Phone: 86-523-86201352
Fax: 86-523-86816890
Email: service@cwbiosciences.com

Ver. A/0

Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing)

For *in vitro* diagnostic use

Emergency Use Authorization (EUA) Only

Rx Only

Product: Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing)

Size: 24 tests/Box, 48 tests/Box, 96 tests/Box

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Intended Use

The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. 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 within the United States and its territories are required to report all results to the appropriate public health authorities.

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

The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays and in vitro diagnostic procedures. The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) is only for use under the Food and Drug Administration's Emergency Use Authorization.

Specimen Collection and Storage

Collection Process

1. Clean hands before sampling, tear open the package of Swabs, and take out the swab. Be careful not to touch the swab head.
2. Wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall with a swab over the base of the tongue.

3. After sampling, put the swab into a SARS-CoV-2 Collection Fluid tube to avoid contact with other parts.
4. Break the swab tip, discard stick and cap the tube containing samples.

Specimen Storage and Transportation

After sampling, swab samples need to be immediately placed in the tube with the SARS-CoV-2 Collection Fluid provided in this kit Swab samples treated with the SARS-CoV-2 Collection Fluid can be stored at 2-8 ℃ or - 80 ℃ for 10 days and can be transported at 2-8 ℃ for no more than 3 days.

Principle

The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) is a real-time reverse transcription-polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect specific ORF1ab and N genes from SARS-CoV-2 in oropharyngeal swabs from patients suspected of COVID-19 by their healthcare provider.

The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) requires collection of oropharyngeal specimens with CoWin swabs followed by immediate placement in a special preservation solution (SARS-CoV-2 Collection Fluid containing Tris buffer, EDTA, NaOH, Tween 20, etc.) that allows testing to be performed without the isolation and purification of nucleic acids from specimens.

Kit Components

Table 1: Kit Components

Reagent	Component	Size and Volume (24 tests/Box)	Size and Volume (48 tests/Box)	Size and Volume (96 tests/Box)
SARS-CoV-2 Collection Fluid	Tris, EDTA, NaOH, etc.	500 μL/tube×24	500 μL/tube×48	500 μL/tube×96
Swabs	CoWin	Swabs	CoWin Bioscience	CW3119M
SARS-CoV-2 Reaction Mixture	Reverse transcriptase, hot start DNA polymerase; specific ORF1ab and N gene primers, probes; internal control primers, and probes; reaction buffer, etc.（Lyophilized powder）	25μL*/tube ×24	25μL*/tube×48	25 μL*/tube×96
SARS-CoV-2 Positive Control	Pseudovirus carrying target genes（Lyophilized powder）	500 μL*/tube×1	500 μL*/tube×1	500 μL*/tube×1
SARS-CoV-2 Negative	Virus particles not carrying target genes（Lyophilized powder）	500 μL*/tube×1	500 μL*/tube×1	500 μL*/tube×1

Reagent	Component	Size and Volume (24 tests/Box)	Size and Volume (48 tests/Box)	Size and Volume (96 tests/Box)
Control				
Positive Control Dissolvent	Tris, EDTA, NaOH	500 μL/tube×1	500 μL/tube×1	500 μL/tube×1
Negative Control Dissolvent	Tris, EDTA, NaOH	500 μL/tube×1	500 μL/tube×1	500 μL/tube×1

* After Reconstitution

Components Required but Not Supplied in Kit

Table 2: Components Required but Not Supplied in Kit

Components Required But Not Included with the Test	Brand	Product model	Manufacturer	Catalog
ABI 7500 Real-Time PCR	ABI	7500	Thermo Fisher	4351105

Other items needed for this test with our kit include:

Gloves

Medical mask

Centrifuge

Benchtop centrifuge

Vortex mixer

Pipette tips

PCR Consumables

Warnings and Precautions

- For *in vitro* diagnostic use
- For Emergency Use Authorization only.
- For prescription use only.
- The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
- The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The experimental personnel should receive professional training (including sample processing, reagent preparation, instrument operation and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratories issued by local regulatory agencies.
- The laboratory should be separated by reagent preparation area, sample preparation area and amplification detection area. All the articles in each area are for special purposes, and they shall not be used for other purposes, as to avoid contamination. The suggested PPE (Personal Protective Equipment) for a laboratory worker are gowns or closed lab coats, hair nets, gloves, eye protection (face shield or goggles) and surgical facemasks or fit-tested N95 masks. Laboratory clothes, hats, shoes, gloves, etc. shall be fully equipped during operation to avoid direct contact of reagents or samples with skin. In case of liquid leakage, wash with plenty of water immediately. In case of contact with skin wounds, inform local health and epidemic prevention department in time.
- The real-time fluorescent quantitative PCR analyzer should be calibrated regularly.

- Please dispose of pipette and kit waste in a designated manner as per company safety policies, and in accordance with local, regional, and federal regulations.
- Clean the working area immediately after the experiment. The areas and surfaces should be disinfected with 1% sodium hypochlorite, 75% alcohol or UV light regularly.
- The quality of the test results (for samples and controls) are related to the collection, transportation, treatment and preservation of samples. If any of the sampling, storage, or testing process is improperly executed, this may lead to false negative or false positive results of the test.
- This product is an in vitro diagnostic reagent kit, a tester should be trained and experienced. Please read the manual carefully before the experiment.
- The laboratory management shall be in strict accordance with the management specifications of the PCR gene amplification laboratory. The laboratory personnel must be trained professionally. The experiment process shall be carried out in strict divisions (reagent preparation area, specimen preparation area, amplification and product analysis area). All consumables are disposable after sterilization. Special instruments and equipment shall be used in each stage of the experiment operation, and the supplies in each zone shall not be used in the cross.
- Sample processing shall be carried out in the biosafety cabinet to protect the safety of testers and prevent environmental contamination.
- The sample should be completely added to the reaction mixture. After adding the sample, cover the tube lid as soon as possible and spin the tube. After amplification, take out the reaction tubes, seal them in a special plastic bag, and discard it in a designated place.
- Do not loosen the lid after amplification in case of aerosol contamination.
- The used tips shall be directly put into the waste tank containing 10% sodium hypochlorite and disposed of together with other waste stuff.
- The worktable and various experimental stuff should be regularly disinfected with 75% alcohol and an ultraviolet lamp.
- The reagent should be recovered to room temperature before use and avoid repeated freezing and thawing. Avoid cross-contamination between reagents.
- Avoid reuse.

