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

【 Reference Number 】

S3102E

【 Package Specification 】

24 tests/kit, 48 tests/kit

【 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 swabs, alveolar lavage fluid, sputum, serum, whole blood and feces from individuals who are suspected of COVID-19 infection.

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.

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 professional, qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

For in vitro diagnostic use only. For professional use only.

【 Test Principle 】

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test. The 2019-nCoV primer and probe sets are designed to detect RNA from SARS-CoV-2 in respiratory and other specimens from patients who are suspected of COVID-19 infection. This kit is used for qualitative detection of the ORF1ab and N genes of SARS-CoV-2 RNA.

An internal control targeting the RNase P gene monitors the sample collection, sample handling and RT-qPCR process to avoid false-negative results.

【 Components of the Diagnostic Kit 】

This kit is an amplification reaction reagent and contains the following components:

No.	Reagent Name	Spec. & Qty.		Main Ingredients
		24 T	48 T	
1	2019-nCoV-PCR Mix	624 μL/ tube x 1	1248 μL/ tube x 1	Primers(4.62%), Probes(1.15%), dNTPs(3.85%), $MgCl_2$(0.77%), Rnasin(0.48%), PCR buffer(89.13%)
2	2019-nCoV-PCR-Enzyme Mix	96 μL/ tube x 1	192 μL/ tube x 1	RT Enzyme(62.5%), Taq Enzyme (37.5%)
3	2019-nCoV-PCR-Positive Control	500 μL/tube x 1	500 μL/tube x 1	In vitro transcribed RNA containing target genes (ORF1ab, N gene) and internal control gene fragments (Rnase P)
4	2019-nCoV-PCR-Negative Control	500 μL tube x 1	500 μL tube x 1	Saline

Note:

1. 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. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kit lots.
2. All biological samples in this diagnostic kit have been inactivated.
3. Materials required but not provided:

a. 1.5 mL DNase-free and RNase-free microfuge tubes
b. 0.2 mL PCR reaction tubes or strip
c. Various models of pipettes and pipette tips (10μL, 200μL and 1000μL tips with filters)
d. Microcentrifuge
e. Vortex mixer

【 Storage and Stability 】

1. This kit should be stored in its original box at -20 ± 5°C and protected from light. The kit is valid for 12 months.
2. Please refer to the date of manufacture and expiry date printed on the outside of the box.
3. Unopened reagents are valid and stable until the expiry date.
4. Once the reagents are opened, the maximum number of freeze/thaw cycles should not exceed three.

【 Compatible Instrument 】

This diagnostic kit has been validated on the following Real-Time PCR instruments:

- Applied Biosystems 7500 System
- Thermofisher QuantStudio™ 5 Real-Time PCR System
- Roche LightCycler® 480 Instrument II
- Molarray MA6000 Real-time quantitative PCR system
- Hongshi SLAN 96P Real Time PCR System
- Sansure iPonatic Portable Molecule Workstation (Model S-Q31A)
- Biorad CFX96 (needs to be evaluated before use)

【 Specimen Requirements 】

1. Applicable specimen type: nasopharyngeal swab, oropharyngeal swab, alveolar lavage fluid, sputum, serum, whole blood, feces.
2. Collection of specimen

Nasopharyngeal swab/oropharyngeal swab: Collect samples in accordance with the relevant local procedures for COVID 19 Laboratory Testing. Collection swabs should have a synthetic tip, such as nylon or Dacron®, and an aluminium or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.

Nasopharyngeal swab: The specimen collection tube should be pasted with the barcode first; the nasopharyngeal swab should be collected within 3 days after the onset of the disease as far as possible. Use swab to measure the length between apex nasi and earlobe, then mark with finger. Insert the swab into the nasal cavity in direction of perpendicular to the nose (face). The swab should be inserted at least half of the length from the earlobe to the apex nasi. Make the swab stops in the nasal for 15 ~ 30 s, gently rotate 3 ~ 5 times, quickly put swab into specimen collection tube containing 2 mL Lysis Buffer (same as Lysis Buffer in the Sample Release Reagent) or Sample Storage Reagent containing RNA enzyme inhibitor. Insert the swab, then break the sterile swab rod near the top, tighten tube cap and seal with sealing film.

