Influenza A Virus / Influenza B Virus/ Respiratory Syncytial Virus Diagnostic Kit (PCR-Fluorescence)

REF 106-0052-01

Product Name
Influenza A Virus / Influenza B Virus/ Respiratory Syncytial Virus Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended use
The kit uses real-time multiplex fluorescent PCR technology that combine with Automated Fully Enclosed qPCR Instrument to qualitative detect influenza A virus,influenza B virus and respiratory syncytial virus in Oropharyngeal swab and Nasal swab specimens in vitro.The results are available for the auxiliary diagnosis of respiratory virus infection.
Influenza viruses include types A (A), B (B), and C (C) which are the pathogens that cause influenza. The type A is the most likely to cause epidemics among them. The influenza A viruses are prone to large-scale genetic mutations which may cause increased infectiousness and pathogenicity. Therefore, it often causes large-scale and even worldwide periodic influenza epidemics, spreads widely in the population, and causes death.The influenza B viruses often cause local outbreaks of influenza. The susceptible population of influenza virus is mostly the elderly and infants. 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 which will cause serious infections and complications. It has a wide epidemic and high incidence. Therefore, the rapid detection of influenza virus and respiratory syncytial virus is conducive to epidemic surveillance as well as the development of effective prevention and control measures, especially to monitor and prevent susceptible infants and the elderly.
The kit uses molecular biology methods for testing. It requires that laboratory operators should have received professional training in gene amplification or molecular biology methods, They should have relevant experimental operation qualifications. The laboratory should have reasonable biological safety precautions and protective procedures.

Test Principle
Automated Fully Enclosed qPCR Instrument uses supplementary icassette technology to automatically perform nucleic acid extraction nucleic acid amplification data reading and result analysis. The instrument includes nucleic acid extraction and multiplex fluorescence PCR. At the same time, the built-in two-dimensional code scanner can automatically identify the execution process corresponding to this kit. The instrument use the uniquely designed software to perform the extraction and PCR process of the kit, display results and analysis results. The kit contains 12 single-use Flu-RSV smart boxes. The reagents stored in icassette include nucleic acid extraction reagents and PCR reaction reagents, which are suitable for Automated Fully Enclosed qPCR Instrument. Because of the instrument is fully enclosed to automatically perform nucleic acid extraction and PCR reaction, the cross-contamination of the specimen can be reduced. Please refer to the user manual of the corresponding instrument before using.
The kit includes nucleic acid extraction reagents and PCR reaction reagents. The nucleic acid extraction reagents contain internal standards for monitoring the entire process of nucleic acid extraction that confirm whether the specimen is fully processed,monitor the presence of PCR inhibitors and avoid false PCR negatives. At the same time, before the PCR reaction starts the instrument will first test the fluorescent signal of the probe to monitor whether the PCR reagent is reconstituted sufficiently ensure that whether the PCR reaction tube is added to the specimen and the integrity and stability of the probe Happening.
The kit uses the influenza A virus M gene (FAM labeled), influenza B virus NS gene (CY5.5 labeled) and respiratory syncytial virus F gene (TEXAS RED labeled) as the detection target area and designs specific primers and fluorescent probes respectively to start one-step RT-PCR reaction. At the same time, the primers and probes (CY5 labeled) of the built-in quality control specimens are designed, and through the detection of quality control specimens, it is confirmed whether the target virus is fully processed, and whether there are PCR reaction inhibitors exists.

Components

Kit Components			Content	Quantity
Flu-RSV icassette (12 pcs)	Nucleic Acid Extraction Kits	Flu-RSV Lyophilization A	Proteinase K	1 pc/icassette
		Flu-RSV Lyophilization B	Pseudovirus 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 Eluent	Tris-HCl	100μL/icassette
		Magnetic beads	Magnetic Microspheres	40μL/icassette
	Flu-RSV PCR Kits	Flu-RSV PCR Cosolvent Solution	PCR Buffer、MgCl2	35μL/icassette
		Flu-RSV Lyophilization	Specific Primer Probes, dNTP, Enzymes	1 pc/icassette
Quality Control	Flu-RSV Positive Control		Pseudovirus containing gene fragments of influenza A virus, influenza B virus and respiratory syncytial disease	1 tube (200μL)
	Flu-RSV Negative Control		Sterilized purified water	1 tube (200μL)

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 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 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.

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 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 Flu-RSV lyophilization, observe whether the Flu-RSV lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the Flu-RSV 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 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.

