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

Product: Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)
Manufacturer: Daan Gene Co., Ltd.
EUL Number: EUL 0493-141-00
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.

The Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) with product codes DA0930, DA0931 and DA0932, Rest-of-World regulatory version manufactured by Daan Gene Co., Ltd., No.19, Xiangshan Road, Science Park, High & New Technology Development District Guangzhou, Guangdong 510665, China was listed as eligible for WHO procurement on 14 May 2020[1].

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the product accepted under EUL for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Change of manufacturer's name from Da An Gene Co., Ltd of Sun Yatsen University to Daan Gene Co., Ltd. Labelling was amended to include the new name.	22-Nov-2021

[1] EUL renewal assessment is in progress.

Intended use:

According to the claim of intended use from Daan Gene Co., Ltd., "*the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal swab and sputum specimens collected from individuals who are suspected of COVID-19 by their healthcare provider. The kit is manually operated and it is intended for use by clinical laboratory personnel specifically trained in PCR in level 2 biosafety laboratory.*

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories shall follow jurisdiction regulations to report all positive results to the appropriate competent 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 evaluated in combination with clinical observations, patient history, and epidemiological information.

The Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures."

Specimen types that were validated:

Oropharyngeal swab and sputum specimens.

Test kit contents:

Component	**24 tests (product code DA0930)**	**48 tests (product code DA0931)**	**96 tests (product code DA0932)**
(ORF1ab/N) PCR reaction solution A	1 vial x 900 µL	1 vial x 900 µL	2 vials x 900 µL
NC (ORF1ab/N) PCR reaction solution B	1 vial x 100 µL	1 vial x 200 µL	2 vials x 200 µL
NC (ORF1ab/N) negative control	1 vial x 400µL		
NC (ORF1ab/N) positive control	1 vial x 400 µL		

Items required but not provided:

Extraction/Purification:

Extraction reagents:

- YHXB No. 20170583, YHXB No. 20150302 products manufactured by DAAN.
- QIAamp Viral RNA Mini Kit, 52906.

Amplification and detection instruments:

- ABI 7500, LightCycler480

Storage:
Store all reagents at -20±5℃.

Shelf-life upon manufacture:

12 months, real-time stability study is ongoing.

Warnings/limitations:

Refer to the instructions for use (IFU).

Product dossier assessment

Daan Gene Co., Ltd. submitted a product dossier for the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and an external assessor appointed by WHO.

Post listing Commitments for EUL:

1. The manufacturer will submit the assessment of the the traceability of the product's calibrators with the WHO interim International Reference material when this becomes available.

2. The manufacturer will submit the completed stability study report by 21 August 2021, and the interim report by 30 September 2021. Submitted evidence is under review.

3. The manufacturer will submit additional clinical evidence will be submitted by 27 July 2020. Submitted evidence is under review.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Daan Gene Co., 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 and external technical experts (assessors), it was established that sufficient information was provided by Daan Gene Co., 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 *"Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"* (ISBN 978-92-4-001531-9.

Daan Gene Co., Ltd is also required to report complaints related to the product. 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 "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"*[2]

Scope and duration of procurement eligibility

[2] https://www.who.int/publications/i/item/9789240015319

Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA with product codes DA0930, DA0931 and DA0932 manufactured by Daan Gene Co., 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 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, Daan Gene Co., Ltd. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. Daan Gene Co., Ltd. is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days.

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. Labels

1 Package specification

The kits have three specifications: Large package, 24test/kit, large package, 48test/kit, large package, 96test/kit, corresponding to product code DA0930, DA0931, DA0932.

2 Component labels

1) Large package, 24 tests/kit

NC (ORF1ab/N) PCR reaction solution A Lot：　　450μl/tube Expiry Date：

NC (ORF1ab/N) PCR reaction solution B Lot：　　100μl/tube Expiry Date：

2) Large package, 48 tests/kit and 96 tests/kit

NC (ORF1ab/N) PCR reaction solution A Lot：　　900μl/tube Expiry Date：

NC (ORF1ab/N) PCR reaction solution B Lot：　　200μl/tube Expiry Date：

Note: two tubes will be placed into the 96 tests/kit.

3) Controls for three specifications

NC (ORF1ab/N) negative control Lot：　　400μl/tube Expiry Date ：

NC (ORF1ab/N) positive control Lot：　　400μl/tube Expiry Date：

3 Outer labels

Detection Kit for 2019 Novel Coronavirus (2019-nCoV)RNA
(PCR-Fluorescence Probing)

DaAn Gene

Detection Kit for 2019 Novel Coronavirus (2019-nCoV)RNA
(PCR-Fluorescence Probing)

DaAn Gene

Production License number: YSYJXSCX No.2004099
Medical Device Registration Certificate No.GXZZ No.20203400063

Manufacturer: DA AN GENE CO., LTD.
Production address: No.19, Xiangshan Road, Science Park, HighNew Technology Development Distrct, Guangzhou, Guangdong, China; No. 6, Lizhishan Road, Science Park, High & New Technology Development Distrct, Guangzhou, Guangdong, China.
Postal code: 510665
Tel: 8008304008 +86-20-32290789
Fax: +86-20-32068820
Website:www.daangene.com

DaAn Gene

IVD

REF DA0932

LOT

-15°C -25°C

96

Detection Kit for 2019 Novel Coronavirus (2019-nCoV)RNA
(PCR-Fluorescence Probing)

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Postal code: 510665
Tel: 8008304008 +86-20-32290789
Fax: +86-20-32068820
Website:www.daangene.com

DaAn Gene

IVD

REF DA0931

LOT

-15°C -25°C

48

Detection Kit for 2019 Novel Coronavirus (2019-nCoV)RNA
(PCR-Fluorescence Probing)

DaAn Gene

Detection Kit for 2019 Novel Coronavirus (2019-nCoV)RNA
(PCR-Fluorescence Probing)

DaAn Gene

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Postal code: 510665
Tel: 8008304008 +86-20-32290789
Fax: +86-20-32068820
Website:www.daangene.com

DaAn Gene

IVD

REF DA0930

LOT

-15°C -25°C

24

Detection Kit for 2019 Novel Coronavirus (2019-nCoV)RNA
(PCR-Fluorescence Probing)

DaAn Gene

2. Instructions for use[3]

[3] 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.

Instructions for Use of Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA

(PCR-Fluorescence Probing)

DA0930
DA0931
DA0932

[Product Name]

Generic name: Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)

[Package Specifications]

Large package, 24 tests/kit; large package, 48 tests/kit; large package, 96 tests/kit;

[Intended Use]

The Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal swab and sputum specimens collected from individuals who are suspected of COVID-19 by their healthcare provider. The kit is manually operated and it is intended for use by clinical laboratory personnel specifically trained in PCR in level 2 biosafety laboratory.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories shall follow jurisdiction regulations to report all positive results to the appropriate competent 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 evaluated in combination with clinical observations, patient history, and epidemiological information.

The Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time

PCR and in vitro diagnostic procedures.

[Summary and Explanation]

The 2019 novel coronavirus (SARS-CoV-2) is a new beta coronavirus (in the same family as SARS-CoV and MERS-CoV). Infection with SARS-CoV-2 causes respiratory symptoms, fever, fatigue and in severe cases, pneumonia, severe acute respiratory syndrome, organ failure and even death. The recent increase in reported cases of pneumonia potentially associated with SARS-CoV-2 throughout the world necessitates preparedness and response in healthcare and lab facilities. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies, and surveillance studies.

The Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) is a multiplexed real-time RT-PCR test for the qualitative detection of SARS-CoV-2 RNA in respiratory specimens (e.g., oropharyngeal swab and sputum specimens) from individuals suspected of COVID-19 by their healthcare providers. The product contains oligonucleotide primers, labeled oligonucleotide probes, and control material used in real-time RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA.

[Test Principle]

The Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The 2019-nCoV primer and probe sets is designed to detect ORF1ab and nucleocapsid protein (N) gene sequences from the SARS-CoV-2 in oropharyngeal swab and sputum specimens collected from patients with signs and symptoms of infection who are suspected of COVID-19. Further, Human housekeeping gene RNP (Ribonuclease P) was developed as the target gene for the internal control (the internal control probe is labeled with Cy5) for monitoring the specimen collection, nucleic acid extraction process and PCR amplification process which can reduce the occurrence of false negative results.

[Warnings and Precautions]

1. This product is used for in vitro test only. Please read the instructions carefully before test;

2. Bring all reagents to room temperature before commencing IVD.

3. Follow standard precautions: All patient specimens and positive controls should be considered infectious and/or biohazardous and handled accordingly with safe laboratory procedures.

4. Use powder-free gloves, shoe covers, hats, eye shields and personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious specimens.

5. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled, and should avoid any behavior that may lead to infection.

6. In order to avoid any potential biological hazards in the specimens, the test specimens should be regarded as infectious and avoid contact with human skin and mucosa; the specimens should be handled in a biosafety cabinet that prevents aerosol outflow. The test tubes and tips used in the specimen preparation area should be poured into a container containing disinfectant and sterilized with the medical wastes before discarding; specimen handling and processing must comply with relevant regulations: including the General Biosafety Standard for Microbiological and Biomedical Laboratories and Regulations on the Administration of Medical Wastes issued by the Ministry of Health;

7. Product processing: After PCR, the product is likely to cause contamination. All reaction tubes should be put into a biosafety garbage disposal bag or other container by the person who is no longer involved in the testing that day, and then discarded after these reaction tubes are completely sealed;

8. Avoid RNase contamination during the whole process. Wear work clothes, disposable gloves and masks during the testing. Complete the operation in a well-ventilated chemical hood or biosafety cabinet that is clean. The chemical hood or biosafety cabinet should be disinfected and sterilized by ultraviolet light to prevent any harmful substances from entering the respiratory tract;

9. Use autoclaved disposable centrifuge tubes and tips or purchase DNase-free and RNase-free centrifuge tubes and tips;

10. Thaw PCR detection reagents completely before use, and use after centrifugation at 8000 rpm for 10 seconds, but avoid repeated freezing and thawing, and cycles of the repeated freezing and thawing should not exceed 7 times;

11. False positive result may appear if cross contamination is not well controlled

during specimen processing;

12. The laboratory management shall be in strict accordance with the management practice of PCR gene amplification laboratory. The laboratory personnel must receive professional training. The testing process shall be strictly divided into different areas (reagent preparation area, specimen preparation area, amplification test area). All consumables shall be sterilized for single use. Special instruments and equipments shall be used at each stage of testing. No cross-utilization of the supplies of each area in each stage shall be allowed;

13. After conducting the test, 10% hypochlorite or 75% alcohol should be used to disinfect the worktable and pipette, followed by exposing them in ultraviolet light for 20~30 minutes;

14. After the RNA specimen extraction is completed, it is recommended to proceed to the next test run immediately; otherwise, please store the extract at -20±5°C for use (within 24 h);

15. Quality control must be performed over each test run.

16. Avoid exposure of the IVD to certain environmental (e.g. The kit must be stored at -15°C or colder when not in use and keep away from the light.) and ensure that it is stored on a flat surface.

17. A variety of factors may cause performance changes during the storage, transportation, and use of reagents, such as improper storage and transportation, non-standard specimen collection, specimen processing and testing. Please strictly follow the Instructions. Due to the characteristics of the specimen collection process such as sampling with swabs and the virus infection process itself, there may be false negative results caused by insufficient specimens collected. Therefore, the test results should be comprehensively judged in combination with other clinical diagnosis and treatment information and re-testing should be carried out if necessary.

18. When an indication of instability or deterioration of the control value is outside the expected range, retest is needed.

19. All contents in this package are prepared and validated for the intended testing purpose. Replacement of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

[Main Components]

Component name		Specification	Quantity	Main constituents
PCR detection reagents (large package, 24 tests/kit, Cat#DA0930)	NC (ORF1ab/N) PCR reaction solution A	450 μL/ tube	1	Specific primers, probes, tris(hydroxymethyl)amino methane-hydrochloric acid buffer
	NC (ORF1ab/N) PCR reaction solution B	100 μL/ tube	1	Hot start Taq DNA polymerase, c-MMLV reverse transcriptase, etc.
PCR detection reagents (large package, 48 tests/kit, Cat#DA0931)	NC (ORF1ab/N) PCR reaction solution A	900 μL/ tube	1	Specific primers, probes, tris(hydroxymethyl)amino methane-hydrochloric acid buffer
	NC(ORF1ab/N) PCR reaction solution B	200 μL/ tube	1	Hot start Taq DNA polymerase, c-MMLV reverse transcriptase
PCR detection reagents (large package, 96 tests/kit, Cat#DA0932)	NC (ORF1ab/N) PCR reaction solution A	900 μL/ tube	2	Specific primers, probes, tris(hydroxymethyl)amino methane-hydrochloric acid buffer
	NC (ORF1ab/N) PCR reaction solution B	200 μL/ tube	2	Hot start Taq DNA polymerase, c-MMLV reverse transcriptase

Controls (all packages)	NC (ORF1ab/N) negative control	400 μL/ tube	1	Pseudovirus with internal standard fragment
	NC (ORF1ab/N) positive control	400 μL/ tube	1	Pseudovirus containing target fragments, pseudovirus containing internal standard fragments

The above components in different batches of kits cannot be interchangeable.

Description of negative/positive control materials: the positive control material is pseudovirus containing the target fragments and internal standard fragments, while the negative control material is pseudovirus containing an internal standard fragments. During use, they should be involved in extraction and should be considered as an infectious substance. They shall be handled and disposed in accordance with relevant regulations. When handling the positive control, please take precautions to avoid contamination of the specimen. Failure to take proper precautions when handling the positive control could result in a false positive result.

[Other materials that are required but not provided]

- QIAamp Viral RNA Mini Kit (52904/52906) by QIAGEN, RNA extraction or purification reagents(Cat#DA0623, YHXB No. 20170583; Cat#DA0900, YHXB No. 20150302) by Daan.

- 1.5 mL microcentrifuge tubes (DNase/RNase free), RNase/DNase-free tips for pipettes, 8-tube strips for real-time PCR, 0.1 mL PCR tube 8-cap strips, White (LightCycler 480 II(V1.5)).

- ABI 7500 Real-Time PCR Systems with V2.4 software (Applied Biosystems) or LightCycler 480 II with V1.5 software (Roche).

- Disposable gloves, shoe covers, hats, eye shields and personal protective equipment (PPE).

- Racks for 1.5mL microcentrifuge tubes, Benchtop centrifuge, Vortex mixer, Transparent Multi-well Plate 96 for Applied Biosystems® 7500 Real-Time PCR

System, Adjustable calibrated pipettes, Automatic Nucleic Acid Extractor (Smart32(V1.0)) (used with the Cat#DA0623, YHXB No. 20170583; Cat#DA0900, YHXB No. 20150302).

- Saline
- 1×PBS
- Notes: Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

[Storage Conditions and Validity Date]

The kit is stored at -20±5°C, and the validity period is 12 months.

The reagent can be stored at 2~8°C for 7 days; the kit can be stored at 37°C for 3 days; repeated freezing and thawing should be avoided; repeated freezing and thawing cycles should not exceed 7 times and the times of decapping the reagent should not exceed 7.

Properties of all the components of the kit are stable during transport under low temperature by cold chain.

Upon receipt of test kits, immediately store them at -20±5°C, avoiding repeated freezing and thawing.

The kit is stable until expiration date when operating according to the Instruction for Use. Do not use kit reagents after their labeled expiration date.

See the product label for the date of manufacture and validity of the kit.

[Applicable Instruments]

ABI 7500(V2.4), LightCycler480 Ⅱ(V1.5)

[Specimen Requirements]

1. Applicable specimen types: oropharyngeal swabs, sputum.

2. Specimen collection (aseptic technique)

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/lab/guidelines-clinical-specimens.html.

Follow specimen collection devices manufacturer instructions for proper collection

methods

Oropharyngeal swab: Wipe the tonsil and posterior pharyngeal wall with two swabs at the same time, the swabs with a synthetic tip, such as nylon or polypropylene fiber (for example, CY-93050(oropharyngeal) from Shenzhen Huachenyang Technology Ltd. and 93050P clinical sampling swab from Shenzhen Mairuikelin Technology Ltd.). Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 1~3 mL of viral transport media, or equivalent.

Sputum: After the patient has a deep cough, collect the coughed sputum in the sterile screw-cap sterile test tube containing 3 mL saline or 1×PBS preservation solution.

3. Specimen storage and transportation

The specimens for virus isolation and RNA detection should be tested as soon as possible.

- Samples can be stored at 2~8°C for 72 hours after collection prior to extraction. If samples need be transported, maintain 2~8°C on ice packs for overnight shipment.
- For longer term storage, unextracted samples can be stored at ≤-70°C. If samples need be transported, maintain ≤-70°C on dry ice for overnight shipment.
- Extracted nucleic acids can be stored at ≤-70°C. If samples need be transported, maintain ≤-70°C for overnight shipment.