Storage Condition and Valid Period

The main component of the kit is a lyophilized powder, which can be stored and

transported at room temperature for 12 months before use. After the kit is opened for use, it can be stored at room temperature for one month or stored at 4 ℃ or - 20 ℃ for 2 months. The production date and expiration date of the kit are shown in the outer packing box, and the validity period is 12 months (at room temperature). If the kit is reconstituted and stored at -20 ℃, the number of repeated freeze-thaw cycles should not exceed 5 times.

Instrument Compatibility

ABI 7500 (software version: 7500_Software_v2 series).

Sample Requirements

The applicable samples are CoWin oropharyngeal swabs. CoWin swab samples need to be immediately treated with the SARS-CoV-2 Collection Fluid of this kit.

Detection Methods

1. Reagents Preparation

1.1. Before first use, the SARS-CoV-2 Positive Control (Lyophilized powder) and the SARS-CoV-2 Negative Control (Lyophilized powder) need to be reconstituted. Centrifugate SARS-CoV-2 positive control (Lyophilized powder) and SARS-CoV-2 negative control (Lyophilized powder) at 12000 rpm for 2 minutes. After centrifugation, carefully open the lid of the SARS-CoV-2 Negative Control (Lyophilized powder) first, and add 500 μL of Negative Control Dissolvent, shake and mix several times until the lyophilized powder is completely dissolved, then spin the tube. After that, open the lid of the SARS-CoV-2 Positive Control (Lyophilized powder), add 500 μL of Positive Control Dissolvent, shake and mix several times until the lyophilized powder is completely dissolved, then spin the tube.

Notes: ①To avoid potential loss of dry powder or contaminate the environment, do not open the lid before centrifugation; ②Reconstitute the Negative Control first, and then reconstitute the Positive Control; ③Do not mix the two different dissolvent reagents or misuse.

1.2 Calculate total reactions N: calculate the number of reactions according to the number of test samples. If the number of samples is n, N =n + 2. Prepare N tubes

containing SARS-CoV-2 Reaction Mixture (Lyophilized powder) out of the kit, centrifugate them at 12000 rpm for 2 minutes, and then put them on ice box for further use. Transfer the tubes to the sample preparation area.

2 Sample Preparation

2.1 Immediately after sampling with the oropharyngeal swab, place the swab head in the tube containing 500 μL of SARS-CoV-2 Collection Fluid. Shake and mix thoroughly at least 1 minute at room temperature before use.

2.2 Transfer 25 μL from each tube of collection fluid to be tested, SARS-CoV-2 Positive Control and SARS-CoV-2 Negative Control to the individual tubes of SARS-CoV- 2 Reaction Mixture (Lyophilized powder) one by one. Cap the tubes tightly, shake and mix thoroughly, then short centrifugation. Transfer the tubes to the nucleic acid amplification area for detection.

3 Running a Test

See below for step-by-step operation of ABI 7500:

3.1 Start ABI 7500 real time PCR system: Turn on the computer connected to the system first, and then turn on ABI 7500 real time PCR system.

3.2 Load the instrument: Push the tray door to open it, load the prepared tubes containing samples and controls into the plate holder in the instrument. Ensure that the tubes are properly aligned in the holder. Close the tray door. Apply pressure to the right side of the tray and at an angle. (Figure 1)

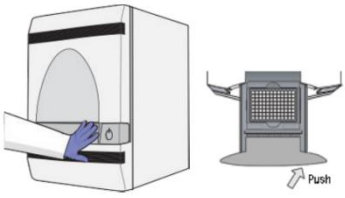

Figure 1: Load the Instrument

3.3 Set up the experiment run:

Double-click7500 icon (7500 software v2 series) or select Start>>All Programs>> Applied Biosystems>>7500 software v2 series.(Figure 2)

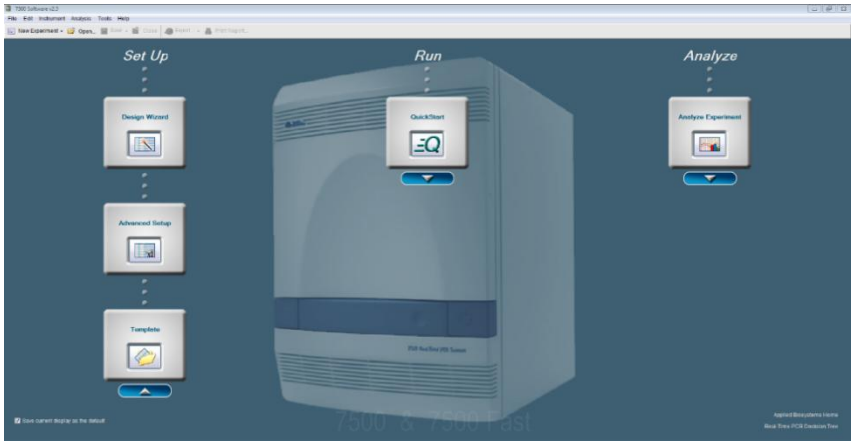

Figure 2: Set up the Experiment Run

3.4 Click New Experiment to enter the Experiment menu. In the Experiment Properties screen, enter identifying information for the experiment; you can leave other fields empty. (Figure 3)

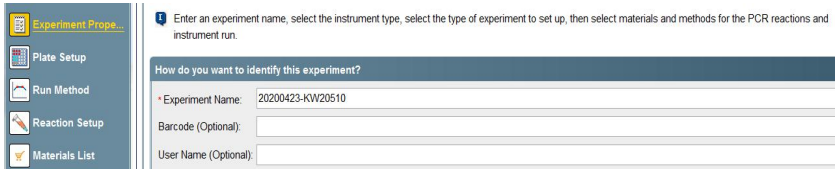

Figure 3: Click New Experiment to Enter the Experiment Menu

3.5 Select 7500 (96 Wells); Quantitation-Standard Curve (for the experiment type); TaqMan Reagents (for reagent); and standard (for ramp speed).(Figure 4)

