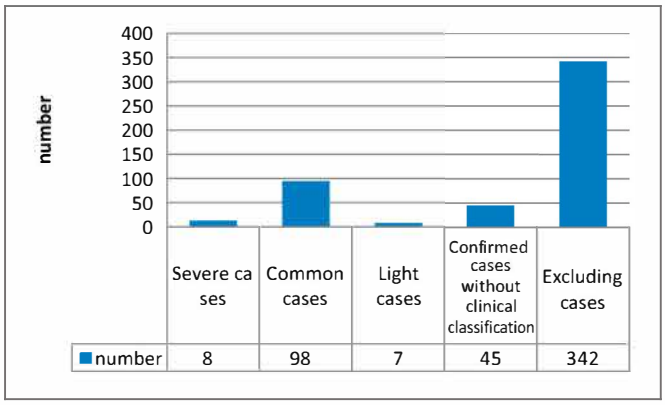


Table 2 Clinical evaluation with 500 cases

	Agreements	95% CI
Clinical sensitivity	99.41%	96.71%-99.90%
Clinical specificity	99.71%	98.36%-99.95%
Total coincidence rate	99.61%	98.58%-99.89%
Kappa=0.991	P <0.05	

12.Reference

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- *Clinical and Laboratory Standards Institute (CLSI). Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. CLSI Document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.*

- *Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.*

13.Additional Information

13.1 Key test Features

Sample type	oropharyngeal swab, nasopharyngeal swab specimen
Minimum amount of sample required	300 µL
RNA processing volume	5 µL

13.2 Labels

The following labels are used in COVID-19 Real-Time PCR Kit

IVD	In Vitro Diagnostic medical device
REF	Catalogue number
	Consult instructions for use
EC REP	Authorized representative in the European community
LOT	Batch Code
	Use-by date

	Temperature limit
	Contains sufficient for <n> tests
	Manufacturer
D	Distributed by
SN	Serial number
	Date of Manufacture
COVID-19 RT-PCR Mix	COVID-19 RT-PCR Mix
COVID-19 Enzyme Mix	COVID-19 Enzyme Mix
Positive Control	Positive Control
Blank Control	Blank Control

13.3 Contact and Representatives

Chaozhou Hybribio Biochemistry Ltd.

D5-3-3-4, High And New Area,
Economic Development Experimental Zone
521000 Chaozhou, Guangdong
PEOPLE'S REPUBLIC OF CHINA

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