Note: Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.

[Test Method]

1. Specimen processing and RNA extraction (specimen processing area)

1.1 Specimen pre-processing

1.1.1 Sputum specimens

The collected SARS-COV-2 sputum specimen is added to a sampling tube containing 3 mL of physiological saline or 1×PBS preservation solution, shaken and mixed, and left to stand for 30 minutes. After centrifugation at 3000 rpm for 30 seconds, the supernatant is taken as the specimen to be tested spare.

Note: The pretreatment of the sputum specimen in the DA0623 extraction kit is a treatment method for bacterial specimens, and is not applicable to SARS-COV-2 specimens.

1.1.2 Oropharyngeal swab specimens

The oropharyngeal swab specimen is added to the virus storage solution, and the nucleic acid can be extracted directly after shaking and mixing.

1.2 RNA Extraction

RNA should be collected from a fresh specimen to ensure suitable RNA quality and quantity. The NC (ORF1ab/N) positive control and NC (ORF1ab/N) negative control should be processed simultaneously, alongside the specimen RNA should be extracted using QIAamp Viral RNA Mini Kit (Cat#52904 or 52906) or Nucleic Acid Extraction Kit (Cat#DA0623 or Cat#DA0900) produced by Daan Gene Co., Ltd. according to the manufacturer's instructions. Extraction with Daan Gene Nucleic Acid Extraction Kit (Cat#DA0623 or Cat#DA0900) uses 200 μL of clinical specimen or control and the final elution volume is 70μL. Extraction with QIAgen's QIAamp Viral RNA Mini Kit uses 140 μL of clinical specimen or control and the final elution volume is 50 μL. For specific steps, please follow the Instruction for Use of each extraction kit. The extracted RNA should be used immediately or stored at -70°C for use later. When handling the positive control, please take precautions to avoid contamination of the specimen. Failure to take proper precautions when handling the positive control could result in a false positive result.

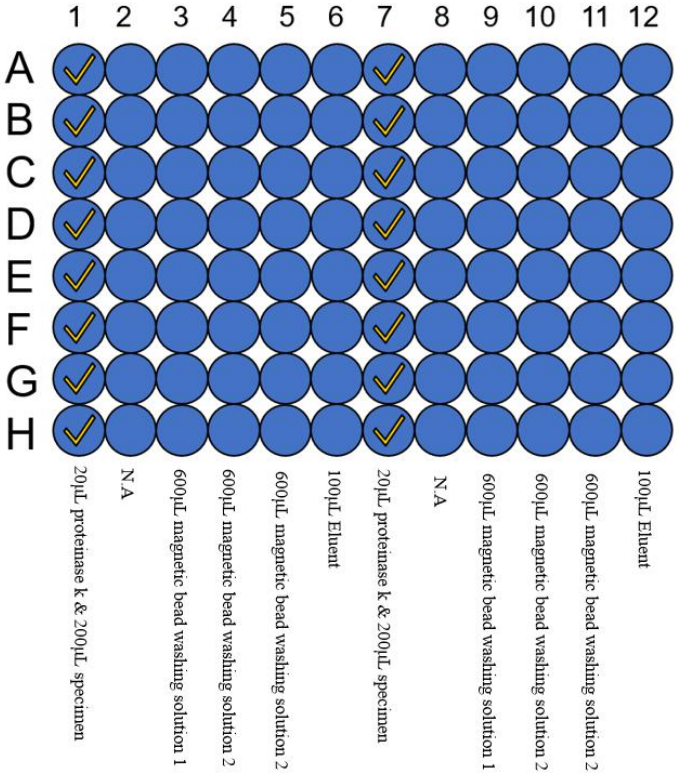
Following is the process mapping steps using the 3 different extraction kits.

1.2.1 DA0623 extraction kit (Pre-packaged: 32 reactions/kit):

Take the pre-packaged 96-well plate from the kit and mix it upside down for about 10 times. Centrifuge at 500 rpm for 1 minute by 96-well plate centrifuge or shake the reagent to the bottom of the deep well plate manually. Tear off the sealing film carefully to prevent liquid spill.

Use pipettor to add 200 μL of specimen (Sputum or oropharyngeal swab), 20 μL of proteinase K that was packaged in the kit to the first and seventh columns (Rows A to H) of the 96 deep-well reagent plate. Rows A to H in the first column contains 8 samples, and rows A to H in the seventh column contains another 8 samples, in total 16 samples can be extracted with one 96 deep-well reagent plate. Please see diagram as follows.

96 hole deep hole plate



Note: Proteinase K is a proteolytic enzyme. Its main function is the enzymolysis of nucleic acid binding histone during the extraction process, and to dissociate the nucleic acid to solution. It is a key reagent for nucleic acid extraction.

The 2nd column and the 8th column are blank, no reagents added to these two columns, and these two columns do not take part in extraction.

Place the deep-well plate into the Smart32 nucleic acid extraction instrument (software version No.: V1.0) for automated extraction. The automated extraction procedure is as follows:

Step	Well	Name	Waiting time (min)	Mixing time (min)	Magnetization number	Mixing speed	Volume (μL)	Temperature status	Temperature (°C)
1	2	Magnetic bead	0	1	3	Medium	400	Close	0
2	1	Magnetic bead bonding solution	0	15	3	Fast	750	The first well	70
3	3	Magnetic bead washing solution 1	0	2	2	Fast	600	Close	0
4	4	Magnetic bead washing solution 2	0	2	2	Medium	600	Close	0
5	5	Magnetic bead washing solution 2	0	2	2	Fast	600	Close	0
6	6	Elution	5	0	0	Slow	100	Close	0
7	6	Elution	0	5	3	Medium	100	The 6th well	70
8	3	Discard magnetic bead	0	1	0	Medium	600	Close	0

Note: “Close” in the column of temperature status means that the temperature control program is switched off.

After the running program is accomplished, the liquid in column 6 and 12 is nucleic acid solution, which is recommended to be used immediately. If the solution is going to be stored, use pipettor to transfer to 1.5 mL microcentrifuge tubes (DNase/RNase free) at -20±5°C for preservation.

1.2.2 DA0900 extraction kit (Pre-packaged: 32 reactions/kit):

Take the pre-packaged 96 deep-well plate out of the kit; invert it for multiple times, and gently shake it to concentrate the reagents and magnetic beads to the bottom of the well plate (or centrifuge with a 96-well plate centrifuge at 500 rpm for 1 minute). Carefully tear off the sealing film during use to prevent the liquid from splashing out.

Use pipettor to add 200 μL specimen and 20 μL proteinase K that was packaged in the kit to columns 1 and 7 (Rows A to H) of the 96 deep-well plate in sequence.

Procedure setting:

Step	Well	Description	Waiting Time (min)	Mixing Time (min)	Times of Absorption of Magnetic Beads	Mixing Speed	Volume (μL)	Temperature Status	Temperature (°C)
1	2	Magnetic beads	0	1	2	High	400	Closed	0
2	1	Lysis	0	15	3	High	940	Well 1	70
3	3	Wash 1	0	2	2	High	600	Closed	0
4	4	Wash 2	0	2	2	High	600	Closed	0
5	5	Wash 3	0	2	2	High	600	Closed	0
6	6	Elution	3	0	0	Medium	60	Well 6	50
7	6	Elution	0	5	3	Medium	60	Well 6	70
8	3	Abandoning of magnetic beads	0	1	0	Medium	600	Closed	0

After the running program is accomplished, the liquid in columns 6 and 12 is the nucleic acid solution, which is recommended to be used immediately. If the solution is going to be stored, use pipettor to transfer to 1.5 mL microcentrifuge tubes (DNase/RNase free) at -20±5°C for preservation.

1.2.3 QIAamp 52904 extraction kit:

1. Pipet 560 μL prepared Buffer AVL containing carrier RNA into a 1.5 mL microcentrifuge tube.

2. Add 140 μL specimen to the Buffer AVL–carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 s.

3. Incubate at room temperature for 10 min.

4. Briefly centrifuge the tube to remove drops from the inside of the lid.

5.Add 560 μL ethanol (96~100%) to the specimen, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the tube to remove drops from inside the lid.

6. Carefully apply 630 μL of the solution from step 5 to the QIAamp Mini column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 × g (8000 rpm) for 1 min. Place the QIAamp Mini column into a clean 2 mL collection tube, and discard the tube containing the filtrate.

7. Carefully open the QIAamp Mini column, and repeat step 6. If the specimen volume was greater than 140 µL, repeat this step until all of the lysate has been loaded onto the spin column.

8. Carefully open the QIAamp Mini column, and add 500 µL Buffer AW1. Close the cap, and centrifuge at 6000 × g (8000 rpm) for 1 min. Place the QIAamp Mini column in a clean 2 mL collection tube (provided), and discard the tube containing the filtrate.

9. Carefully open the QIAamp Mini column, and add 500 µL Buffer AW2. Close the cap and centrifuge at full speed (20,000 × g; 14,000 rpm) for 3 min. Continue directly with step 11; or to eliminate possible Buffer AW2 carryover, perform step 10 and then continue with step 11.

10. Place the QIAamp Mini column in a new 2 mL collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

11. Place the QIAamp Mini column in a clean 1.5 mL microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QIAamp Mini column and add 60 µL Buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min.

12. Centrifuge at 6000 × g (8000 rpm) for 1 min.

2. PCR reagent preparation (reagent preparation area)

Take out the NC (ORF1ab/N) PCR reaction solution A and NC (ORF1ab/N) PCR reaction solution B from the kit. After thawing at room temperature, shake and mix. Centrifuge at 8,000 rpm for 10 s before use.

Take N (N = number of specimens to be tested + NC (ORF1ab/N) negative control + NC (ORF1ab/N) positive control) PCR reaction tube. A NC single-reaction amplification system is prepared as follows:

Test run	NC(ORF1ab/N) PCR reaction solution A	NC(ORF1ab/N) PCR reaction solution B	Reaction solution volume
1 test	17 µL	3 µL	20 µL
24 tests	408 µL	72 µL	480 µL
48 tests	816 µL	144 µL	960 µL

96 tests	1632 μL	288 μL	1920 μL

After thoroughly mix the components, centrifuge at 8,000 rpm for 10 s to cause all the liquid on the tube wall to fall to the bottom of the tube, and then aliquot 20 μL of the amplification system into the PCR tubes.

3. Sampling (specimen preparation area)

Add 5 μL each of the negative control material, the RNA of specimens to be tested, and the positive control material processed into the PCR reaction tubes, cover the tubes tightly, and transfer them to the amplification detection area after centrifugation at 8,000 rpm for 10 s.

4. PCR amplification (amplification detection area)

4.1 Place the reaction tube in the specimen sink of the instrument.

4.2 Setting of ABI 7500 Instrument(software V2.4)

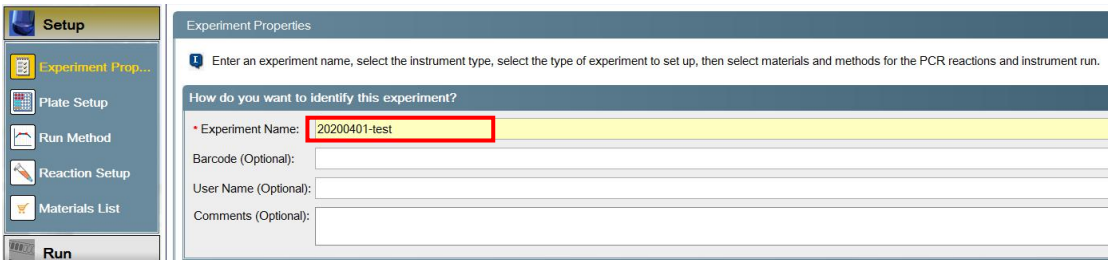
4.2.1 Set up PCR Machine

(1) Ensure that the ABI 7500 Real-Time PCR Instrument(V2.4) is set up and well calibrated before you prepare specimen for PCR run

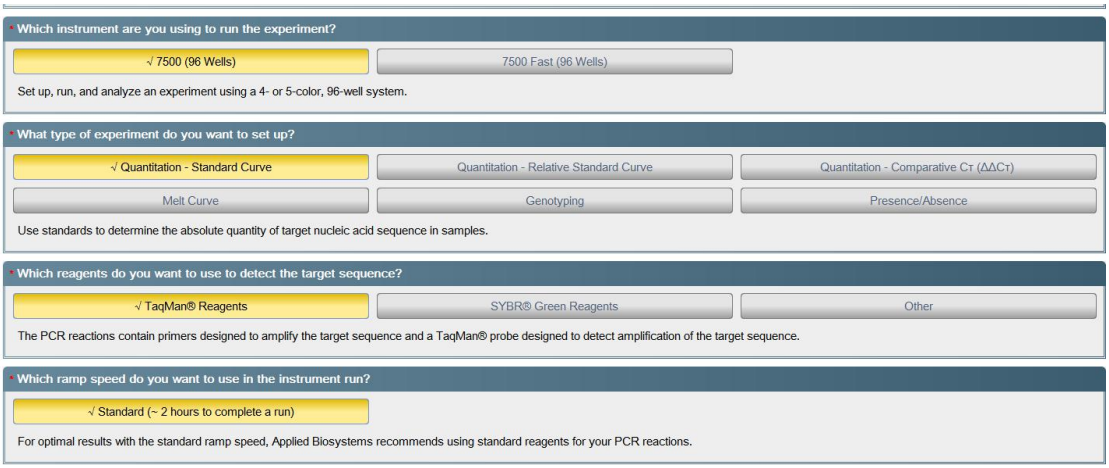
(2) Ensure the correct PCR amplification protocol is used

4.2.2 Double-click (7500 software V2.4) or select Start>>All Programs>>Applied Biosystems>>7500 Software V2.4.

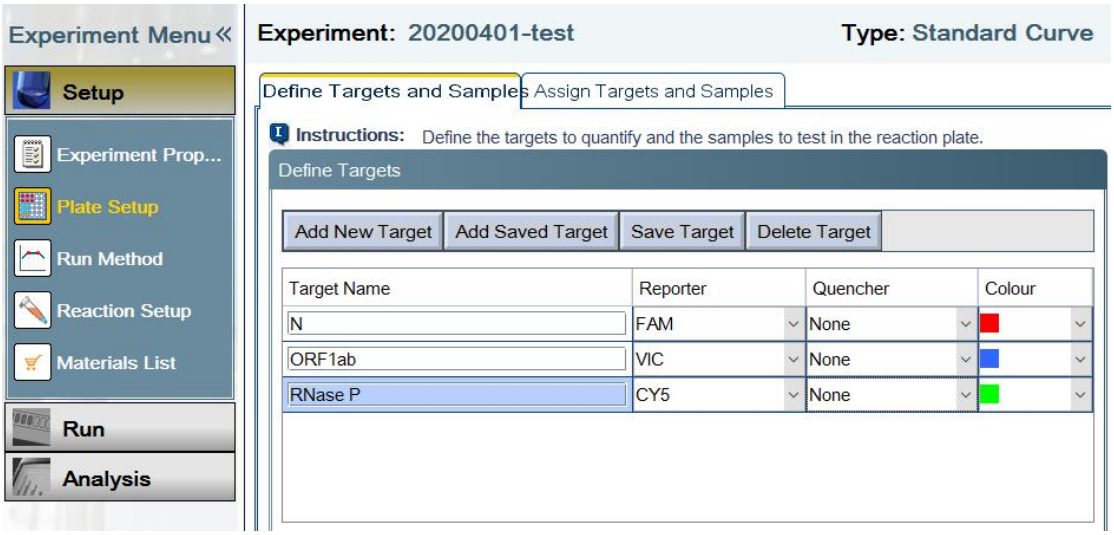
4.2.3 **Click** New Experiment to enter Experiment menu. In the Experiment Properties screen, **enter** identifying information for the experiment; you can leave other fields empty.