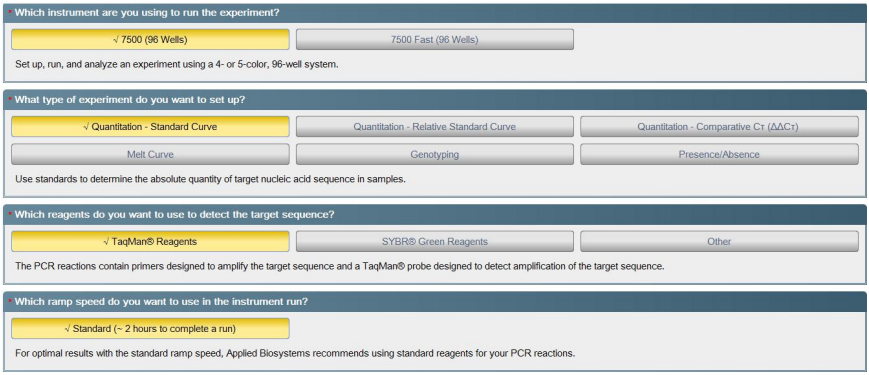

Figure 4: Parameters Setup

3.6 Click Plate Setup, in the Targets screen, under the tab Define Targets and Samples, enter targets as shown in the figure. Input target name: ORF1ab, N, IC, corresponding to fluorescence report group: FAM, ROX, VIC respectively, All quencher group select none, Color selection are blue, red, green respectively. (Figure 5)

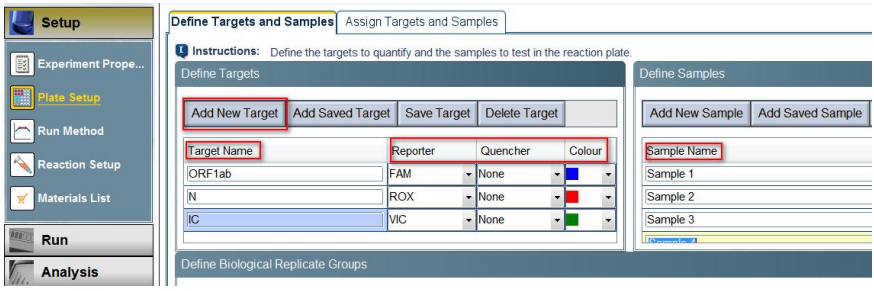

Figure 5: Parameters Setup

3.7 Click Assign Targets and Sample tab, in the Samples screen, enter the name of samples and controls to include in the reaction plate in corresponding well, and select the sample/target reactions to set up. Select None for passive reference. (Figure 6)

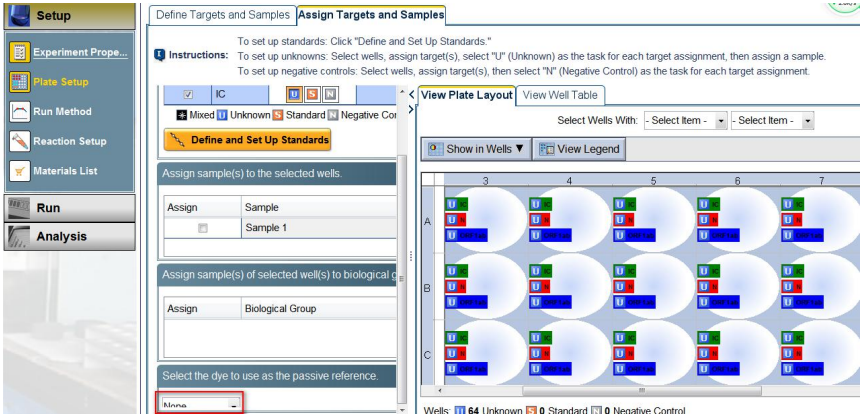

Figure 6: Parameters Setup

3.8 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 30 µL in the Reaction Volume Per Well field. The FAM channel (Reporter: FAM, Quencher: None) will be set up for detection of ORF1ab Gene of SARS-CoV-2; The ROX channel (Reporter: ROX, Quencher: None) will be set up for detection of N Gene of SARS-CoV-2; and the VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) will be set up for the detection of the internal control (ACTB); Reference Dye: None. Configure the PCR protocol as shown in Table 3.

Table 3: PCR Parameters

Procedure	Temperature	Time	Cycle
Reverse transcription	55°C	1 min	1
Pre-denaturation	95°C	20 sec	1
Denaturation	95°C	10 sec	45
Annealing, extension and Collect fluorescence signal	58°C	30 sec	

