SARS-CoV-2 and Influenza A/B Virus Multiplex Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3113E-24, S3113E-48

Product Name

SARS-CoV-2 and Influenza A/B Virus Multiplex Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification

24 tests/kit, 48 tests/kit

Intended Use

The SARS-CoV-2 and Influenza A/B Virus Multiplex Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for the qualitative detection of nucleic acids of the SARS-CoV-2, Influenza A and Influenza B in oropharyngeal swab and sputum from individuals meeting WHO SARS-CoV-2 clinical criteria (e.g., clinical signs and symptoms associated with SARS-CoV-2 infection) in conjunction with WHO SARS-CoV-2 epidemiological criteria (e.g., history of residence in or travel to a geographic region with active SARS-CoV-2 transmission at the time of travel, or other epidemiologic criteria for which SARS-CoV-2 testing may be indicated), suspected cases of influenza A virus and influenza B virus infection and other persons requiring the diagnosis or differential diagnosis of febrile respiratory infection.

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

Summary

The novel virus, now known as SARS-CoV-2 (previously known as 2019-nCoV), is a RNA virus of the beta coronavirus family. The WHO has named the disease caused by SARS-CoV-2 as coronavirus disease 2019 (abbreviated “COVID-19”). It’s demonstrated that SARS-CoV-2 has the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption. The potential public health threat posed by COVID-19 is globally high.

Influenza Virus is a kind of RNA virus in the Orthomyxoviridae family which leading to human and animal influenza. It causes acute upper respiratory tract infection, spreads rapidly through the air and has periodic pandemics around the world. Human influenza virus are influenza pathogens which can be classified into three types, namely A , B and C. Among them, influenza A is the most harmful, while influenza B and influenza C have weak pathogenicity and are not easy to mutate. Influenza A Virus (Inf. A) has many subtypes. So far, there are 16 subtypes of HA and 9 subtypes of NA. Influenza B Virus (Inf. B) can be divided into two phylogenetic lineages: Yamagata family and Victoria family.

Test Principle

By applying real-time fluorescence reverse-transcription PCR (RT-PCR) technology on the fluorescence quantitative PCR instrument, this kit utilizes the conserved sequence of ORF 1ab and N gene of novel coronavirus (SARS-CoV-2), the conserved sequence of M gene of influenza A virus and the conserved sequence of NP gene of influenza B virus as multiplex RT-PCR amplification target regions to realize the detection of SARS-CoV-2 and influenza A/B virus via fluorescent signal changes.

The multiplex RT-PCR detection system contains primers and probes for internal control (IC) which can be used to monitor the sample collection, sample handling and RT-PCR process 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	SARS-CoV-2/Inf A/B-PCR Mix	624 μL/ tube x 1	1248 μL/ tube x 1	Primers, Probes, dNTPs, $MgCl_2$, Rnasin, PCR buffer
2	SARS-CoV-2/Inf A/B-Enzyme Mix	96 μL/ tube x 1	192 μL/ tube x 1	RT Enzyme, Taq Enzyme
3	SARS-CoV-2/Inf A/B-Positive Control	1000 μL/tube x 1	1000 μL/tube x 1	Armored RNA containing the target genes (ORF1ab, N, M, NP gene) and internal control Gene fragments (Rnase P)
4	SARS-CoV-2/Inf A/B-Negative Control	1000μL/tube x 1	1000μL/tube x 1	Physiological saline

Note:

1. Do not mix or exchange components from different kit lots.
2. Self-prepared materials: 4% NaOH for sputum liquefaction.
3. All biological samples in the diagnostic kit have been inactivated.
4. 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, Sample Release Reagent (Reference Number: S1014E) or Nucleic Acid Extraction-Purification Kit (Reference Number: S10016E) manufactured by Sansure Biotech Inc. for nucleic acid extraction, 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 valid for 12 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 five.

Compatible Instrument

The diagnostic kit is applicable to SLAN-96P PCR instrument, Portable Molecule Workstation (S-Q31A), Portable Molecule Workstation (S-Q31B), ABI 7500, Real-time Quantitative Thermal Cycler (MA6000) and Quant Gene 9600.

Specimen Requirements

1. Applicable specimen type: Oropharyngeal swab, sputum.
2. Collection of specimen: 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".