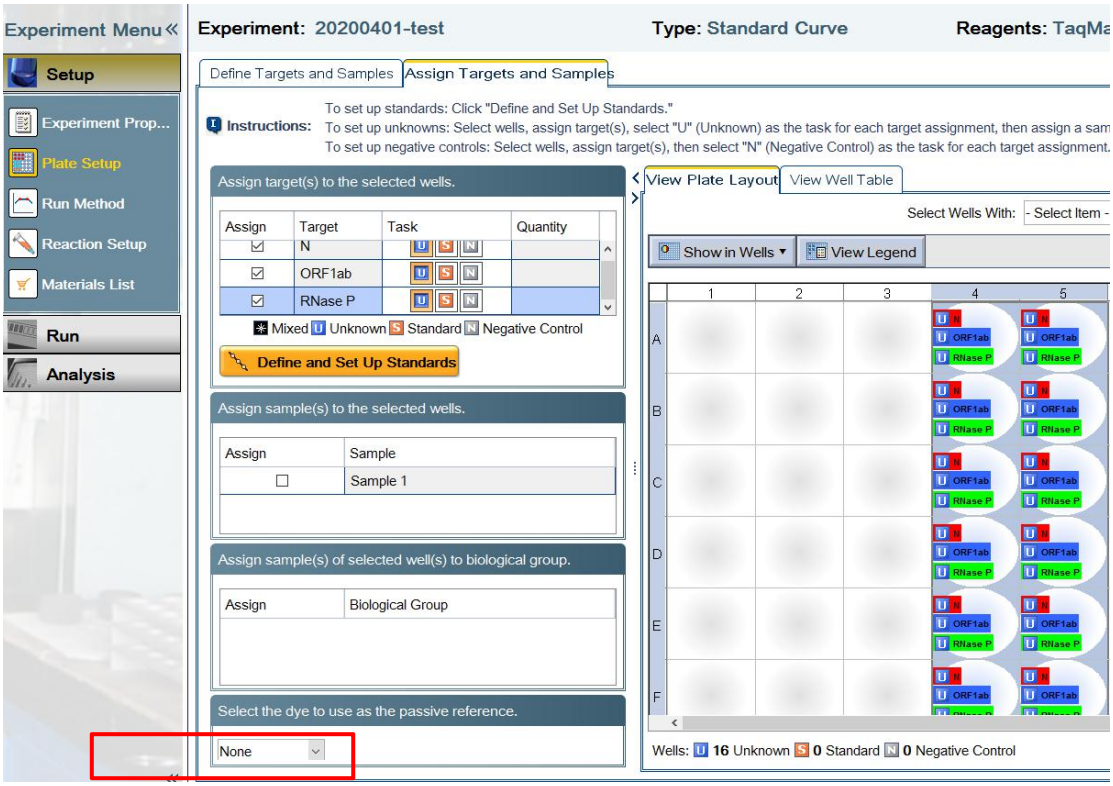
4.2.4 Select **7500 (96 Wells)**; **Quantitation-Standard Curve** (for the experiment type); **TaqMan Reagents** (for reagent); and **standard** (for ramp speed).



4.2.5 **Click** Plate Setup, in the Targets screen, under the tab Define Targets and Specimens, enter targets as showed in the figure.



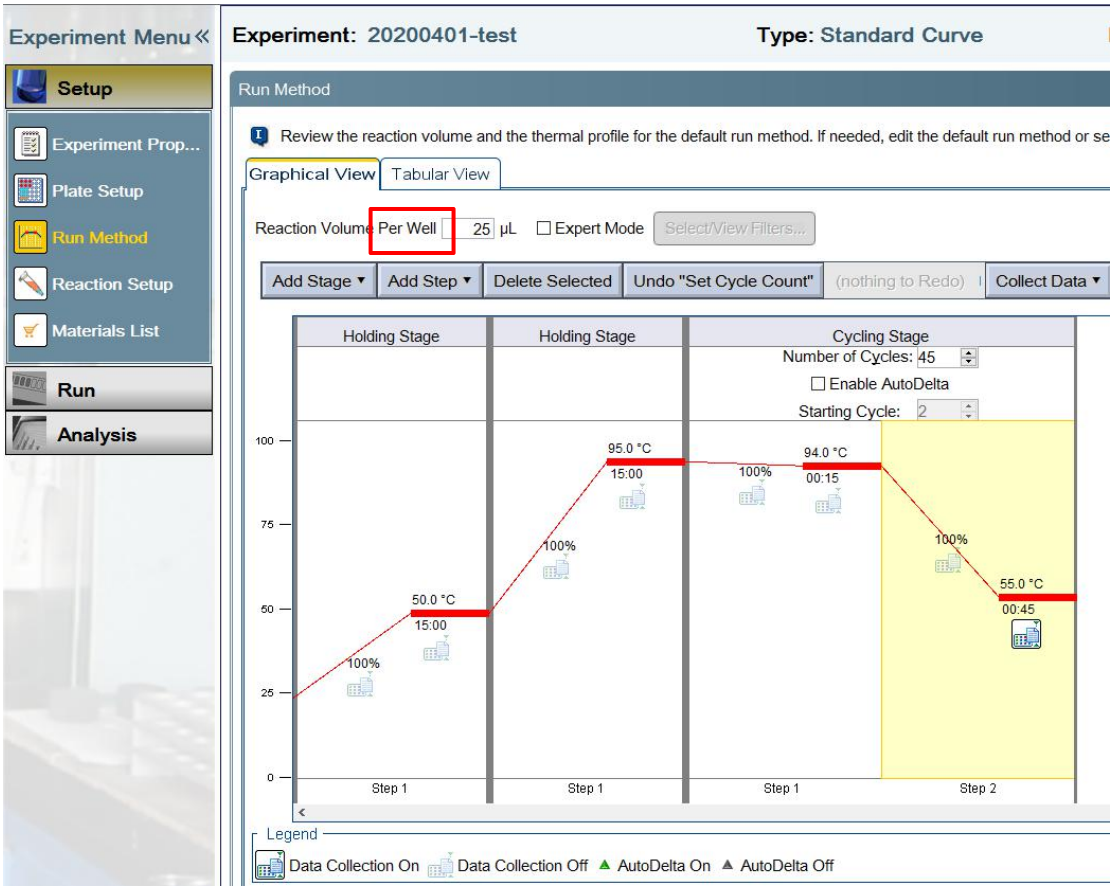
4.2.6 **Click** Assign Targets and Specimen tab, in the specimens screen, **enter** the name of specimens and controls to include in the reaction plate in corresponding well, and **select** the specimen/target reactions to set up. **Select** None for passive reference.



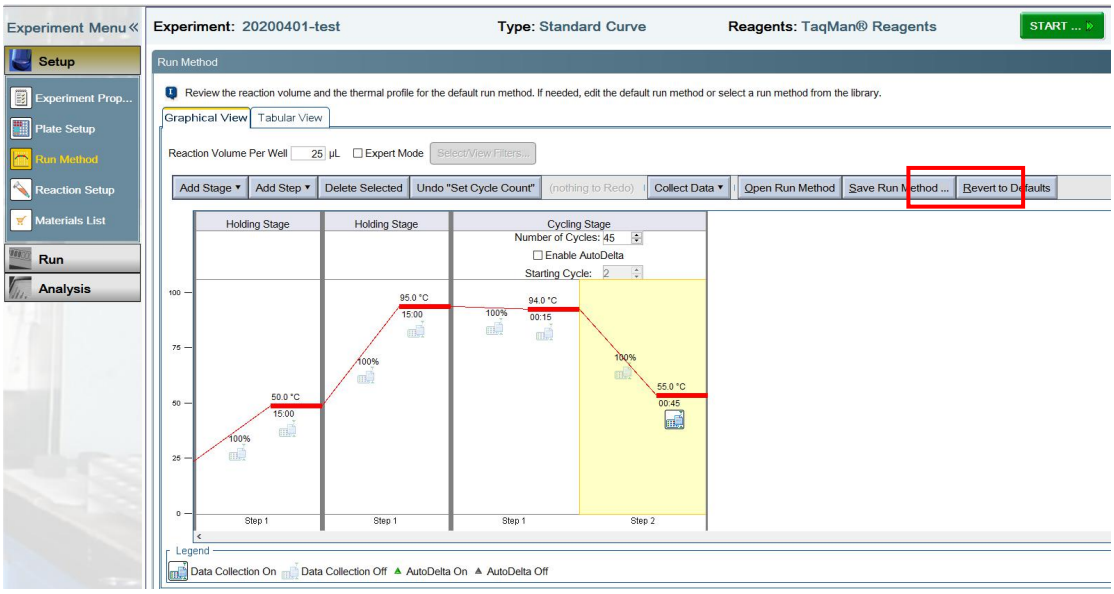
4.2.7 **Click** Run Method On the Run Method screen, **select** either the Graphical View tab (default) or the Tabular View to edit the run method Make sure the thermal profile displays the holding and cycling stages shown below. Enter **25 µL** in the Reaction Volume Per Well field. The FAM channel (Reporter: FAM, Quencher: None) will be set up for detection of SARS-CoV-2 N gene; the VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) will be set up for the detection of SARS-CoV-2 ORF1ab gene ; the Cy5 channel (Reporter: Cy5, Quencher: None) will be set up for the detection of the internal reference (human RNase P)Reference Dye: None. Configure PCR protocol as shown in Table 1.

Table 1 Thermal cycler protocol

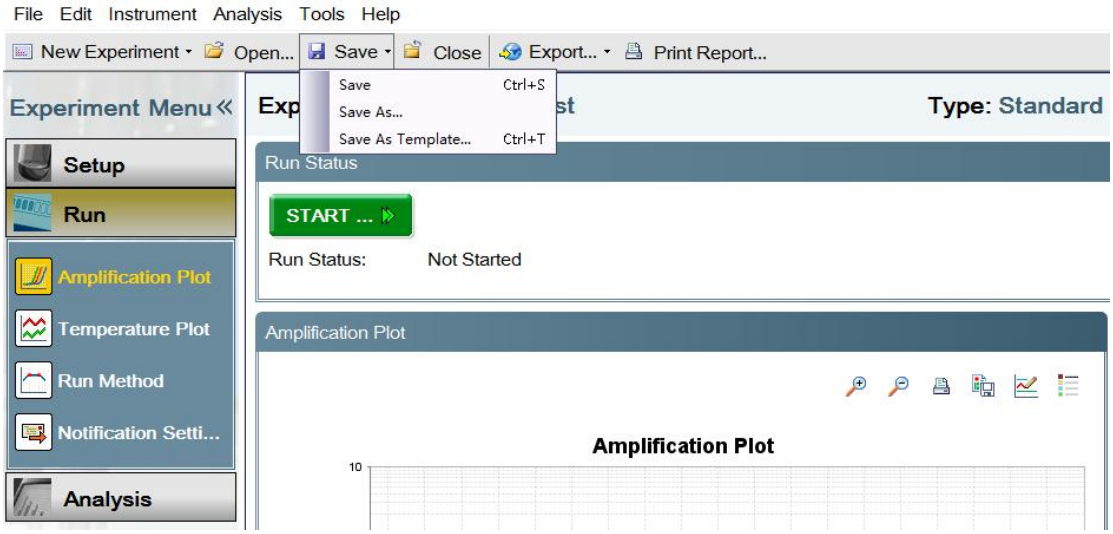
Stage	Reps	Target（℃）	Running Time	Fluorescence measured (Y/N?)
1	1	50	00:15:00	N
2	1	95	00:15:00	N
3	45	94	00:00:15	N
		55	00:00:45	Y



4.2.8 You may save a run method as shown in the figure below and use the method for future experiments.



4.2.9 **Click** Run, in the Run screen, save the experiment.



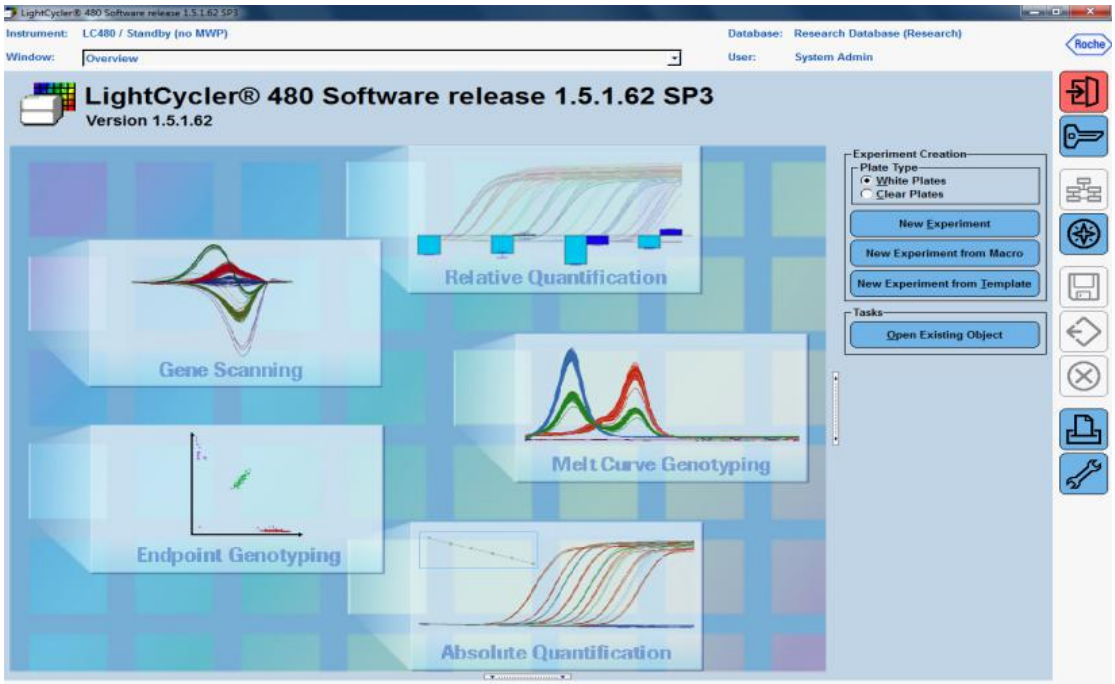
4.3 Setting of LightCycler480 Ⅱ(software V1.5)

4.3.1 Set up PCR Machine

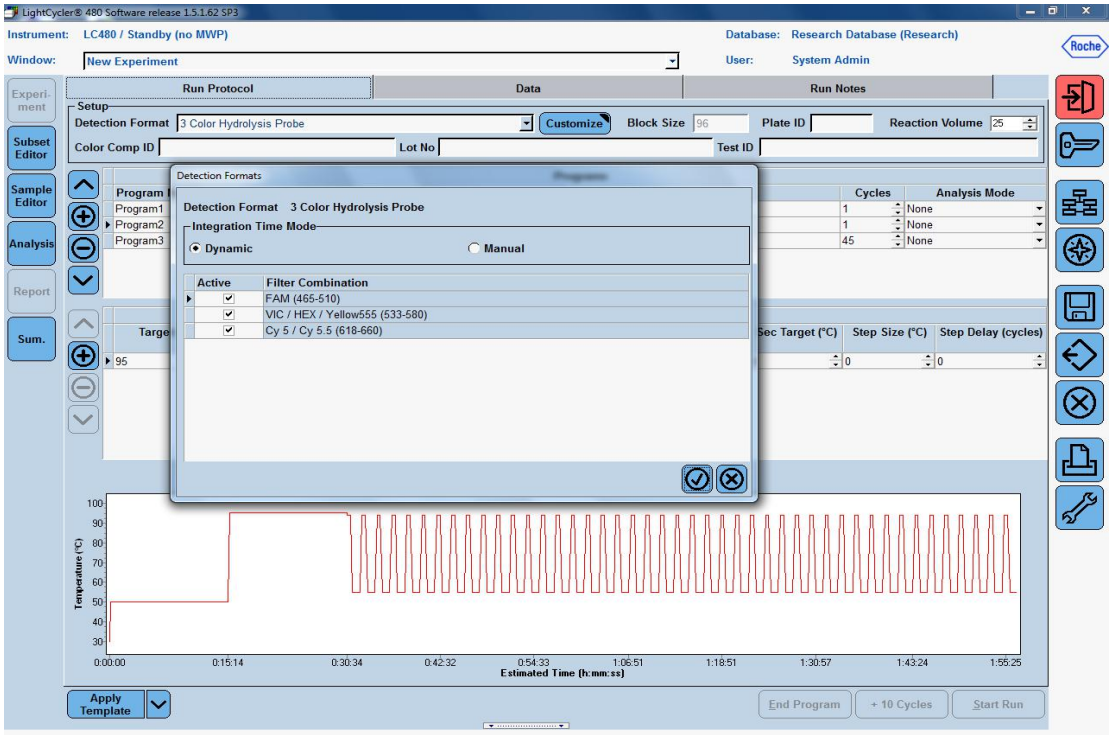
(1) Ensure that the LightCycler 480 II Real-Time PCR Instrument (V1.5) is set up before you prepare specimen for PCR run

