

【Product Name】

Respiratory Pathogens Nucleic Acid Detection Kit (Isothermal Amplification on Disk Chip)

【Packing Specifications】

12 tests/kit

【Intended Use】

Respiratory Pathogens Nucleic Acid Detection Kit (Isothermal Amplification on Disk Chip) is intended for the qualitative clinical detection of 13 common respiratory pathogens including *Streptococcus pneumoniae*, *Staphylococcus aureus*, methicillin-resistant *Staphylococci*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycobacterium tuberculosis complex*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. The detected samples of the kit are nucleic acid extracted from secretions of patients' lower respiratory tract. The target population are suspected patients of lower respiratory tract infections.

The testing results of the kit serve as auxiliary diagnosis of common lower respiratory tract infections, and also are used in the field of epidemiological investigations, etc. At present, the clinical diagnosis of lower respiratory tract infections mainly adopts the method of bacterial culture.

【Principles of the Test】

Respiratory Pathogens Nucleic Acid Detection Kit (Isothermal Amplification on Disk Chip) employs isothermal amplification technology for PCR amplification reaction at isothermal temperature (65℃) and utilizes fluorescent dye incorporation method for real-time fluorescent detection. The testing results of positive samples will produce similar real-time fluorescent S-shape amplification curve. It can complete the amplification and detection of target genes in one step. The method can simultaneously do high-throughput and parallel detection of multiple nucleic acid target genes combining with microfluidic chip technology. See Figure 1: Disk Chip Structure: each chip is equipped with 24 reaction chambers, counter clockwise number, where the sample input & output port No.1 is the reaction chamber No.1. 1 set of primers embedded and fixed in the specific reaction chamber of each chip are used for the amplification and detection of 1 nucleic acid sequence, See Table 1: Detection Index Information in each reaction chamber.

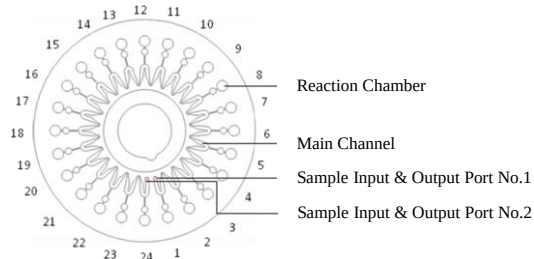


Figure1 Disk Chip Structure

Table1 Detection Index in Each Reaction Chamber

Reaction Chamber	Detection Index	Reaction Chamber	Detection Index
1	Negative control	13	Negative control
2	<i>Streptococcus pneumoniae</i>	14	<i>Stenotrophomonas maltophilia</i>
3	<i>Staphylococcus aureus</i>	15	<i>Haemophilus influenzae</i>
4	Negative control	16	Negative control
5	Methicillin-resistant <i>staphylococci</i>	17	<i>Legionella pneumophila</i>
6	<i>Escherichia coli</i>	18	<i>Mycobacterium tuberculosis complex</i>
7	Negative control	19	Negative control
8	<i>Klebsiella pneumoniae</i>	20	<i>Mycoplasma pneumoniae</i>
9	<i>Pseudomonas aeruginosa</i>	21	<i>Chlamydia pneumoniae</i>
10	Negative control	22	Negative control
11	<i>Acinetobacter baumannii</i>	23	Positive external control
12	Positive internal control	24	Negative control

Note : Please do not directly touch the areas of reaction chamber and sample input & output port of chip!

【Main Components】

Table 2 Contents of the Kit

Name of Contents	Main Components	Specification	Quantity
Chips	Primers	1 test/slice	12slices
Parafilm	/	12 tests/piece	1 piece
Isothermal PCR Amplification Mix	Fluorescent dye,enzyme	270μL/tube	1tube
Positive Control	Genome DNA of <i>Escherichia coli</i>	160μL/tube	1tube

Note: Each component in different LOTs of kits can not be interchanged for use. Negative control is purified water; the users need to prepare this by themselves.

【Storage and Period of Validity】

The kit should be stored at -20℃ in dark. The kit remains stable for at least 8 months from the date of manufacture if handled properly.

Note: the chips have been packed in their own individual packages. Please open the packages at room temperature.

【Applicable Instrument】

The kit is applicable for the instrument: RT-isoAmplifier (Cat.No.: 170010).