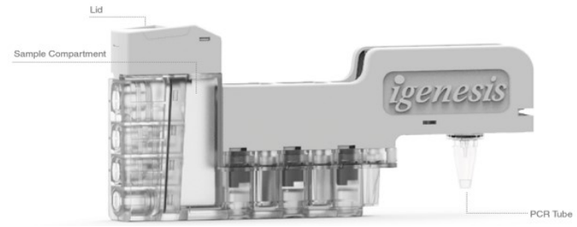


Figure 1. Flu-RSV iCassette (Lateral View)

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
Internal control: In the effective range of Ct≤37 of CY5 channel, the internal control is qualified.
Probe quality control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.
Positive control test results: FAM, CY5.5 and TEXAS RED channel are all positive.
Negative control test results: FAM,CY5.5, TEXAS RED channel are all negative, and CY5 channel is positive.

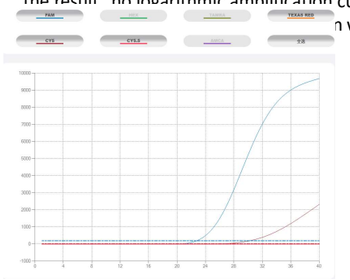
Reference Interval
Based on the analysis of clinical sample test results, using the ROC curve method, the final determined Ct positive threshold value for this reagent kit is 37.

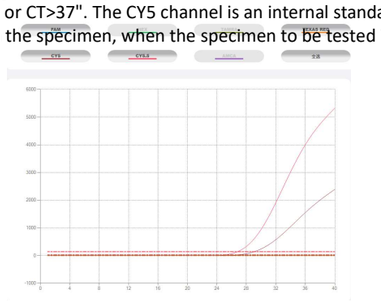
Interpretation of test results
The sample to be tested is judged according to the standards in the table below：

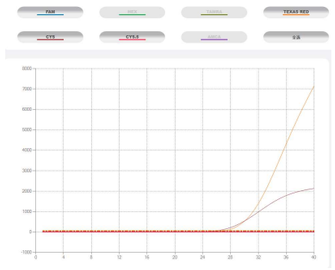
FAM Channel	CY5.5 Channel	TEXAS RED Channel	CY5 Channel	Results
√	×	×	√	Influenza A virus positive (Figure 2)
×	√	×	√	Influenza B virus positive (Figure 3)
×	×	√	√	Respiratory syncytial virus positive (Figure 4)
√	√	×	√	Influenza A virus and influenza B virus positive
√	×	√	√	Influenza A virus and respiratory syncytial virus positive
×	√	√	√	Influenza B virus and respiratory syncytial virus positive

√	√	√	√	1. Positive control 2. Influenza A virus, influenza B virus and respiratory syncytial virus positive
×	×	×	√	Negative (Figure 5)
×	×	×	×	Invalid

Note：“√”It means the result "has obvious logarithmic amplification curve, and Ct≤37"; "×" means the result "no logarithmic amplification curve or CT>37". The CY5 channel is an internal standard [illegible] with the specimen, when the specimen to be tested is







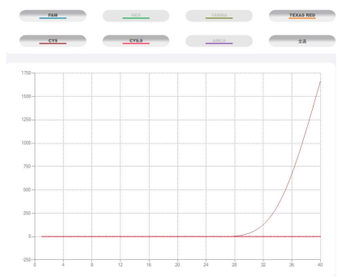


Figure 4. Respiratory syncytial virus positive

Figure 5. Negative

Limitations of Test Method

1. The test results of this reagent should be comprehensively analyzed in combination with the patient's clinical symptoms and other relevant medical examination results, and should not be used alone as the basis for patient management.
2. If the positive result is influenza A virus, it is recommended to further experiments to confirm the subtype of influenza A virus, and consult the local public health prevention agency for consultation and treatment.
3. Unreasonable specimen collection, transportation and handling and low virus titers and improper experimental operation and experimental environment may lead to false negative results.
4. The test target sequence variation of influenza virus, respiratory syncytial virus or the sequence changes caused by other reasons may lead to false negative results.
5. The optimal specimen type for detection and the optimal specimen time after infection may not be confirmed, so multiple specimens from the same patient will reduce the possibility of false positive results for a sudden new influenza virus.
6. During specimen collection, patients who received live attenuated vaccines may result in a positive test result.
7. Inconsistent positive rates of specimen at different stages of the disease course.
8. The nucleic acid sequence to be tested may appear in the body for a long time, regardless of viral activity. A positive nucleic acid test does not necessarily mean that you are currently infected with the corresponding virus or that it is the causative agent of clinical symptoms.
9. Positive and negative predictive values depend largely on prevalence. The test performance is established during the epidemic season (eg 2018/2019). Test performance for some viruses may vary with test prevalence and test population.

