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

【 Reference Number 】

S3102E

【 Package Specification 】

24 tests/kit, 48 tests/kit

【 Intended Use 】

Novel Coronavirus(2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is used for qualitative detection of the ORF1ab and N genes of novel coronavirus (2019-nCoV) in nasopharyngeal swab, oropharyngeal swab, alveolar lavage fluid, sputum, serum, whole blood and feces from suspected pneumonia cases with novel coronavirus infection, patients with suspected clusters of novel coronavirus infection, and other patients requiring diagnosis or differential diagnosis of novel coronavirus infection.

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

【 Summary 】

The definitions of "suspected cases" and "suspected clusters of patients" shall be defined by referring to the "Pneumonia Diagnosis and Treatment Program for Novel Coronavirus Infection" and "Pneumonia Case Monitoring Program for Novel Coronavirus infection" issued by China CDC (the current version).

Novel Coronavirus(2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is only used for the auxiliary diagnosis of related cases and the emergency reserve for in vitro diagnosis during the pneumonia outbreak of novel Coronavirus (2019-nCoV) infection since December 2019, this kit shouldn't be used as routine in vitro diagnostic in clinical practice. Please follow the relevant requirements of the "Pneumonia Diagnosis and Treatment Program for Novel Coronavirus Infection", "Pneumonia Prevention and Control Program for Novel Coronavirus infection" and other documents in use.

The novel Coronavirus nucleic acid tests should comply with "Technical Guidelines for Laboratory Testing of Novel Coronavirus in China CDC " and keep good biosafety.

【 Test Principle 】

By applying Real-time fluorescence quantitative RT-PCR technology on the fluorescence quantitative PCR instrument, this test utilizes the novel coronavirus (2019-nCoV) ORF 1ab and the specific conserved sequence of coding nucleocapsid protein N gene as the target regions which are designed for the conserved sequence of the double-target genes, to achieve detection of sample RNA through fluorescent signal changes.

The PCR detection system uses the positive internal control, which monitors the presence of PCR inhibitors in test specimens by detecting whether the internal control signal is normal, to avoid a false negative result.

【 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	Premiers(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 transcriptional RNA containing target genes (ORF1ab, N gene) and internal standard gene fragments (Rnase P)
4	2019-nCoV-PCR-Negative Control	500 μL tube x 1	500 μL tube x 1	Normal saline

Note:

1. Do not mix or exchange components from different kit lots.
2. All biological samples in the diagnostic kit have been inactivated.
3. Materials required but not provided: 1.5 mL DNase-free and RNase-free centrifuge tubes, 0.2 mL PCR reaction tubes, pipette tips (10 μL, 200 μL and 1000 μL tips with filters are preferred), desktop centrifuge, desktop vortex mixer various models of pipette guns.
4. Self-prepared reagent: 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) manufactured by Sansure Biotech Inc. or QIAamp Viral RNA Mini Kit (50) manufactured by QIAGEN. Sample Storage Reagent, such as Sample Storage Reagent (Reference Number :X1002E) manufactured by Sansure Biotech Inc.

【 Storage and Stability 】

1. The diagnostic kit should be stored in a sealed pouch at -20±5°C and protected from light. The kit is provisionally valid for 6 months.
2. Please refer to the date of manufacture and expiry date on the outer package.
3. The reagents keep valid and stable within the expiry date if not used. As long as the container of the reagent is opened, the freeze/thaw cycles should not exceed three.

【 Compatible Instrument 】

The diagnostic kit is applicable to SLAN-96P, ABI7500, Life Technologies QuantStudio ™ 5 , Roche Cobas 480, MA-6000 PCR instrument.

【 Specimen Requirements 】

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

2. Collection of specimen

Nasopharyngeal swab/oropharyngeal swab: Collect sample in accordance with the relevant provisions of "Specimen Collection Method" in the "Pneumonia Laboratory Technical Guide for Novel Coronavirus Infection" from "Pneumonia Prevention and Control Plan for Novel Coronavirus Infection". It is proved that the swab made of nylon sampling head and ABS sampling rod can be selected for sample collection.

