SARS-2-CoV (Covid-19)/Influenza A Virus/Influenza B Virus Respiratory Syncytial Virus Diagnostic Kit (PCR-Fluorescence)

REF 106-0057-01

Product Name
SARS-2-CoV (Covid-19)/Influenza A Virus/Influenza B Virus/Respiratory Syncytial Virus Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
The Influenza viruses include three types, A, B, and C which are pathogens that cause influenza. Among them, the influenza A virus is most likely to cause epidemic outbreaks. Because the influenza A viruses are prone to large-scale genetic mutations, it may increase infectivity and pathogenicity. Therefore, it can often cause large-scale and even worldwide periodic influenza epidemics and spread to large areas and caused death. The influenza B viruses often cause local outbreaks of influenza. The susceptible population of influenza virus is mostly the elderly, infants and children. The respiratory syncytial virus is the most common and important pathogen of lower respiratory tract infections in infants and young children. It is easy to cause bronchiolitis and pneumonia, resulting in serious infections and complications, and has a wide spread and high incidence.
The SARS-CoV-2 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the SARS-CoV-2 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. Because it is similar to the symptoms caused by respiratory pathogens such as influenza virus, the initial outbreak of SARS-CoV-2 is often mistaken for influenza virus infection and there have been cases of influenza virus and SARS-CoV-2 infection. Rapid detection is conducive to epidemic monitoring and the development of effective prevention and control measures.
The kit uses real-time multiple fluorescent PCR technology that combined with an Automated Fully Enclosed qPCR Instrument for the qualitative detection of SARS-CoV-2, influenza A virus, influenza B virus and respiratory syncytial virus in human Oropharyngeal swab, nasal swab and Sputum samples in vitro. The results can be used for auxiliary diagnosis of virus infection.

Test Principle
The kit uses real-time multiplex fluorescent PCR technology to qualitatively detect the SARS-CoV-2, influenza A virus, influenza B virus and respiratory syncytial virus in Oropharyngeal swab, nasal swab. The kit combines the SARS-CoV-2 ORF1ab gene (CY5 labelled) and N gene (TEXAS RED labelled), influenza A virus M gene (FAM labelled), influenza B virus NS gene (CY5.5 labelled) and respiratory syncytial virus F gene (AMCA labelled) as the detection target area and design specific primers and fluorescent probes respectively for one-step RT-PCR test. At the same time, the kit contains primers and probes (HEX labelled) for the quality control specimens. Through the detection of the quality control specimens, it is confirmed whether the target virus has been adequately processed and whether there is a PCR reaction inhibitory substance.
The kit uses iCassette technology in combination with supporting instruments to automatically perform nucleic acid extraction and nucleic acid amplification by the instrument throughout the entire process, reducing direct cross-contamination of samples.

Components

Kit Components			Content	Quantity
nCoV-Flu-RSV iCassette (12 pcs)	Nucleic acid extraction reagent	nCoV-Flu-RSV lyophilization A	Proteinase K	1pc/iCassette
		nCoV-Flu-RSV lyophillization B2	Pseudovirus lyophilization contains internal standard fragments	1 pc/iCassette
		Virus lysate solution	Guanidine isothiocyanate	450μL/iCassette
		Virus binding solution	Guanidine isothiocyanate	175μL/iCassette
		Virus washing solution	Sodium chloride	900μL/iCassette
		Virus eluate	Tris-HCl	100μL/iCassette
		Magnetic beads	Magnetic microsphere	11μL/iCassette
	nCoV-Flu-RSV PCR reagent	nCoV-Flu-RSV-F PCR cosolvent solution	PCR Buffer, $MgCl_2$	15μL/iCassette
		nCoV-Flu-RSV-F lyophillization	Specific primer probe, dNTP, enzyme	1 pc/tube
	Control materials	nCoV-Flu-RSV positive control	Pseudovirus contains the nCoV-Flu-RSV gene amplicon	1tube（200μL）
		nCoV-Flu-RSV negative control	Sterilizing purified water	1tube（200μL）

Note: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 9 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 Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : 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.