Oropharyngeal swab: The specimen collection tube should be pasted with the barcode first; the oropharyngeal swab should be collected within 3 days after the onset of the disease as far as possible. A sterile flocking swab should be used for sampling, moderately wipe the posterior pharyngeal wall, avoid touching the tongue. Quickly place a sterile swab into the collection tube used for collection of oropharyngeal swabs. Break the sterile swab rod near the top, tighten tube cap and seal with sealing film.

Alveolar lavage fluid: Severe patients or patients with pneumonia who progress rapidly. Clinician extract ⩾5 mL BALF into a 50 mL aseptic container labeled with sample bar code and screw cap by aseptic operation. Collect specimen, then tighten tube cap and seal with sealing film.

Sputum: The specimen collection tube should be pasted with the barcode first. Do not open the airway to collect specimens when collecting sputum. Collect deep cough sputum into a disposable aseptic sampling cup with screw cap, load 2 mL protease K (1g/L) into sampling cup. Collect sputum, then tighten tube cap and seal with sealing film. Send to detection within 30 min as far as possible. Protease K should not be added first if specimens need to be transported over long distances.

Whole blood: Blood samples can be collected within 7 days after the onset or critical patients, or patients considered with viremia. The specimen collection tube should be pasted with the barcode first. Collect 2~4 mL of blood samples into vacuum blood collection tube containing EDTA anticoagulant.

Feces: For patients with gastrointestinal symptoms such as diarrhea at the early stage of the disease, preserve 3~5 g (soybean size) feces. The specimen collection tube should be pasted with the barcode first. Collect sample into specimen collection tube with screw cap containing 2 mL normal saline (RNA enzyme inhibitor can be added when conditions permit) then seal with sealing film.

After sample collection, it is recommended to place into Sample Storage Reagent for preservation.

It has been proved that preservation solution, such as normal saline, TE buffer, 2-4M containing guanidine (such as guanidine hydrochloride) can also be used as Sample Storage Reagent for sample preservation. The Sample Storage Reagent containing guanidine cannot be directly adapted to Sample Release Reagent manufactured by Sansure Biotech Inc. for nucleic acid extraction. If necessary, it is recommended to use Nucleic Acid (DNA/RNA) Extraction or Purification Kit (Magnetic beads method) (Reference Number : S1002E) or Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number : S1006E) manufactured by Sansure Biotech Inc. or the QIAamp Viral RNA Mini Kit (50) manufactured by QIAGEN for nucleic acid extraction.

3. Storage and delivery of specimens:

Specimens to be tested can be immediately processed, specimens to be tested within 24 hours can be stored at 4℃. Specimens that cannot be detected within 24 hours should be stored at -70℃ or below (in the absence of -70℃ storage conditions, specimens to be tested 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 container with ice or in a sealed foam box with ice packs. The inactivation of samples at 56℃ can be performed before sample lysis or extraction for 30min, which will not affect the PCR detection performance.

【 Test Method 】

1. Please process according to the following steps for SLAN-96P, ABI7500, QuantStudio ™ 5, Roche 480, MA-6000 PCR instrument:

1.1 Preparation of reagent (performed at "reagent preparation region")

1.1.1 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.

1.1.2 Prepare the 2019-nCoV-PCR Master Mix according to following table. The volume required is based on the total number of specimens, plus a 2019-nCoV-PCR-Positive Control and a 2019-nCoV-PCR-Negative Control. Mix thoroughly then centrifuge it for later use. The remaining reagent must be stored at -20°C immediately.

	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.				

1.2. Processing and loading of specimens (performed at "specimen processing region")

1.2.1 Use Sample Release Reagent (Reference Number : S1014E) or Sample Release Reagent (Reference Number : S1015E) or Nucleic Acid (DNA/RNA) Extraction or Purification Kit (Magnetic beads method) (Reference Number : S1002E) or Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number : S1006E) manufactured by Sansure Biotech Inc. to extract the nucleic acid according to corresponding manual.

