SARS-2-CoV(Covid-19) nucleic acid detection cartridge (Real-time reverse transcription PCR method)

REF 106-0050-01

Product Name
SARS-2-CoV(Covid-19) nucleic acid detection cartridge (Real-time reverse transcription PCR method)

Specification
12 reactions/kit

Intended use
The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.
This manual describes the use of real-time RT-PCR (RT-PCR) assays for the in-vitro qualitative detection of SARS-CoV-2 via throat swab, nasal swab, Sputum. The primer and probe sets are designed specifically for detection of the N and ORF1ab genes of SARS-CoV-2.

Test Principle
The kit utilizes the real-time reverse transcription PCR method to qualitatively detect novel coronavirus in nasopharyngeal swab and sputum samples of patients in-vitro. This product tests two gene targets of the novel coronavirus ORF1ab gene (FAM labelled) and the N gene (TEXAS RED labelled) according to the primers and fluorescent probes for novel coronavirus detection published by China's CDC, and detects whether they contain novel coronavirus (SARS-CoV-2). At the same time, primers and probes (CY5 labelled) with built-in quality control samples are designed. Through the detection of quality control samples, whether the target virus has been adequately treated is confirmed, and whether PCR reaction inhibitor exists is detected.
The reagent combines with supporting instruments to use totally enclosed detection technology, and the instrument automatically performs nucleic acid extraction and nucleic acid amplification in the whole process, thus reducing direct cross contamination of samples.

Components

Kit Components			Content	Quantity
nCoV iCassette (12 pcs)	Nucleic acid extraction reagent	nCoV lyophilization A	Proteinase K	1 pcs/iCassette
		nCoV lyophilization B	lyophilized product contains Armour virus	1 pcs/ iCassette
		Virus lysis solution	Guanidine Isothiocyanate	450 μL/iCassette
		Virus binding solution	Guanidine Isothiocyanate	175 μL/iCassette
		Virus washing solution	Sodium chloride	900 μL/iCassette
		Virus eluent	Tris-HCl	100μL//iCassette
		Magnetic beads	Magnetic microspheres	11 μL//iCassette
		nCoV Complex solvent	PCR Buffer, $MgCl_2$	35 μL//iCassette
	nCoV PCR Reagent	nCoV lyophilization	Specific primer and probe, dNTP, Enzyme	1 pcs//iCassette
Quality Control	nCoV Positive Control		contain Armour virus	1 tube (200 μL)
	nCoV Negative Control		Sterilized purified water	1 tube (200 μL)

Note: The components in kits with different batch numbers are not interchangeable.

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 6 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette cover before adding the sample. If you open the iCassette cover, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Nano, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Lite or Galaxy Pro.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Vortex instrument.
- Pipettes.
- Sterile pipette tips.
- 2% DTT.

Sample Requirements
1.Sample types: Oropharyngeal swab,nasal swaband Sputum
2. Sample collection
2.1 Oropharyngeal swab sample: Wipe both pharyngeal tonsils and posterior pharyngeal wall with two plastic rod swabs with polypropylene fiber heads and immerse the swab tips in virus preservation solution (isotonic saline solution, tissue culture medium or phosphate buffer solution can also be used) and discard the tail then tighten the tube lid. All collected samples should be divided into duplicates at the time of collection in the hospital and one of them should be kept separately for review.
2.2 Nasal swab sample: Insert gently a plastic rod swab with a polypropylene fiber head into the nasal palate in the nasal passage, stay for a while and then slowly turn to exit. Collect the other nostril in the same way. Immerse two swabs into the same tube containing preservation solution, discard the tail and tighten the tube lid.
2.3 Sputum samples
2.3.1 Collect the sputum samples: After the patient has a deep cough, collect the coughed sputum in the 50mL screw-cap test tube containing 3mL of sampling fluid.
2.3.2 Add double of the sample's volume of 2% DTT to the sample. Then shake the tube vigorously for 1 minute. Once shaken, keep it at room temperature for 15 minutes to be balanced, shaking the tube for 10 seconds every 5 minutes. The sample should be fully liquified and free of tiny lumps of unliquified sputum.
3. Sample storage and transportation
The specimens used for virus isolation and nucleic acid detection should be tested as soon as possible. If the specimen can be tested within 24 hours, it can be stored at 2-8℃.If the specimens cannot be detected within 24 hours, it should be stored at -70℃ or below (if there is no storage condition at -70℃, it can be stored in the refrigerator at -20±5℃).Special libraries or special counters should be set up to store specimens separately. Avoid repeated freezing and thawing during specimen transportation.
4. Principles of Biosafety Protection:
All operations should comply with relevant national laws and regulations.