(2) Ensure the correct PCR amplification protocol is used

4.3.2 Click LightCycelr 480(V1.5) Software to open.



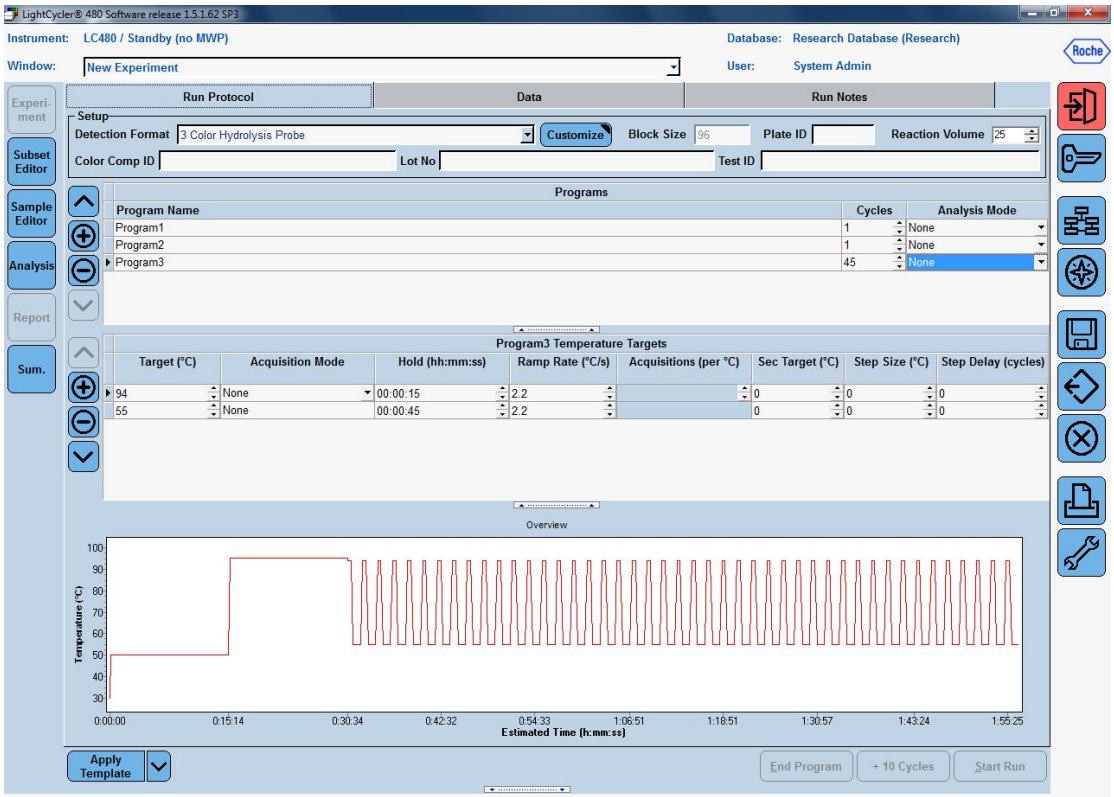
4.3.3 **Click** New Experiment to enter Experiment, Detection Format select 3 color hydrolysis probe, the fluorescence channels: FAM, VIC, Cy5



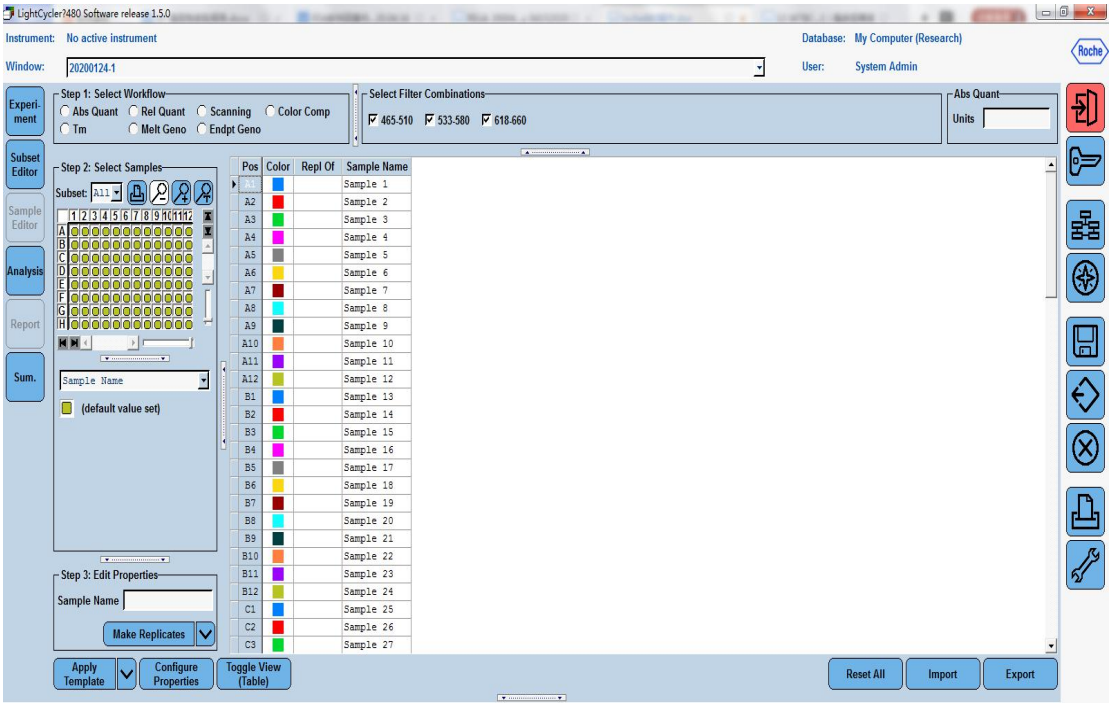
4.3.4 Set and confirm the thermal cycles protocol, Enter **25** μL in the Reaction Volume. Configure PCR protocol as shown in Table 2.

Table 2 Thermal cycler protocol

Stage	Reps	Target（℃）	Running Time	Data Collection
1	1	50	00:15:00	
2	1	95	00:15:00	
3	45	94	00:00:15	
		55	00:00:45	√



4.3.5 **Select** Specimen Editor to set the Specimen name, save the file after setting



4.4 Run PCR

Close the lid or seal the plate immediately to avoid contamination. Spin down briefly and add plate to the Applied Biosystems 7500(V2.4), or LightCycler 480 II(V1.5) Real-Time PCR Instrument Click run button to start reaction.

4.5 After the run completes, unload the instrument and proceed to data analysis

5. Data Analysis

5.1 Setting data analysis parameters

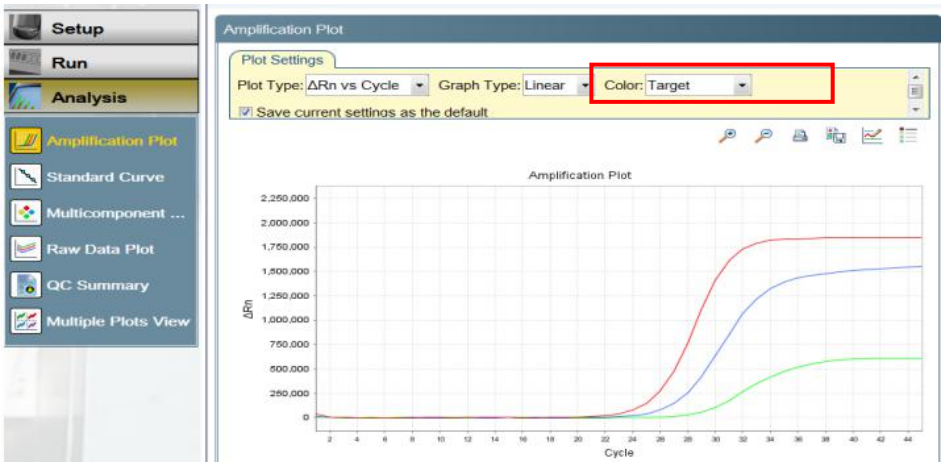
5.1.1 ABI 7500 Real-Time PCR Instrument using 7500 software V2.4

5.1.1.1 **Click** Analysis in the Amplification Plot screen under Plot Settings tab

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

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

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



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

5.1.1.3 Manually set thresholds

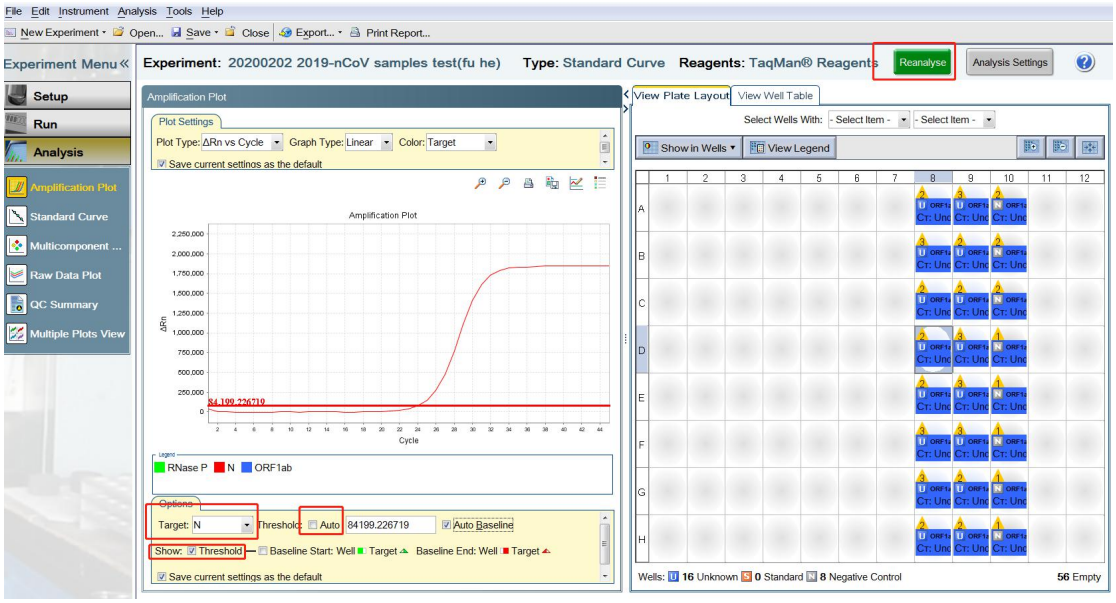
5.1.1.3.1 In the Target drop-down list, select N.

5.1.1.3.2 **Uncheck** "Auto" as shown in the figure below.

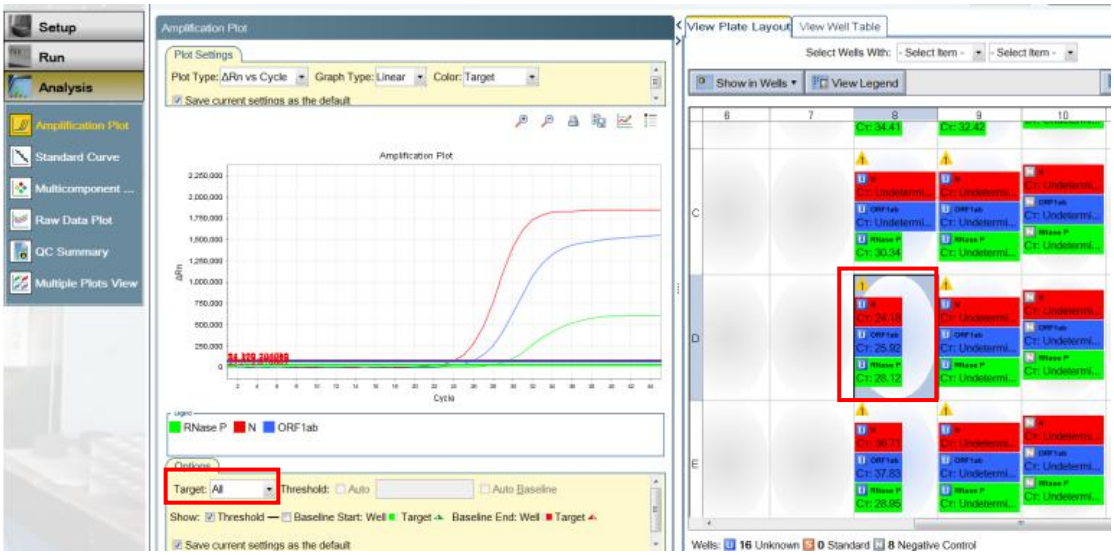
5.1.1.3.3 The threshold should be set to cross the amplification cure at the beginning of exponential phase, the amplification curve of the negative control to be straight or lower than the threshold line

5.1.1.3.4 Repeat the steps above for ORF1ab and RNase P.

5.1.1.4 **Click** Analyze The software analyzes the data with the settings.



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



5.1.1.6 Example of a positive specimen amplification curve (e.g N gene FAM in red, ORF1ab gene VIC in blue and internal reference Cy5 in green).

5.1.1.7 Amplification curves float or decline

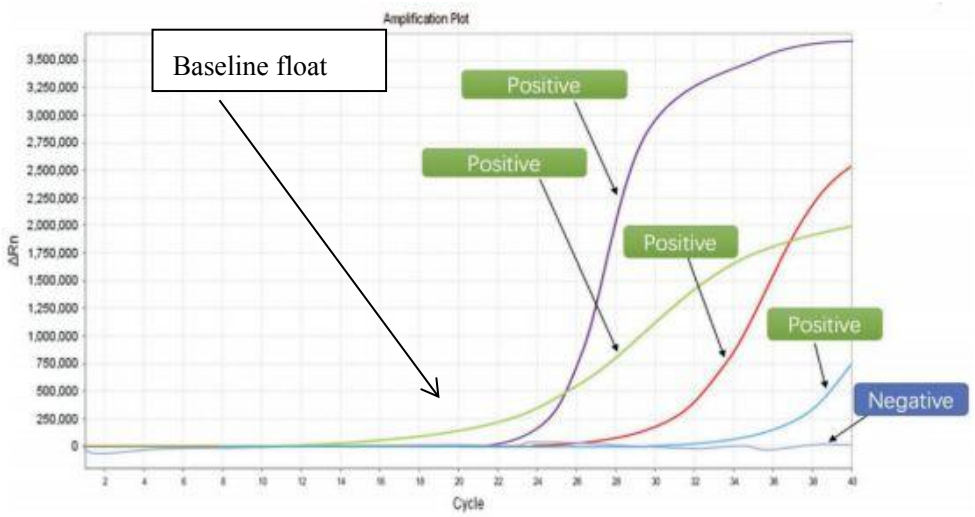
Amplification curves float will lead the interpreted Ct value to be lower than the actual Ct value. In the case:

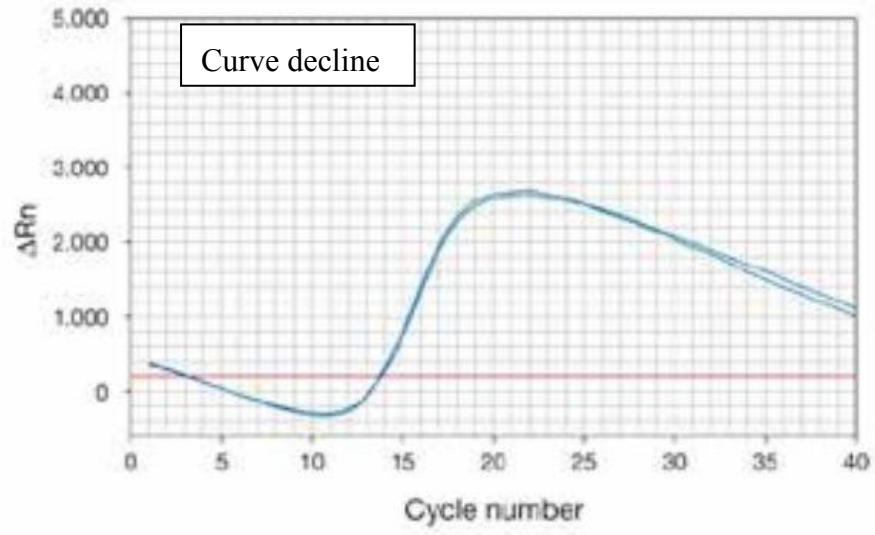
The baseline setting for data analysis software may be too low.

If you set the end cycle of the baseline setting too low, insufficient background signal will be subtracted from some or all samples. As a result, the precision of affected curves will be lowered.

The Start cycle and End cycle should be increased (the Start cycle should be adjusted within the range of 3~15 and the End cycle should be adjusted within the range of 15~20)

Amplification curves decline can be solved by reducing the End value. It should be adjusted within the range of 5~15.

