Sansure Biotech

Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

For Emergency Use Only

Instructions for Use

(24 Tests/kit and 48 Tests/kit)

For in vitro Diagnostic (IVD) Use
For Prescription Use only
For Emergency Use Authorization only

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1. Reference Number

S3104E

2. Package Specification

24 tests/kit, 48 tests/kit

3. Intended Use

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate swabs, nasal washes and nasal aspirates from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. 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 (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for use by qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is only for use under the Food and Drug Administration's Emergency Use Authorization (EUA).

4. Product Overview/Test Principle

During the 2019-nCoV pneumonia epidemic that happened in China, Sansure Biotech developed a fast and simple NAT kit based on its advanced RNA fast-releasing technology. The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe sets are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients who are suspected of COVID-19 by their healthcare provider. This kit is used for qualitative

detection of the ORF1ab and N genes of SARS-CoV-2 RNA. By one simple step of centrifugation and lysis, the sample mixture can be directly added to the 2019-nCoV-PCR master mix (2019-nCoV-PCR Mix + 2019-nCoV-PCR-Enzyme Mix) to carry out rRT-PCR amplification. QIAamp Viral RNA Mini Kit (50, Catalogue No. 52904) can be used as alternative extraction method. Internal control targeting the RNase P gene monitor the sample collection, sample handling and rRT-PCR process to avoid false-negative results. The LoD of the kit is 200 copies/mL.

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe set(s) is designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients with signs and symptoms of infection who are suspected of COVID-19.

5. Components Included within the Kit

No.	Reagent Name	Spec. & Qty.		Main Ingredients
		24 T	48 T	
1	Sample Release Reagent	1200 μL/tube x 1	1200 μL/tube x 2	Lysis buffer(S03)
2	2019-nCoV-PCR Mix	624 μL/ tube x 1	1248 μL/ tube x 1	Primers, Probes, dNTPs, MgCl2, Rnasin, PCR buffer
3	2019-nCoV-PCR-Enzyme Mix	96 μL/ tube x 1	192 μL/ tube x 1	RT Enzyme, Taq Enzyme
4	2019-nCoV-PCR-Positive Control	500 μL/tube x 1	500 μL/tube x 1	In vitro transcriptional RNA for ORF1ab, N gene and internal control RNase P gene
5	2019-nCoV-PCR-Negative Control	500 μL/tube x 1	500 μL/tube x 1	Saline
6	Sample Storage Reagent	2.0 mL/tube × 24	2.0 mL/tube × 24 × 2	0.9% saline, Rnasin

6. Reagent Stability and Transportation

The diagnostic kit (in small box) should be stored at -20 ±5℃ in the dark and should be transported in a sealed foam box with ice packs. The Sample Storage Reagent (in big and white box) should be stored and transported at room temperature or 2 - 8℃ or below. The kit should be stored at -20 ±5℃. Unpacked kits should avoid repeated freeze-thaw cycles.

7. Components Required But Not Included within the Test

Alternative extraction reagents:

QIAamp Viral RNA Mini Kit (50, Catalogue No. 52904, Qiagen), which is equivalent to the QIAamp DSP Viral RNA Mini Kit in the U.S.A.

Consumables not supplied:

- Swab specimens with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft.

- 1.5 mL DNase-free and RNase-free Eppendorf tube
- 0.2 mL PCR tube or strip
- Various models of pipettes and pipette tips (10μL, 200μL and 1000μL tips with filters)
- Centrifuge (can reach to 12,000 rpm)
- Microcentrifuge
- desktop vortex mixer
- 0.9% saline
- -20℃ cold blocks
- 10% bleach
- DNAZap™ (Ambion, cat. #AM9890)
- Disposable powder-free gloves and surgical gowns

Real-Time PCR Instrument(s):
ABI 7500 Real-Time PCR System

8. Warnings and Precautions

Federal Law restricts this device to sale by or on the order of a licensed practitioner.

For emergency use only.
For in vitro diagnostic use only (IVD).

Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

This test has not been FDA cleared or approved; This test has been authorized by FDA under an EUA for use by authorized laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.
This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens.
This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Please read the package insert carefully prior to operation. The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is only for emergency use with a prescription, as an *in vitro* diagnostic (IVD) test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.

Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. a) 1st Area: Preparation Area—Prepare testing reagent: b) 2nd Area: specimen processing—Process the specimen and controls: c) 3rd: Amplification Area—PCR conducted.

Sample Release Reagent has not been validated with specimens stored in U.S.A commercialized sample storage, preservation, or transport media (VTM/UTM) and may cause false negative results.

The clinical laboratory should be equipped with instruments and operators in strict accordance with relevant requirements outlined in local, state and national regulations. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.

All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit and is in violation of the product Emergency Use Authorization. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kit lots. Prior to begin each assay, each component must be thoroughly thawed and briefly centrifuged. Avoid repeated freeze-thaw cycles.

All pipette tips and centrifuge tubes in the assay should be sterile and DNase/RNase-free. To prevent contamination, filtered pipette tips are required and should be replaced after the addition of each reagent or sample.

Dispose of waste in compliance with local, state, and federal regulations.

All lab workbench and supplies should be cleaned and disinfected regularly using 70% Ethanol or 10% bleach.

Avoid exposure to light of the 2019-nCoV-PCR Mix.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

9. Controls Materials

9.1 2019-nCoV-PCR-Negative Control: A “no template” (negative) control is used to monitor whether there is contamination for the rRT-PCR process and is used in each detection run.

9.2 2019-nCoV-PCR-Positive Control: A positive template control is used to monitor whether the rRT-PCR process works properly and is used in each detection run.

9.3 An internal control for RNase P gene is used to monitor the sample collection, handling and rRT-PCR process and is used in each sample amplification.

10. Sample Collection, Storage and Transportation

10.1 Equipment preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents, such as 10% bleach, 70% ethanol, and DNAzap™, should be used to minimize the risk of nucleic acid contamination.

10.2 Specimen collection

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens.

Collection should avoid possible contamination in collection, storage, and transportation. The specimen should be presumed contagious and be handled according to relevant regulations.

Collection swabs should have a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. After sample collection, swabs should be stored in Sample Storage Reagent immediately. When using the Sample Storage Reagent provided by the manufacturer, the user is able to directly lyse the sample using the Sample Release Reagent RNA fast-releasing technology provided in this kit.

10.3 Storage and delivery of specimens:
Specimens can be immediately processed. Specimens should be tested within 24 hours if stored at 4℃. Specimens that cannot be tested within 24 hours should be stored at -70℃ or below (in the absence of -70℃ storage conditions, specimens can be stored at -20℃ for 10 days, nucleic acid can be stored at -20±5℃ for 15 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

11. Laboratory Procedure

11.1 Sample extraction
11.1a: Fast and simplified sample extraction method:
For clinical specimens preserved in Sample Storage Reagent, sample processing can use the Sample Release Reagent RNA fast-releasing technology provided in the kit. Pipet 200 μL of specimen into 1.5 mL EP tube, centrifuge at 12,000 rpm for 5 min, and then discard the supernatant fluid carefully, avoid removing the precipitation in the bottom. Add 50 μL Sample Release Reagent into each tube, vortex for 5 second. The lysed sample can be directly added to the rRT-PCR reaction.

Precautions: Sample Release Reagent has not been validated with specimens stored in U.S.A commercialized sample storage, preservation, or transport media (VTM/UTM) and may cause false negative results.

11.1b: Qiagen QIAamp Viral RNA Mini Kit extraction method:
Qiagen Viral RNA Mini kit may be used as an alternative extraction method using specimens preserved in Sample Storage Reagent provided or other U.S. commercialized sample storage, preservation, or transport media (e.g., VTM/UTM). The extraction procedure should be performed according to the manufacturer's instructions: load 140 μL of specimen to each column and eluted with 50 μL solution. The extracted RNA can be directly added to the rRT-PCR reaction immediately or store at -70℃.