1.2.2 Add 20 μL of the extracted RNA to the PCR tubes in the following order: 2019-nCoV-PCR-Negative Control, patient specimen(s), and 2019-nCoV-PCR-Positive Control.

1.2.3 Add 30 μL of 2019-nCoV-PCR Master Mix into each well. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into the Real-Time PCR system.

1.3 PCR Amplification (Refer to user manual of each instrument to adjust the settings.)

1.3.1 Place PCR reaction tubes into the specimen wells of the amplification equipment. Set up the 2019-nCoV-PCR-Positive Control, 2019-nCoV-PCR-Negative Control and specimens to be tested in order and input specimen name.

1.3.2 Select PCR test channel:

a) Select FAM (ORF-1ab region) and ROX (N gene) channels to test 2019-nCoV nucleic acid.

b) Select CY5 channel to test internal control.

3.3 Set cycle parameters

	Steps	Temperature	Time	Cycle No.
1	Reverse transcription	50°C	30 min.	1
2	cDNA pre-denaturation	95°C	1 min.	1
3	Denaturation	95°C	15 sec.	45
	Annealing, extension and fluorescence collection	60°C	30 sec.	
4	Device cooling	25°C	10 sec.	1

When the settings are completed, save the settings and carry out the reaction procedure.

2. Please process according to the following steps for Portable Molecule Workstation (Model: S-Q31A):

2.1 Preparation of reagent strip

2.1.1 Add each component in the kit and Sample Release Reagent (Reference Number: S1014E) or Sample Release Reagent (Reference Number: S1015E) manufactured by Sansure Biotech Inc. into the 4-tube strip as shown in **Figure 1**:

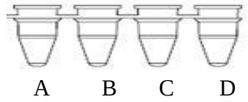

Figure 1 Schematic diagram of the 4-tube strip

1) Add 20μL specimen to be tested or 2019-nCoV-PCR-Positive Control or 2019-nCoV-PCR-Negative Control to the **Tube A** in the 4-tube strip.
2) Add 20μL Sample Release Reagent to the **Tube C** in the 4-tube strip, add 26μL 2019-nCoV-PCR Mix and 4μL 2019-nCoV-PCR-Enzyme Mix to the **Tube D** in the 4-tube strip.

2.1.2 Place the 4-tube strip on the corresponding hole on the reagent strip of the instrument in the direction from A to D;

2.1.3 Take out the **PCR reaction tube** and place it in the PCR hole of the reagent strip, take out the **Tips** and place them in the H hole of the reagent strip.

2.2 Test Procedure

2.2.1 Start up the power supply of Portable Molecule Workstation (Model: S-Q31A), then log in the software.

2.2.2 Place the reagent strips that have been put into the reagent and consumables in the reagent carrier tank of Portable Molecule Workstation (Model: S-Q31A).

2.2.3 Click **"LabTask"** and enter **"TaskName"**. Select 2019-nCoV under LabProject ComboBox for Novel Coronavirus test.

2.2.4 Click **"Submit"** and **"Run"** successively.

2.2.5 When the Portable Molecule Workstation (Model: S-Q31A) shows **"Please transfer the PCR tube"** on the interface, take out the PCR tube and cover it well, then centrifuge it instantaneously.

2.2.6 Insert the PCR tube into the PCR amplification module, close the outer cover of the amplification module, then click **"Ok"** for amplification detection.

3. Result Analysis (Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Analyze amplification curve of target of detection and internal control. Adjust Start, End and Threshold values of Baseline of the graph according to analysis result (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click “Analyze” to implement the analysis, make sure each parameter satisfies the requirements given in “4. Quality Control”. Go to “Plate” window to record qualitative results.

4.Quality Control

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 positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples.

2019-nCoV-PCR-Positive Control			2019-nCoV-PCR-Negative Control			Results	Actions
ORF 1ab (FAM)	N (ROX)	IC (CY5)	ORF 1ab(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.							