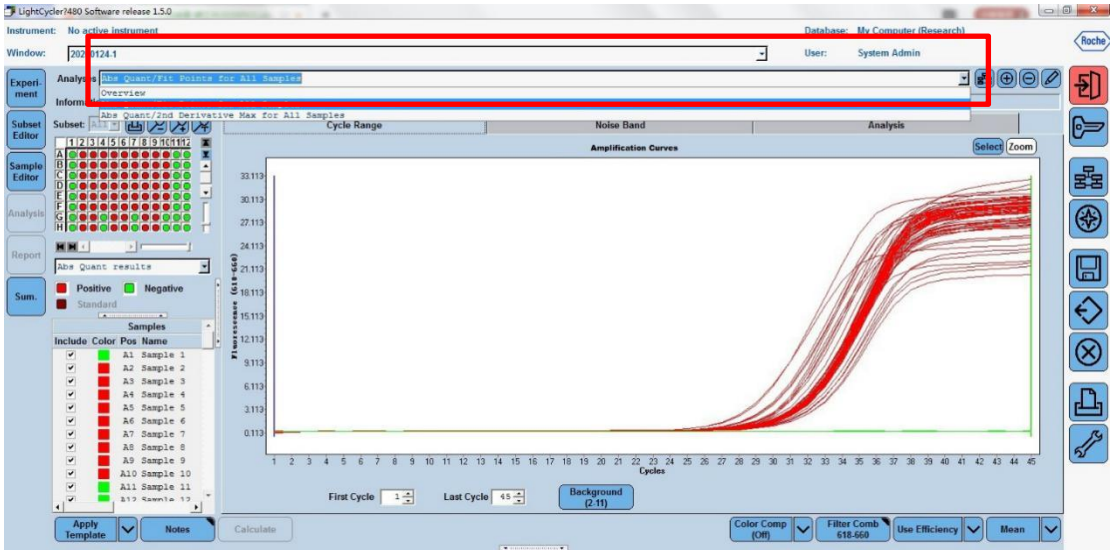




5.1.2 LightCycler 480 II Real-Time PCR Instrument using software V1.5

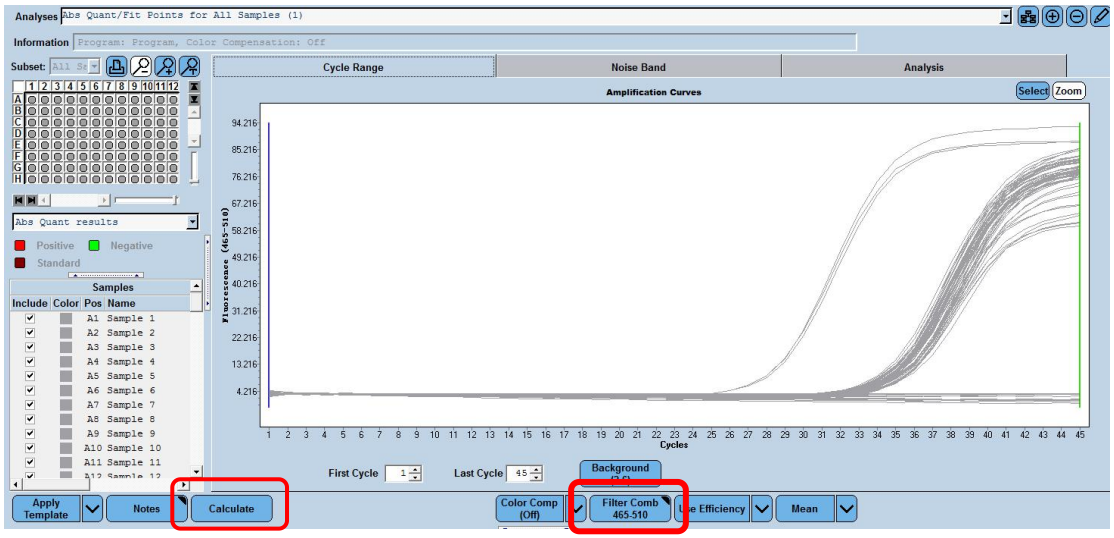
5.1.2.1 Click "Abs Quant/Fit Points for All Specimens", the software will

automatically analyze the results.

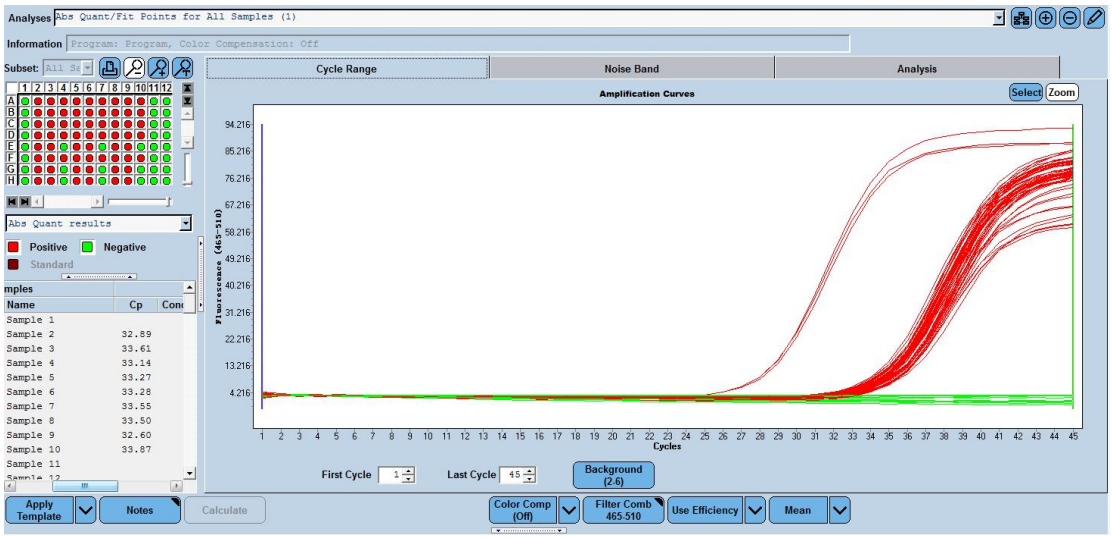


5.1.2.2 The instrument can't analyze all channels at the same time. It needs to analyze each channel separately. Therefore, you need to select channels first and then click calculate to analyze.

For example, select FAM channel (filter comb: 465-510).

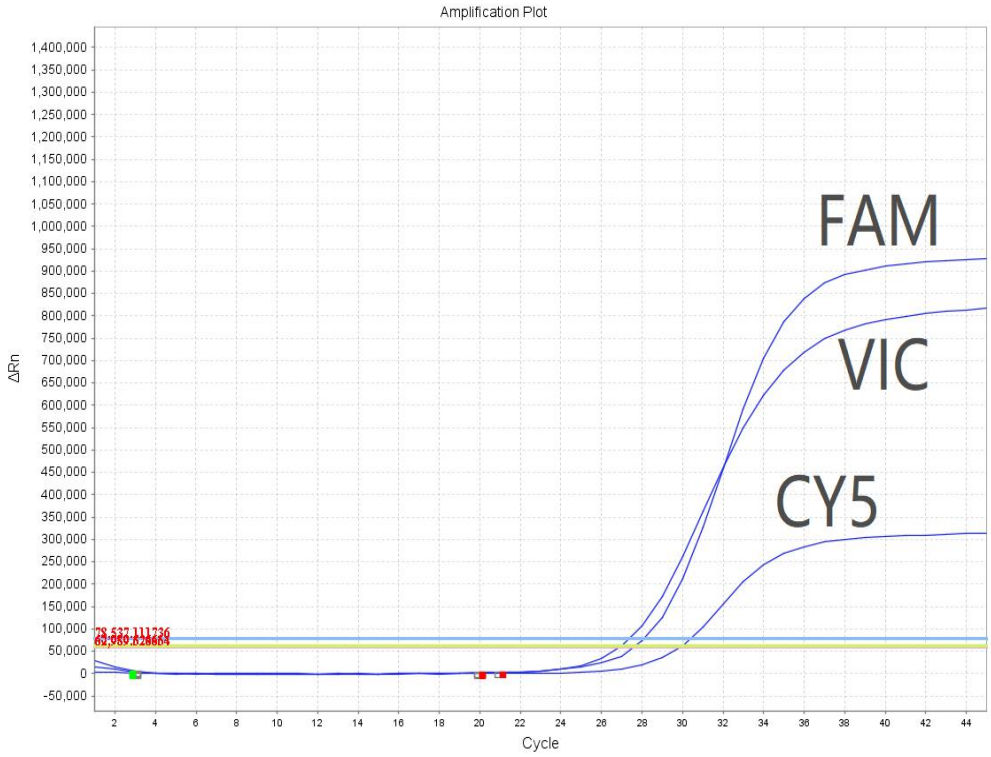


5.1.2.3 Click calculate to analyze.

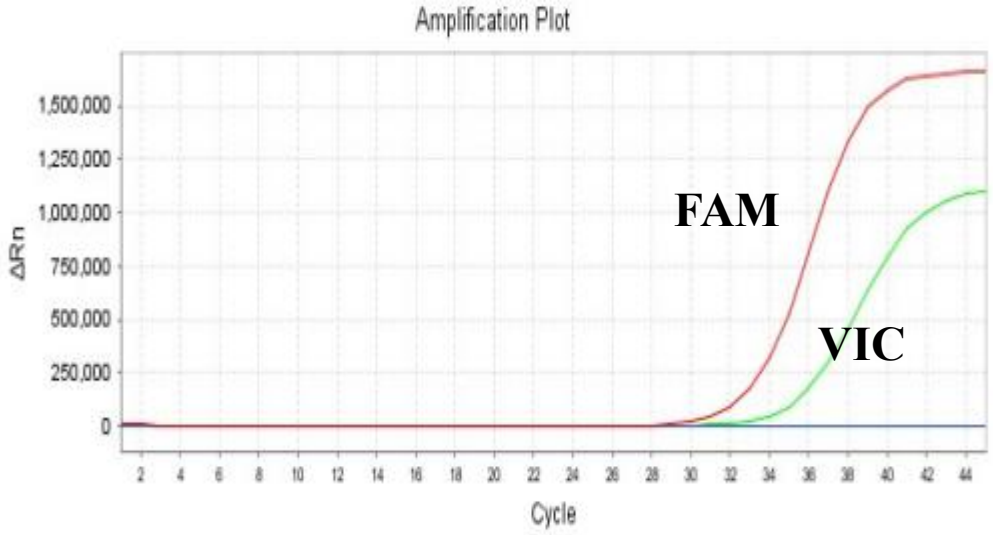


5.2 Example of a positive specimen amplification curve

5.2.1 Example of a positive specimen amplification curve when FAM, VIC and Cy5 channels are all positive.



5.2.2 Example of a positive specimen amplification curve when FAM, VIC and Cy5 channels are positive, Cy5 channel is negative.



6. Quality Control

NC (ORF1ab/N) negative control: no obvious amplification curve for FAM and VIC detection channels, and Cy5 channel's Ct value<35;

NC (ORF1ab/N) positive control: obvious amplification curves for FAM and VIC detection channels and Ct value≤ 32, and amplification curve or no amplification curve for Cy5 channel;

The above requirements must be met at the same time in each test run; otherwise, the test run is invalid and needs to be carried out again.

Note: FAM channel for N gene detection; VIC channel for ORF1ab gene detection; Cy5 channel for internal standard.

7. Interpretation of results

7.1 Positive for SARS-CoV-2: If the test result shows amplification curve in the FAM and VIC with Ct value ≤ 40, the result is interpreted as positive.

Note: When the FAM and VC detection channels are positive, the result from the Cy5 channel (internal standard channel) may be negative due to the competition of the system.

7.2 Negative for SARS-CoV-2: If the test result show amplification curve in Cy5 and no amplification curve in the FAM and VIC, the result is interpreted as negative.

7.3 Indeterminate for SARS-CoV-2: If the test result show amplification curve in

either FAM or VIC, and no amplification curve in Cy5, the result is indeterminate. The specimen should be retested.

a) If the retest result shows amplification curve in the FAM and VIC with Ct value ≤ 40, the result is interpreted as positive.

b) If the retest result does not show amplification curve in the FAM and VIC , the result is interpreted as negative.

c) If the retest result shows Ct value ≤ 40 in either the FAM or VIC, the result is interpreted as positive.

7.4 Invalid: If the test result does not show amplification curve in any of Cy5, FAM, or VIC, the test is invalid.

Note: When the test is invalid, it indicates that the system is inhibited or the operation is wrong. Therefore, the specimen needs to be re-tested.

7.5 The report is recommended to be in the following format:

The format of negative result report: no 2019 Novel Coronavirus (2019-nCoV) RNA was detected in the specimens, and the concentration was lower than the sensitivity of the kit;

The format of the positive result report: 2019 Novel Coronavirus (2019-nCoV) RNA was detected in the specimens.

Table 3 Results interpretation for patient specimens

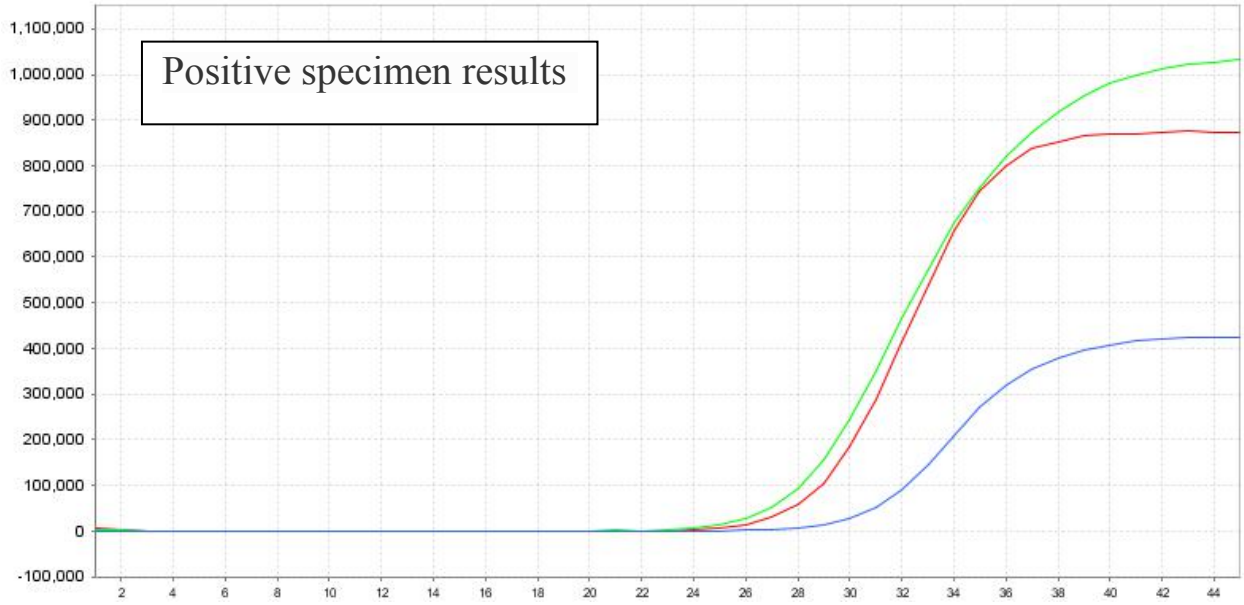
Results (Ct value)			Result	Action
N(FAM)	ORF1ab(VIC)	RNP(Cy5)		
Negative or Ct > 40	Negative or Ct > 40	Negative or Ct > 40	Invalid	Repeat test, If the repeat result remains invalid, consider re-collect new specimen
negative	negative	Ct ≤ 40	SARS-CoV-2 Negative	Report results to healthcare provider Consider testing for other viruses.
Ct ≤ 40	Ct ≤ 40	Negative or Ct ≤ 40	SARS-CoV-2 Positive	Report results to healthcare provider and appropriate public health authorities

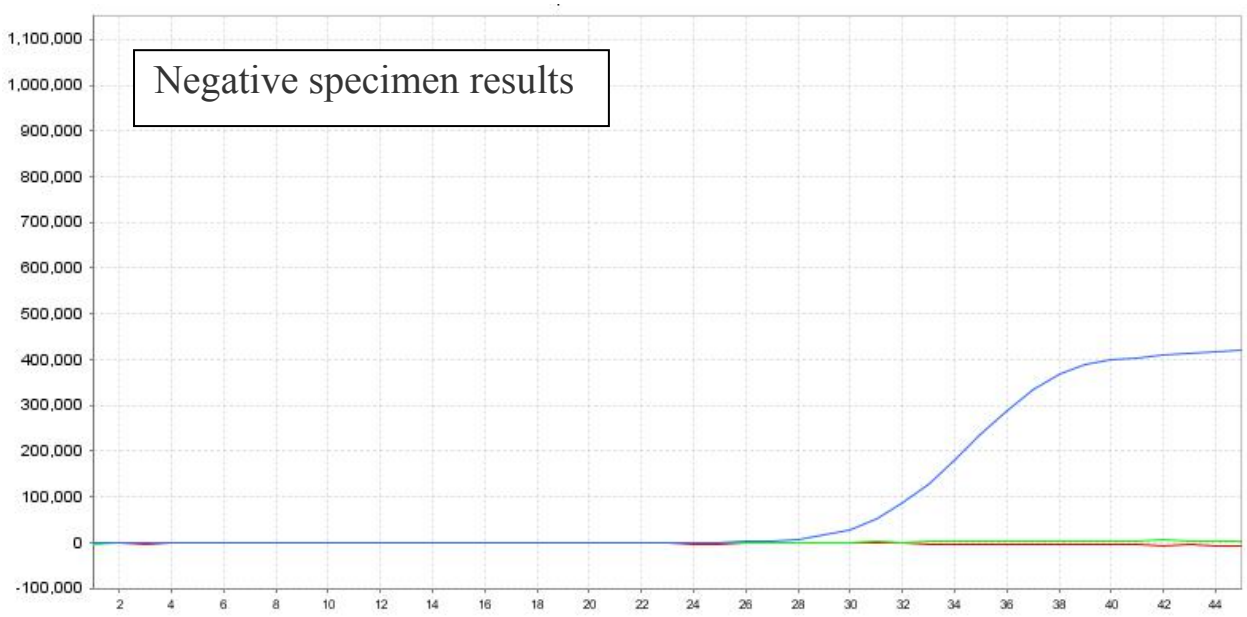
Either FAM or VIC, Ct≤ 40	Negative or Ct ≤ 40	SARS-CoV-2 Inconclusive	Repeat test, If the repeat result remains inconclusive, report SARS-CoV-2 Positive.
Either FAM or VIC, 40＜Ct＜45	Negative or Ct ≤ 40	SARS-CoV-2 Inconclusive	Repeat test, If the repeat result remains inconclusive, consider re-collection of new specimen

Note: 1. Negative means no amplification curve.