11.2. Preparation of reagents

11.2.1 Take out each component from the diagnostic kit and place them at room temperature. Allow the reagents to equilibrate at room temperature, then vortex each of them respectively for later use.

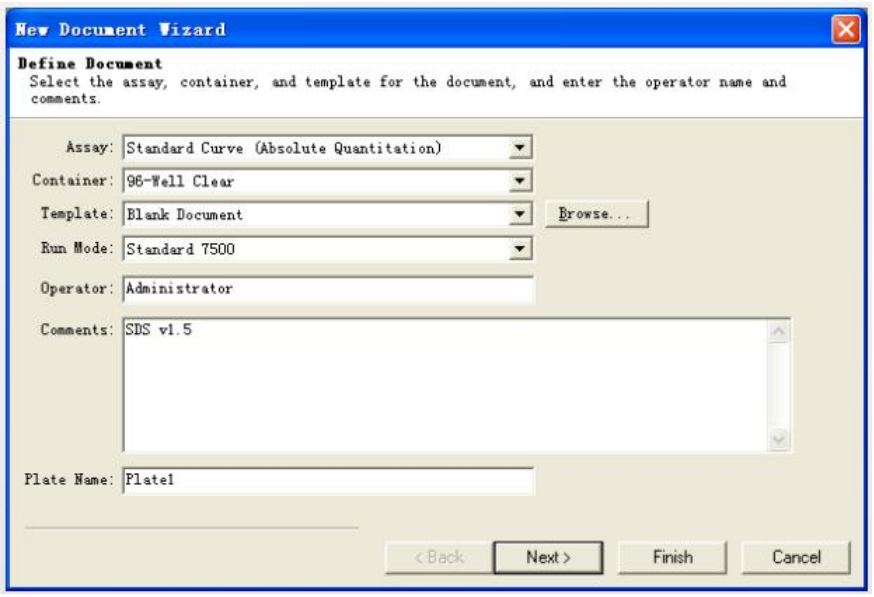
11.2.2 Prepare the 2019-nCoV-PCR Master Mix (26 μL 2019-nCoV-PCR Mix + 4 μL 2019-nCoV-PCR-Enzyme Mix) based on the total number of specimens, 2019-nCoV-PCR-Positive Control and 2019-nCoV-PCR-Negative Control and mix thoroughly. The remaining reagent must be stored at -20°C immediately.

Table 1. Master mix preparation

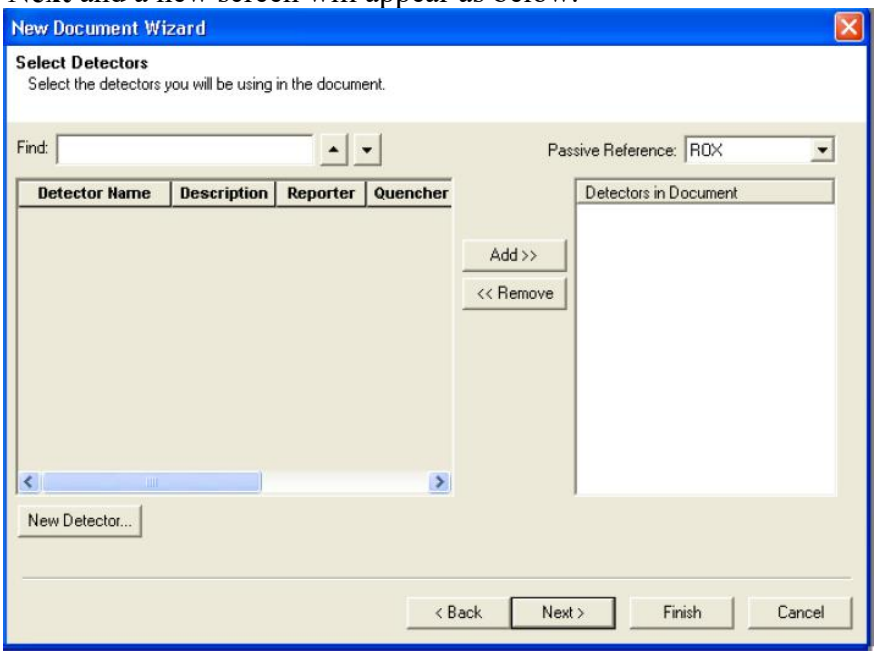
	1 sample	10 samples	24 samples	48 samples
2019-nCoV-PCR Mix (μL)	26	260	624	1248
2019-nCoV-PCR-Enzyme Mix (μL)	4	40	96	192
Note: The above configuration is for reference only.				

11.2.3 Add 30 μL of 2019-nCoV-PCR Master Mix into each well. Cover the wells and transfer to the sample processing area. Add 20 μL of the extracted RNA to the well pre-filled with reagent mix in the following order: 2019-nCoV-PCR-Negative Control, patient specimen(s), and 2019-nCoV-PCR-Positive Control. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into Applied Biosystems ABI 7500 real-time RT-PCR system and record the exact location of controls and each specimen.

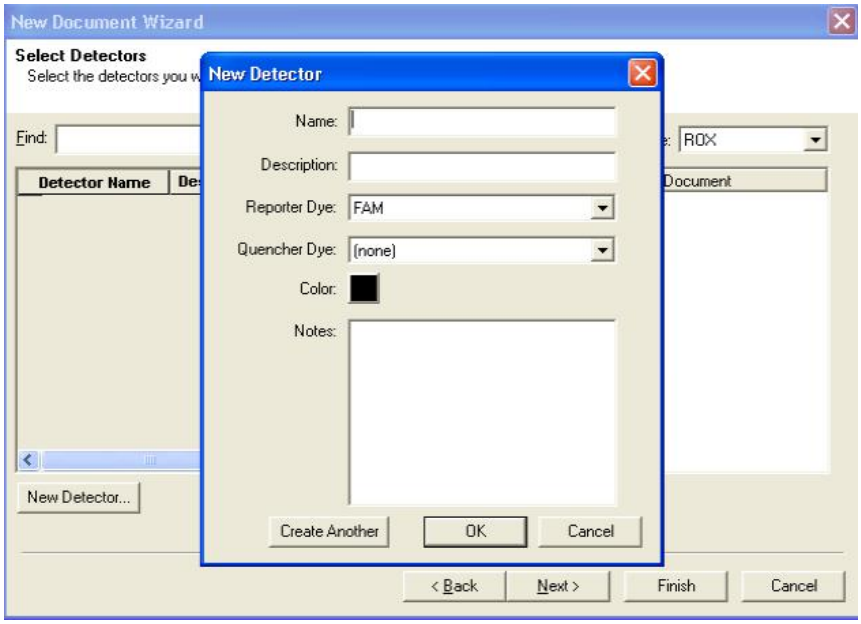
11.3. Running a PCR amplification on ABI 7500 using 7500 software v1.5:
11.3.1. Start ABI 7500 real time PCR system: Turn on the computer connected to the system first, then turn on ABI 7500 real time PCR system.
11.3.2. Load the instrument: Push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door.
11.3.3. Set up the experiment run:
11.3.3.1. Double-click ABI 7500 icon (7500 software v1.5) on the desktop. A new window should appear, select Create New Document from the menu.