3.9 You may save a run method as shown in the figure below and use the method for future experiments. (Figure 7)

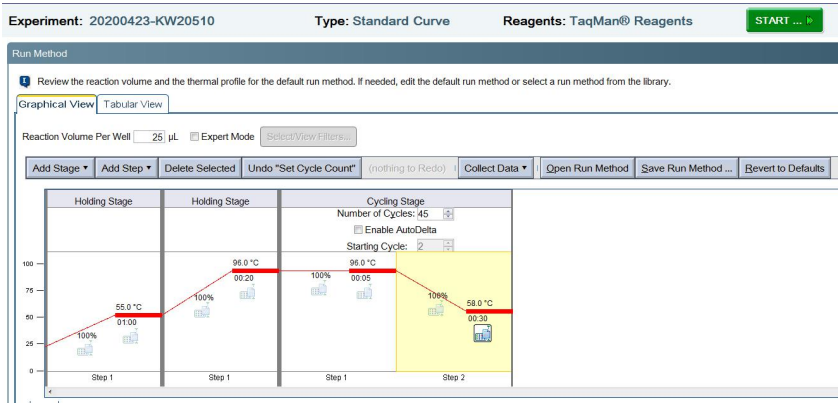

Figure 7: Parameters Setup

3.10 Click Run, in the Run screen, save the experiment. Click START. (Figure 8)

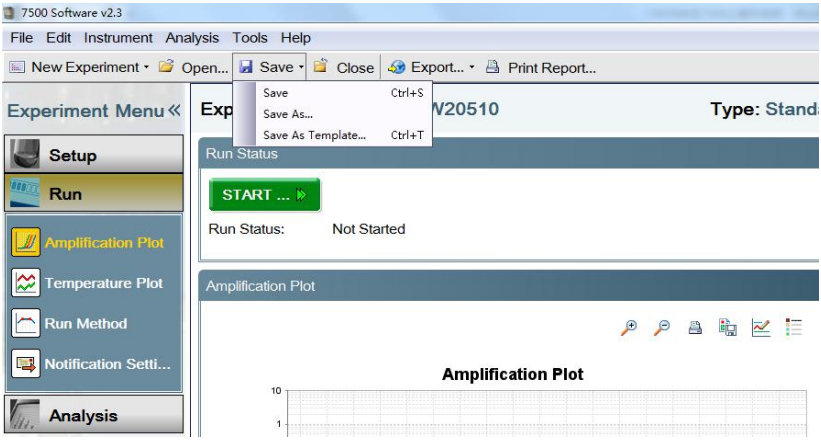

Figure 8: Click START

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

4 Data Analysis

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

4.1 Click Analysis. In the Amplification Plot screen under Plot Settings tab:(Figure 9)

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

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

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

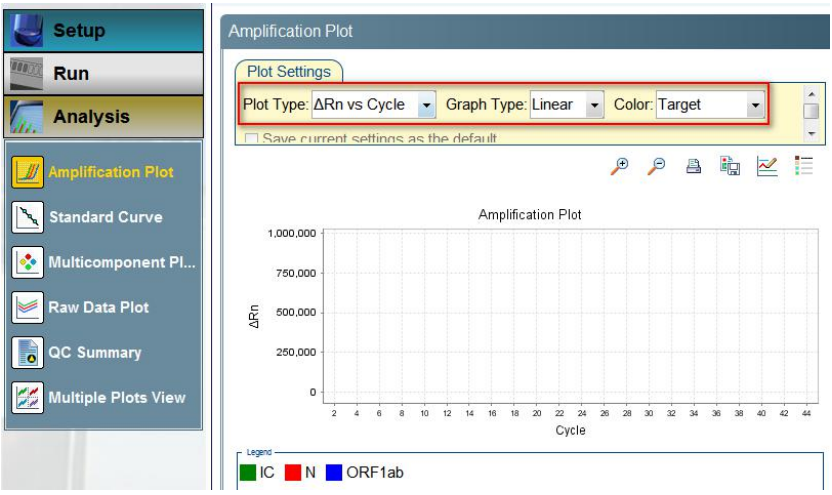

Figure 9: Analysis

4.2 Set Auto baseline.

4.3 Manually set thresholds:(Figure 10)

a. In the Target drop-down list, select Target ORF.

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

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

d. Repeat the steps for Target N and IC.

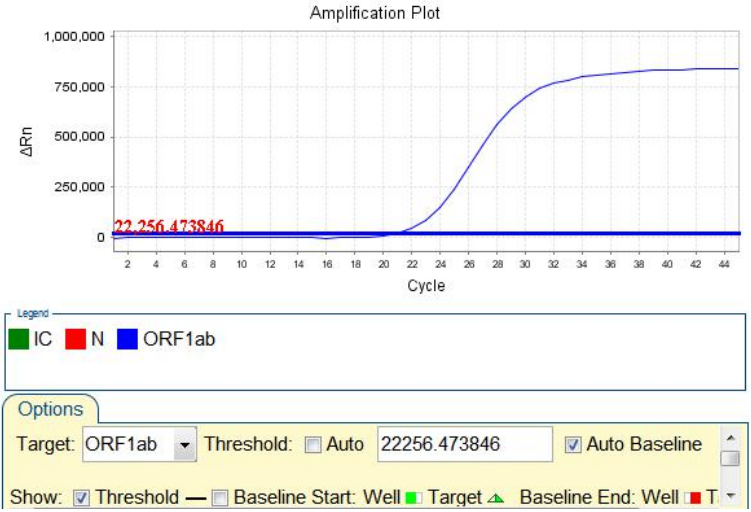

Figure 10: Manually Set Thresholds

4.4 Click Analyze. The software analyzes the data with the settings.(Figure 11)

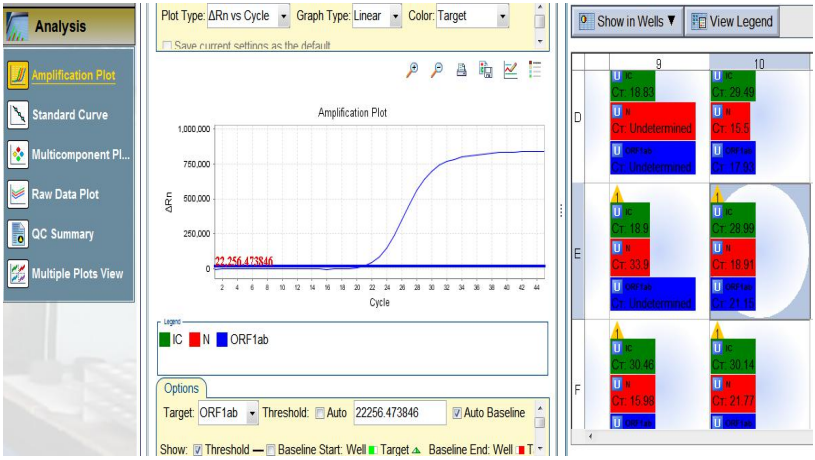

Figure 11: Click Analyze

4.5 To review a Ct value of a sample, click the well containing the sample as shown in the figure below. In the Target drop down, select the target for review.(Figure 12)