2. Inconclusive means the Ct values are >40 (due to the potential that the detected result may be an anomaly/contamination)

Table 4 Figure example of test results





[Positive Judgment Value]

The ROC curve method is used to determine both the reference Ct value of the kit and the internal standard reference value are 40.

[Limitations of Test Method]

1. The specimen test results are related to the quality of specimen collection, processing, transportation and storage. Unreasonable specimen collection, transfer, storage and processing may lead to incorrect test results;

2. Cross contamination is not well controlled during specimen processing, and a false positive result may appear;

3. The genetic mutation of the virus during the epidemic period may also lead to false negative results;

4. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment response.

5 The positive result detected by this kit does not definitively indicate the presence of viable SARS-CoV-2 virus but does indicate the presence of SARS-CoV-2 RNA..

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

7 There is a risk of false negative values due to the presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.

8 This device has been evaluated for use with human specimen material only.

9 The performance of Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) was established using oropharyngeal swab specimens and sputum specimens.

[Performance Characteristics]

1. Limit of Detection (LoD)

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 19/20 (95%) replicates test are positive. And each specimen replicate was extracted independently with the resulting RNA extract used in the real-time RT-PCR reaction. The LoD was determined by limiting dilution studies using characterized specimens.Nucleic Acid Extraction Kit for estimation of LoD, determination of LoD and evaluation of LoD is DA0623.

LoD study followed as three steps: estimation, determination and confirmation.

1.1 Estimation of LoD

- A pseudovirus (containing the N gene and ORF1ab gene targets) and quantitated viral genomic RNA obtained from 3 positive clinical specimens from different regions in Guangdong Province, China were used as the initial specimens for LoD estimation. Specimens were prepared in using clinical sputum matrix that was determined to be negative for SARS-CoV-2 RNA. specimens were prepared at 10^5 copies/mL, 10^4 copies/mL and10^3 copies/mL and 200 copies/mL. The test kit lot were used to test on the ABI7500 (V2.4). Three duplicates were tested for each lot at each concentration to estimate the lower limit of detection of this test kit.

- Acceptance criteria: Use the lowest detectable concentration level of 100% as the estimated detection line.

- For the three lots evaluated, detection rates were 100% (3/3) for specimens prepared at 10^5 copies/mL, 10^4 copies/mL and10^3 copies/mL. For specimens containing 200 copies/mL, the positive detection rate varied between 33.3% (1/3) to 100% (3/3), The study results established that the LoD of the assay is between

200 copies/mL and 1000 copies/mL.

Table 5 Results of estimation of LoD for 3 lots

specimens	lot	positive tests/total tests							
		N（FAM）(copies/mL)				ORF1ab（VIC）(copies/mL)			
		10^5	10^4	10^3	200	10^5	10^4	10^3	200
pseudovirus	2020001	3/3	3/3	3/3	2/3	3/3	3/3	3/3	2/3
	2020002	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
	2020003	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Clinical specimen 1	2020001	3/3	3/3	3/3	1/3	3/3	3/3	3/3	3/3
	2020002	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	2020003	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
Clinical specimen 2	2020001	3/3	3/3	3/3	1/3	3/3	3/3	3/3	2/3
	2020002	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	2020003	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
Clinical specimen 3	2020001	3/3	3/3	3/3	1/3	3/3	3/3	3/3	3/3
	2020002	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	2020003	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3

Table 6 Ct value results of estimation of LoD for 3 lots

specimens	lot	Ct value											
		N(FAM) (copies/mL)				ORF1ab(VIC) (copies/mL)				RNP(Cy5) (copies/mL)			
		10^5	10^4	10^3	200	10^5	10^4	10^3	200	10^5	10^4	10^3	200
pseudovirus	2020001	28.61	32.04	36.31	36.04	28.54	31.90	35.15	36.96	29.72	30.46	30.05	30.19
		28.68	32.42	35.37	38.35	28.57	32.00	35.26	37.89	29.69	30.39	30.02	29.91
		28.36	31.69	35.81	-	28.46	31.70	35.21	-	29.37	29.83	30.15	29.67
	2020002	29.32	32.64	34.33	35.18	29.00	32.20	35.42	36.17	30.82	30.79	30.43	31.05
		29.24	31.85	35.11	33.84	28.97	32.13	35.41	-	30.67	30.60	31.18	32.88
		29.18	32.35	35.12	34.42	28.98	32.25	34.99	36.33	30.65	30.72	30.58	30.89
	2020003	29.61	31.24	34.35	34.76	29.23	30.96	34.80	35.29	29.87	30.55	20.74	29.36
		28.74	31.99	34.01	35.33	28.18	31.63	34.96	36.25	27.98	29.53	25.01	26.83
		29.61	32.14	33.89	34.05	29.13	31.45	34.20	35.14	29.90	29.53	28.44	29.36
Clinical Specimen1	2020001	28.40	32.21	34.92	-	28.35	32.31	35.11	37.87	29.25	30.03	30.03	30.15
		28.67	32.22	36.35	-	28.64	32.09	35.44	38.00	29.55	29.82	30.12	29.92
		28.45	32.08	36.21	38.42	28.70	32.12	34.92	38.01	29.64	29.92	29.88	29.92

	2020002	29.11	33.05	33.55	35.44	28.86	32.48	34.95	36.58	30.60	30.78	30.80	30.81
		29.18	32.66	33.92	34.82	28.90	32.48	34.68	35.94	30.66	30.96	30.17	30.65
		29.20	32.39	34.76	35.68	28.97	32.51	35.24	36.21	30.76	30.91	30.45	30.80
	2020003	28.70	32.26	35.08	35.16	28.74	32.00	35.36	36.32	30.12	31.81	32.44	30.16
		29.32	32.13	34.82	35.60	28.94	32.11	35.57	35.96	31.98	31.78	32.31	21.85
		29.03	32.78	34.13	34.40	28.61	32.15	34.64	-	30.98	31.98	26.15	34.77
Clinical Specimen2	2020001	28.55	31.75	34.83	38.73	28.54	32.03	35.26	38.63	29.52	29.81	30.10	29.98
		28.67	32.20	35.87	38.59	28.65	31.61	35.19	37.39	29.76	29.82	30.11	30.12
		28.63	31.67	36.09	38.52	28.55	31.79	35.33	38.04	29.46	29.76	30.26	30.05
	2020002	29.03	32.42	34.20	35.69	28.86	32.31	35.22	36.68	30.72	29.38	30.58	30.88
		29.24	32.80	34.35	-	28.98	32.36	35.33	36.17	30.80	30.89	30.89	34.08
		29.23	32.24	34.74	-	28.89	32.08	35.19	-	30.88	30.66	30.93	31.26
	2020003	29.64	32.61	34.89	33.99	29.20	31.81	35.17	-	32.08	31.57	24.92	28.49
		29.50	32.72	33.96	35.05	29.15	32.56	35.07	35.62	31.94	32.27	25.17	24.04
		29.53	33.11	34.88	35.65	29.06	32.23	34.75	36.30	32.08	32.23	26.51	32.36
Clinical Specimen3	2020001	28.43	31.88	36.66	41.44	28.19	31.97	35.47	37.89	29.43	29.83	30.10	29.97
		28.54	31.66	34.64	-	28.56	32.00	34.86	37.74	29.57	29.91	29.87	29.97
		28.33	32.13	35.88	36.43	28.37	32.04	34.79	37.42	29.57	30.06	29.95	30.16
	2020002	28.97	32.70	34.60	34.41	28.97	32.23	34.87	35.79	30.67	30.77	30.56	30.63
		29.11	32.67	34.03	36.82	28.89	32.25	34.59	36.21	30.74	30.71	30.35	30.80
		29.29	32.26	34.92	35.35	28.91	31.99	35.40	36.18	30.61	30.69	30.75	30.62
	2020003	29.54	33.32	34.30	37.13	29.06	32.77	34.66	36.58	28.52	32.54	29.71	32.00
		28.73	32.13	34.44	36.63	28.16	31.79	35.27	36.47	25.11	31.43	27.92	32.05
		29.51	32.21	33.78	33.84	29.14	31.68	34.90	-	29.86	31.37	21.82	24.32
Mean Ct		29.00	32.30	34.86	/	28.78	32.05	35.07	/	/	/	/	/
SD		0.41	0.45	0.82	/	0.30	0.35	0.31	/	/	/	/	/
CV%		1.42	1.38	2.36	/	1.04	1.08	0.87	/	/	/	/	/

Note: "-" means negative; "/" means (1) At this concentration, the detection rate is different (different negative and positive appear), so the average Ct value and CV% cannot be counted; (2) RNP (Cy5) is the internal standard channel. Because different specimens are used, the average Ct value and CV% cannot be counted.

1.2 Determination of LoD (LoD using pseudovirus)

- The pseudovirus described above was used to further evaluate the limit of detection of the assay. Specimens were prepared at 1000 copies/mL, 500 copies/mL and 200 copies/mL. All specimens were extracted by DA0623 nucleic

acid extraction kit(lot:2019003). Testing was performed with three kit lots with PCR performed on the ABI7500 (V2.4). Twenty replicates were tested for per lot at each concentration to analyze the detection rate (i.e., positivity rate) of specimens at different concentrations.

- Acceptance criteria: When the detection rate was at or higher than 19/20 (95%), the corresponding concentration was the determined to be the LoD of the assay.
- The detection rates were all 100% (20/20) for 1000 copies/mL and 500 copies/mL for all 3 lots, For specimens with a concentration of 200 copies/mL, the positivity rate was lower than 19/20 (<95%). The LoD was determined to be 500 copies/mL for specimens prepared using pseudovirus.

Table 7 Results of determination of LoD for 3 lots

Concentration	Targets	2020001		2020002		2020003	
		Positives/ tests	Positive rate (%)	Positives/ tests	Positive rate (%)	Positives/ tests	Positive rate (%)
1000copies/mL	N(FAM)	20/20	100%	20/20	100%	20/20	100%
	ORF1ab (VIC)	20/20	100%	20/20	100%	20/20	100%
500copies/mL	N(FAM)	20/20	100%	20/20	100%	20/20	100%
	ORF1ab (VIC)	20/20	100%	20/20	100%	20/20	100%
200copies/mL	N(FAM)	18/20	90%	17/20	85%	16/20	80%
	ORF1ab (VIC)	18/20	90%	17/20	85%	16/20	80%

Table 8 Ct value results of determination of LoD for 3 lots

	N(FAM) copies/mL			ORF1ab(VIC) copies/mL			RNP(Cy5) copies/mL		
	1000	500	200	1000	500	200	1000	500	200
Mean Ct	35.43	35.44	/	35.45	35.46	/	/	/	/
SD	0.19	0.32	/	0.21	0.26	/	/	/	/
CV%	0.52	0.91	/	0.58	0.73	/	/	/	/

Note: "-" means negative; "/" means (1) At this concentration, the detection rate is different (different

negative and positive appear), so the average Ct value and CV% cannot be counted; (2) RNP (Cy5) is the internal standard channel. Because different specimens are used, the average Ct value and CV% cannot be counted.

1.3 Validation of LoD (Using SARS-CoV-2 whole genomic RNA)

- Study description

1) Quantification and dilution of Viral genomic RNA

1.1 Standard curves were prepared using plasmids of 4 gradient concentrations (10^7, 10^6, 10^5 and 10^4 copies/mL) with known concentration quantification. The absolute value of the linear correlation coefficient R of prepared standard curve should greater than 0.9800, which means | R| >0.9800. The concentration quantification was measured by Nanodrop 2000C (Thermo Fisher). The standard curve (y=kx+b) is a linear function of the Ct value and the concentration. The concentration of Viral genomic RNA was automatic obtained on ABI 7500 (V2.4) by comparing the Ct value of nucleic acid with the standard curve.

Note: y= concentration (copies/mL), x= Ct value，K= Linear correlation coefficient，b= intercept.

2) Specimen Preparation

Specimens were prepared by diluting the quantitated viral genomic RNA and spiking into 20 natural negative sputum and 20 natural negative oropharyngeal swab specimens to generate 40 contrived specimens at a final concentration of 500 copies/mL. Specimen aliquots were then prepared for a total of 240 specimens.

Each specimen was extracted individually with the specimen extract independently run through the entire testing process.

3) PCR Testing

A total of 240 specimens were tested on different instruments (ABI7500 (V2.4) and LightCycler480II (V1.5)), 3 test kit lots of Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing*)* were used as shown in table 9.

Table 9 Instruments and reagent lot numbers of 6 groups of specimens

Specimen group	Testing instrument	Reagent lot number
Group 1	ABI7500 (V2.4)	2020001
Group 2	ABI7500 (V2.4)	2020002
Group 3	ABI7500 (V2.4)	2020003
Group 4	LightCycler480 Ⅱ (V1.5)	2020001

Group 5	LightCycler480 Ⅱ (V1.5)	2020002
Group 6	LightCycler480 Ⅱ (V1.5)	2020003

- Study Results

The study results showed positivity rates of 100% (for both oropharyngeal swab and sputum matrices as well as for both PCR instruments evaluated. (See the Table 10 and Table 11 for positive detection rates for the study.

- Conclusion

According to the LoD determination acceptance criteria, when the positive detection rate is greater than 95%, the concentration can be determined as the assay LoD. Therefore, the LoD of the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) as well as the individual N gene and ORF1ab gene targets is confirmed as 500 copies/mL.

Table 10 Summary of the LoD validation

Specimens	positive tests/total tests			
	ABI7500 (V2.4)		LightCycler480 Ⅱ (V1.5)	
	N(FAM)	ORF1ab(VIC)	N(FAM)	ORF1ab(VIC)
sputum	60/60	60/60	60/60	60/60
oropharyngeal swabs	60/60	60/60	60/60	60/60

Table 11 Results of validation of LoD for 240 replicates test on two instruments with 3 lots-DA0623

specimens	positive tests/total tests					
	ABI75000 (V2.4)			LightCylcer480 Ⅱ (V1.5)		
	lot 2020001	lot2020002	lot2020003	lot2020001	lot2020002	lot2020003
oropharyngeal swab	20/20	20/20	20/20	20/20	20/20	20/20
Sputum	20/20	20/20	20/20	20/20	20/20	20/20

Table 12 Statistical table of LoD verification results on two instruments with 3 lots-DA0623

specimens		ABI7500 (V2.4)						LightCylcer480 Ⅱ (V1.5)					
		lot 2020001		lot2020002		lot 2020003		lot2020001		lot 2020002		lot2020003	
		N (FAM)	ORF1ab (VIC)	N (FAM)	ORF1ab (VIC)	N (FAM)	ORF1ab (VIC)	N (FAM)	ORF1ab (VIC)	N (FAM)	ORF1ab (VIC)	N (FAM)	ORF1ab (VIC)
oropharyngeal swab	Mean Ct	36.09	35.39	36.13	35.32	35.79	35.30	35.27	35.14	35.24	35.18	35.60	35.53
	STDEV	0.57	0.16	0.71	0.30	0.66	0.18	0.35	0.48	0.41	0.33	0.43	0.43
	CV%	1.58	0.46	1.95	0.86	1.83	0.51	1.00	1.37	1.15	0.95	1.21	1.20
Sputum	Mean Ct	36.14	35.33	36.46	35.40	36.14	35.32	35.40	35.59	35.56	35.30	35.53	35.70
	STDEV	0.78	0.25	0.90	0.25	0.63	0.25	0.46	0.76	0.41	0.42	0.44	0.67
	CV%	2.15	0.72	2.48	0.71	1.75	0.71	1.31	2.13	1.16	1.20	1.23	1.88

2. Evaluation of additional Extraction Kits

Preliminary evaluation of the following three extraction methods:

- Nucleic Acid Extraction or Purification Kit (Daan, Cat#DA0623)
- Nucleic Acid Extraction Kit (Daan, Cat#DA0900)
- QIAamp Viral RNA Mini Kit (Qiagen, Cat#52904)

After extraction, real-time RT-PCR was performed using ABI7500 (V2.4) specimen replicates were prepared using SARS-CoV-2 whole genomic RNA (from positive clinical specimen) that was quantitated using the standard curve method described above for LoD estimation testing. Specimens were prepared using both oropharyngeal swab and sputum clinical matrices.