11.3.3.2. Click **Next** and a new screen will appear as below.



11.3.3.3. Click **New Detector** and a new screen will appear as below.



11.3.3.4. Start by creating the ORF1ab Detector. Include the following:

a. Name: ORF1ab

b. Description: *leave blank*

c. Reporter Dye: **FAM**

d. Quencher Dye: **(none)**

e. Color: *to change the color of the detector indicator do the following:*

① Click on the color square to reveal the color chart

② Select a color by clicking on one of the squares

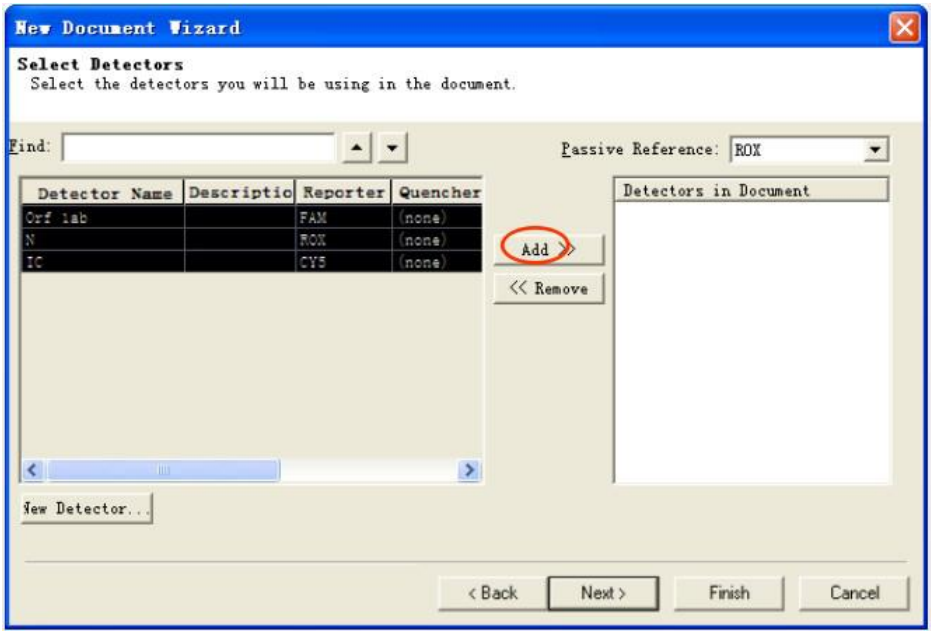
③ After selecting a color click **OK** to return to the New Detector screen

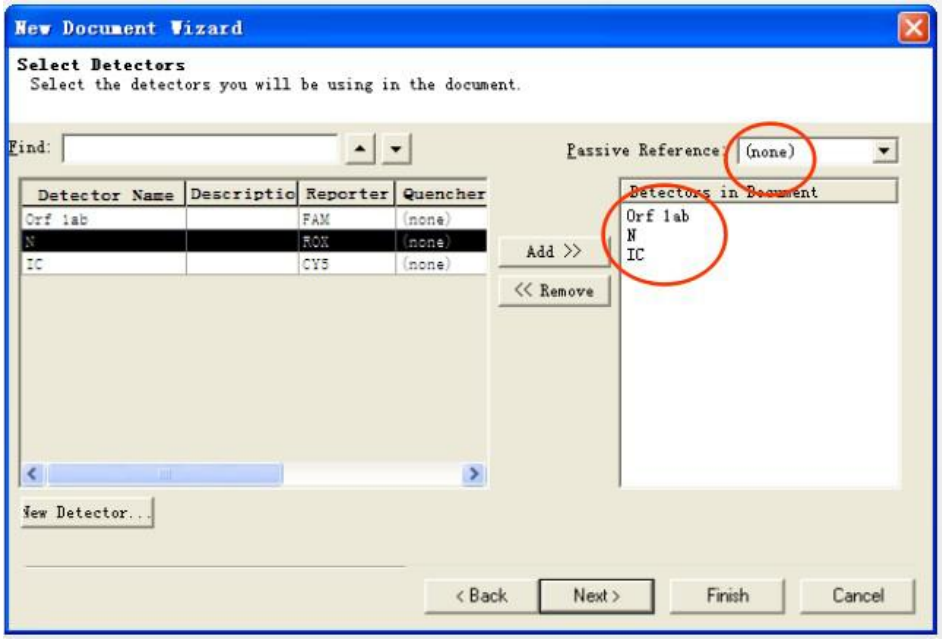
f. Click the OK button of the New Detector screen to return to the screen shown above.

11.3.3.5. Repeat step 11.3.3.3 - 11.3.3.4 for each target in the panel.

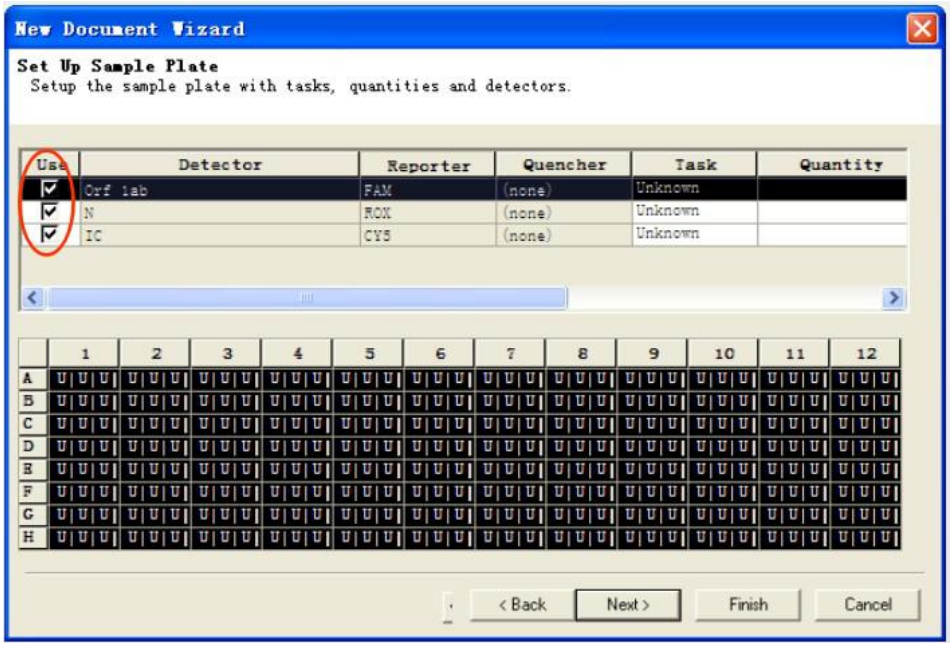
Name	Reporter Dye	Quencher Dye
ORF1ab	FAM	（none）
N	ROX	（none）
IC	CY5	（none）

11.3.3.6. After each Detector is added, the **Detector Name**, **Description**, **Reporter** and **Quencher** fields will become populated in the **Select Detectors** screen. Before proceeding, the newly created detectors must be added to the document. To add the new detectors to the document, click **Add**. Detector names will appear on the right hand side of the **Select Detectors** window. Once all detectors have been added, select **(none)** for Passive Reference at the top right hand drop down menu.





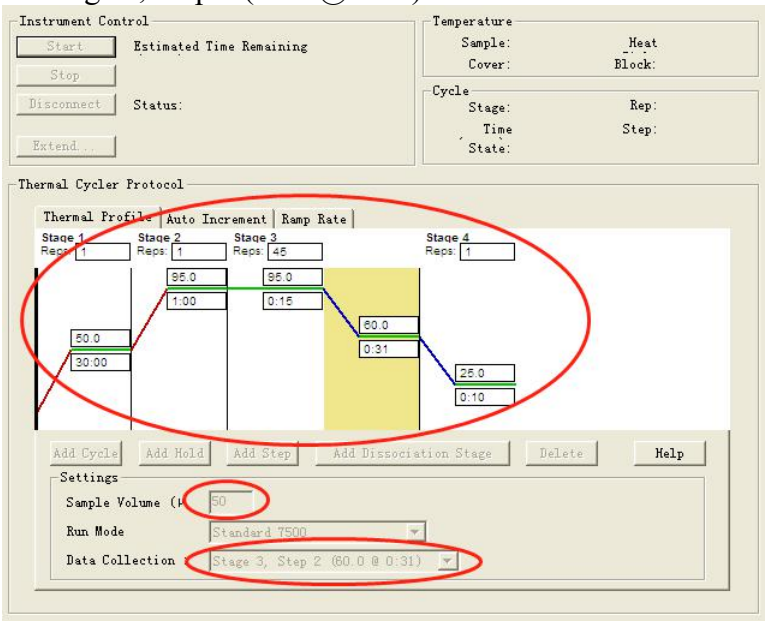
11.3.3.7. Click **Next**, select the well containing the samples and controls, and then click the Detector.