Product Performance Index

1. Sensitivity

The test results of 10 positive reference products in the "Second Generation Influenza A/B Nucleic Acid Detection Reagents National Reference Products" were all positive, with a coincidence rate of 100%. The test results of 9 negative reference products were all negative, and 1 negative reference product (NC09 respiratory syncytial virus type B) was positive, with a coincidence rate of 100%. The test results of 12 positive reference products in the company's reference products were positive, and 9 negative reference products were negative, with a coincidence rate of 100%.

Minimum detection limit: Influenza A virus 1×10^3copies/mL, Influenza B virus 1×10^3copies/mL, Respiratory syncytial virus 5×10^2copies/mL

2. Specificity

The verified pathogenic microorganisms that are the same as the infection site or have similar infection symptoms are negative and have no cross-reaction, and the verified concentrations are as follows:

Microorganism	Concentration	Microorganism	Concentration
Measles Virus	3.2×10^8 $TCID_{50}$ /L	Streptococcus Pneumoniae	1.62×10^6 CFU/mL
Escherichia Coli	$12\sim14\times10^{10}$ CFU/mL	Pertussis	2×10^7 CFU/mL
Pseudomonas Aeruginosa	$24\sim40\times10^{10}$ CFU/mL	Chlamydia Pneumoniae	1×10^6 copies/mL
Staphylococcus Aureus	$14\sim22\times10^{10}$ CFU/mL	Haemophilus Influenzae	3.5×10^7 CFU/mL
Mumps Virus	6.3×10^8 $TCID_{50}$ /L	Legionella Pneumophila	2×10^7 CFU/mL
Rubella Virus	3.2×10^8 $TCID_{50}$ /L	Citrobacter	2.6×10^6 CFU/mL
Respiratory Adenovirus Group B	1.57×10^5 copies/mL	Klebsiella Pneumoniae	3×10^7 CFU/mL
Neisseria Gonorrhoeae	1×10^6 copies/mL	Cryptococcus Neoformans	2.1×10^6 CFU/mL
Mycobacterium Tuberculosis	1×10^4 copies/mL	Candida albicans	2.1×10^6 CFU/mL
Mycoplasma Pneumoniae	2×10^6 copies/mL	Enterovirus EV71	3.6×10^6 copies/mL
Respiratory Adenovirus Type 3	1.57×10^5 copies/mL	Cytomegalovirus	1×10^6 copies/mL
Respiratory Adenovirus Type 7	1.57×10^5 copies/mL	Parainfluenza Virus Type 1	1×10^5 copies/mL
Parainfluenza Virus Type 2	1×10^5copies/mL	Parainfluenza Virus Type 3	1×10^5 copies/mL
Neisseria Meningitidis	1×10^6 copies/mL		

3. Interfering Substances

Endogenous substances: blood (5%), mucin (2mg/mL) do not interfere with the detection of the kit.
Exogenous substances: The sample may contain tobramycin (0.05mg/mL), oxymetazoline hydrochloride (99ug), dexamethasone (0.6mg/L), menthol (13.5mg/L)), mupirocin (20mg/L), budesonide (2.5mg/L), oseltamivir (0.15mg/mL), ribavirin (15mg/mL), cetirizine hydrochloride (2.5 mg/mL), mometasone (1g/mL).

4. Precision

Intra-assay precision coefficient of variation (CV%) ≤ 5%.
The test results of 12 positive reference materials were all positive, and the coincidence rate was 100%.

Precaution

1. Children are more likely to spread the virus on a larger scale and last longer, so the sensitivity of detection for children may be higher than for adults.
2. f the iCassette is oscillated after adding the sample, do not use the iCassette.
3. Each icassette is a single-use test, please do not reuse the tested icassette.
4. The tips used in the experiment should have reasonable cleaning and quality inspection procedures to avoid false negative results caused by RNase contamination or amplification reaction inhibitors.
5. The preparation of specimen should be carried out in a biological safety cabinet, wearing work clothes, wearing disposable gloves, and using a self-unloading pipette during the experiment.
6. The pipette tip that the specimen is ready to be used should be inserted into the container containing the disinfectant. After preparing the experiment, it can be discarded after being sterilized with the icassette.
7. After the experiment, the workbench and pipette should be treated with 10% hypochlorous acid or 75% alcohol and then irradiated with an ultraviolet lamp for 30 minutes.
8. Clinical laboratories should strictly follow the management standards in the local related regulations for molecular biology laboratories and clinical gene amplification laboratories.
9. The performance of this product is only verified for the claimed applicable specimen types and the specimen collection and processing methods (including specimen collection fluid, etc.) described under [Specimen Requirements], other specimen types or specimen collection and processing methods cannot guarantee product performance.

References

1. 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/0
Date of Issue: June ,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
Σ	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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