Influenza A Virus/Influenza B Virus Nucleic Acid Detection Kit

(Fluorescence RT-PCR)

Instructions for Use

For professional use only.

For in vitro diagnostic use only.

REF **BSJ04S1**

IVD CE

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Intended Use

Influenza A Virus /Influenza B Virus Nucleic Acid Detection Kit (Fluorescence RT-PCR) is used for the qualitative detection and differentiation of the influenza A and influenza B Virus RNA extracted form nasopharyngeal swabs from suspected cases. The kit is used for the auxiliary diagnosis and epidemiological surveillance of Influenza A Virus and Influenza B Virus infection The kit does not detect the presence of influenza virus of other type.

Negative result does not preclude influenza virus infection and should not be used as the sole basis for the diagnosis, treatment or other patient management decisions.

For professional in vitro diagnostic use only.

Principle

Influenza A Virus /Influenza B Virus Nucleic Acid Detection Kit (Fluorescence RT-PCR) amplifies and detects viral RNA in swab specimens obtained from suspected patients.

The kit selects the M gene (FAM) region of Influenza A Virus and N gene (HEX) region of Influenza B Virus [1-3], and designs two sets of primers and fluorescent probes that cover two sites of the genes. The two sets of primers and probes can specifically bind to the target sequences. When the RT-PCR amplification reaction is performed, the fluorescent signal(s) can be detected by a full-automatic fluorescent PCR detector to realize real-time online monitoring of the RT-PCR reaction. In order to control the entire extraction and detection process, human Ribonuclease gene (CY5) was act as a non-competitive internal control during the extraction and detection process.

Components

Components		**BSJ04S1**	**Main Ingredients**
Kit size		**32 tests/kit**	
Amplification reagent	2×RT-PCR Buffer	400 μL×1	dNTP, Mg^{2+}, Tris
	Enzyme Mix	38.4 μL×1	DNA polymerase, RT enzyme
	Primer/Probe of FluA & FluB	201.6 μL×1	Specific Primers and Probes
Control	Positive control	500 μL×1	Pseudovirus mixture containing gene of influenza A/B viruses
	Negative control	500 μL×1	Solution containing internal reference gene plasmid

a. The positive control and negative control need to be set to monitor the test body and the operating environment; the negative and positive control have been packaged in the kit.

b. The components of different lots cannot be mixed for use.

c. Equipment or materials required but not provided: Specimen collection kits, Nucleic acid extraction kits; PCR tubes and caps, etc.

Applied instrument

The kit can be applied to Bioer's Line-Gene series fluorescent quantitative PCR detection system and other manufacturers' similar fluorescent quantitative PCR detection systems. The instrument should contain three channels of FAM, HEX (VIC/JOE), and CY5.

Warnings and Precautions

- For *in vitro diagnostic* use (IVD). For professional use only.
- Read the Instructions for Use carefully before operation. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory

management.

- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC). Handling samples in the biosafety cabinet, to ensure operator safety and avoid environmental pollution. Place harmful samples and reagents properly. Discard the waste in special containers. Wipe the table, centrifuge, and equipment frequently with 1.0% sodium hypochlorite or 70 % ethanol. The laboratory and the ultra-clean workbench need UV-treated periodically and after each experiment.
- All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment
- Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents, while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product.
- Separate laboratory areas are recommended to performing predefined procedures of the assay.
  Area I: Reagent preparation area-reagent required for preparing amplification.
  Area II: Sample processing area-processing of tested samples and controls.
  Area III: PCR detection region-PCR amplification detection.
- The separation of the reaction solution should avoid the generation of air bubbles as far as possible. Before the amplification, pay attention to check whether the caps of each reaction tube are tightened to avoid contaminating instrument.
- Samples should be completely put into the reaction solution when adding samples. No samples should adhere to the tube wall and the cap should be tightened as soon as possible after adding samples.
- The storage of the kit, the specimen and nucleic acid products should follow the requirement(s). Before using, they should be fully thaw out at room temperature, mixed and then instantaneous briefly centrifugation. RNA should be maintained on cold-block or on ice during preparation and use to ensure stability.
- After amplification, please take out the reaction tube immediately, seal it in the s pecial plastic bag, put it in the designated place, and wait for unified treatment.

- Dispose of used /unused kit reagents and human specimens according to local, state, and federal regulations.

Storage and period of validity

1. The kit should be stored at -25℃~-15℃ away from light, and avoid repeated freeze-thaw. The kit can be stored for 3 days at 2~8 ℃ after opening.
2. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not uses after the stated expiry date.
3. The kit can be transported in foam box sealed with ice bags or dry ice at 2~8℃ or lower.