11.3.3.8. Click **Finish**.

11.3.3.9. Select the **Instrument** tab. Set the parameters as follows:

- Stage 1: 50°C for 30 min, 1 cycle;
- Stage 2: 95°C for 1 min, 1 cycle;
- Stage 3: 95°C for 15 sec, 60°C for 31 sec, 45 cycles.
- Stage 4: 25°C for 10 sec, 1 cycle;
- Sample Volume: 50
- Data Collection at Stage 3, Step 2 (60.0 @ 0:31)

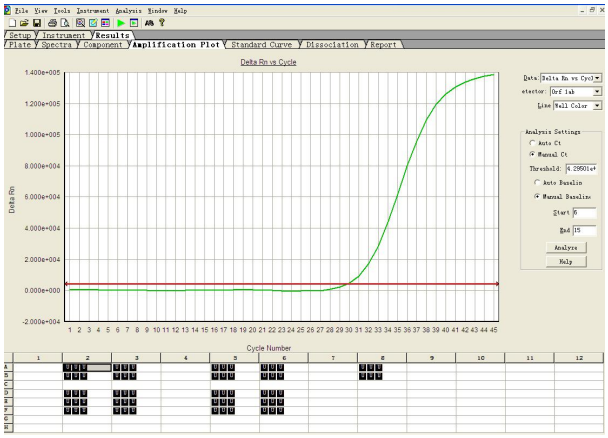


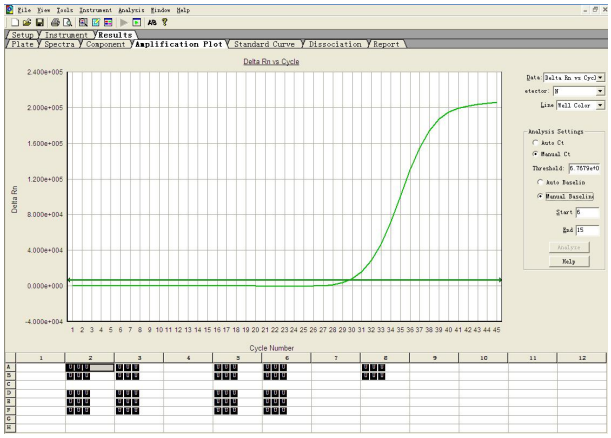
11.3.3.10.  Save the document and then click **Start** to run the evaluation.

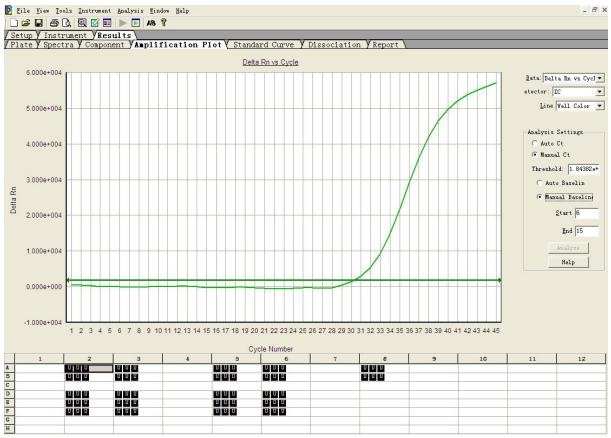
11.4 Data Analysis

See below for step-by-step operation of ABI 7500 using 7500 software v1.5 for Data analysis:
11.4.1 After the run is completed, click **Results**. Click **Amplification Plot** tab and view and adjust the raw data.

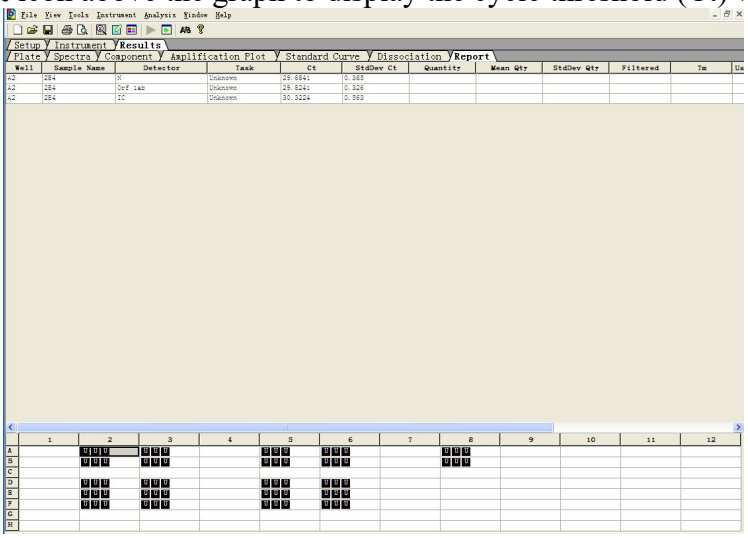
- In the **Data** window, **Delta Rn vs Cycle** should be selected.
- In the **Detector** window, "ORF1ab" "N" and "IC" should be selected.
- The **Start (cycle)** window should be "3-15." The **End (cycle)** window should be 5-20. Users can adjust the values according to the actual situation.
- Adjust the threshold just above the curve from NTC (noise) .
- Lastly, be sure to click "Analyze" icon to update the analysis.







11.4.2 Click **Report** icon above the graph to display the cycle threshold (Ct) values.



12. Interpretation of Results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The Ct cutoff value of this kit is set as 40 and the end user is required to review fluorescent curves before final interpretation. All the positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples.

1) Positive and Negative Controls

The positive control and negative control for each run are interpreted as described in Table 2 below.

Table 2. Positive and Negative Control Interpretation.

2019-nCoV-PCR-Positive Control			2019-nCoV-PCR-Negative Control			Results	Actions
ORF1ab (FAM)	N (ROX)	IC (CY5)	ORF1ab (FAM)	N (ROX)	IC (CY5)		
+	+	+	-	-	-	Valid	Continue to result interpretation
Any one of them shows negative			Not considered			Invalid	rRT-PCR failed, re-run
Not considered			Any one of them shows positive				Extraction, rRT-PCR contaminated, re-run
Result of (-): Ct value >40 or Undetermined Result of (+): Ct value ≤ 40 If there is contamination for the re-run, please perform decontamination procedures.							

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. **Table 3** below describes the results interpretation concerning the use of the controls provided with the test. The Ct cutoff value of this kit is set as 40 and the end user is required to review fluorescent curves before final interpretation. All

positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples (38≤Ct≤40).

Table 3. Interpretation of Results based on Controls.

ORF1ab (FAM)	N (ROX)	IC (CY5)	Results
+	+	Not considered	2019-nCoV Positive
+	-		
-	+		
-	-	+	2019-nCoV Negative
-	-	-	Invalid
Result of (-): Ct value >40 or Undetermined Result of (+): Ct value ≤ 40 Invalid Result: There is no typical S-shape amplification curve or Ct > 40 or No Ct detected for ORF1ab gene (FAM), N gene (ROX) and internal control (CY5), indicating that the specimen concentration is too low, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.			