   Oropharyngeal swab: Use a sterile flocking swab (nylon sampling head and ABS sampling rod) to wipe the bilateral pharyngeal tonsils and posterior pharyngeal wall. After sampling, quickly place the sterile swab into the sample storage reagent (Reference Number: X1002E) manufactured by Sansure Biotech Inc. for storage.

   It has been proved that storage solution, such as sterile virus sampling solution, physiological saline and 2-4 M 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 (Reference Number: S1014E) manufactured by Sansure Biotech Inc. for nucleic acid extraction. If necessary, it is recommended to use Nucleic Acid Extraction-Purification Kit (Reference Number: S10016E) manufactured by Sansure Biotech Inc. for nucleic acid extraction.

   Sputum: After rinsing the patient’s mouth with clean water, collect deep cough sputum into a sample collection cup that containing the sampling solution. The sampling solution is Physiological saline, PBS buffer and Sample Storage Reagent (Reference Number: X1002E) from Sansure Biotech Inc.
3. Storage and delivery of specimens:

   The test specimens can be processed immediately and the specimens to be tested within 24 hours can be stored at 4 °C. Specimens that cannot be detected within 24 hours should be stored at -70 °C or below for long-term storage (in the absence of -70 °C storage conditions, the test specimens can be stored at -20 °C for 9 months, and nucleic acid can be stored at -20 ± 5 °C for 9 months). Multiple freeze/thaw cycles should not exceed five times. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

Test Method

1. Please refer to the following procedures when using SLAN-96P 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 According to the quantity of test specimens, SARS-CoV-2/Inf A/B-Positive Control and SARS-CoV-2/Inf A/B-Negative Control, pipette appropriate quantity of SARS-CoV-2/Inf A/B-PCR Mix and SARS-CoV-2/Inf A/B-Enzyme Mix (SARS-CoV-2/Inf A/B-PCR Mix 26 μL/test + SARS-CoV-2/Inf A/B-Enzyme Mix 4 μL/test), mix them thoroughly to make a PCR-Mastermix, centrifuge it instantaneously for later use.

Components	1 sample	10 samples	24 samples	48 samples
SARS-CoV-2/Inf A/B-PCR Mix (μL)	26	260	624	1248
SARS-CoV-2/Inf A/B-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.				

1.1.3 Transfer the above-prepared reagents to the “specimen processing region” for later use.

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

1.2.1 Processing of specimens

It is recommended to use the Sample Release Reagent (Reference number: S1014E) and Nucleic Acid Extraction-Purification Kit (Reference number: S10016E) manufactured by Sansure Biotech Inc. to extract the nucleic acid as per the product manual.

1.2.2 Add 30 μL PCR-Mastermix into the PCR reaction tube with 20 μL above processed sample. Carry out fluorescence quantitative PCR detection on fluorescence PCR instrument.

1.3. PCR Amplification (performed at “amplification and analysis region”) (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 SARS-CoV-2/Inf A/B-Positive Control, SARS-CoV-2/Inf A/B-Negative Control and the test specimens in the corresponding sequence and input specimen names.

1.3.2 Select PCR test channel:

a) Select FAM channel to test SARS-CoV-2 nucleic acids, HEX channel to test Influenza A nucleic acids, and ROX channel to test Influenza B nucleic acids.

b) Select CY5 channel to test internal control.

1.3.3 Set cycle parameters

No.	Steps	Temperature	Time	Cycle No.
1	Reverse transcription	50 °C	5 min	1
2	Predenaturation	95 °C	1 min	1
3	Denaturation	95 °C	10 sec	41
	Annealing, extension and fluorescence collection	60 °C	20 sec*	

Note:Due to the ABI 7500 instrument itself, it is necessary to set 31s or 32s at 60°C.

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

2. Please refer to the following procedures when using the Portable Molecule Workstation (S-Q31A or S-Q31B)

2.1 Reagent Strip preparation

2.1.1 Transfer the corresponding components in the kit and the Sample Release Reagent (Reference number: S1014E) manufactured by Sansure Biotech Inc. to the 4-tube-strip for use as shown in Figure 1.