【 Reference Range 】

Through the research on reference values, the Ct reference value of target gene is determined to be 40, the Ct reference value of internal control is determined to be 40.

【 Explanation of Detection Result 】

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. The table below describes the result interpretation concerning the use of the controls mentioned above. The end user is required to review fluorescent curves before final interpretation. All the positive curve should be typical S-shape amplification curve or without plateau for weakly positive samples (38≤Ct≤40).

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 ORF 1ab 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.			

【 Limitations of Detection Method 】

1. 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.
2. Mutation in the target sequence of SARS-CoV-2 or change in the sequence due to virus evolution may lead to false negative results.
3. Improper reagent storage may lead to false negative results.
4. Use of this assay is limited to personnel who are trained in the procedure.
5. 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.
6. 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.
7. Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.
8. 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.
9. 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.

【 Product Performance Index 】

1. Inclusivity

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 (1545 sequences), NGDC 2019nCoVR (166 sequences), NCBI (184 sequences) database accessed on March 25, 2020. The primer/probe sets for ORF1ab gene and N gene sequencing alignment analysis demonstrate 100% homology for SARS-CoV-2 sequences identified from patient samples.

2. Specificity

Cross-reactivity of the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) was evaluated by both in silico analysis based on NCBI database and by wet testing. There are no cross-reactions with coronavirus (NL63, HKU1, 229E,OC43), SARS coronavirus, MERS coronavirus, influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus 1, Human Metapneumovirus, parainfluenza virus Type 1, 2,3 and 4, enterovirus (EV-C95), Rhinovirus, Chlamydia pneumonia, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Streptococcus pneumoniae, Streptococcus pyogenes, Bordetella pertussis, Mycoplasma pneumoniae, Pneumocystis jirovecii (PJP), Pooled human nasal wash. 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.

3. **Limit of detection:** The limit of detection of this kit is 200 copies/mL.

4. **Precision:** The coefficient of variation (CV%) of Ct value of the within-run precision is ≤ 5%.

5. **Possible interfering substances in specimens: Mucin: bovine submaxillary gland, type I-S (20μg/mL), Blood (human, 5%(v/v)), Nasal sprays or drops (100μg/mL), Nasal corticosteroids (50μg/mL), FluMist (100μg/mL), Homeopathic allergy relief medicine (200μg/mL), Anti-viral drugs (300U/Ml), Antibiotic, nasal ointment (100μg/mL), Antibacterial, systemic (100μg/mL) have no significant interference with the detection results of the kit.**

6. **Clinical evaluation：A total of 643 samples were collected for clinical evaluation. The total sensitivity: 99.48 % (95%CI: 97.1% ~100.0 %), specificity:100 % (95%CI: 99.2% ~100.00 %), total compliance rate:99.84 % (95%CI: 99.1% ~100.00 %), and Kappa value: 0.9963.**

【 Precautions 】

1. For in vitro diagnostic use only (IVD).
2. Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
3. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
4. 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 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.
5. 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.

【 Bibliography 】

1. Aslak Widerøe Kristoffersen, Svein Arne Nordbø, Rognlien A G W , et al. Coronavirus Causes Lower Respiratory Tract Infections Less Frequently Than RSV in Hospitalized Norwegian Children[J]. The Pediatric Infectious Disease Journal, 2010, 30(4):279-283.

2. E. Moës, Vijgen L , Keyaerts E , et al. A novel pancoronavirus RT-PCR assay: frequent detection of human coronavirus NL63 in children hospitalized with respiratory tract infections in Belgium[J]. BMC Infectious Diseases, 2005, 5.

【 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
Σ	Number of Tests		Any warnings and/or precautions to take

Sansure Biotech Inc.

Add.: No. 680, Lusong Road, Yuelu District, 410205 Changsha, Hunan Province, PEOPLE’S REPUBLIC OF CHINA

Tel.: +86-731-88883176

Fax: +86-731-88884876

Web: www.sansure.com.cn

For Professional Use Only

IVD