13. Limitations

The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.

Mutation in the target sequence of SARS-CoV-2 or change in the sequence due to virus evolution may lead to false negative results. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Improper reagent storage may lead to false negative results.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was established using nasopharyngeal/oropharyngeal swabs. Nasal swabs, mid-turbinate nasal swabs, and bronchoalveolar lavage fluid specimens are also considered acceptable specimen types for use with the kit. but performance has not been established.

Test results of the diagnostic kit can only be used as an aid in clinical diagnosis. Symptoms and physical signs, disease history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered for the clinical diagnosis and treatment.

Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

The fast-releasing technology using Sample Releasing Reagent has been evaluated only for use in combination with the Sample Storage Reagent provided in the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit. Sample Release Reagent used with specimens stored in other storage, preservation, or transport media (VTM/UTM) not provided in the kit has not been fully validated and may cause false negative results.

The Orf1ab and N gene primer/probes may detect bat coronaviruses and the N gene primer/probes may detect pangolin coronaviruses based on *in silico* analysis.

14. Troubleshooting

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use.
	PCR tube not properly sealed	Ensure plates are sealed correctly.
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of RNA samples carrying interferences	Repeat the test with the neat extracted RNA and 1:10 dilution of the extracted RNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

15. Conditions of Authorization for the Laboratory

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic 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/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic 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 (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use 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 You (Young Wang, (443)538-5780)) 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 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. Sansure Biotech Inc., 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, to perform high complexity tests" as "authorized laboratories."

16. Performance Evaluation

1) Limit of Detection (LoD) - Analytical Sensitivity:

LoD studies were used to determine the lowest detectable concentration of SARS-CoV-2 RNA at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples.

Preparation of the manufacturer's standards:
RNA was extracted from a clinical specimen positive for SARS-CoV-2 RNA (*oropharyngeal swab, confirmed by gene sequencing*) and from a 1:10 dilution of the same specimen using the QIAamp Viral RNA Mini Kit (50, Catalogue No. 52904). The RNA concentration of the neat and diluted specimen was determined by the median value of six replicates using digital

PCR (TD-1 digital PCR system). The final concentration of the positive sample was set as 5.4×10^5 copies/mL using the median value of ORF1ab gene and N gene.

LoD with Clinical Specimen:
The LoD of the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was estimated by testing standardized dilutions of the positive specimen serially diluted to 2.0×10^4 copies/mL, 2.0×10^3 copies/mL, 2.0×10^2 copies/mL, and 20 copies/mL (n = 5 each) using negative specimen matrix (a negative oropharyngeal swab specimen in Sample Storage Reagent). The lowest concentration at which all *5* replicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing *20 replicates* with concentrations at the tentative limit of detection. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of *19 out of 20 replicates*.

The LoD of the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was established using both extraction methods: the Sample Release Reagent RNA fast-releasing technology (**Tables 4** and **5**) and Qiagen QIAamp Viral RNA Mini Kit (**Table 6**). The results demonstrated that the LoD of the two extraction methods are equivalent, which is 200 copies/mL.

Table 4. Tentative LoD Detection Results of 2019-nCoV Using Sample Release Reagent

2019-nCoV Strain Tested	Stock 2019-nCoV Titer	Serial 10-Fold Dilution Factor	Concentration in Dilution Tested [copies/mL]	Replicate 1 Ct	Replicate 2 Ct	Replicate 3 Ct	Replicate 4 Ct	Replicate 5 Ct	Call Rate	Lowest Concentration with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
2019-nCoV positive specimen	*ORF1ab gene 5.4×10^5 copies/mL*	*2.7×10^{-1}*	*20000*	*30.89*	*30.96*	*31.14*	*31.57*	*31.24*	*100%*	*200 copies/mL*	*200 copies/mL*
		2.7×10^{-2}	*2000*	*33.67*	*34.26*	*34.55*	*35.11*	*35.02*	*100%*		
		2.7×10^{-3}	*200*	*36.63*	*39.07*	*36.65*	*36.65*	*37.62*	*100%*		
		2.7×10^{-4}	*20*	*38.07*	*Undet*	*Undet*	*40.43*	*Undet*	*40%*		
	N gene 5.4×10^5 copies/mL	*2.7×10^{-1}*	*20000*	*29.97*	*30.53*	*30.21*	*31.03*	*30.66*	*100%*	*200 copies/mL*	*200 copies/mL*
		2.7×10^{-2}	*2000*	*34.36*	*34.04*	*33.61*	*33.76*	*33.62*	*100%*		
		2.7×10^{-3}	*200*	*35.60*	*37.11*	*38.02*	*35.67*	*36.23*	*100%*		
		2.7×10^{-4}	*20*	*37.88*	*Undet*	*Undet*	*37.34*	*Undet*	*40%*		

Table 5. LoD Detection Results of 2019-nCoV Using Sample Release Reagent

Test No.	Concentration(copies/mL)					
	ORF1ab gene			N gene		
	400	200	100	400	200	100
1	36.78	36.26	39.55	35.64	36.50	38.28
2	36.32	36.52	38.65	38.61	36.93	38.19
3	36.84	37.36	37.05	36.19	37.27	37.50
4	36.18	37.59	Undet	35.81	36.72	Undet
5	36.51	36.28	37.81	36.08	36.64	37.23
6	34.75	36.73	Undet	35.56	36.13	Undet
7	36.79	38.18	39.13	35.91	37.44	38.42
8	35.59	38.27	Undet	35.59	36.99	Undet
9	35.27	36.74	Undet	35.54	36.88	Undet
10	36.86	37.85	37.09	35.87	36.89	42.13
11	36.99	36.85	Undet	36.04	36.86	36.71
12	36.76	Undet	Undet	35.43	Undet	Undet
13	37.38	36.90	39.07	35.90	37.33	38.47
14	35.76	37.88	Undet	36.27	37.81	Undet
15	36.51	38.03	Undet	35.23	36.54	Undet
16	36.54	36.06	38.33	35.49	39.35	Undet
17	36.32	37.63	37.20	36.13	36.00	38.65
18	36.23	37.92	Undet	35.00	38.54	Undet
19	35.46	37.95	Undet	35.30	36.04	Undet
20	36.35	37.95	39.08	36.67	37.65	38.25
Call rate	100%	95%	50%	100%	95%	45%

Table 6. LoD Detection Results of 2019-nCoV Using Qiagen QIAamp Viral RNA Mini Kit (50, Catalogue No. 52904)

Test No.	Concentration（copies/mL）					
	ORF1ab gene			N gene		
	400	200	100	400	200	100
1	35.83	38.49	37.93	35.77	37.39	37.87
2	36.22	36.35	Undet	37.00	35.99	Undet
3	37.56	36.82	37.45	35.75	39.59	37.66
4	36.72	36.44	Undet	36.10	36.69	Undet
5	36.41	38.29	35.52	35.92	37.27	37.67
6	35.59	Undet	Undet	36.01	Undet	Undet
7	35.16	36.54	38.59	34.84	36.97	36.22
8	36.34	36.86	38.80	35.10	37.29	37.46
9	35.63	36.59	39.01	36.03	38.05	36.78
10	35.72	38.42	Undet	35.53	36.03	Undet
11	37.41	39.39	Undet	36.10	38.08	Undet
12	35.10	35.66	Undet	36.07	37.03	Undet
13	35.29	37.35	Undet	35.01	36.03	Undet
14	36.32	36.03	36.73	35.13	36.11	Undet
15	35.94	36.01	37.11	34.34	36.40	36.65
16	35.38	36.43	38.06	34.49	36.32	37.54
17	36.01	36.70	Undet	34.72	35.32	Undet
18	38.02	36.82	Undet	34.91	37.05	Undet
19	35.89	35.55	Undet	35.45	36.70	38.10
20	36.03	34.75	Undet	36.69	35.60	41.39
Call rate	100%	95%	45%	100%	95%	45%