Figure 12: Analysis

4.6 Example of a positive sample amplification curve (ORF1ab gene (FAM) in blue, N gene (Rox) in red, and internal control (VIC) in green).(Figure 13-15)

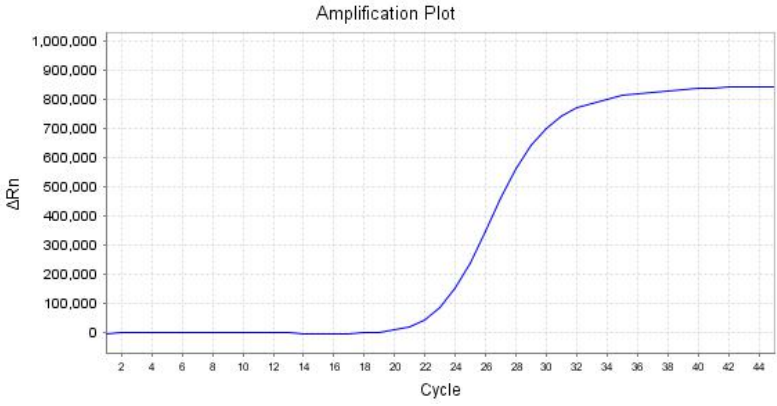

Figure 13: Example of a Positive Sample Amplification Curve

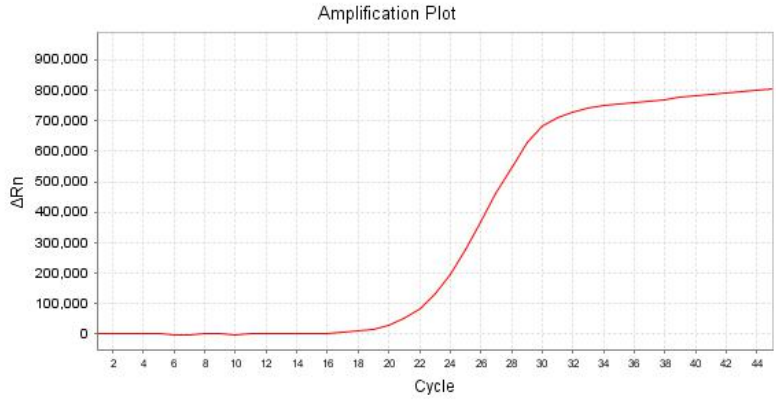

Figure 14: Example of a Positive Sample Amplification Curve

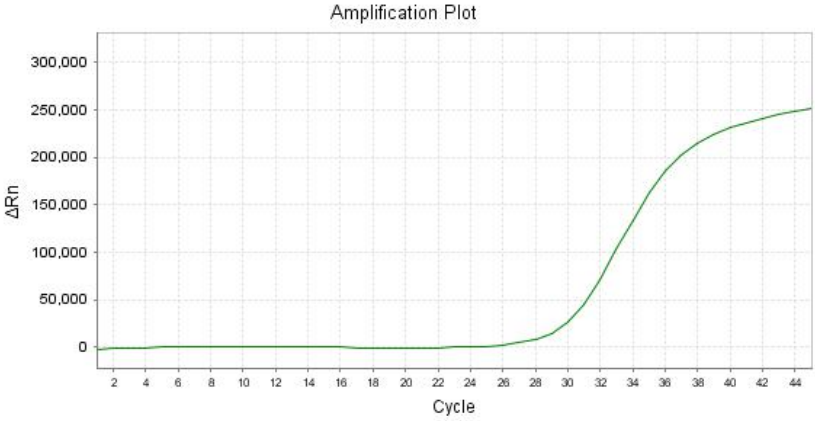

Figure 15: Example of a Positive Sample Amplification Curve

Experimental Validity

There are three different controls in this kit, the description of the controls used in this kit are listed below.

A. SARS-CoV-2 Positive Control: A positive template control is a pseudovirus that targets the SARS-CoV-2 N gene and ORF1ab gene. It is used on every plate and is needed to verify the assay's amplification and detection functions, and monitors gross reagent failure, such as reagent degradation, or incorrect assay set-up.

B. SARS-CoV-2 Negative Control: The Negative Control (Virus particles not carrying target genes) is needed to check for contamination, a negative result indicates that the reagents have not become contaminated. If a positive result is obtained, the results should be analyzed and checked if a correct amplification curve was obtained. When a clear amplification curve is obtained, repeat the assay to confirm whether the sample was contaminated or not. Negative control should be tested with the samples at the same time in each experiment.

C. Internal Control: An internal control consists of the human ACTB (β-actin) gene which is needed to monitor the quality of respiratory nucleic acid and PCR reaction system to avoid blank samples and false negative results. A positive result indicates that PCR reaction system worked correctly, and the quality of respiratory nucleic acid is acceptable. Since specific primers and probes of internal control are added to SARS-CoV-2 Reaction Mixture, every tested sample should have a positive internal control. The plasmid containing the ACTB (β-actin) gene has also been added to the SARS-CoV-2 Negative Control and SARS-CoV-2 Positive Control and should have a positive internal control result.

Note: ORF1ab gene reported fluorescence is FAM, N gene reported fluorescence is ROX, and the internal control gene reported fluorescence is VIC in this product.

1. SARS-CoV-2 Positive Control: FAM and ROX channels have a typical S-type amplification curve and CT value < 35. The internal control VIC channel has a typical S-type amplification curve and CT value < 35.

2. SARS-CoV-2 Negative Control: FAM and ROX channel value CT > 35 or no CT value, the amplification curve is a straight line or slight oblique line, no exponential growth period.

3. Experimental sample: the internal control VIC channel has a typical S-type amplification curve and CT value < 35.

4. The experiment result is qualified only if the conditions of 1, 2 and 3 above are all satisfied at the same time, otherwise it is invalid.

