Streptococcus Pneumoniae/ Klebsiella Pneumoniae/ Legionella Pneumophila/ Haemophilus influenzae/ Bordetella Pertussis Diagnostic Kit (PCR-Fluorescence)

REF 106-0056-01

Product Name
Streptococcus Pneumoniae/ Klebsiella Pneumoniae/ Legionella Pneumophila/ Haemophilus influenzae/ Bordetella Pertussis Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
Streptococcus pneumonia (SP), *Klebsiella pneumonia* (KP), *Legionella pneumophila* (LP), *Haemophilus influenzae* (HI) and *Bordetella pertussis* (BP) are the main pathogens of respiratory tract infections, often causing epidemic community acquired pneumonia in infants and children. The kit uses real-time multiple fluorescent PCR technology, performed on the Automated Fully Enclosed qPCR Instrumentfor the in vitro qualitative detection of *Streptococcus pneumoniae, Klebsiella pneumoniae, Legionella pneumoniae, Haemophilus influenzae* and *Bordetella pertussis* in human sputum samples, the test results can be used to assist in the diagnosis of respiratory infections.

Test Principle
The Automated Fully Enclosed qPCR Instrument, uses Intelligent cassette (iCassette) technology to automatically perform nucleic acid extraction, amplification, data reading and result analysis. The instrument includes two parts: nucleic acid extraction and multiple fluorescent PCR. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this kit. The uniquely designed software is used to execute the extraction and PCR process of the kit, display the results and analyze the results. The kit contains 12 disposable SP/KP/LP/HI/BP iCassettes. The reagents prefilled in the iCassette include nucleic acid extraction reagents and PCR reaction reagents, which can be performed on the Automated Fully Enclosed qPCR Instrument, Since the Automated Fully Enclosed qPCR Instrument is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, it reduces direct cross-contamination of samples. For a complete description of the instrument, please refer to the user manual of the corresponding instrument.

The kit includes nucleic acid extraction reagents and PCR reaction reagents. The nucleic acid extraction reagents contain internal control which is used to monitor the whole process of nucleic acid extraction, confirm whether the sample is adequately processed, and monitor whether there are PCR inhibitors to avoid PCR false negatives. At the same time, before the start of the PCR reaction, the Automated Fully Enclosed qPCR Instrument, will first test the fluorescent signal of the probe to monitor whether the PCR reagents are fully reconstituted, whether the samples are added to the PCR reaction and the integrity and stability of the probe.

The kit uses the highly conserved regions encoded by each bacterial genome to design specific primers and fluorescent probes (FAM-labeled *Streptococcus pneumoniae*, HEX-labeled *Klebsiella pneumoniae*, TEXAS RED-labeled *Legionella pneumophila,* CY5 labeled *Bordetella pertussis*, CY5.5 labeled *Haemophilus influenz*a, and TAMRA labeled internal control). The kit can simultaneously detect 5 kinds of bacteria in one PCR reaction tube.

Components

Component Name			Main ingredients	Quantity
SP/KP/LP/HI/BP iCassette (12 pcs)	Nucleic acid extraction reagent	Sample lysate solution	Guanidine isothiocyanate	800μL/iCassette
		Sample binding solution	Guanidine isothiocyanate	200μL/iCassette
		Sample washing solution	Sodium chloride	900μL/iCassette
		Sample elution	Tris-HCl	90 μL/iCassette
		Magnetic beads	Magnetic microspheres	11 μL/iCassette
	SP/KP/LP/HI/BP PCR reagent	SP/KP/LP/HI/BP PCR cosolvent solution	PCR Buffer, $MgCl_2$	35 μL/iCassette
		SP/KP/LP/HI/BP lyophillization E	Specific primer probe, dNTP, enzyme	1 pc/iCassette
	Pretreatment solution		NaOH	2 x 28mL/bottle
	Enhancer		/	1 tube (1mL)
Quality Control	SP/KP/LP/HI/BP positive control		Including the target fragments of SP/KP/LP/HI/BP	1 tube (250μL)
	SP/KP/LP/HI/BP negative control		Sterilizing purified water	1 tube (250μL)

Note: Components in different lot numbers are not interchangeable.