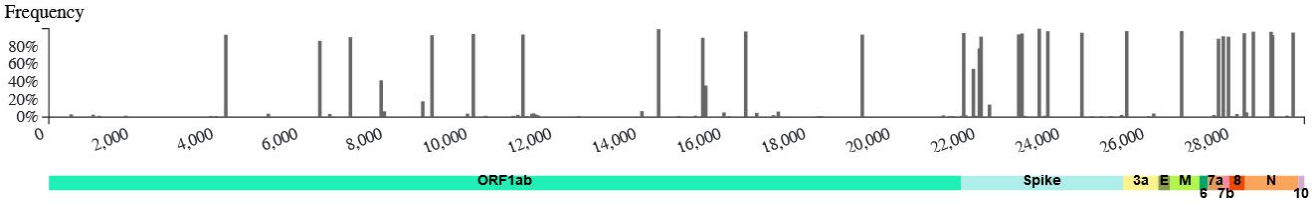
2) Inclusivity (analytical sensitivity):

Inclusivity of the primer/probe set used in the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was analyzed *in silico* based on SARS-CoV-2 sequences from GISAID (6442390 sequences), NGDC 2019nCoVR (6563698 sequences), NCBI (2957763 sequences) database accessed on December 23, 2021. The primer/probe sets for ORF1ab gene and N gene sequencing alignment analysis demonstrate 100% inclusivity for SARS-CoV-2 sequences identified from patient samples. The representative alignment results for both genes are shown in **Table 7**.

Table 7. Representative results of *In Silico* Analysis for 2019-nCoV primers/probes against the reported 2019-nCoV sequences by 2021-12-23.

Strain	Target	Accession	% Homology Test FP%	% Homology Test RP%	% Homology Test Probe%
BetaCoV/Wuhan/WH-01/2019	ORF1ab gene	CNA0007332	100	100	100
BetaCoV/Wuhan/WH-01/2019	N gene	CNA0007332	100	100	100
hCoV-19/Wuhan/IVDC-HB-01/2019	ORF1ab gene	EPI_ISL_402119	100	100	100
hCoV-19/Wuhan/IVDC-HB-01/2019	N gene	EPI_ISL_402119	100	100	100
hCoV-19/Wuhan/WIV04/2019	ORF1ab gene	EPI_ISL_402124	100	100	100
hCoV-19/Wuhan/WIV04/2019	N gene	EPI_ISL_402124	100	100	100
hCoV-19/Guangdong/20SF012/2020	ORF1ab gene	EPI_ISL_403932	100	100	100
hCoV-19/Guangdong/20SF012/2020	N gene	EPI_ISL_403932	100	100	100
hCoV-19/Nonthaburi/61/2020	ORF1ab gene	EPI_ISL_403962	100	100	100
hCoV-19/Nonthaburi/61/2020	N gene	EPI_ISL_403962	100	100	100
hCoV-19/USA/IL1/2020	ORF1ab gene	EPI_ISL_404253	100	100	100
hCoV-19/USA/IL1/2020	N gene	EPI_ISL_404253	100	100	100
hCoV-19/USA/CA1/2020	ORF1ab gene	EPI_ISL_406034	100	100	100
hCoV-19/USA/CA1/2020	N gene	EPI_ISL_406034	100	100	100
hCoV-19/France/IDF0372/2020	ORF1ab gene	EPI_ISL_406596	100	100	100
hCoV-19/France/IDF0372/2020	N gene	EPI_ISL_406596	100	100	100
hCoV-19/Australia/VIC01/2020	ORF1ab gene	EPI_ISL_406844	100	100	100
hCoV-19/Australia/VIC01/2020	N gene	EPI_ISL_406844	100	100	100
hCoV-19/Germany/BavPat1/2020	ORF1ab gene	EPI_ISL_406862	100	100	100
hCoV-19/Germany/BavPat1/2020	N gene	EPI_ISL_406862	100	100	100
hCoV-19/Singapore/1/2020	ORF1ab gene	EPI_ISL_406973	100	100	100
hCoV-19/Singapore/1/2020	N gene	EPI_ISL_406973	100	100	100
hCoV-19/England/01/2020	ORF1ab gene	EPI_ISL_407071	100	100	100
hCoV-19/England/01/2020	N gene	EPI_ISL_407071	100	100	100
hCoV-19/Finland/1/2020	ORF1ab gene	EPI_ISL_407079	100	100	100
hCoV-19/Finland/1/2020	N gene	EPI_ISL_407079	100	100	100
hCoV-19/Japan/AI/I-004/2020	ORF1ab gene	EPI_ISL_407084	100	100	100
hCoV-19/Japan/AI/I-004/2020	N gene	EPI_ISL_407084	100	100	100
hCoV-19/South Korea/KCDC03/2020	ORF1ab gene	EPI_ISL_407193	100	100	100
hCoV-19/South Korea/KCDC03/2020	N gene	EPI_ISL_407193	100	100	100

Figure 1. The schema of mutations with frequency higher than 1% until December 2021.



Specifically, an in silico inclusivity analysis of the primer/probe sets was performed using complete genomes with high coverage in the GISAID database from 23 Oct 2021 to 23 Dec 2021,

which includes the following variants of concerns currently designated by WHO:

- 18,195 genomes of the Alpha variant
- 1,816 genomes of the Beta variant
- 126,1398 genomes of the Delta variant
- 18,002 genomes of the Gamma variant
- 33,288 genomes of the Omicron variant

The analysis demonstrated that all genomes for each variant were predicted unlikely to impact the detection of SARS-CoV-2.

The wet testing of inclusivity using the Sample Release Reagent RNA fast-releasing technology was evaluated as supplemental data by testing three SARS-CoV-2 positive specimens from different areas in China. These specimens were confirmed positive by China CDC suggested rRT-PCR kit. Each specimen was diluted to 1×LoD in negative specimen matrix (oropharyngeal swab specimen in Sample Storage Reagent) and tested in triplicate (**Table 8**).

Table 8. Reactivity: Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

2019-nCoV Strain/Isolate	Source/Sample Type*	Concentration	Ct of ORF1ab gene	Ct of N gene
Specimen 1 from Wuhan	oropharyngeal swab, inactivated	200 copies/mL	35.24	37.77
			34.30	36.65
			36.11	35.99
Specimen 2 from Beijing	oropharyngeal swab, inactivated	200 copies/mL	35.42	38.34
			35.61	36.73
			35.14	36.48
Specimen 3 from Hunan	oropharyngeal swab, inactivated	200 copies/mL	37.36	37.44
			34.93	37.85
			34.95	35.26

*Samples were inactivated at 50 ℃ for 30 minutes.

3) Cross-reactivity (Analytical Specificity):

Cross Reactivity: Cross-reactivity of the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was evaluated by both *in silico* analysis and by wet testing potentially cross-reactive whole pathogens or purified nucleic acid from clinical specimens. For wet-testing, each sample was diluted to a specific concentration in negative specimen matrix (a negative oropharyngeal swab specimen in Sample Storage Reagent) and tested in triplicate using Sample Release Reagent RNA fast-releasing technology (**Table 9**). No cross-reactivity was detected. The *in silico mapping* analysis of each primer/probe against 27 pathogens is based on the NCBI nr/nt database accessed March 25, 2020 using the online BLASTN 2.10.0+ and the representative results are shown in **Table 10**. The Orf1ab and N gene primer/probes may detect bat coronaviruses and the N gene primer/probes may detect pangolin coronaviruses based on this *in silico* analysis. No cross reactivity was observed for other listed respiratory pathogens based on both *in silico* and wet-testing.