Table 4: Control Reaction Results Interpretation

<table>
<tr><th colspan="3">Positive Control</th><th colspan="3">Negative Control</th><th>Results</th><th>Actions</th></tr>
<tr><td>ORF1ab (FAM)</td><td>N (ROX)</td><td>IC (VIC)</td><td>ORF1ab (FAM)</td><td>N (ROX)</td><td>IC (VIC)</td><td rowspan="2">valid</td><td rowspan="2">Proceed to Examination and Interpretation of Patient Specimen Results</td></tr>
<tr><td>+</td><td>+</td><td>+</td><td>−</td><td>−</td><td>+</td></tr>
<tr><td colspan="3">At least one of them (FAM, ROX, VIC) shows negative</td><td colspan="3">Not considered</td><td rowspan="2">Invalid</td><td>rRT-PCR failed, re-run sample</td></tr>
<tr><td colspan="3">Not considered</td><td colspan="3">FAM or ROX channel shows positive</td><td>rRT-PCR contaminated, re-run sample</td></tr>
<tr><td colspan="8">Results are (-) when Ct value >35 or Undetermined.
Results are (+) when Ct value <35.
If there is contamination for the re-run, perform decontamination procedures.</td></tr>
</table>

Evaluation of Test Results

Automatically save the result after the reaction, adjust the start value, end value and a threshold value of the baseline according to the image after analysis (The user can adjust it according to the actual situation, set the value of threshold in the log graph pane to make the threshold line in the exponential phase of the amplification curve, and the amplification curve of the negative control is flat or lower than the threshold line). Click analysis to get the analysis results automatically and read the test results in the report window.

Table 5: Interpreting Test Results of Each Target

Test Results	IC Results	Interpreting Test Results	Action
ORF1ab gene (+), N gene (+)	(+) or (-)	SARS-CoV-2 Positive	Report results to healthcare provider and CDC.

Test Results	IC Results	Interpreting Test Results	Action
Only ORF1ab gene (+)	(+) or (-)	SARS-CoV-2 Presumptive Positive	Repeat test one more time. If the repeat result remains presumptive positive, additional confirmatory testing may be conducted, if it is necessary between SARS-CoV-2 and other SARS-like virus currently unknown to infect humans, for epidemiological purposes or clinical management.
Only N gene (+)			
ORF1ab gene (-), N gene (-)	(+)	SARS-CoV-2 Negative	Report results to healthcare provider. Consider testing for other respiratory pathogens.
ORF1ab gene (-), N gene (-)	(-)	Invalid	Repeat test one more time. If the repeat result remains invalid, consider collecting new specimen.
Results are (-) when Ct value >35 or Undetermined. Results are (+) when Ct value < 35. If there is contamination for the re-run, perform decontamination procedures.			

Limitations of Test Methods

1. The performance of the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) was established using Oropharyngeal swabs samples.

2. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

3. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction kits have not been evaluated.

4. Results from the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) should be used as an adjunct to clinical observations and other information available to the physician.

5. This test is intended for detection of SARS-CoV-2. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.

6. Although the detected target sequences of this kit are the conservative region of SARS-CoV-2's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.

7. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined.

Conditions of Authorization for the Laboratory

The Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) Instructions for Use (Handbook). Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Jiangsu CoWin Biotech Co., Ltd (via email: https://www.cwbiotech.com or via phone: (86) 523-86201352), any suspected occurrence of false positive or false negative results and significant deviations

from the established performance characteristics of your product of which they become aware.

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

G. You, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

Product Performance Evaluation

1. Analytical Sensitivity

To determine the Limit of Detection (LoD) and analytical sensitivity of the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing), studies were performed using serial dilutions of analyte and the LoD was determined to be the lowest concentration of analyte that could reliably be detected with 95% of all tested positive.

LoD of each target assay in the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) was determined and confirmed using ddPCR quantified SARS-CoV-2 RNA virus that was spiked in oropharyngeal swab specimens that were confirmed negative for SARS-CoV-2.

Each dilution was tested in triplicate. The minimum concentration that the positive detection rate greater or equal to 95% was considered as the preliminary LoD. The results are summarized in the Table below:

Table 6: Preliminary LoD Data

Sample ID	concentration（copies/mL）	ORF1ab	N	Internal Control	Result	Overall % Positivity
		FAM	ROX	VIC		
S1-1	2700	30.88	30.08	26.52	+	100%
S1-2	2700	31.08	30.62	26.75	+	
S1-3	2700	31.35	30.84	26.67	+	
S2-1	900	32	31.86	26.05	+	100%
S2-2	900	32.46	32.22	27.83	+	
S2-3	900	33.09	32.33	27.36	+	
S3-1	300	34.64	33.9	26.01	+	100%
S3-2	300	34.64	34.62	26.16	+	
S3-3	300	34.06	34.01	28.7	+	
S4-1	100	36.88	35.81	27.6	−	0%
S4-2	100	37.4	35.69	26.66	-	
S4-3	100	38.71	35.36	26.3	-	
S5-1	33.3	Undetermined	Undetermined	28.28	−	0%
S5-2	33.3	40.22	Undetermined	26.54	−	
S5-3	33.3	Undetermined	Undetermined	27.94	−	
N-1	0	Undetermined	Undetermined	27.44	−	0%
N-2	0	Undetermined	Undetermined	27.98	−	
N-3	0	Undetermined	Undetermined	25.9	−	

The preliminary LoD of the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) was confirmed by testing 20 replicates of a sample prepared by spiking the quantified SARS-CoV-2 RNA virus at the preliminary LoD, i.e., 300 copies/mL in oropharyngeal swab specimens that were confirmed negative for SARS-CoV-2. Contrived specimens were processed utilizing the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) according to the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) workflow.