Sample Requirements
1.Sample types: Oropharyngeal swab,nasal swab.
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.
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-Flu-RSV 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-Flu-RSV-F lyophilization, observe whether the nCoV-Flu-RSV-F lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the nCoV-Flu-RSV-F lyophilization, 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-Flu-RSV 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.

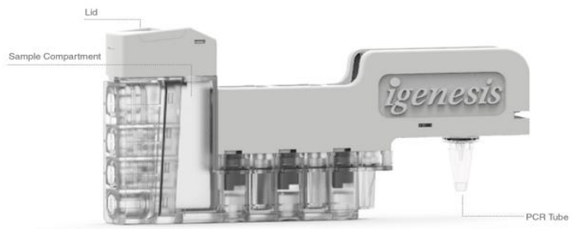

Figure 1. nCoV-Flu-RSV iCassette (Lateral View)

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
The results of positive control are positive in all six channels (FAM,HEX,AMCA,CY5.5,CY5 and TEXAS RED) .
The results of negative control are negative.
Internal Standard control: The CT value of HEX channel is within the effective range of ≤37 which is qualified for internal standard.
In the same batch of reagent experiments, the above requirements must be met at the same time, otherwise, this experiment is invalid and needs to be retested.

Reference Interval
Determine according to the ROC curve method, Negative (There is no Ct value or the Ct value is more than 39); Positive (Ct value is less than 37); Suspicious (Ct value is between 37 and 39, it is recommended to retest the experiment. If the Ct value is less than 39 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 value greater than 39
Positive	Ct value is less than 37
Suspicious	If the Ct value is between 37-39, it is recommended to repeat the test. If the retested Ct value is less than 39 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	CY5.5 Channel	AMCA Channel	HEX Channel	Results
×	√	√	×	×	√	SARS-CoV-2 positive(Figure 2)
√	×	×	×	×	√	Influenza A virus positive (Figure 3)
×	×	×	√	×	√	Influenza B virus positive (Figure 4)
×	×	×	×	√	√	Respiratory syncytial virus positive(Figure 5)

√	√	√	×	×	√	SARS-CoV-2 and influenza A virus positive
×	√	√	√	×	√	SARS-CoV-2 and influenza B virus positive
×	√	√	×	√	√	SARS-CoV-2 and respiratory syncytial virus positive
√	×	×	√	×	√	Influenza A virus and influenza B virus positive
√	×	×	×	√	√	Influenza A virus and respiratory syncytial virus positive
×	×	×	√	√	√	Influenza B virus and respiratory syncytial virus positive
√	√	√	√	×	√	SARS-CoV-2, influenza A virus and influenza B virus positive
√	√	√	×	√	√	SARS-CoV-2, influenza A virus and respiratory syncytial virus positive
×	√	√	√	√	√	SARS-CoV-2, influenza B virus and respiratory syncytial virus positive
√	×	×	√	√	√	Influenza A virus, influenza B virus and respiratory syncytial virus positive
√	√	√	√	√	√	SARS-CoV-2, influenza A virus, influenza B virus and respiratory syncytial virus positive
×	×	×	×	×	√	Negative (Figure 6)
√	√	√	√	√	√	Positive control
×	×	×	×	×	√	Negative control
If only the Ct value of the CY5 or TEXAS RED channel is less than 37 and the other channel has no amplification curve, the result needs to be re-examined. If the result of re-test is consistent, the SARS-CoV-2 is suspected to be positive; If the result of re-test is negative, it can be judged that no SARS-CoV-2 has been detected.						

Note："√" means that the result "has an obvious logarithmic amplification curve"；"×" indicates the result "no logarithmic amplification curve"."√/×" means that the result "may be positive or negative". The HEX channel is an internal standard channel. Because of its specific competition with the sample, when the test sample is positive, it may be tested negative.