Table 9. Cross-Reactivity of Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Virus/Bacteria/Parasite	Strain	Source/ Sample type	Concentration	Ct Value (ORF1ab gene/N gene)
Human coronavirus 229E	229E	Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Human coronavirus OC43	OC43	Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Human coronavirus HKU1	HKU1	Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Human coronavirus NL63	NL63	Clinical specimen	1.0×10^6copies/mL	Undet/Undet
SARS-coronavirus		RNA	1.0×10^6copies/mL	Undet/Undet
MERS-coronavirus		Pseudovirus	1.0×10^6copies/mL	Undet/Undet
Adenovirus 1		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Human Metapneumovirus (hMPV)		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Parainfluenza virus 1-4		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Influenza A		National standard	$1.0\times10^6TCID_{50}$/mL	Undet/Undet
Influenza B		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Enterovirus (EV-C95)		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Respiratory syncytial virus		Clinical specimen	1.0×10^6copies/mL	Undet/Undet

Rhinovirus		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Chlamydia pneumonia		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Haemophilus influenzae		National standard	1.0×10^6CFU/mL	Undet/Undet
Legionella pneumophila		National standard	1.0×10^6CFU/mL	Undet/Undet
Mycobacterium tuberculosis		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Streptococus pneumoniae		National standard	1.0×10^6CFU/mL	Undet/Undet
Streptococcus pyogenes		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Bordetella pertussis		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Mycoplasma pneumoniae		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Pneumocystis jirovecii (PJP)		Clinical specimen	1.0×10^6copies/mL	Undet/Undet
Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract*		Clinical specimen	15ng/µL	Undet/Undet

Table 10. The *In Silico* Specificity Analysis of Primer and Probe Sets for Other Respiratory Pathogens.

Pathogen (Taxonomy ID)	Strain	Target	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human coronavirus 229E (11137)	camel/Abu Dhabi/B38	N gene	MF593473.1	55.00%	46.20%	60.90%
Human coronavirus 229E (11137)	camel/Abu Dhabi/B38	ORF1ab gene	MF593473.1	50.00%	52.90%	36.00%
Human coronavirus OC43 (31631)	HCoV_OC43/Seattle/USA/SC9428/2018	N gene	MN310476.1	45.00%	38.50%	No Sig.
Human coronavirus OC43 (31631)	HCoV_OC43/Seattle/USA/SC9428/2018	ORF1ab gene	MN310476.1	41.70%	76.50%	40.00%
Human coronavirus HKU1 (290028)	HKU1 SC2628	N gene	KY983584.1	40.00%	42.30%	43.50%
Human coronavirus HKU1 (290028)	HKU1 SC2628	ORF1ab gene	KY983584.1	45.80%	52.90%	36.00%
Human coronavirus NL63 (277944)	HCoV_NL63/Seattle/USA/SC0179/2018	N gene	MN306018.1	40.00%	42.30%	43.50%
Human coronavirus NL63 (277944)	HCoV_NL63/Seattle/USA/SC0179/2018	ORF1ab gene	MN306018.1	41.70%	52.90%	40.00%
MERS-CoV (1335626)	BtVs-BetaCoV/SC2013	N gene	KJ473821.1	No Sig.	42.30%	No Sig.
MERS-CoV (1335626)	BtVs-BetaCoV/SC2013	ORF1ab gene	KJ473821.1	41.70%	No Sig.	No Sig.
Adenoviridae (10508)	53/FS161/Fukui/2004	N gene	AB568098.1	55.00%	42.30%	47.80%
Adenoviridae (10508)	ITA/2018/251170-16	ORF1ab gene	MK625182.1	58.30%	70.60%	No Sig.
Human metapneumovirus (162145)	bj0154	N gene	MN745086.1	50.00%	No Sig.	47.80%
Human metapneumovirus (162145)	C-85473	ORF1ab gene	KM408077.1	45.80%	No Sig.	No Sig.

Pathogen (Taxonomy ID)	Strain	Target	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Paramyxoviridae (11158)	MVs/Venezia.ITA/22.17/3[D8]	N gene	MK513627.1	No Sig.	73.10%	No Sig.
Paramyxoviridae (11158)	HPIV3/MEX/2822/2006	ORF1ab gene	KF687324.1	No Sig.	76.50%	No Sig.
Orthomyxoviridae (11308)	A/Homo sapien/China/LS314/2019	N gene	MT106847.1	No Sig.	No Sig.	56.50%
Orthomyxoviridae (11308)	A/sanderling/New Jersey/756/1986	ORF1ab gene	CY117434.1	No Sig.	76.50%	No Sig.
Influenza A virus (11320)	A/Homo sapien/China/LS314/2019	N gene	MT106847.1	No Sig.	No Sig.	56.50%
Influenza A virus (11320)	A/sanderling/New Jersey/756/1986	ORF1ab gene	CY117434.1	No Sig.	76.50%	No Sig.
Influenza B virus (11520)	B/New York/20/2018	N gene	MK999210.1	No Sig.	61.50%	No Sig.
Influenza B virus (11520)	B/Alabama/12/2019	ORF1ab gene	MT029398.1	50.00%	No Sig.	No Sig.
Enterovirus (12059)	Donovan	N gene	AY421766.1	No Sig.	69.20%	No Sig.
Enterovirus (12059)	PS87/Belfast; ATCC VR-774	ORF1ab gene	DQ092794.1	70.80%	No Sig.	No Sig.
Respiratory syncytial virus (12814)	B/WI/629-Q0306/10	N gene	JN032121.2	45.00%	No Sig.	47.80%
Respiratory syncytial virus (12814)	99-901	ORF1ab gene	MK947359.1	No Sig.	47.10%	No Sig.
Rhinovirus (12059)	Donovan	N gene	AY421766.1	No Sig.	69.20%	No Sig.
Rhinovirus (12059)	PS-87	ORF1ab gene	X79368.1	70.80%	No Sig.	No Sig.
Chlamydia pneumoniae (83558)	-	N gene	LN847257.1	65.00%	46.20%	47.80%
Chlamydia pneumoniae (83558)	-	ORF1ab gene	LN847257.1	54.20%	64.70%	52.00%
Haemophilus influenzae (727)	-	N gene	CP043770.1	No Sig.	53.80%	47.80%
Haemophilus influenzae (727)	-	ORF1ab gene	CP043770.1	No Sig.	76.50%	48.00%
Legionella pneumophila (446)	-	N gene	CP011105.1	No Sig.	No Sig.	65.20%
Legionella pneumophila (446)	-	ORF1ab gene	CP011105.1	62.50%	No Sig.	No Sig.
Mycobacterium tuberculosis (1773)	-	N gene	CP045962.1	60.00%	46.20%	52.20%
Mycobacterium tuberculosis (1773)	-	ORF1ab gene	CP045962.1	No Sig.	76.50%	48.00%
Streptococcus pneumoniae (1313)	-	N gene	CP038808.1	60.00%	53.80%	52.20%
Streptococcus pneumoniae (1313)	-	ORF1ab gene	CP038808.1	54.20%	64.70%	No Sig.
Streptococcus pyogenes (1314)	-	N gene	CP036530.1	65.00%	50.00%	No Sig.
Streptococcus pyogenes (1314)	-	ORF1ab gene	CP036530.1	No Sig.	No Sig.	68.00%
Bordetella pertussis (520)	-	N gene	CP033419.1	65.00%	No Sig.	52.20%
Bordetella pertussis (520)	-	ORF1ab gene	CP033419.1	No Sig.	70.60%	No Sig.

