SARS-CoV-2 /Influenza A Virus /Influenza B Virus Nucleic Acid Detection Kit (Fluorescence RT-PCR)

Instructions for Use

Effective Date: Feb 18, 2022

For professional use only.

For in vitro diagnostic use only.

IVD -25℃ -15℃ CE

REF BSJ17S1 / BSJ17M1

INTENDED USE

SARS-CoV-2 /Influenza A Virus /Influenza B Virus Nucleic Acid Detection Kit (Fluorescence RT-PCR) is used for the qualitative detection of the SARS-CoV-2, Influenza A Virus and Influenza B Virus nucleic acid in specimens of nasopharyngeal swabs, oropharyngeal swabs and sputum specimens from suspected cases. The kit is used for the auxiliary diagnosis and epidemiological surveillance of SARS-CoV-2, Influenza A Virus and Influenza B Virus infection, cannot be used as the basis for the diagnosis or exclusion of cases alone.

For professional use only.

For in vitro diagnostic use only.

PRINCIPLE

This product selects the ORF1ab (FAM) and N gene (HEX) regions of SARS-CoV-2[1-3], the specific regions of Influenza A Virus (ROX) and Influenza B Virus (CY5) [4], and designs four sets of primers and fluorescent probes that cover four sites of the genes. The four 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.

COMPONENTS

Components		Main Ingredients	BSJ17S1	BSJ17M1
			24 tests/kit	48 tests/kit
Amplification reagent	2×RT-PCR Buffer	dNTP, Mg^{2+}	300μL×1	600μL×1
	Enzyme Mix	DNA polymerase, RT enzyme	31.2μL×1	62.4μL×1
	Primer/Probe Mix	Specific Primers and Probes	148.8μL×1	297.6μL×1
Control	Positive	Plasmid with specific	1000μL×1	1000μL×1

	Control	genes		
	Negative Control	Purified water	1000μL×1	1000μL×1

a. *The positive control and negative control need to be set to monitor the test body and the operating environment; the negative control 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 Fluorescence Quantitative Detection System, LineGene 9600 Plus (FQD-96A). The instrument should contain at least four channels of FAM, HEX , ROX and CY5.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use (IVD).
- 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.
- Both the kit and nucleic acid products are all stored at -20 °C. 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 special 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°C ~ -15°C away from light, and avoid repeated freeze-thaw. The kit can be stored for 3 days at 2-8 °C after opening.
2. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not use after the stated expiry date.
3. The kit can be transported in foam box sealed with ice bags or dry ice at 2-8°C or lower.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION

1. Specimens: nasopharyngeal swabs, oropharyngeal swabs or sputum specimens

from suspected cases.

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 °C for up to 24 hours. If a delayed processing is expected, the specimens should be stored at -70°C or lower. Specimens should not be frozen and thawed frequently.
4. Transportation: Specimen should be transported with 0°C 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 MagaBio plus Virus DNA/RNA Purification Kit II (Cat: BSC71) or MagaBio plus Virus DNA/RNA Purification Kit III (Cat: BSC86) to purify the nucleic acid with Gene Pure Series Nucleic acid extractor.

For Manual extraction: It is recommended to use Biospin Virus DNA/RNA Extraction Kit (Cat: BSC77).

⚠ **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 and controls):

Reagents	2×RT-PCR Buffer	Enzyme Mix	Primer/Probe Mix
Dosage/ test	12.5μL	1.3μL	6.2μL
Dosage	(N+1) ×12.5μL	(N+1) ×1.3μL	(N+1) ×6.2μL

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

⚠ **Note: Do not label on the scanned area of the reaction tubes!**

3) RT-PCR reaction

Place the reaction tubes on a PCR instrument.