Specimen Collection, Storage, and Transportation

1. Specimens: nasopharyngeal swabs.
2. Collection: Specimens of all types are collected by conventional methods.
3. Storage: It is recommended that specimens be processed as soon as possible after collection. If specimens are not processed immediately they should be stored at 2~8 ℃ for up to 24 hours. If a delayed processing is expected, the specimens should be stored at -70℃ or lower. Specimens should not be frozen and thawed frequently.
4. Transportation: Specimen should be packaged and transported in accordance with the requirements of infectious agents. Specimen should be transported with 0℃ curling bottle or foam box sealed with ice.

Specimen pretreatment (specimen disposal area)

Follow the instructions of the nucleic acid extraction and purification kit.

For Automatic extraction: It is recommended to use nucleic acid extraction or purification kit produced by Hangzhou Bioer Technology Co. Ltd with Gene Pure Series Nucleic acid extractor for Specimen nucleic acid purification.

For Manual extraction: It is recommended to use Biospin Virus DNA/RNA Extraction Kit.

Note: The negative control, positive control and unknown specimen need to be tested in the same experiment.

It's recommended to prepare the reagent ahead of specimen pretreatment to ensure that the reagents are not contaminated.

Using of the kit PCR reaction (PCR test area)

1) Reagent prepares

Thaw out the reagents at room temperature. Mix gently and centrifuge all reagents for a few seconds.

Make RT-PCR reagents according to the quantity of specimens and controls as below (***N means the number of specimens to be tested + negative control substance + positive control substance + estimated loss.***):

Reagents	2×RT-PCR Buffer	Enzyme Mix	Primer/Probe of FluA & FluB
Dosage/test	12.5 μL	1.2 μL	6.3 μL
Dosage	N×12.5 μL	N×1.2 μL	N×6.3 μL

Distribute 20 μL mixed RT-PCR reagents into each PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

Add 5μL negative control, 5 μL extracted product, 5 μL positive control into different PCR tube. Cap the PCR tubes immediately to prevent cross contamination.

DO NOT LABEL ON THE SCANNED AREA OF THE REACTION TUBES!

3) RT-PCR reaction

Place the reaction tubes on a RT-PCR instrument.

It is recommended to choose FAM, HEX and CY5 channels to collect fluorescent signals.

Set fluorescent signals detecting at 60 ℃, liquid volume is 25 μL.

Set reaction procedure as below:

Step	Stage	Temperature	Duration	Number of cycles	Pattern
1	Reverse transcription	50℃	5 min	1	Fast
2	Predenaturation	95℃	1 min	1	
3	Denaturation	95℃	5 sec	45	
	Annealing, extension and fluorescence detection	60℃	10 sec(♦)		

♦ represents fluorescence collection detection.

Quality control standards

Expected performances of controls are as below:

	FAM	HEX	CY5	Interpretation of Test Results
Positive Control	All the three channels should yield Ct values, Ct≤30, and the amplification curve with "S" curve			All requirements are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct Value	No Ct Value	Ct Value≤30, and the amplification curve with "S" type	

Result Analysis and Judgments

Cut off Value According to the results of clinical experiments, draw the Receiver Operating Curve（ROC）to determine the Cut off value of influenza A virus in this kit: 41.16; Cut off value of influenza B virus in this kit: 40.69; Cut off value of Reference gene in this kit: 40.12.

	FAM (Influenza A virus)	HEX (Influenza B virus)	CY5 (Reference)	Result
1	Ct≤ 41.16，with "S" curve	NoCt	With or without Ct	Influenza A virus nucleic acid Positive
2	NoCt	Ct≤ 40.69，with "S" curve	With or without Ct	Influenza B virus nucleic acid Positive
3	Ct≤ 41.16，with "S" curve	Ct≤ 40.69，with "S" curve	With or without Ct	Influenza A virus and Influenza B virus nucleic acid Positive
4	NoCt	NoCt	Ct≤ 40.12	Influenza A virus and Influenza B virus nucleic acid Negative
5	NoCt	NoCt	Ct≥ 40.12 or NoCt	Abnormal. It is recommended to re-collect, extract and test

NOTE:

1. If FAM or HEX has a Ct value and the amplification curve with “S” curve, but it is greater than the Cut off value, it’s recommended to take another specimen from the patient to perform are-tested. If the re-test result still has a Ct value, and the amplification curve with “S” curve, then it can be judged as the corresponding Viral nucleic acid positive, otherwise it should be judged as corresponding viral nucleic acid negative.
2. When the specimen test result is suspicious, it needs to be re-extracted and re-tested. If the re-test results are still within this range, it is positive. Otherwise, it is negative.