【Requirement of Samples】

1. Type of Samples
Nucleic acid extracted from the secretions of lower respiratory tract.
2. Samples Collection
Samples are collected from outpatients or inpatients. Collect the secretions of lower respiratory tract from suspected infected patients, the volume of sample should not be less than 0.6 mL, the samples should be stored in the sample collection tube.
3. Storage of Samples
The extracted nucleic acid should be detected immediately. If not immediately detected, the extracted nucleic acid can be stored at -20℃ for up to 2 months and avoid repeated freezing and thawing.
4. Transportation of Samples
Samples for short-term transportation should use ice storage pot, foambox with ice or dry ice for transportation.

【Assay Procedure】

1. Preparation before Testing

Prepare the corresponding quantity of kits according to the requirements of testing.

- Note : 1. The following procedures of testing are required to operate in a clean bench. Firstly clean the bench with 75% ethanol and then use ultraviolet sterilization for 15 minutes.**
2. Please do not put the chip in the range of UV irradiation during sterilization in order to avoid damaging the structure of primers.

2. Requirements of Equipment and Materials

- Vortex
- Water bath
- Microcentrifuge (e.g. Eppendorf 5424)
- RT-isoAmplifier (Cat.No.: 170010).
- Low Speed Centrifuge (Specification: Centrifugate the isothermal amplification chips)
- Micropipettes for 10 -1000μL
- Sterilized pipette tips (Specification: 20-1000 μL)
- Centrifuge tube (Sterilized, specification: 200 μL, 1.5mL, 15mL, 50mL)
- Various specifications of centrifuge tube racks

3. Nucleic Acid Extraction

3.1 Turn on the water bath and set it to 95℃.

3.2 Centrifugate the DNA Extraction Tube at 10000rpm for 1 minute.

3.3 Add certain volume (usually equal to the volume of the sample) of 10% NaOH solution into the sample. Vortex the mixed product for 2 minutes and then incubate the mixed product at 37℃ for 30-60 minutes. If necessary, extend the liquefaction time or increase the volume of 10% NaOH solution by 0.5-1ml.

3.4 Pipette the liquefied product into a 1.5ml tube and centrifugate it at 12000 rpm for 5 minutes. Remove the supernatant.

3.5 Pipette 1ml Washing Buffer into the tube. Vortex well and centrifugate it at 12000 rpm for 5 minutes. Remove the supernatant.

3.6 Pipette 50μl DNA Extraction Buffer into the tube and pipette the precipitate repeatedly. Pipette the liquid and the precipitate into a DNA Extraction Tube.

3.7 Place the DNA Extraction Tube on the vortex and oscillate it at the maximum speed for 5 minutes. Incubate the oscillated product at 95℃ for 5 minutes.

3.8 Centrifugate the incubated product at 10000 rpm for 1 minute.

4. Loading of Chips

- 4.1 Preparation of isothermal amplification reaction solution

Take isothermal amplification reaction solution from the kit. Let it thaw in room

temperature. Shake gently. Briefly centrifuge to collect contents at bottom.

At the area of storage and preparation of reagents, add 20 μ L isothermal amplification reaction solution into the prepared 200 μ L centrifuge tube. After covering the tube, move it to the sample preparation area. Each sample is limited to use 1 centrifuge tube.

At the area of sample preparation, add 34.5 μ L extracted nucleic acid sample (or negative/positive control) into the microtube and shake gently to let it mix evenly. Briefly centrifuge to the bottom of tube. The total volume of each nucleic acid reaction solution is 54.5 μ L (Table 3)

Table 3 Components of PCR reaction solution

Component	Volume(μ L)
Reaction solution	20
Template DNA	34.5
Total	54.5

4.2 Loading of chips

At the area of sample preparation, take chips from the kit and let them adjust to room temperature. Open the package on the clean bench and put the sample input & output port of the chip upward. Use micropipette to take 50 μ L prepared nucleic acid amplification reaction solution and add it into the main channel of the chip from the sample input & output port No.1, and stop loading when the main channel is fully filled. Take a piece of parafilm and cover the sample input & output ports, Then take 1 clean pipetter tip and press the parafilm in one direction till they are firmly sealed.