Table 7: LoD Confirmation Data

Sample ID	Concentration (copies/mL)	ORF1ab	N	Internal Control	Result	Overall % Positivity
		FAM	ROX	VIC		
S3-1	300	34.07	33.48	26.75	+	100%
S3-2	300	33.37	32.84	26.64	+	
S3-3	300	34.25	33.61	27.32	+	
S3-4	300	33.54	33.33	27	+	
S3-5	300	34.28	33.37	27.04	+	
S3-6	300	33.87	33.92	27.27	+	
S3-7	300	33.44	33.64	27.77	+	
S3-8	300	34.36	32.85	28.18	+	
S3-9	300	34.08	33.7	27.9	+	
S3-10	300	34.29	33.63	28.04	+	
S3-11	300	33.64	33.92	27.81	+	
S3-12	300	34.17	34.29	27.72	+	
S3-13	300	33.77	33.7	27.62	+	
S3-14	300	34.16	33.36	27.21	+	
S3-15	300	34.12	33.61	27.69	+	
S3-16	300	34.78	33.78	27.99	+	
S3-17	300	34.09	34.72	27.96	+	
S3-18	300	33.01	33.58	27.26	+	
S3-19	300	34.03	34.07	27.2	+	
S3-20	300	33.97	34.82	26.56	+	

Table 8: LoD Confirmation Summary

Concentration (copies/mL)	ORF1ab		N		Internal Control	
	Mean Ct	Detection Rate	Mean Ct	Detection Rate	Mean Ct	Detection Rate
300 copies/mL	33.97	100% (20/20)	33.71	100% (20/20)	27.45	100% (20/20)

The confirmed LoD for the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) is 300 copies/mL.

2. Analytical Specificity

Inclusivity:

An alignment was performed on July 15, 2020 with the oligonucleotide primer and probe sequences of the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) with 34,250 publicly available SARS-CoV-2 sequences from the China National Center for Bioinformation (contains NCBI and GISAID sequences) to demonstrate the predicted inclusivity of the assay. All the alignments show 99.88% identity of the assay to the SARS-CoV-2 sequences. Therefore, it is determined that the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) will detect 100% of available SARS-CoV-2 target analyte sequences.

Cross-reactivity:

In-silico Analysis

In-silico analysis for the N and ORF1ab primer/probe set of the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) was conducted to assess cross-reactivity. *In-silico* cross-reactivity is defined as greater than 80% homology between primer and probe set and any sequence present in the targeted microorganism. The minimal conditions under which cross-reaction can occur are established as being capable of producing an amplicon (<500 bp) and are limited to more than 80% homology of all the oligos that bind to the microorganism.

Table 9: *In-Silico* Analysis of Cross-reactivity

Pathogen	N gene			ORF1ab		
	Forward Primer	Probe	Reverse Primer	Forward Primer	Probe	Reverse Primer
Parainfluenza virus 3	50%	65%	59.10%	57.40%	57.10%	78.90%
Parainfluenza virus 2	68.20%	70%	63.60%	57.10%	57.10%	73.70%
Parainfluenza virus 1	63.60%	55%	59.10%	66.70%	53.60%	57.90%
Parainfluenza virus 4	57.90%	65%	63.60%	61.90%	46.40%	68.40%
Respiratory syncytial virus	63.60%	65%	63.30%	61.90%	57.10%	63.20%
Adenovirus	63.60%	75%	63.60%	61.90%	57.10%	68.40%
Human coronavirus 229E	54.50%	65%	54.50%	76.20%	64.30%	63.20%
Human coronavirus NL63	63.60%	70%	63.60%	81%	60.70%	68.40%
Respiratory syncytial virus	77.30%	65%	63.60%	61.90%	57.10%	68.40%
Human coronavirus HKU1	54.50%	60%	63.60%	66.70%	60.70%	57.90%
Enterovirus	59.10%	65%	68.20%	66.70%	64.30%	63.20%
Rhinovirus	59.10%	60%	54.50%	71.40%	53.60%	68.40%
SARS-coronavirus	90.90%	85%	54.50%	90.50%	96.40%	68.40%
Influenza A	63.60%	60%	63.60%	71.40%	53.60%	68.40%
MERS-coronavirus	63.60%	60%	63.60%	61.90%	50%	73.70%

Pathogen	N gene			ORF1ab		
	Forward Primer	Probe	Reverse Primer	Forward Primer	Probe	Reverse Primer
Human Metapneumovirus (hMPV)	59.10%	60%	63.6	61.90%	57.10%	57.90%
Human coronavirus OC43	63.60%	70%	68.20%	61.90%	57.10%	57.90%
Influenza B	59.10%	55%	72.70%	61.90%	53.60%	57.90%
Pseudomonas aeruginosa	68.20%	65%	63.60%	57.10%	60.70%	68.40%
Chlamydia pneumoniae	68.20%	75%	63.60%	71.20%	60.70%	73.70%
Staphylococcus epidermis	68.20%	65%	59.10%	71.40%	60.70%	73.70%
Candida albicans	63.60%	75%	68.20%	63.20%	67.90%	73.70%
Mycobacterium tuberculosis	59.10%	65%	59.10%	52.40%	67.90%	73.70%
Bordetella pertussis	63.60%	65%	68.20%	71.40%	67.90%	63.20%
Streptococcus pneumoniae	77.30%	70%	72.70%	61.90%	60.70%	68.40%
Haemophilus influenzae	59.10%	75%	63.60%	66.70%	60.70%	73.70%
Pneumocystis jirovecii (PJP)	68.20%	65%	63.60%	66.70%	53.60%	78.90%
Streptococcus pyogenes	68.20%	75%	59.10%	61.90%	53.60%	63.20%

Pathogen	N gene			ORF1ab		
	Forward Primer	Probe	Reverse Primer	Forward Primer	Probe	Reverse Primer
Legionella pneumophila	68.20%	70%	59.10%	71.40%	53.60%	63.20%
Mycoplasma pneumoniae	72.70%	75%	72.7	76.20%	64.30%	78.90%

In-silico analysis demonstrated that there was no cross-reactivity with tested organisms except sequences targeting SARS-coronavirus. Therefore, SARS-coronavirus along with some additional respiratory organisms were additionally wet-tested, and no cross-reactivity was observed as shown below.

Wet Testing

Wet testing against high risk pathogenic organisms of the respiratory tract, selected based on disease prevalence, disease risk, homology to assay specific targets and homology to the SARS-CoV-2 genome, was performed to confirm the results of the *in silico* analysis. Each organism identified in the Table below was tested with the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit by spiking organism stock ($1x10^6$ copies/mL) into in oropharyngeal swab specimens that were confirmed negative for SARS-CoV-2. Results demonstrated that tested non-target respiratory organisms were not detected by the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing).