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

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

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	50°C	10 min	1
2	95°C	1 min	1
3	95°C	10 sec	45
	60°C	30 sec	

QUALITY CONTROL STANDARDS

Expected performances of controls are as below:

Control	FAM	HEX	ROX	CY5	Interpretation of Test Results
Positive Control	Ct Value≤35, and the amplification curve with "S" type				All requirements are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct Value				

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

Fluorescent signals	Ct≤40, with "S" curve	40＜Ct＜45, with "S" curve	No Ct	Result
FAM and HEX	√			SARS-CoV-2 nucleic acid Positive
		√		SARS-CoV-2 nucleic acid Suspicious
			√	SARS-CoV-2 nucleic acid Negative
ROX	√			Influenza A Virus nucleic acid Positive
		√		Influenza A Virus nucleic acid Suspicious
			√	Influenza A Virus nucleic acid Negative

CY5	√			Influenza B Virus nucleic acid Positive
		√		Influenza B Virus nucleic acid Suspicious
			√	Influenza B Virus nucleic acid Negative

NOTE:

1. Among the two fluorescence signals of FAM and HEX, any one has a Ct value ≤ 40 with "S" type amplification curve, the other has no Ct value or Ct ＞40 , it needs to take different specimen of the same person to be tested again. If the re-test results are still within this range, and it is positive.
2. When the specimen test result is suspicious, it needs to be re-extracted and tested again, and the re-test results are still within this range, and it is positive. Otherwise, it is negative.
3. Two or more of SARS-CoV-2, Influenza A Virus, Influenza B Virus test results are positive, indicating that it may be infected by multiple pathogens at the same time.

LIMITATIONS

1. The kit is only used for the qualitative detection the presence of SARS-CoV-2, 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.
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. For the positive result or any suspected cases, it's recommended 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 Bioer's LineGene 9600 Plus Fluorescence Quantitative (FQD-96A). Since positive specimen of SARS-CoV-2 was unavailable, positive control was prepared for the validation. The positive control was purchased from a commercial company, which contains the target

fragments of the ORF1ab and N genes of SARS-CoV-2, M gene of Influenza A Virus and NP gene of Influenza B Virus.

★ Limit of Detection (LoD): The positive reference standard was diluted into 1000 copies/mL, 500 copies/mL and 250 copies/mL, then were tested by 3 lots of kits. Each concentration was tested with 20 replicates. The testing data demonstrated that the kit can detect SARS-CoV-2, Influenza A Virus and Influenza B with detection rate equal or higher than 95% at the concentration equal or higher than 500 copies/mL.

★ Analytical sensitivity: 5 positive reference standards and 5 negative reference standards 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 Coronavirus OC43, Coronavirus HKU1, Coronavirus 229E, Coronavirus NL63, Respiratory syncytial virus A/B, Human rhinovirus and Adenovirus. No cross reactivity has been observed by testing the British QCMD sample of the Middle East respiratory syndrome coronavirus.

★ Analytical specificity: The potentially interfering substances were spiked into positive control, then tests were performed with 3 lots of kits. The tested substances Blood (10%), Mucins (0.2mg/mL) and Nasal secretions (15%) showed no influence on the detection.

★ Precision: Positive controls, weak positive controls and negative controls were tested by 3 lots of kits with 10 replicates by 2 operators for 7 days. The results showed that the variation coefficient (CV) of within-lot, between-lots, between-operators and between-days were less than 5%.

REFERENCES

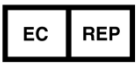
[1] Shijie Q, Xinyi X, Xuejia S, et al. Mechanistic insights into SARS-CoV-2 epidemic via revealing the features of SARS-CoV-2 coding proteins and host responses upon its infection[J]. Bioinformatics, 2020, 36(21).

[2] Mary L, Guerrino M, Niamh M, et al. Whole-genome sequencing to track SARS-CoV-2 transmission in nosocomial outbreaks[J]. Clinical Infectious Diseases, 2020.

[3] Divinlal H, Sathishkumar R, Tom L, et al. SARS-CoV-2 Whole Genome Amplification and Sequencing for Effective Population-Based Surveillance and Control of Viral Transmission[J]. Clinical Chemistry, 2020.

[4] Cui D , Zhao D , Xie G , et al. Simultaneous detection of influenza A subtypes of H3N2 virus, pandemic (H1N1) 2009 virus and reassortant avian H7N9 virus in humans by multiplex one-step real-time RT-PCR assay[J]. Springerplus, 2016, 5(1):2054.

SYMBOL DESCRIPTION

	Manufacturer		Catalogue number
	CE mark		Authorized representative in the European community
	Batch code		Consult instructions for use
	In vitro diagnostic medical device		Temperature limitation
	Caution		Use by date
	Positive Control		Negative Control

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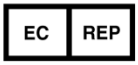

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