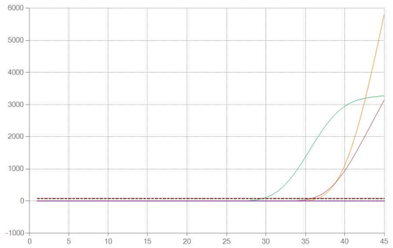

Figure 2. SARS-CoV-2 positive

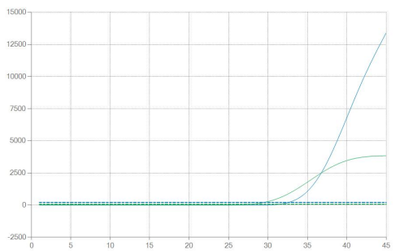

Figure 3. Influenza A virus positive

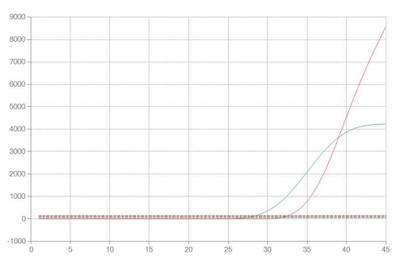

Figure 4. Influenza B virus positive

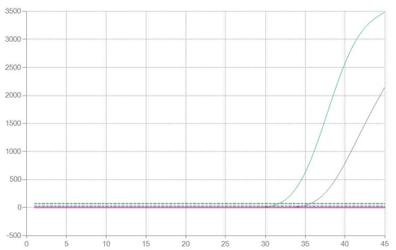

Figure 5. Respiratory syncytial virus positive

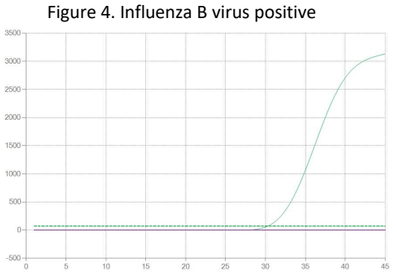

Figure 6. Negative

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 LoD of the kit

SARS-2-CoV (Covid-19)	200copies/mL
Influenza A Virus	500copies/mL
Influenza B Virus	500copies/mL
Respiratory Syncytial Virus	500copies/mL

2. Analysis Specificity

Through the specificity test: No cross-reaction with pathogenic microorganisms that are the same as the infection site or have similar symptoms of infection, such as measles virus, mumps virus, rubella virus, Staphylococcus aureus, Pseudomonas aeruginosa, respiratory adenovirus (type 1, 3, 7), parainfluenza virus type 2, EB virus, Mycoplasma pneumoniae, Legionella, Bordetella pertussis, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Mycobacterium tuberculosis.

3. Interfering Substances

The endogenous substances such as whole blood and mucus that may be present in the oropharyngeal or nasal swab samples do not interfere with the test results of the kit.

4 Precision

The coefficient of variation of intra-assay 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

1. Clinical management of severe acute respiratory infection when novel coronavirus(nCoV-Flu-RSV)infection is suspected——Interim guidance.2020.
2. WHO.Manual for the laboratory diagnosis and virological surveillance of influenza[J/OL].
3. WHO.Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1)virus infection[J/OL].
4. WHO.WHO information for molecular diagnosis of influenza virus - update[J/OL].
5. Kuypers J, Wright N, Ferrenberg J, et al. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children.[J]. Journal of Clinical Microbiology, 2006, 44(7):2382-8.

Instruction Version

Version: A/1
Date of Issue: January,2021
Last revised: March, 2024

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		

IGENESIS(SHANGHAI)CO. , LTD.
Address: Floor 3, building 1, Lane 500, Furonghua Road, Pudong New Area, 201318 Shanghai, P.R. China.
Tel: +86-21-38016598

EC REP **Lotus NL B.V.**
Address: Koningin Julianaplein 10, 1e Verd,2595AA, The Hague, Netherlands.
Email：peter@lotusnl.com