Results from the study showed 100% detected (5/5) for specimen concentrations of 10^5, 10^4 and 10^3 copies/mL and 80% detection for specimen concentrations of 200 copies/mL, demonstrating equivalent performance between extraction kits. Results are presented in Table 13.

Table 13 Determination of LoD by different extraction methods

Specimen		N gene (FAM)				ORF1ab (VIC)			
		10^5 copies/mL	10^4 copies/mL	10^3 copies/mL	200 copies/mL	10^5 copies/mL	10^4 copies/mL	10^3 copies/mL	200 copies/mL
oropharyngeal swab	Daan Gene DA0623	5/5	5/5	5/5	4/5	5/5	5/5	5/5	4/5
	Daan Gene DA0900	5/5	5/5	5/5	4/5	5/5	5/5	5/5	4/5
	QIAGEN Cat No.: 52904	5/5	5/5	5/5	4/5	5/5	5/5	5/5	4/5
sputum	Daan Gene DA0623	5/5	5/5	5/5	4/5	5/5	5/5	5/5	4/5
	Daan Gene DA0900	5/5	5/5	5/5	4/5	5/5	5/5	5/5	4/5
	QIAGEN Cat No.: 52904	5/5	5/5	5/5	4/5	5/5	5/5	5/5	4/5

Additional LoD confirmation testing was conducted using the ABI7500 V2.4 instrument for each extraction method and specimen matrix. A total of 20 specimen replicates prepared at a concentration of 500 copies/mL, 250 copies/mL and 100 copies/mL using the same SARS-CoV-2 whole genomic RNA material were evaluated. Results were 100% detection of both assay targets for specimens with 500 copies/mL while lower concentrations generated <95% positivity, confirming the assay LoD of 500 copies/mL for each specimen matrix and all three extraction kits.

Table 14 Summary of validation of LoD of the 3 extraction kits- ABI 7500V2.4

Specimen	Extraction Kits	Positive number/specimen number			
		N gene (FAM)		ORF1ab (VIC)	
		500 copies/mL	250 copies/mL	500 copies/mL	250 copies/mL
oropharyngeal swab	Daan Gene DA0623	20/20	15/20	20/20	17/20
	Daan Gene DA0900	20/20	18/20	20/20	18/20
	QIAGEN Cat No.: 52904	20/20	17/20	20/20	17/20
sputum	Daan Gene DA0623	20/20	16/20	20/20	16/20
	Daan Gene DA0900	20/20	16/20	20/20	16/20
	QIAGEN Cat No.: 52904	20/20	17/20	20/20	18/20

3. Cross-reactivity

3.1 Inclusivity (analytical sensitivity)

As of 26 May 2020, we have collected the 2678 full-length gene sequences for N gene and 2672 full-length gene sequences for ORF1ab gene of SARS-CoV-2 published by NCBI, then after multiple alignment with mega 6.0 software (developed by Kumar, The Pennsylvania State University), it can be seen that the sequence N gene designed for this product has a single base mutation at the 1st and 15th positions of forward primer, and another single base mutation in the 10th and 12th positions of reverse primer, as well as a mutation in the 5th position of ORF1ab gene

（EPI_ISL_411929）probe. All the mutations were located in the middle of the primer and probe sequence, which did not affect the detection sensitivity of the target gene. All existing base mismatches did not appear on the first base at the 3'end, which has little influence on amplification. The N gene of the >MT326140.1 Specimen has a mismatch at the second base at the 3'end. This mismatch needs to be evaluated for the effect of primer amplification efficiency. In addition, from the current known sequences, there is only one mismatch in 2678 specimen sequences. The mutation frequency is low, which has little influence on the detection rate of the kit.

In the future, we will conduct sequence analysis every 3 months. If there are significant mutations that may affect the detection effect of the kit, Daan will immediately report to WHO and discuss with WHO about the solution.

3.2 In silico analysis of cross-reactivity

- Study description

A total of 40 potential cross-reacting microorganisms (listed in Table 15 and Table 16 in page 41) complete genomic sequences was downloaded from NCBI and evaluated by in silico analysis. Microorganisms evaluated were from the same virus family, may be present in respiratory specimens, and/or induce similar symptoms as SARS-CoV-2. The predicted specificity of the N gene and ORF1ab gene amplified sequences was analyzed by BioEdit software.

- Acceptance criteria:

In silico cross-reactivity lower than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. If the silico cross-reactivity is higher than 80%, wet-testing should be performed.

- Results

The analysis of demonstrated comparison results, the assay primers of the N gene and ORF1ab gene targets have high homology to SARS-CoV; but the probes have <80% homology (6~8 base mismatch). The homology of ORF1ab gene primers have close to or greater than 80% homology for Coronaviruses NLL63, HKU1, OC43, MERS, SARS-CoV and 229E . Significant homology was not observed for all other pathogens evaluated in the analysis. Results of in silico analysis are presented in Table 15 and Table 16.

- Conclusion

Based on results from in silico analysis, wet testing was performed for coronaviruses NLL63, HKU1, OC43, MERS, SARS-COV and 229E (See Section 16.2 below). There was no high homology found for other pathogens evaluated.

Table 15 Other high priority pathogens from the same genetic family in silico analysis of cross-reactivity

microorganisms	N	ORF1ab
Human coronavirus NL63	No alignment was found	F:83.3%; R:61.5%; P: 50%(12/24)
Human coronavirus OC43	No alignment was found	F:83.3%; R:57.7%; P: 79.2%(19/24)
Human coronavirus HKU1	No alignment was found	F79.2%; R:65.4%; P: 70.8%(17/24)
Human coronavirus 229E	No alignment was found	F:83.3%; R:66.7%; P: 75%(18/24)
SARS-CoV	F:100%; R:91.3%; P: 69.2%(18/26)	F:100%; R:96.2%; P: 75%(18/24)
MERS-CoV	No alignment was found	F:79.2%; R:65.4%; P: 66.7%(18/24)

Note: 1."F" means upstream primer, "R" means downstream primer, "P" means probe. "X%" means the similarity between the cross pathogen sequence obtained by computer simulation and the SARS-CoV-2 sequence. 2." No alignment was found" means no homology.

Table 16 High priority organisms likely in the circulating area in silico analysis of cross-reactivity

Pathogen	Subtypes (if applicable)	N	ORF1ab
Influenza A	H1N1	No alignment was found	No alignment was found
	H1N1(2009)	No alignment was found	No alignment was found
	H3N2	No alignment was found	No alignment was found
	H5N1	No alignment was found	No alignment was found
	H7N9	No alignment was found	No alignment was found
Influenza B	IVB-Y	No alignment was found	No alignment was found
	IVB-V	No alignment was found	No alignment was found
Respiratory syncytial virus	RSVA	No alignment was found	No alignment was found
	RSVB	No alignment was found	No alignment was found
Parainfluenza virus 1-4	PIV1	No alignment was found	No alignment was found
	PIV2	No alignment was found	No alignment was found
	PIV3	No alignment was found	No alignment was found
	PIV4	No alignment was found	No alignment was found
Adenovirus	ADV1	No alignment was found	No alignment was found

Pathogen	Subtypes (if applicable)	N	ORF1ab
	ADV2	No alignment was found	No alignment was found
	ADV3	No alignment was found	No alignment was found
	ADV4	No alignment was found	No alignment was found
	ADV5	No alignment was found	No alignment was found
	ADV7	No alignment was found	No alignment was found
	ADV55	No alignment was found	No alignment was found
Enterovirus	EV-A	No alignment was found	No alignment was found
	EV-B	No alignment was found	No alignment was found
	EV-C	No alignment was found	No alignment was found
	EV-D	No alignment was found	No alignment was found
Human Metapneumovirus (hMPV)	NA	No alignment was found	No alignment was found
EBV	NA	No alignment was found	No alignment was found
measles virus	NA	No alignment was found	No alignment was found
Cytomegalovirus	NA	No alignment was found	No alignment was found
Rotavirus	NA	No alignment was found	No alignment was found
Norovirus	NA	No alignment was found	No alignment was found
Mumps virus	NA	No alignment was found	No alignment was found
Varicella zoster virus	NA	No alignment was found	No alignment was found
Mycoplasma pneumoniae	NA	No alignment was found	No alignment was found
Chlamydia pneumoniae	NA	No alignment was found	No alignment was found
Legionella pneumophila	NA	No alignment was found	No alignment was found
Bordetella pertussis	NA	No alignment was found	No alignment was found
Haemophilus influenzae	NA	No alignment was found	No alignment was found
Staphylococcus aureus	NA	No alignment was found	No alignment was found
Streptococcus pneumoniae	NA	No alignment was found	No alignment was found
Streptococcus	NA	No alignment was found	No alignment was found

Pathogen	Subtypes (if applicable)	N	ORF1ab
pyogenes			
Klebsiella pneumoniae	NA	No alignment was found	No alignment was found
Mycobacterium tuberculosis	NA	No alignment was found	No alignment was found
Aspergillus fumigatus	NA	No alignment was found	No alignment was found
Candida albicans	NA	No alignment was found	No alignment was found
Candida glabrata	NA	No alignment was found	No alignment was found
Cryptococcus neoformans	NA	No alignment was found	No alignment was found
human genome	NA	No alignment was found	No alignment was found
Rhinovirus	Rhinovirus A	No alignment was found	No alignment was found
	Rhinovirus B	No alignment was found	No alignment was found
	Rhinovirus C	No alignment was found	No alignment was found
Pseudomonas aeruginosa	NA	No alignment was found	No alignment was found
Staphylococcus epidermis	NA	No alignment was found	No alignment was found
Streptococcus salivarius	NA	No alignment was found	No alignment was found
Pneumocystis jirovecii (PJP)	NA	No alignment was found	No alignment was found

Note：" No alignment was found" means no homology found.

3.3 Cross-Reactivity/Microbial Interference Studies

- Study description

Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) was evaluated with a diverse selection of viruses and microorganisms for potential cross-reactivity or microbial interference. The pathogens evaluated (Table 17) were chosen based on the virus family, the potential presence in respiratory specimens, and/or based on their ability to cause similar symptoms to SARS-CoV-2 infection. All specimens were prepared with the potential cross-reacting/interfering microorganism at a high concentration. Two specimens were prepared: one specimen prepared with the potential cross-reacting/interfering microorganism together with SARS-CoV-2

pseudovirus (1×~2×LoD) to evaluate for potential microbial interference, the other specimen prepared with the potential cross-reacting organism only to evaluate for potential cross-reactivity.

- Acceptance criteria

The test results of specific specimens without SARS-CoV-2 pseudovirus should be negative, and the test results of specific specimens with SARS-CoV-2 pseudovirus (1×~2×LoD) should be positive.

- Results

The results of pathogens without SARS-CoV-2 pseudovirus were all negative.

The results of pathogens with 1×~2× LoD SARS-CoV-2 pseudovirus were all positive for both N gene and ORF1ab gene targets.

- Conclusion

Microbial interference or cross-reactivity was not observed in the study.

Table 17 Cross-Reactivity/Microbial Interference, Pathogens Evaluated

Pathogens	Subtypes (if applicable)	concentration	source
Influenza A	H1N1	$1*10^5$ PFU/mL	ATCC
	H1N1(2009)	$1*10^5$ PFU/mL	State Key Laboratory of Virology（wuhan）
	H3N2	$1*10^5$ PFU/mL	ATCC
	H5N1	$1*10^5$ PFU/mL	State Key Laboratory of Virology（wuhan）
	H7N9	$1*10^5$ PFU/mL	State Key Laboratory of Virology（wuhan）
Influenza B	IVB-Y	$1*10^5$ PFU/mL	State Key Laboratory of Virology（wuhan）
	IVB-V	$1*10^5$ PFU/mL	State Key Laboratory of Virology（wuhan）
Respiratory syncytial virus	RSVA	$1*10^5$ PFU/mL	ATCC
	RSVB	$1*10^5$ PFU/mL	ATCC
Parainfluenza virus 1-3	PIV1	$1*10^5$ PFU/mL	ATCC
	PIV2	$1*10^5$ PFU/mL	ATCC
	PIV3	$1*10^5$ PFU/mL	ATCC
Adenovirus	ADV1	$1*10^5$ PFU/mL	ATCC
	ADV2	$1*10^5$ PFU/mL	ATCC
	ADV3	$1*10^5$ PFU/mL	ATCC
	ADV4	$1*10^5$ PFU/mL	ATCC

	ADV5	1*10^5 PFU/mL	ATCC
	ADV7	1*10^5 PFU/mL	ATCC
	ADV55	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
Enterovirus	EV-A	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
	EV-B	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
	EV-C	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
	EV-D	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
HMPV	NA	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
EBV	NA	1*10^6 IU/mL	WHO
measles virus	NA	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
Cytomegalovirus	NA	1*10^6 IU/mL	WHO
Rotavirus	NA	1*10^5 PFU/mL	YUNKANG (Regional Medical Laboratory)
Norovirus	NA	1*10^{6}copies/mL	YUNKANG (Regional Medical Laboratory)
Mumps virus	NA	1*10^5 PFU/mL	National Institute for Food and Drug Control, China
Varicella zoster virus	NA	1*10^5 PFU/mL	ATCC
Mycoplasma pneumoniae	NA	1*10^5 PFU/mL	ATCC
Chlamydia pneumoniae	NA	1*10^5 PFU/mL	ATCC
Legionella	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Bordetella pertussis	NA	1*10^6 CFU/mL	ATCC
Haemophilus influenzae	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Staphylococcus aureus	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Streptococcus pneumoniae	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Streptococcus pyogenes	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Klebsiella pneumoniae	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Mycobacterium tuberculosis	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Aspergillus fumigatus	NA	1*10^6 CFU/mL	ATCC
Candida albicans	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China
Candida glabrata	NA	1*10^6 CFU/mL	National Institute for Food and Drug Control, China

Cryptococcus neoformans	NA	1*10^6 CFU/mL	ATCC
Human coronavirus NL63	NA	1*10^5 PFU/mL	State Key Laboratory of Virology（wuhan）
Human coronavirus OC43	NA	1*10^5 PFU/mL	State Key Laboratory of Virology（wuhan）
Human coronavirus HKU1	NA	1*10^5 PFU/mL	State Key Laboratory of Virology（wuhan）
Human coronavirus 229E	NA	1*10^5 PFU/mL	State Key Laboratory of Virology（wuhan）
SARS-CoV (pseudovirus)	NA	1*10^{6}copies/mL	Daan Gene Co., Ltd.
MERS-CoV (pseudovirus)	NA	1*10^{6}copies/mL	Daan Gene Co., Ltd.
human genome	NA	50ng/μL	Daan Gene Co., Ltd.

Note: “copies/mL” is calculated by digital PCR or optical density. “ng/μL” is calculated by optical density. “PFU/mL” and “CFU/mL” are calculated by culture.