Table 10: Wet Testing of Potential Cross-Reactive Respiratory Organisms

Virus/Bacteria/Parasite	ORF1ab	N	IC	Result
	FAM	ROX	VIC	
Human coronavirus 229E	Undetermined	Undetermined	30.12	–
Human coronavirus OC43	Undetermined	Undetermined	30.43	–
Human coronavirus HKU1	Undetermined	Undetermined	30.23	–
Human coronavirus NL63	Undetermined	Undetermined	23.49	–

Virus/Bacteria/Parasite	ORF1ab	N	IC	Result
MERS-coronavirus	Undetermined	Undetermined	29.82	–
SARS-coronavirus	41.68*	Undetermined	30.4	–
Parainfluenza virus 1-4 （Pooled）	Undetermined	Undetermined	29.98	–
Adenovirusv1,2,5 （pooled）	Undetermined	Undetermined	30.39	–
Respiratory Syncytial Virus	Undetermined	Undetermined	30.14	–
Enterovirus	Undetermined	Undetermined	30.1	–
Rhinovirus	Undetermined	Undetermined	29.96	–
Influenza A	Undetermined	Undetermined	30.63	–
Human Metapneumovirus (hMPV)	Undetermined	Undetermined	29.98	–
Influenza B	Undetermined	Undetermined	29.51	–
Pseudomonas aeruginosa	Undetermined	Undetermined	29.72	–
Chlamydia pneumoniae	41.67*	Undetermined	30.27	–
Staphylococcus epidermis	Undetermined	Undetermined	30.08	–
Candida albicans	Undetermined	Undetermined	30.16	–
Mycobacterium tuberculosis	Undetermined	Undetermined	30.24	–
Bordetella pertussis	Undetermined	Undetermined	30.22	–
Streptococcus pneumoniae	Undetermined	Undetermined	29.45	–
Haemophilus influenzae	Undetermined	Undetermined	30.67	–
Pneumocystis jirovecii	Undetermined	Undetermined	28.91	–
Streptococcus pyogenes	Undetermined	Undetermined	29.84	–
Legionella pneumophila	Undetermined	Undetermined	30.04	–

Virus/Bacteria/Parasite	ORF1ab	N	IC	Result
Mycoplasma pneumoniae	Undetermined	Undetermined	30.04	−
Enterovirus (EV-C95)	Undetermined	Undetermined	30.47	−
Rhinovirus	Undetermined	Undetermined	30.52	−

**ORF1ab target CT value is above the expected CT (ORF1ab expected CT cut off is <35), the test result is negative.*

Interference Substance Study:

An Interference Substance study was performed with the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing) to access the performance of the assay with common respiratory substances. Contrived specimens were prepared using quantified SARS-CoV-2 RNA virus that was spiked (3x LoD) in oropharyngeal swab specimens that were confirmed negative for SARS-CoV-2 and tested with interfering substances at clinically relevant concentrations in triplicate. The results are summarized in the Table below:

Table 11: Interference Substances Studies

Interference Substances preparation		Results		
		Test 1	Test 2	Test 3
Contrived virus sample with SARS-CoV-2 RNA concentration at 900 copies/mL	5%(v/v) Human blood	+	+	+
	100 μ g/mL Nasal sprays	+	+	+
	20 μ g/mL Mucin: bovine submaxillary gland, type I-S	+	+	+
	50 μ g/mL Nasal corticosteroids	+	+	+
	200 μ g/mL Homeopathic allergy relief medicine	+	+	+
	100 μ g/mL FluMist	+	+	+
	500 μ g/mL Throat lozenges	+	+	+
	300U/mL Anti-viral drugs Zanamivir	+	+	+
	100 μ g/mL Nasal ointment Mupirocin	+	+	+
	100 μ g/mL Tobramycin	+	+	+

Interference Substances preparation		Results		
		Test 1	Test 2	Test 3
Contrived virus sample with SARS-CoV-2 RNA concentration at 0 copies/mL	5%(v/v) Human blood	—	—	—
	100 μ g/mL Nasal sprays	—	—	—
	20 μ g/mL Mucin: bovine submaxillary gland, type I-S	—	—	—
	50 μ g/mL Nasal corticosteroids	—	—	—
	200 μ g/mL Homeopathic allergy relief medicine	—	—	—
	100 μ g/mL FluMist	—	—	—
	500 μ g/mL Throat lozenges	—	—	—
	300U/mL Anti-viral drugs Zanamivir	—	—	—
	100 μ g/mL Nasal ointment Mupirocin	—	—	—
	100 μ g/mL Tobramycin	—	—	—

3. Clinical Evaluation

A clinical study was performed to evaluate the performance of the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing). Comparator FDA EUA real-time PCR test results obtained from a total of 96 clinical oropharyngeal swab samples (56 negatives and 40 positives for SARS-CoV-2 each collected with a CoWin swab and placed in SARS-CoV-2 Collection Fluid and a second oropharyngeal swab for comparator testing) from Taizhou GeneWill Medical Laboratory Co., Ltd were compared to results obtained with the Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit (PCR-Fluorescence Probing). Data is summarized in the Table below:

Table 12: Clinical Evaluation

Novel Coronavirus (SARS-CoV-2) Fast Nucleic Acid Detection Kit	FDA EUA RT-PCR Test		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	40	0	40	PPA 100%	91.2-100%
Not Detected	0	56	56	NPA 100%	93.6-100%
Total	40/40	56/56	96		

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Manufacturer

Jiangsu CoWin Biotech Co., Ltd

Add：No. 58 G52 Building, East of Lujia Road, West Side of Tai Road, China medical city, Taizhou, Jiangsu, 225300

Phone：86-523-86201352 Fax：86-523-86816890

Web: https://www.cwbiotech.com E-mail： service@cwbiotech.com

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