Pathogen (Taxonomy ID)	Strain	Target	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Pneumocystis jirovecii (42068)	RU7	N gene	XM_018372654.1	No Sig.	50.00%	No Sig.
Pneumocystis jirovecii (42068)	RU7	ORF1ab gene	XM_018373664.1	No Sig.	No Sig.	60.00%
Candida albicans (5476)	-	N gene	CP032019.1	60.00%	53.80%	60.90%
Candida albicans (5476)	-	ORF1ab gene	CP032019.1	54.20%	No Sig.	48.00%
Pseudomonas aeruginosa (287)	-	N gene	CP047697.1	70.00%	No Sig.	No Sig.
Pseudomonas aeruginosa (287)	-	ORF1ab gene	CP047697.1	No Sig.	No Sig.	52.00%
Staphylococcus epidermidis (1282)	-	N gene	CP035643.1	55.00%	53.80%	56.50%
Staphylococcus epidermidis (1282)	-	ORF1ab gene	CP035643.1	No Sig.	70.60%	48.00%
Streptococcus salivarius (1304)	-	N gene	CP018186.1	65.00%	50.00%	52.20%
Streptococcus salivarius (1304)	-	ORF1ab gene	CP018186.1	58.30%	70.60%	48.00%
SARSr-CoV (694009)	SARS coronavirus Frankfurt 1	N gene	AB257344.1	45.00%	80.80%	78.30%
SARSr-CoV (694009)	SARS coronavirus Frankfurt 1	ORF1ab gene	AB257344.1	45.80%	64.70%	64.00%
hCoV-19/bat/Yunnan/RaTG13/2013	-	N gene	EPI_ISL_402131	100	100	86.956
hCoV-19/bat/Yunnan/RaTG13/2013	-	ORF1ab gene	EPI_ISL_402131	100	100	100
hCoV-19/pangolin/Guangxi/P4L/2017	-	N gene	EPI_ISL_410538	100	100	95.652
hCoV-19/pangolin/Guangxi/P4L/2017	-	ORF1ab gene	EPI_ISL_410538	95.83	88.24	-

b) *Interference Studies:* The following potential interfering substances were investigated. Each potential interfering substance was added to a contrived positive and a negative oropharyngeal specimen in the Sample Storage Reagent and tested in triplicate using Sample Release Reagent RNA fast-releasing technology. No interference was detected. **Tables 11** through **13** provide the results of these studies.

Table 11. Interfering Substances: Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) Evaluation Test Using Negative Specimen

Potential Interfering Substance	Concentration	Results (Detected X/3)
Mucin: bovine submaxillary gland, type I-S	20μg/mL	0/3
Blood (human)	5%(v/v)	0/3
Nasal sprays or drops	100μg/mL	0/3
Nasal corticosteroids	50μg/mL	0/3

Potential Interfering Substance	Concentration	Results (Detected X/3)
FluMist	100μg/mL	0/3
Homeopathic allergy relief medicine	200μg/mL	0/3
Anti-viral drugs	300U/mL	0/3
Antibiotic, nasal ointment	100μg/mL	0/3
Antibacterial, systemic	100μg/mL	0/3

Table 12. Interfering Substances: Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) Evaluation Test Using Positive Specimen

Potential Interfering Substance	Concentration	Viral Strain Level (In multiples of LoD)	Results (Detected X/3)
Mucin: bovine submaxillary gland, type I-S	20μg/mL	3xLoD	3/3
Blood (human)	5%(v/v)	3xLoD	3/3
Nasal sprays or drops	100μg/mL	3xLoD	3/3
Nasal corticosteroids	50μg/mL	3xLoD	3/3
FluMist	100μg/mL	3xLoD	3/3
Homeopathic allergy relief medicine	200μg/mL	3xLoD	3/3
Anti-viral drugs	300U/mL	3xLoD	3/3
Antibiotic, nasal ointment	100μg/mL	3xLoD	3/3
Antibacterial, systemic	100μg/mL	3xLoD	3/3

Table 13. Ct Values for Interfering Substances Evaluation Using Positive Specimen

Interfering substances	Mean Ct Value of 3 replicates	
	ORF1ab gene	N gene
Mucin: bovine submaxillary gland, type I-S	34.89	35.29
Blood (human)	36.05	35.68
Nasal sprays or drops	35.92	35.69
Nasal corticosteroids	35.86	35.54
FluMist	35.66	35.52
Homeopathic allergy relief medicine	35.09	35.25
Anti-viral drugs	35.45	35.85
Antibiotic, nasal ointment	35.73	34.93
Antibacterial, systemic	35.08	35.66

4). Matrix Equivalency

The matrix equivalency between oropharyngeal swabs and nasopharyngeal swabs stored in the Sample Storage Reagent was carried out using an oropharyngeal swab positive specimen (confirmed by gene sequencing). The positive specimen was diluted to 1×LoD and 5×LoD using oropharyngeal swab and nasopharyngeal swab negative specimens collected from the same patient (20 patients in total). An additional 10 pairs of oropharyngeal swab and nasopharyngeal swab negative specimens were collected and tested using the Sample Release Reagent RNA fast-releasing technology. The results in **Table 14** show that the specimen matrices are equivalent.

Table 14. The matrix equivalency evaluation between oropharyngeal and nasopharyngeal swabs.

Sample Type	Concentration	No. of Test	ORF1ab gene		N gene	
			Positive rate %	Average Ct Value	Positive rate %	Average Ct Value
NPS	1xLoD	20	100	36.07	100	36.74
OPS			100	36.05	100	36.30
NPS	5xLoD	20	100	34.33	100	34.14
OPS			100	34.15	100	33.80
NPS	Negative	10	0	n/a	0	n/a
OPS			0	n/a	0	n/a

5). Clinical Evaluation

The clinical performance of Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was established using 246 oropharyngeal swab specimens (in Sample Storage Reagent) collected from patients who were suspected of COVID-19. The comparator method was the Real-Time Fluorescent RT-PCR kit for Detecting SARS-2019-nCoV from BGI Genomics, which received Emergency Use Authorization from the US Food and Drug Administration on March 26, 2020. The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) used the Sample Release Reagent RNA fast-releasing technology and BGI Genomics comparator assay extraction method was the Qiagen QIAamp Viral RNA Mini Kit. Both assays were run on Applied Biosystems ABI 7500 with SDS software version 1.5. The results are summarized in **Table 15** and demonstrated a PPA of 94.34% and NPA of 98.96%.

Table 15. Clinical evaluation between Sansure Biotech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) and BGI Genomics Comparator Method

Test		BGI Genomics Comparator		Total
		Positive	Negative	
Sansure Biotech	Positive	50	2	52
	Negative	3	191	194
Total		53	193	246

Positive Agreement Rate: 50/53 ×100%＝94.34% (95% CI: 84.34% ~ 98.82%);
Negative Agreement Rate: 191/193×100%= 98.96 % (95% CI: 96.31% ~ 99.87 %);

17. Symbols

Symbols	Meanings	Symbols	Meanings
IVD	In Vitro Diagnostic Medical Device		Date of Manufacture
	Use By		Consult Instructions for Use

	Temperature Limitation		Manufacturer
LOT	Lot Number	REF	Reference Number
	Number of Tests		Any warnings and/or precautions to take
Rx only	Prescription only		

18. Contact Information and Product Support

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Revision History

Version	Revision Date	Remarks
V00	05-04-2020	Initial Release Version
V01	December 23, 2021	1. Doc.Version: V00 updated to V01. Revision date: 05-04-2020 updated to December 16, 2021. 2. Add the limitation description of performance test in section 13 and "RX Prescription only" in section 17. 3. Add “Revision History”. 4. Update the Inclusivity analysis
V02	March 24, 2022	1. Update the Inclusivity analysis. 2. Updated to include viral mutation limitations per EUA Conditions of Authorization 3. Updated to include authorized laboratories statements as per letter of authorization.