Storage condition and Shelf life
The kit must be stored at 2-8℃and the shelf life of the kit is 9 months.
The transportation temperature range of the kit should be kept at 2-8℃.
Please do not open the iCassette lid before adding the sample. If you open the iCassette lid, 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.
- Ultrosonic machine: Galaxy USL.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Timer.
- Pipettes.
- Sterile pipette tips.
- Saline.

Sample Requirements
1. Sample type: Sputum or Nasopharyngeal swab
2. Sample collection
2.1 Sputum collection: Collecting 1-3ml of sputum coughed up from the deep lungs of the patients and immediately sealed it for examination.
2.2 Nasopharyngeal swab：Using a nasopharyngeal swab to remove the nasopharyngeal secretions, place them in a sterile tube, and immediately sealed for test.
3. Storage and transportation of samples
3.1 Sample storage: The samples should be stored at 2-8℃ and used for testing within 12 hours or placed at -20±5℃ as soon as possible, but it should not be used exceed 1 month.
3.2 Sample transportation: The samples should be transported under cooling at 0℃.
4. Principles of Biosafety Protection
All the operations should be in accordance with the local relevant laws and regulations.

Test Method and Operation
1. Prepare SP/KP/LP/HI/BP iCassette
1.1 Samples processing
A. Process sputum samples:
Add twice the volume of pre-processing liquid, then add the appropriate amount of enhancer in the proportion indicated in Table 1, shake vigorously for 1 minute, and leave it for 15 minutes at room temperature to liquify, shake for 10 seconds every 5 minutes. The sample should be fully liquified and free of tiny lumps of unliquified sputum. However,the pre-processing liquid can be appropriately increased if their are unliquified sputum..

Table 1 Proportion of Added Enhancer

Sputum specimen	Pre-processing liquid	Total volume	Enhancer
1mL	2 mL	3mL	30μL
2mL	4 mL	6mL	60μL

B. Process the nasopharyngeal swab:
Pipette 3 mL sterilized saline to the sterile tube and shake and mix well for use as samples.
C. Ultrasonic Crushing
Open the SP/KP/LP/HI/BP iCassette package and observe whether the lyophilization in the tube are intact. Pipette 1mL of the sample to be tested and slowly add it to the SP/KP/LP/HI/BP iCassette sample compartment as shown in Figure1, close the lid tightly, put it into the ultrasonic machine, and sonicate for 20 seconds. (Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
D. Place the ultrasonicated iCassette into the instrument.

1.2. Process negative and positive controls:
A. Pipette 750 μL of saline and 250 μL of negative control or positive control and slowly add it to the SP/KP/LP/HI/BP iCassette sample compartment as shown in Figure1, close the lid tightly, put it into the ultrasonic machine, and sonicate for 20 seconds.
B. Place the ultrasonicated iCassette into the instrument.

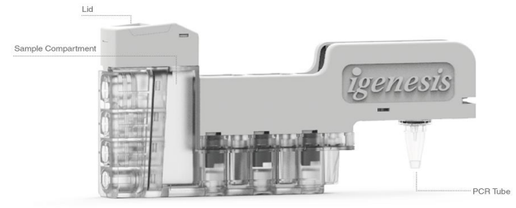


Figure 1 SP/KP/LP/HI/BP iCassette (Lateral View)

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: Within the effective range of Ct ≤ 38 for the TAMRA channel , which is qualified for internal control.
The results of positive control are positive in all channels, TAMRA can be positive or negative.
The results of negative control are negative, only TAMRA can be positive.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.

Result judgment
The assay was positive when there was a significant amplification curve for any of the channels FAM, HEX, TEXAS RED, CY5, and CY5.5 and Ct ≤ 38.