Limitations

1. The kit is only used for the qualitative detection the presence of Influenza A Virus and Influenza B Virus in specimens. Neither the quantitative value nor the rate of increase can be determined by the qualitative test.
2. The results of the test are just for clinical reference. The test should not be used as sole criteria for diagnosis. Results should be considered in conjunction with the clinical information and other data available to the physician. A positive result may be raised by (residual) nucleic acid of influenza virus, which loses the activity on viral infectivity.
3. An incorrect result may occur by incorrect operation in sample collection, transportation or processing.
4. A false negative result may occur by very low concentration of target virus in the specimens, mutations within the viral genome covered by the kit’s primers and/or probe, and unproved external interference factors, such as PCR inhibitor.
5. A false positive result may occur by aerosol pollution or operating errors.
6. A false positive result may occur if the patient has received a live attenuated influenza virus vaccine recently.
7. For the positive result or any suspected cases, it’s recommend to re-extract and/or retest with a new lot of kit or confirmed with another available method.

Performance Indicators

Performance validation was conducted with the Line-Gene series fluorescent quantitative PCR detection system from Bioer. The kit can be applied to Bioer’s Line-Gene series fluorescent quantitative PCR detection system and other manufacturers' similar fluorescent quantitative PCR detection systems. The Specimens includes influenza A viruses (H1N1, H1N1(2009), H3N2, H5N1, H7N9) and Influenza B Virus(Victoria and Yamagata). The Specimens was purchased from a research institute in China.

★ Limit of Detection (LoD): The positive specimen was diluted into 500 copies/mL, 200 copies/mL, 100 copies/mL and 50 copies/mL, then were tested by 3 lots of kits. Each control was tested with 20 replicates. The testing data demonstrated that the kit can detect Influenza A Virus and Influenza B with detection rate equal or higher than 95% at the concentration equal or higher than 200 copies/mL.

★ Analytical sensitivity: 7 positive references, 5 LoD positive references and 10 negative references were tested by 3 lots of kits. The positive coincidence rate was 100%, and the negative coincidence rate was 100 %.

★ Analytical specificity: No cross reactivity has been observed by testing the clinical positive specimens such as Meningococcus, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, rubella virus, mumps virus, respiratory adenovirus (type 1, 2, 3, 5, 7, 11), respiratory syncytial virus (A, B), parainfluenza virus (1, 2, 3), coronavirus (OC43, HKU1, 229E, NL63), SARS-CoV-2, Bacillus pertussis, human rhinovirus (A, B, C), enterovirus (A, B, C, D), human metapneumovirus, cytomegalovirus, measles virus, Moraxella catarrhalis, Boca virus, Coxsackie virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, suppurative Streptococcus, Oral Streptococcus, Pseudomonas aeruginosa.

★ Analytical specificity: The potentially interfering substances were incorporated into 7 different types of influenza virus clinical positive specimens, then tests were performed by 3 lots of kits. The tested substances blood, mucin and nasal secretions showed no influence on the detection. Common medications for colds or other similar symptoms do not interfere with testing.

★ Precision: Precision references (CV1-CV4) and negative reference were tested by 3 lots of kits with 10 replicates by 2 operators for 20 days. The results showed that the variation coefficient (CV) of within-lot, between-lots, between-operators and between-days were less than 5%.

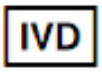
References

[1] Blair RH, Dawson ED, Taylor AW, et al. Clinical validation of the FluChip-8G Influenza A+B Assay for influenza type and subtype identification[J]. Journal of Clinical Virology, 2019, 118:20-27.

[2] Kakar A, Gogia A, Gangwani A. Risk factors associated with severe outcomes in adult hospitalized patients according to influenza type and subtype[J]. Current Medicine Research and Practice, 2019, 9(4):162-163.

[3] Tong SX, Zhu XY, Li Y, et al. New World Bats Harbor Diverse Influenza A Viruses[J]. Plos Pathogens, 2013, 9(10):e1003657.

Symbol Description

	CE MARK		CATALOGUE NUMBER
	IN VITRO DIAGNOSTIC MEDICAL DEVICE		BATCH CODE
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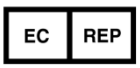	AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY		DO NOT REUSE
	NEGATIVE CONTROL		POSITIVE CONTROL

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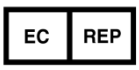

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