Test Method and Operation
1.**Prepare the nCoV iCassette**
1.1 Processing samples or control materials in the samples preparation room,first vortex the collection tube for 10-15 seconds.
1.2 Open the package of nCoV lyophilization, observe whether the nCoV lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the nCoVlyophilization, and make sure the PCR tube is screwed up.
1.3 Open the lid of the iCassette, and pipette 200 μl of sample or controls to the nCoV iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly.(Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
1.4 Place the iCassette into the instrument.

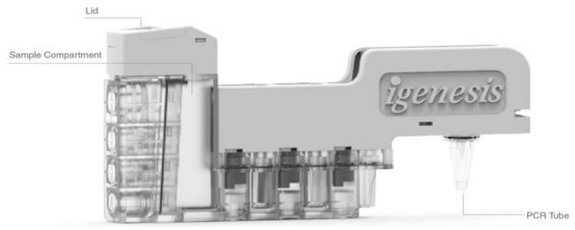

Figure 1. nCoV iCassette (Lateral View)

2. Test Operation
2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.
2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis
After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Positive control test results: FAM, TEXAS RED channel are all positive.
Negative control test results: FAM,TEXAS RED channel are all negative, and CY5 channel is positive.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.

Reference Interval
Determine according to the ROC curve method, Negative (There is no Ct value or the Ct value is more than 29); Positive (Ct value is less than 27); Suspicious (Ct value is between 27 and 29, it is recommended to retest the experiment. If the Ct value is less than 29 and the amplification curve has obvious peaks, the sample is judged as positive, otherwise it is negative）

Interpretation of Test Results

Negative	No Ct value or Ct＞29
Positive	Ct＜ 27
Suspicious	If the Ct value is between 27-29, it is recommended to repeat the test. If the retested Ct＜29 and the amplification curve has a clear peak, the sample is judged as positive; otherwise, it is negative.

The sample to be tested is judged according to the standards in the table below：

FAM Channel	TEXAS RED Channel	CY5 Channel	Results
√	√	√/×	SARS-CoV-2 positive
×	×	√	SARS-CoV-2 negative
×	×	√	Negative control.
√	√	√/×	Positive control
If the Ct value of the test sample is less than 27 in only FAM or TEXAS RED channel, and the other channel has no amplification curve, it is recommended to repeat the test. The repeat test result was unanimously judged to be positive for the suspected novel coronavirus (SARS-CoV-2), the repeat test result was negative and could be judged as no novel coronavirus (SARS-CoV-2).			

Note:"√"indicates that the result "has an obvious logarithmic amplification curve"; "×"indicates the result "no logarithmic amplification curve"; "√/×"Indicates that the result "may be positive or negative."; CY5 channel is an internal control channel. Due to specific competition with the sample, when the test sample is positive, it may be tested negative.

Limitations of Test Method
1. Unreasonable sample collection, transportation and treatment, low bacterial content in the sample, excessive nucleic acid degradation or target concentration in the amplification reaction system below the detection limit may lead to false negative results.
2. The test results of this product cannot be directly used as the basis for clinical diagnosis or case exclusion, and should be combined with other relevant medical examination results for comprehensive analysis.

Product performance index
1. The Sensitivity of kit is 2×10^2copies/mL
2. Analysis Specificity
Specific tests show that pathogenic microorganisms (influenza B virus Yamagata Victoria, H1N1, H3N2, H5N1, H7N9, respiratory adenovirus (1,3,7), respiratory syncytial virus A, B, parainfluenza

virus 2 ,EB virus, *Mycoplasma pneumoniae*, Legionella, pertussis bacillus, *Hemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Mycobacterium tuberculosis*) with the same infection site or similar infection symptoms have no cross reactions.
3. Endogenous interfering substance:
Endogenous substances that may exist in throat swab and nasal swab such as brand-new whole blood and mucus, do not interfere with the detection results of the kit.
4. Precision
The coefficient of variation of intra-batch precision is less than or equal to 5%.

Precautions

1.If the iCassette is oscillated after adding the sample, do not use the iCassette.
2. Clinical laboratories should strictly follow the management standards in the local related regulations for molecular biology laboratories and clinical gene amplification laboratories.
3.Each iCassette is a single-used, please do not reuse it.
4. In the sample processing stage, use a negative pressure ultra-clean workbench.
5. During the experiment, the medical staff must wear work clothes, disposable gloves, and use self-discharging pipettes.
6.After the experiment, the workbench and pipette were treated with 2% sodium hypochlorite or 75% alcohol, and then irradiated with a UV lamp for 30 minutes.

References

Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. Interim guidance.2020.

Instruction Version

Version: A/0
Date of Issue: March,2020

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

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