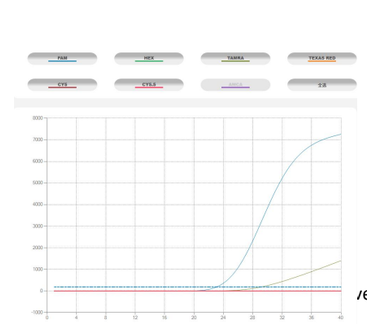
Reference Interval
Through the analysis of clinical sample test results, the ROC curve method is used to finally determine the Ct reference value of this kit is 38.

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

FAM Channel	HEX Channel	TEXAS RED Channel	CY5 Channel	CY5.5 Channel	TAMRA Channel	Result
√	×	×	×	×	√	*Streptococcus pneumoniae* Positive(Figure 2)
×	√	×	×	×	√	*Klebsiella pneumonia* Positive(Figure 3)
×	×	√	×	×	√	*Legionella pneumophila* Positive(Figure 4)
×	×	×	√	×	√	*Bordetella pertussis* Positive(Figure 5)

×	×	×	×	√	√	*Haemophilus influenzae* Positive(Figure 6)
×	×	×	×	×	√	Negative retest (Figure 7)
×	×	×	×	×	×	Invalid
×	×	×	×	×	√	Negative control
√	√	√	√	√	√	Positive control

Note:“√”indicates that the result "has an obvious amplification curve" and Ct≤38;“×”indicates the result "No amplification curve or Ct≥38".



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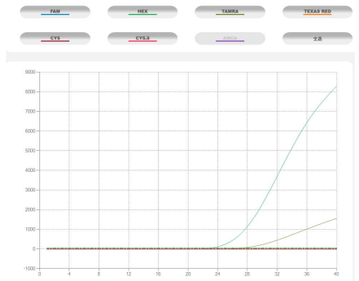

Figure 3. *Klebsiella pneumoniae* positive

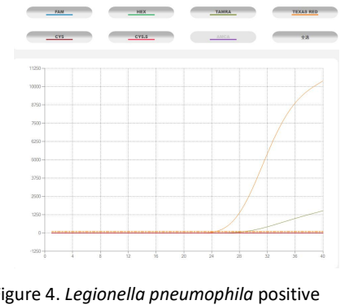

Figure 4. *Legionella pneumophila* positive

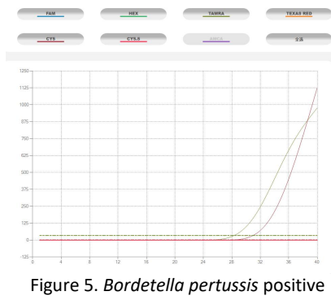

Figure 5. *Bordetella pertussis* positive

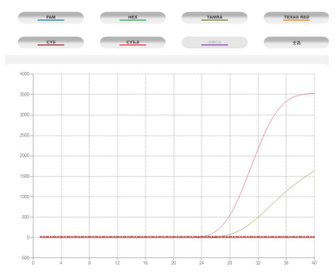

Figure 6. *Haemophilus influenzae* positive

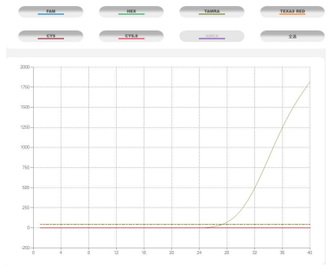

Figure 7. Negative samples

Limitations of Test Methods

1.The test results of this kit are for clinical reference only, and the clinical diagnosis and treatment of patients should be comprehensively considered in conjunction with their symptoms/signs, medical history, other laboratory tests, and treatment responses.

2.Unreasonable sample collection, transportation and processing, as well as improper experimental operation and experimental environment may lead to false negative or false positive results.

Product Performance Index

1.Analysis Sensitivity

Take *Streptococcus pneumoniae* (ATCC 49619), *Klebsiella pneumoniae* (ATCC 49117), *Legionella pneumoniae* (ATCC 33152), *Bordetella pertussis* (ATCC 9340) and *Haemophilus influenzae* (ATCC 49766) single colonies to 0.9% Physiological saline is made into a bacterial suspension, diluted with 0.9% saline, and coated. After 48 hours of incubation, count the number of colonies and calibrate the concentration (see the table below for details).