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 cotaining 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 swab. 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 cotaining 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) 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 pitcher with ice or in a sealed foam box with ice. The inactivation of samples at 56℃ for 30min will not affect the detection of this kit.

【 Test Method 】

1. Preparation of reagent (performed at "reagent preparation region")

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.

1.2 According to the quantity of test specimens, 2019-nCoV-PCR-Positive Control and 2019-nCoV-PCR-Negative Control, pipette appropriate quantity of 2019-nCoV-PCR Mix and 2019-nCoV-PCR-Enzyme Mix (2019-nCoV-PCR Mix 26 μL/test + 2019-nCoV-PCR-Enzyme Mix 4 μL/test), mix them thoroughly to make a PCR-Mastermix, centrifuge it instantaneously for later use.

	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 just for your reference and to ensure enough volume of the PCR-Mastermix, more volume of the actual pipetting may be required.				

1.3 Transfer the above-prepared reagents to the "specimen processing region" for later use.

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

2.1 Use Sample Release Reagent (Reference Number : S1014E), Sample Release Reagent (Reference Number : S1015E), Nucleic Acid (DNA/RNA) Extraction or Purification Kit (Magnetic beads method) (Reference Number : S1002E) manufactured by Sansure Biotech Inc. to extract the nucleic acid as per the product manual.

2.2 Add 30 μL PCR-Mastermix into PCR reaction tube with 20 μL above processed sample. Carry out fluorescence quantitative PCR detection on fluorescence PCR instrument. The fluorescent PCR tube can be sealed with 15 μL paraffin oil before PCR amplification.

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

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 the corresponding sequence and input specimen name.

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 predenaturation	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.

4. 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 satisfy the requirements given in "5. Quality Control". Go to "Plate" window to record qualitative results.

1. Quality Control

	2019-nCoV-PCR-Negative Control	2019-nCoV-PCR-Positive Control
Ct value	No Ct or Ct > 40 at channel FAM, ROX and CY5 (internal control)	≤ 35 at channel FAM, ROX and CY5 (internal control)

The test result is treated as valid if all the conditions in the above-mentioned are met for the same test. Otherwise the test result is treated as invalid and needs to be re-tested.

【 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 】

Conclusion	Amplification results
2019-nCoV Positive	There is typical S-shape amplification curve detected at FAM and/or ROX channel, and the amplification curve which is detected at CY5 channel, Ct≤40.
2019-nCoV Negative	There is no typical S-shape amplification curve(No Ct) or Ct ＞ 40 detected at FAM and ROX channel, and the amplification curve which is detected at CY5 channel, Ct ≤ 40.

There is no typical S-shape amplification curve detected at FAM, ROX and CY5 channel (No Ct), or Ct > 40. It is indicated that the specimen' s concentration is too low, or there are interfering substances that inhibit the reaction. The test result is invalid. An investigation should be performed to find out and exclude the reasons, please collect specimen again and retest the specimens. (If repeated tests still produce invalid results, please contact Sansure Biotech.)

Note: For virus cultures, there is no requirements fpr internal control test results.

【 Limitations of Detection Method 】

1. Test results of the diagnostic kit can be used only for clinical reference. The symptoms and physical signs, disease history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered during their clinical diagnosis and treatment.

2.The possibility analysis of false negative results:

2.1 The unreasonable of specimen collection, delivery, processing and specimen in low concentrations may lead to false negative results.

2.2 The mutation in the target sequence of 2019-nCoV novel coronavirus to be measured or the change in the sequence due to other causes may lead to false negative results.

2.3 The unreasonable of reagent storage may lead to false negative results.

2.4 Unverified interferences or PCR inhibitors may lead to false negative results.

2.5 Cross-contamination occurring in the specimen processing may lead to false positive results.

2.6 The clinical laboratory should be equipped with instruments and operators in strict accordance with relevant requirements outlined in local, state and national regulations. Operate in strict accordance with the product manual.

