Bordetella Pertussis Nucleic Acid Detection Kit (Fluorescent PCR)

Instructions for Use

Effective Date: Jan 10, 2022

For professional use only.

For in vitro diagnostic use only.

IVD -25℃ -15℃ CE

REF BSJ10S1 / BSJ10M1

INTENDED USE

Bordetella Pertussis Nucleic Acid Detection Kit (Fluorescent PCR) is used for the qualitative detection of *Bordetella Pertussis* nucleic acid in pharyngeal swabs from cases of people under 18 years of age. The kit is used for the auxiliary diagnosis and epidemiological surveillance of *Bordetella Pertussis* infection, cannot be used as the basis for the diagnosis or exclusion of cases alone.

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PRINCIPLE

This product selects the IS 481 region (FAM) of *Bordetella Pertussis*[1-3], and designs one set of primers and fluorescent probe. The one set of primers and probe can specifically bind to the target sequences. When the PCR amplification reaction is performed, the fluorescent signal can be detected by a full-automatic fluorescent PCR detector to realize real-time online monitoring of the PCR reaction. In order to control the entire extraction and detection process, human gene was act as a non-competitive internal control during the extraction and detection process.

COMPONENTS

Components		Main Ingredients	BSJ10S1	BSJ10M1
			24 tests/kit	48 tests/kit
Amplification reagent	BP Buffer A	dNTP, Mg2+, hot-start DNA polymerase	312μL×1	624μL×1
	BP Buffer B	Specific primers and probes	48μL×1	96μL×1
Control	Positive Control	Plasmid with specific genes and internal reference genes	1000μL×1	1000μL×1

	Negative Control	Plasmid with Internal reference gene	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 Hangzhou Bioer Technology Co.,Ltd. Fluorescence Quantitative Detection System, LineGene 9600 Plus (FQD-96A) or QuantGene 9600 (FQD-96C). The instrument should contain at least two channels of FAM 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.
- 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 5 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: Pharyngeal swab
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 III (Cat: BSC86) to purify the nucleic acid with Gene Pure Series Nucleic acid extractor.

⚠ **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	BP Buffer A	BP Buffer B
Dosage/ test	13μL	2μL
Dosage	(N+1) ×13μL	(N+1) ×2μL

Distribute 15 μL mixed PCR reagents into each PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

Add 10μL negative control, 10μL extracted product, 10μL 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) PCR reaction

Place the reaction tubes on a PCR instrument.

It is recommended to choose FAM 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	37°C	2 min	1
2	95°C	1 min	1
3	95°C	15 sec	45
	60°C	30 sec	

QUALITY CONTROL STANDARDS

Expected performances of controls are as below:

Control	FAM (BP)	CY5 (IC)	Interpretation of Test Results
Positive Control	All the three channels yield Ct Value≤35 with "S" amplification curve		All requirements are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct Value	Ct Value≤35 with "S" amplification curve	

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

FAM (NG)	CY5 (IC)	Result Judgment
Ct Value ≤39, with "S" curve	No specific requirement	*Bordetella Pertussis* nucleic acid Positive.
Ct Value >39, or no Ct Value	Ct Value ≤40, with "S" curve	*Bordetella Pertussis* nucleic acid Negative.
Ct Value >39, or no Ct Value	Ct Value >40; with "S" curve; or no Ct Value	Re-sample & re-test.

NOTE:

If the Ct value of the FAM channel is greater than the Cut off value with an "S" type amplification curve or no Ct value, while the Ct value of the CY5 channel is greater than 40 with an "S" type amplification curve or no Ct value, it's recommended to re-sample from the same patient and re-test.

LIMITATIONS

1. The kit is only used for the qualitative detection the presence of *Bordetella Pertussis* nucleic acid 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. Negative result does not preclude *Bordetella pertussis* or *Bordetella parapertussis*

infection and should not be used as the sole basis for the diagnosis, treatment or other patient management decisions. The result should not use for monitoring treatment of *Bordetella pertussis.*

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 infecting organism in the specimens, mutations within the infecting organism genome covered by the kit's primers and/or probe, and unproved external interference factors, such as PCR inhibitor.
5. False-negative results may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.
6. A false positive result may occur by aerosol pollution or operating errors.
7. 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.
8. The performance of this test has not been established for patients without symptoms of *Bordetella pertussis* infection

PERFORMANCE INDICATORS

Performance validation was conducted with Bioer's Fluorescence Quantitative Detection System, LineGene 9600 Plus (FQD-96A) or QuantGene 9600 (FQD-96C). Since clinical positive specimen 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 IS 481 gene of *Bordetella Pertussis*.

★ Limit of Detection (LoD): The positive reference standard was diluted into 5 CFU/mL, 2.5CFU/mL, 1.25 CFU /mL and 1 CFU /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 *Bordetella Pertussis* with detection rate equal or higher than 95% at the concentration equal or higher than 2.5CFU/mL.

★ Analytical sensitivity: 5 positive reference standards and 12 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 Influenza A H1N1 virus, Respiratory syncytial virus A/B, Parainfluenza virus, Adenovirus, Enterovirus, Epstein-barr virus, Human cytomegalovirus, Staphylococcus aureus, Klebsiella pneumoniae, Monilia albicans, Cryptococcus neoformans, Aspergillus fumigatus, Candida glabrata, Mycoplasma pneumonia, Legionella pneumophila, Neisseria meningitidis, Pseudomonas aeruginosa, Haemophilus influenzae,

Streptococcus pneumoniae, Bordetella Parapertussis, Bordetella bronchiseptica, Influenza A virus, Influenza B virus, Adenoviridae 7, Staphylococcus aureus, TE buffer and Human genomic DNA.

★ Analytical specificity: The potentially interfering substances were spiked into positive control, then tests were performed by 1 lots of kits. The tested substances Blood (10%), Mucins (0.2mg/mL) ,Nasal secretions (15%), Oxymetazoline (0.5mg/L), Sodium chloride (0.09%), Dexamethasone (0.1mg/L), Triamcinolone acetonide (105ng/mL), Budesonide (3nmol/L), Mometasone (0.03%), Fluticasone (0.5ng/mL), Ribavirin (3680ng/mL), Oseltamivir (1275μg/L), Levofloxacin (5μg/mL), Azithromycin (0.4mg/L), Tobramycin (3.7μg/mL), Phenylephrine (0.5mg/mL), Beclomethasone (0.2mg/L), Flunisolide (1mg/mL), Histamine hydrochloride (1mg/mL), Zanamivir (142ng/mL), Peramivir (100μg/mL), Lopinavir (25μg/mL), Ritonavir (25μg/mL), Arbidol (614.1ng/mL), Ceftriaxone (80μg/mL), Meropenem (100μg/mL) showed no influence on the detection.

★ Precision: Positive controls and low positive controls 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 positive reference standards of within-day, between-day, within-batch and between-batch were less than 5%, the variation coefficient (CV) of weak positive reference standards of within-day, between-day, within-batch and between-batch were less than 10%.

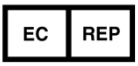
REFERENCES

[1] Van der Zee A,Schellekens JF,Mooi FR. Laboratory Diagnosis of Pertussis [J].Clin Microbiol Rev,2015,28(4):1005-1026.

[2] Wang K,Bettiol S,Thompson MJ, et al.Symptomatic treatment of the coughin whooping cough[J].Cochrane Database Syst Rev,2014,(9):CD003257.

[3] Caro V,Elomaa A,Brun D,Mertsola J,He Q,Guiso N.Bordetella pertussis,Finland and France.Emerg Infect Dis.2006,12(6):987-9.

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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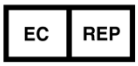

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