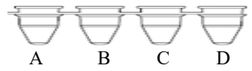

Figure 1 Diagram of the 4-tube-strip

1) Add 20 μL of the above-mentioned processed specimens, SARS-CoV-2/Inf A/B-Positive Control or SARS-CoV-2/Inf A/B-Negative Control to well A of the 4-tube-strip;
2) Add 20 μL Sample Release Reagent to well C of the 4-tube-strip, and add 26 μL SARS-CoV-2/InfA/B-PCR Mix and 4 μL SARS-CoV-2/InfA/B-Enzyme Mix to D well of the 4-tube-strip;

2.1.2 Place the 4-tube-strip in the corresponding hole on the reagent strip of the instrument according to the direction of 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 it in the H hole of the reagent strip.

2.2. Detection process

2.2.1 Turn on the power of the instrument and log in to the software;

2.2.2 Place the above reagent strips with reagents and consumables in the reagent strip carrier slot of the instrument;

2.2.3 Click "Experimental Task" and type in the "Task Name". Select SARS-CoV-2/Inf A/B in the "Experimental Project" drop-down menu;

2.2.4 Click "Submit" and "Run" in turn;

2.2.5 When the interface prompts "Please transfer PCR reaction tube", take out the PCR reaction tube, cover the cap, and then centrifuge it briefly;

2.2.6 Insert the PCR reaction tube into the thermal cycling module, close the outer cover of the thermal cycling module, and click "OK" to carry out the amplification test.

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

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

4. Quality Control

SARS-CoV-2/Inf A/B-Negative Control	SARS-CoV-2/Inf A/B-Positive Control

Ct value	No Ct or Ct > 40 at channels FAM, HEX, ROX and CY5 (internal control)	Ct ≤ 35 at channels FAM, HEX, ROX and CY5 (internal control)

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

Reference Range

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

Explanation of Detection Result

Conclusion	Amplification results
SARS-CoV-2 Positive	There is typical S-shape amplification curve detected at FAM channel, and Ct ≤ 40.
Influenza A Positive	There is typical S-shape amplification curve detected at HEX channel, and Ct ≤ 40.
Influenza B Positive	There is typical S-shape amplification curve detected at ROX channel, and Ct ≤ 40.
SARS-CoV-2, Influenza A and Influenza B Negative	There is no typical S-shape amplification curve (No Ct) or Ct > 40 is detected at channels FAM, HEX and ROX, but the amplification curve is detected at CY5 channel and Ct ≤ 40.

If there is no typical S-shape amplification curve detected at FAM, HEX, 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. Then the test result is treated as invalid. An investigation should be performed to find out and exclude the reasons, please collect specimen again and retest the specimens. (If repeated tests results are still invalid, please contact info@sansure.com.cn)

Note: For virus cultures, there is no requirements for 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 inappropriate specimen collection, delivery, processing and specimen in low concentrations may lead to false negative results.
   2.2 The mutation in the target sequence of virus to be measured or the changes in the sequence due to other causes may lead to false negative results.
   2.3 The inappropriate 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. **Accuracy**
   Test enterprise positive references and the results are all positive.
2. **Specificity**
   For SARS-CoV-2 and Influenza A/B Virus Multiplex Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), there are also no cross-reaction with positive samples of coronavirus (NL63, HKU1, 229E,OC43), SARS coronavirus, MERS coronavirus, 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, B, C (EV-C95) and D(EV-D70), partial pulmonary virus, Human metapneumoviru, cryptococcus neoformans, pyogenic streptococcus, acinetobacter baumannii, pneumocystis yersinensis, klebsiella pneumoniae, streptococcus pneumoniae, haemophilus influenzae, pseudomonas aeruginosa, legionella pneumophilia, bordetella pertussis, staphylococcus aureus, mycoplasma pneumoniae, chlamydia pneumonia, 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 and etc. Test the enterprise negative references, and the results 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 inter/inner batch, inter/inner day 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, 2%(v/v) anhydrous ethanol, and 5%(v/v) human whole blood have no significant interference with the detection results of the kit.

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	EC REP	Authorized representative in the European Community
	Any warnings and/or precautions to take	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

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

EC REP

Obelis S.A

Bd. Général Wahis 53, 1030 Brussels, BELGIUM

Tel: + (32) 2.732.59.54

Fax: + (32) 2.732.60.03

E-Mail : mail@obelis.net

CE IVD

For Professional Use Only