Reference number	Type	Concentration（CFU /mL）
S1	*Streptococcus*	1×10^1
S2	*Klebsiella*	1.2×10^3
S3	*Legionella*	1.2×10^3
S4	*Bordetella*	1.0×10^3
S5	*Haemophilus*	1.5×10^2

The test results of the 5 sensitivity reference products are all positive and the coincidence rate was 100%.

2.Analysis Accuracy

Take *Streptococcus pneumoniae* (ATCC 49619), *Klebsiella pneumoniae* (ATCC 49117), *Legionella pneumoniae* (ATCC 33152), *Bordetella pertussis* (ATCC 9340) and *Haemophilus influenzae* (ATCC 49766) single colonies to 0.9% Physiological saline is made into a bacterial suspension, diluted with 0.9% saline, and coated. After 48 hours of incubation, count the number of colonies and calibrate the concentration (see the table below for details).

Reference number	Type	Concentration(CFU /mL)
P1	*Streptococcus*	1×10^2
P2	*Klebsiella pneumoniae*	1.2×10^4
P3	*Legionella pneumophila*	1.2×10^4
P4	*Bordetella pertussis*	1.0×10^4
P5	*Haemophilus*	1.5×10^3

The test results of the 5 positive reference products are all positive and the coincidence rate was 100%.

3.Analysis Specificity

The kit has passed the specific test to draw the following conclusions: pathogenic microorganisms that are the same as the infected site or have similar symptoms of infection (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, respiratory adenovirus, influenza A virus, influenza B virus, respiratory syncytial virus, *Candida albicans*), measles virus, mumps virus, rubella virus, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) have no cross reaction.

Precautions

1.If you find that the iCassette is oscillated and leaked after adding the sample, do not continue to use it.

2.Each iCassette is single-used please do not reuse the tested iCassette.。

3.In order to avoid cross-contamination, traditional PCR tests must be carried out in the reagent preparation area, sample preparation area, amplification and product analysis area during the experiment. This iCassette is fully enclosed, and it is sufficient to add samples in the sample preparation area. The subsequent nucleic acid extraction, amplification detection and result analysis processes are all automated with the instrument, avoiding cross-contamination to the greatest extent.

4.The pipette used in the experiment should have reasonable cleaning and quality inspection procedures to avoid contamination or amplification reaction inhibitors causing false negative results.

5.Sample preparation should be carried out in a biological safety cabinet, wear overalls, wear disposable gloves, and use tipping pipettes during the experiment.

6.The used tip of the sample should be put into the container containing the disinfectant. After the preparation experiment is completed, it can be discarded after being sterilized with the iCassette.

7.After the experiment, treat the bench and pipette with 10% hypochlorous acid or 75% alcohol, and then irradiate it with a UV lamp for 30 minutes.

8.This kit uses molecular biology methods and requires experimental operators to have received professional training in gene amplification or molecular biology method detection, have relevant experimental operating qualifications, and the laboratory should have reasonable biosafety facilities and protective procedures.

9.This kit is only used for in vitro diagnosis.

References

1.Corless C E , Guiver M , Borrow R , et al. Simultaneous Detection of Neisseria meningitidis, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in Suspected Cases of Meningitis and Septicemia Using Real-Time PCR[J]. Journal of Clinical Microbiology, 2001, 39(4):1553-1558..

2. Benitez A J , Winchell J M . Clinical Application of a Multiplex Real-Time PCR Assay for Simultaneous Detection of *Legionella Species*, *Legionella pneumophila,* and *Legionella pneumophila* Serogroup 1[J]. Journal of Clinical Microbiology, 2013, 51(1):348-351.

3.Chen Z , Liu M , Cui Y , et al. A novel PCR-based genotyping scheme for clinical\r, *Klebsiella pneumoniae*[J]. Future Microbiology, 2014, 9(1):21-32.

Instruction Version

Version: A/3

Date of Issue: July,2019

Last revised: June, 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
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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