【 Product Performance Index 】

1. Accurancy

Test enterprise positive reference, the results are all positive.

2. Specificity

For Novel Coronavirus(2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), there are also no cross-reaction with coronavirus (NL63, HKU1, 229E,OC43), SARS coronavirus, MERS coronavirus, influenza A virus, influenza B virus Type Yamagata and Type Victoria, influenza A (H1N1) virus, influenza A (H3N2) virus, influenza A (H5N1) virus, influenza A (H7N9) virus, respiratory syncytial virus type A and Type B, nasal virus Type A, Type B and Type C, adenovirus Type 1, 2, 3, 4, 5, 7 and 55, parainfluenza virus Type 1, 2 and 3, intestinal virus type A, type B, type C (EV-C95), type D(EV-D70), partial pulmonary virus, human lung virus, cryptococcus neoformans, pyogenic streptococcus, acinetobacter baumannii, pneumocystis carinii, klebsiella pneumoniae, streptococcus pneumoniae, haemophilus influenzae, pseudomonas aeruginosa, legionella pneumophilia, bordetella pertussis, staphylococcus aureus, mycoplasma pneumoniae pneumonia, streptococcus pneumoniae, klebsiella pneumoniae, chlamydia, EB virus, human cytomegalo virus, aspergillus fumigatus, candida albicans, candida glabrata, mycobacterium tuberculosis, non-tuberculous mycobacterium, norovirus, rotavirus, varicella zoster virus, measles virus, mumps virus, human genome DNA etc. positive samples. Test the enterprise negative reference, the result are all negative.

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:** 100 ug/mL hydroxymezoline hydrochloride, 50 ug/mL dexamethasone, 50 ug/mL cefmenoxime hydrochloride, 100 ug/mL oseltamivir, 100 ug/mL zanamivir, 100 ug/mL ribavirin, 100 ug/mL azithromycin, 300U/mL α-interferon, 320 ug/mL budesonide, 125 ug/mL beniferin, 100 ug/mL tobramycin, 50 ug/mL beclometrasone, 100 ug/mL flunicasone, 100 ug/mL momethasone, 200 ug/mL fluticasone, 200 ug/mL histamine dihydrochloride, 100 ug/mL peramivir, 100 ug/mL lopenavir, 100 ug/mL mupiroxacin, 100 ug/mL triamcinolone, 100 ug/mL litonavir, 100 ug/mL abidor, 60 ug/mL sodium chloride, 100 ug/mL urea, 10 ug/mL heme, 20 ug/mL purified mucin, 20%(v/v) anhydrous ethanol, and 20%(v/v) human whole blood have no significant interference with the detection results of the kit.

6. **Clinical evaluation** is based on the recommend method of "Novel Coronavirus Infection Pneumonia Laboratory Testing Technology Guide", "Novel Coronavirus Infection Pneumonia Cases Monitoring Programme (second edition)" to diagnosis/exclusion result as a comparision, in the Military Cademy of Military Medical Research Institute, Hunan Disease Control and Prevention Center, Hunan Province People's Hospital, Central South University Xiangya 2nd hospital, according to clinical data collected from the four institutions, such as statistical analysis, the preliminary evaluation, basic clinical confirmed the product performance can meet the emergency needs.The types of samples for clinical evaluation included pharyngeal swabs and alveolar lavage.Further clinical data will be collected after marketing to confirm the clinical performance of the product.

【 Precautions 】

1. The product can only be used for in vitro diagnosis. Please read the product manual carefully before operation.

2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control for each test.

3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory, laboratory personnel must receive professional training, test processes must be performed in separated regions, all consumables should be for single use only after sterilization, special instruments and devices should be used for every process, all lab devices used in different processes and regions should not be cross-used.

4. All specimens for detection should be handled as if infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.

5. Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized, please strictly follow the instructions.

Due to the characteristics of swab and other sample collection process and viral infection process itself, false negative results may be caused by insufficient sample volume, which should be combined with other clinical diagnosis and treatment information for comprehensive judgment, retest when necessary.

【 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

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