Table 18 The results (Ct value) of microbial interference studies with 1×~2×LoD SARS-COV-2 pseudovirus

Pathogens	Ct value								
	lot2020001			lot2020002			lot2020003		
	N (FAM)	ORF1ab (VIC)	RNP (Cy5)	N (FAM)	ORF1ab (VIC)	RNP (Cy5)	N (FAM)	ORF1ab (VIC)	RNP (Cy5)
H1N1	34.57	33.92	29.79	34.91	34.25	28.97	34.09	33.63	29.42
H1N1(2009)	35.04	34.01	28.66	35.95	34.16	29.32	36.82	33.61	29.67
H3N2	35.51	33.65	29.65	35.02	33.90	29.37	35.53	33.81	29.65
H5N1	35.16	33.81	30.18	34.46	33.65	28.71	34.66	33.61	29.67
H7N9	35.25	33.87	28.95	34.71	34.34	29.38	34.78	33.55	29.77
IVB-Y	34.47	34.24	30.07	35.05	34.75	28.90	34.88	34.19	29.64
IVB-V	35.30	33.90	30.63	34.97	34.15	29.65	35.22	34.16	29.81
RSVA	34.27	33.74	29.77	34.51	34.21	29.11	33.34	32.82	29.63
RSVB	34.82	33.84	30.12	35.15	34.01	29.43	34.38	33.71	29.82
PIV1	35.67	34.00	29.63	34.50	34.10	29.75	35.12	33.86	29.74
PIV2	35.31	33.91	29.92	35.26	34.67	28.90	35.91	33.80	29.82
PIV3	35.51	33.94	30.25	34.50	34.38	29.72	35.34	33.86	29.86

Pathogens	Ct value								
	lot2020001			lot2020002			lot2020003		
	N (FAM)	ORF1ab (VIC)	RNP (Cy5)	N (FAM)	ORF1ab (VIC)	RNP (Cy5)	N (FAM)	ORF1ab (VIC)	RNP (Cy5)
ADV1	34.87	34.23	30.61	34.47	34.40	29.65	35.06	33.96	29.89
ADV2	35.92	34.24	30.70	34.92	34.67	29.81	35.12	33.86	29.90
ADV3	36.22	33.99	29.59	35.58	34.60	29.01	35.31	34.14	29.65
ADV4	33.66	34.02	29.93	34.19	34.30	29.35	34.23	33.14	29.79
ADV5	34.30	34.09	29.99	36.13	34.73	29.40	34.25	33.58	29.86
ADV7	35.77	34.57	29.88	34.92	34.58	29.10	34.71	33.39	29.69
ADV55	35.74	34.28	30.09	35.72	34.27	29.39	35.83	33.96	29.75
EV-A	35.44	34.15	30.41	34.83	34.42	29.46	34.31	33.69	29.95
EV-B	35.03	34.60	30.35	34.94	34.38	29.63	35.03	34.18	29.78
EV-C	34.93	34.37	29.74	34.63	34.41	29.09	34.82	34.00	29.80
EV-D	35.69	34.50	29.88	34.70	33.99	29.24	35.63	34.07	29.72
HMPV	33.26	32.53	30.03	35.35	34.42	29.36	34.39	33.78	29.88
EBV	35.08	34.24	30.03	34.42	34.18	29.53	34.81	33.52	29.87
measles virus	35.62	34.00	30.55	36.31	34.73	29.55	34.87	33.35	29.91
Cytomegalovirus	34.60	33.90	30.25	35.18	34.44	29.56	35.03	33.85	29.91
Rotavirus	35.91	34.11	30.71	35.27	34.48	29.38	36.37	33.82	29.86
Norovirus	35.17	34.64	29.71	35.63	34.31	29.10	35.45	34.39	29.81
Mumps virus	36.73	34.08	30.32	38.66	34.54	29.48	35.30	34.00	29.83
Varicella zoster virus	35.01	34.81	30.05	34.96	34.23	29.25	35.21	33.59	29.97
Mycoplasma pneumoniae	34.92	33.70	29.89	34.33	34.67	29.34	34.48	33.98	29.94
Chlamydia pneumoniae	35.18	34.70	30.41	35.90	34.51	29.49	35.49	33.27	29.81
Legionella	36.15	34.80	30.44	35.56	34.84	29.28	35.46	33.87	29.92
Bordetella pertussis	34.67	33.94	29.46	34.48	34.14	29.14	35.00	33.63	29.83
Haemophilus influenzae	36.28	34.41	30.02	35.44	34.44	29.38	35.17	33.95	29.98
Staphylococcus aureus	35.39	34.29	29.97	35.34	34.85	29.36	35.41	33.75	30.00
Streptococcus pneumoniae	36.57	34.25	30.38	34.72	34.34	29.50	34.63	33.62	29.76
Streptococcus pyogenes	35.47	34.77	29.94	36.88	34.46	29.47	34.98	33.81	29.87
Klebsiella pneumoniae	35.97	34.07	30.38	34.71	34.63	29.66	35.34	33.68	29.86
Mycobacterium tuberculosis	35.46	34.52	29.75	34.95	34.77	29.34	33.77	33.08	29.81
Aspergillus fumigatus	36.74	34.83	29.86	35.10	34.71	29.53	34.29	33.79	29.99

Pathogens	Ct value								
	lot2020001			lot2020002			lot2020003		
	N (FAM)	ORF1ab (VIC)	RNP (Cy5)	N (FAM)	ORF1ab (VIC)	RNP (Cy5)	N (FAM)	ORF1ab (VIC)	RNP (Cy5)
Candida albicans	36.30	34.33	30.19	35.11	34.52	29.10	35.79	33.97	29.91
Candida glabrata	36.10	34.80	30.33	35.53	34.64	29.50	34.69	34.25	29.66
Cryptococcus neoformans	36.18	34.96	30.49	34.98	34.61	29.81	34.83	33.54	29.78
Human coronavirus NL63	35.51	34.27	30.29	34.99	34.65	29.82	35.14	34.18	29.73
Human coronavirus OC43	35.50	34.36	29.57	35.35	34.72	28.79	34.55	33.71	28.37
Human coronavirus HKU1	35.79	34.63	29.71	34.59	34.80	29.24	34.46	33.52	29.17
Human coronavirus 229E	35.07	34.66	29.34	36.53	34.74	29.47	35.35	33.59	29.89
SARS-CoV(pseudovirus)	36.35	34.88	29.77	35.67	34.42	29.54	35.51	33.98	29.49
MERS-CoV(pseudovirus)	35.69	34.51	29.96	36.41	34.05	29.71	35.50	33.93	29.92
human genome	35.78	34.71	30.36	34.65	34.60	29.68	35.23	33.97	29.63
NTC	-	-	30.01	-	-	29.13	-	-	29.15
PTC	27.93	26.96	28.85	28.20	27.54	28.07	28.79	27.90	28.34

Note: "-" means FAM and VIC negative, Cy5 positive. NTC means negative control, PTC means positive control.

3.4 Interfering substances

- Study description

Performance of the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) was evaluated for various potentially interfering substances that may be present in respiratory specimens. The potentially interfering substances and concentrations are presented in Table 19.

Specimens tested were prepared with negative clinical sputum matrix as follows (See Table 20 for additional details):

- Negative sputum matrix
- Negative sputum matrix with interfering substance

- Negative sputum matrix with interfering substance and SARS-CoV-2 pseudovirus at 1000 copies/mL (2×LoD).

- Acceptance criteria:

The test results of negative sputum and negative sputum with interfering substances specimens should be negative. The test results of sputum with interfering substances and 1000 copies/mL (2×LoD) pseudovirus specimens should be positive.

Table 19 Information for interfering substances

substances	concentration	substances	concentration
Human blood	10%	zanamivir	100 μg/mL
mucus	20 μg/mL	ribavirin	100 μg/mL
Phenylephrine Hydrochloride	100 μg/mL	oseltamivir	100 μg/mL
oxymetazoline	100 μg/mL	peramivir	100 μg/mL
sodium chloride	0.9%	lopinavir	100 μg/mL
beclomethasone	100 μg/mL	mupirocin	100 μg/mL
dexamethasone	100 μg/mL	levofloxacin	100 μg/mL
fluticasone propionate	100 μg/mL	azithromycin	100 μg/mL
triamcinolone	100 μg/mL	tobramycin	100 μg/mL
budesonide	100 μg/mL	ritonavir	100 μg/mL
mometasone	100 μg/mL	meropenem	100 μg/mL
fluticasone	100 μg/mL	Arbidol	100 μg/mL
histamine hydrochloride	100 μg/mL	ceftriaxone	100 μg/mL
interferon	300 U/mL	/	/

Table 20 three situations of Specimens of Interfering substances

substances	Specimen 1	Specimen 2	Specimen 3
Human blood	negative sputum	negative sputum+10% Human blood	negative sputum+10% Human blood+2×LoD pseudovirus
mucus		negative sputum+20 μg/mL mucus	negative sputum+20 μg/mL mucus+2×LoD pseudovirus
Phenylephrine Hydrochloride		negative sputum+100 μg/mL Phenylephrine Hydrochloride	negative sputum+100 μg/mL Phenylephrine Hydrochloride+2×LoD pseudovirus
oxymetazoline		negative sputum+100 μg/mL oxymetazoline	negative sputum+100 μg/mL oxymetazoline+2×LoD pseudovirus
sodium chloride		negative sputum+0.9% sodium chloride	negative sputum+0.9% sodium chloride+2×LoD pseudovirus
beclomethasone		negative sputum+100μg/mL beclomethasone	negative sputum+100 μg/mL beclomethasone+2×LoD pseudovirus
dexamethasone		negative sputum+100 μg/mL	negative sputum+100 μg/mL beclomethasone+2×LoD pseudovirus
fluticasone propionate		negative sputum+100 μg/mL fluticasone propionate	negative sputum+100 μg/mL fluticasone propionate+2×LoD pseudovirus
triamcinolone		negative sputum+100 μg/mL triamcinolone	negative sputum+100 μg/mL triamcinolone+2×LoD pseudovirus
budesonide		negative sputum+100 μg/mL budesonide	negative sputum+100 μg/mL budesonide+2×LoD pseudovirus
mometasone		negative sputum+100 μg/mL mometasone	negative sputum+100 μg/mL mometasone+2×LoD pseudovirus
fluticasone		negative sputum+100 μg/mL fluticasone	negative sputum+100 μg/mL fluticasone+2×LoD pseudovirus
histamine hydrochloride		negative sputum+100 μg/mL histamine hydrochloride	negative sputum+100 μg/mL histamine hydrochloride+2×LoD pseudovirus
interferon		negative sputum+300 U/mL interferon	negative sputum+300 U/mL interferon+2×LoD pseudovirus
zanamivir		negative sputum+100 μg/mL	negative sputum+100

		zanamivir	μg/mLzanamivir+2×LoD pseudovirus
ribavirin		negative sputum+100 μg/mL ribavirin	negative sputum+100 μg/mL ribavirin+2×LoD pseudovirus
oseltamivir		negative sputum+100 μg/mL oseltamivir	negative sputum+100 μg/mL oseltamivir+2×LoD pseudovirus
peramivir		negative sputum+100 μg/mL peramivir	negative sputum+100 μg/mL peramivir+2×LoD pseudovirus
lopinavir		negative sputum+100 μg/mL lopinavir	negative sputum+100 μg/mL lopinavir+2×LoD pseudovirus
mupirocin		negative sputum+100 μg/mL mupirocin	negative sputum+100 μg/mL mupirocin+2×LoD pseudovirus
levofloxacin		negative sputum+100 μg/mL levofloxacin	negative sputum+100 μg/mL levofloxacin+2×LoD pseudovirus
azithromycin		negative sputum+100 μg/mL azithromycin	negative sputum+100 μg/mL azithromycin+2×LoD pseudovirus
tobramycin		negative sputum+100 μg/mL tobramycin	negative sputum+100μg/mL tobramycin+2×LoD pseudovirus
ritonavir		negative sputum+100 μg/mL ritonavir	negative sputum+100 μg/mL ritonavir+2×LoD pseudovirus
meropenem		negative sputum+100 μg/mL meropenem	negative sputum+100 μg/mL meropenem+2×LoD pseudovirus
Arbidol		negative sputum+100 μg/mL Arbidol	negative sputum+100 μg/mL Arbidol+2×LoD pseudovirus
ceftriaxone		negative sputum+100 μg/mL ceftriaxone	negative sputum+100 μg/mL ceftriaxone+2×LoD pseudovirus

- Results

All the results of negative sputum were negative.

All the results of negative sputum with interfering substances were negative.

All the results of sputum with 2×LoD pseudovirus and interfering substances were positive for both N gene and ORF1ab gene targets.

Table 21 Results (Ct value) of interfering substances study in the sputa for 3 lots

Interfering substances	Negative sputa with interfering substances	1000 copies/mL sputum with interfering substances								
		lot2020001			lot2020002			lot2020003		
		N(FAM)	ORF1ab(VIC)	RNP(Cy5)	N(FAM)	ORF1ab(VIC)	RNP(Cy5)	N(FAM)	ORF1ab(VIC)	RNP(Cy5)
Phenylephrine Hydrochloride	-	33.59	33.53	30.63	33.25	33.62	29.65	33.14	33.43	29.86
oxymetazoline	-	32.63	32.71	29.77	32.47	33.82	29.11	32.5	32.14	29.81
sodium chloride	-	32.7	33.71	30.12	33.17	33.11	29.43	32.41	32.21	29.99
beclomethasone	-	33.02	33.09	29.63	33.9	32.71	29.75	32.9	32.41	29.91
dexamethasone	-	33.56	33.59	29.92	32.26	33.18	28.90	33.7	33.04	29.64
fluticasone propionate	-	33.13	32.78	30.25	33.37	33.74	29.72	33.91	33.28	29.42
triamcinolone	-	33.65	33.52	30.61	33.98	32.08	29.65	33.46	32.83	29.67
budesonide	-	33.57	32.48	30.70	33.69	32.36	29.81	33.07	33.89	29.65
mometasone	-	32.13	32.9	29.59	32.06	32.98	29.01	33.89	33.77	29.67
fluticasone	-	32.64	33.51	29.93	32.04	33.01	29.35	32.2	33.4	29.77
histamine hydrochloride	-	33.74	32.47	29.99	32.95	33.83	29.40	33.87	33.87	29.64
interferon	-	33.87	33.06	29.88	33.29	33.73	29.10	33.69	33.85	29.81
zanamivir	-	33.14	33.84	30.09	33.44	32.74	29.39	32.45	32.48	29.63
ribavirin	-	32.28	32.54	30.41	32.9	33.73	29.46	33.08	32.88	29.82
oseltamivir	-	32.24	33.2	30.35	33.31	32.82	29.63	32.31	33.72	29.74
peramivir	-	32.5	33.29	29.74	33.56	32.49	29.09	32.72	32.6	29.82
lopinavir	-	32.21	33.12	29.88	32.29	33.57	29.24	33.72	33.57	29.86
mupirocin	-	32.98	32.47	30.03	33.82	32.21	29.36	33.63	32.03	29.89
levofloxacin	-	33.15	32	30.03	33.1	32.2	29.53	33.08	33.55	29.90
azithromycin	-	33.9	33.78	30.55	32.04	32.22	29.55	33.22	32.22	29.97
ceftriaxone	-	32.08	32.45	30.25	33.06	32.26	29.56	32.72	33.08	29.94
ritonavir	-	33.03	32.71	30.71	33.99	33.23	29.38	32.99	32.09	29.81
tobramycin	-	32.15	32.66	29.71	33.22	33.8	29.10	33.13	32.7	29.92
meropenem	-	33.08	32.87	30.32	32.27	33.2	29.72	33.46	33.38	29.83
Arbidol	-	33.14	33.04	30.05	32.71	32.83	29.88	32.61	32.34	29.98
Human blood	-	33.58	33.96	29.89	32.99	32.59	29.87	34	33	30.00
mucus	-	32.2	33.09	29.65	33.8	33.04	29.76	32.28	32.43	29.76
negative sputa	-	33.62	33.3	29.11	32.45	33.91	29.87	33.55	32.52	29.87
NTC		-	-	30.01	-	-	29.13	-	-	29.15
PTC		27.93	26.96	28.85	28.2	27.54	28.07	28.79	27.9	28.34

Note: "-" means negative result. NTC means negative control, PTC means positive control.

[Clinical Evaluation Characteristics]

In this clinical trial, 736 statistical specimens were finally included, including 318 oropharyngeal swabs and 418 sputum specimens. The test results of 318 oropharyngeal swabs were positive in 18 cases and negative in 300 cases. The sputum specimens of 418 cases were positive in 33 cases and negative in 385 cases. Statistical analysis results of throat swab specimens and sputum specimens are shown in Table 22 and 23. The comparator method was 2019 Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit from Shanghai BioGerm Medical Biotechnology Co., Ltd.

Table 22 Statistical analysis result of oropharyngeal swab specimens

Test		Comparator method		Total
		Positive	Negative	
Assessment reagent	Positive	18	0	18
	Negative	0	300	300
Total quantity		18	300	318

Positive coincidence rate=18/（18+0）×100%=100.00%
Negative coincidence rate＝300/（0+300）×100%=100.00%
Total coincidence rate=（18+300）/（18+0+0+300）×100%=100.00%

Table 23 Statistical analysis result of sputum specimens

Test		Comparator method		Total
		Positive	Negative	
Assessment reagent	Positive	33	0	33
	Negative	0	385	385
Total quantity		33	385	418

Positive coincidence rate=33/（33+0）×100%=100.00%
Negative coincidence rate＝385/（0+385）×100%=100.00%
Total coincidence rate=（33+385）/（33+0+0+385）×100%=100.00%

According to statistical analysis, compared with the comparator method, the test results of oropharyngeal swab specimens are as follows: positive coincidence rate was 100.00%(95%CI82.41%-100%), the negative coincidence rate was 100.00%(95%CI98.74%-100%), and the total coincidence rate was 100.00%(95%CI98.81%-100%); the test results of sputum specimen are as follows: positive coincidence rate was 100.00%(95%CI89.57%-100%), negative coincidence

rate was 100.00%(95%CI99.01%-100%), and total coincidence rate was 100.00%(95%CI99.09%-100%). The consistency of kappa test showed that the consistency of oropharyngeal swab specimens and sputum specimens was good and statistically significant (Kappa values were both 1.000(95%CI1 ~ 1), P<0.001).

[References]

1. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

[Basic Information]

Name of manufacturer/ after-sales service unit: Daan Gene Co., Ltd.

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