- Note :**
- 1. The size of sample input & output ports is small , please make sure that the pipette tip will be well inserted into the sample input port before loading in order to avoiding leakage.**
 - 2. When pressing the cover slip, it should avoid excessive force which could damage the coating and affect the sealing ; Do not cover the reaction chamber when covering the sample input & output ports.**

4.3 Centrifugation of chips

Fix the loaded chip on the low speed centrifuge and centrifuge it with a speed of 6000rpm for 30 seconds, and then take it out.

- Note :** Please make sure that the chip is well fixed during centrifugation , it is recommended that the side of the sample input & output is downward.

5. Nucleic Acid Amplification and Interpretation of Results

5.1 Nucleic Acid Amplification

5.1.1 Turn on the RT-isoAmplifier and corresponding software, click “Open Lamp” button and the RT-isoAmplifier will preheat automatically, preheat needs 10 minutes.

5.1.2 Click the button “Load out”, and place the chip on the tray, align the positioning of center of the tray and the centre hole of chip. The small positioning cylinder of tray goes through the gap of centre hole of chip to fix the chip. Then click the button “Load in” to send the chip into the RT-isoAmplifier.

5.1.3 Input the information of detected samples into the “Sample Information” at the interface of detection, where the Number of Sample, the Number of Chip and the Type of Sample are required, others are optional. After inputting the information of samples, click “Start Testing” button at the operation area to start testing. The instrument will start to detect the sample based on the preset programs (See Table 4). It will display the real-time fluorescent curve at fluorescent curve area” and display real-time monitoring temperature at the left status bar, meanwhile, displaying the remaining time at the detection status area. The software will automatically analyze the data after detection, meanwhile, the instrument will automatically cool down. The sample will automatically load out when the temperature cools down to 37 $^{\circ}$ C. Then load out the chip, please refer to 《User Manual of RT-isoAmplifier》 for more information.

Table 4 Nucleic Acid Amplification Program

Step	1	2
Temperature ($^{\circ}$ C)	37	65
Time (Minutes)	3	47

5.2 Interpretation of Results

The software will automatically analyze the fluorescent data after testing. Fluorescent curve area displays the normalized curve and the area of Testing Results displays the testing results of quality control and each detection index. Please refer to 《User Manual of RT-isoAmplifier》 for more information.

【Interpretation of Positive Value】

The kit employs ROC method to calculate the reference values of 13 detected indexes, the reference value of each index is integrated into the software of interpretation of testing results of the kit. The software will automatically interpret the testing results of the sample. When the value of amplification time of detected index is less than or equal to the reference value of the index, the testing result of interpretation is positive. When the value of amplification UML-301

time of detected index is more than the reference value of the index, the testing result of interpretation is negative.

【Interpretation of Testing Results】

1. Postive and Negative Control in the Chip

Each chip of the kit contains 1 positive control (See Positive External Control in Table 1) and 9 negative controls (See Negative Control in Table 1). According to the standard of interpretation of testing results, the testing result of positive control is positive and the testing results of 9 negative controls are negative. If any testing result is wrong, it is determined that the testing result of the sample is invalid and needs to be re-tested.

Each chip of the kit also contains 1 positive internal control which is the human specific primer, the testing result of positive internal control should be positive when testing the clinical sample. If the concentration of human genome DNA of sample is low, the testing result of positive internal control could be negative, it means that the quantity of human cells of sample is small and it is recommended to re-collect the samples to test.

2. Quality Control of Kit

Positive Control: Escherichia coli should be positive, others are negative.

For the first time to use the kit, it should use the positive control and negative control to test: the testing result of negative controls is negative and the testing result of positive control is positive of Escherichia coli. When doubting the cleanliness of operating environment, it can use the negative control and positive control for quality control.

3. Interpretation of Testing Result

Figure 2 shows a normal amplification result, the right side of the image is the result display area, “The testing result of quality control: Normal tests (normal positive, normal negative)”, the result shows that the testing results are reliable. The testing result “+” indicates that the bacteria in the sample is detected, the testing result “-” indicates that the bacteria in the sample is not detected. The left side of the image shows the normalized fluorescent curve, where the S-shape amplification curve is the positive index, and the horizontal line is the negative index. The interpretations of testing results of 2 indexes of Staphylococcus aureus are as Table 5:

Table 5 Interpretation of Testing Results of Staphylococcus Aureus

		Methicillin-Resistant Staphylococcus Aureus (MRS)	
		+	-
Staphylococcus Aureus	+	Methicillin-Resistant Staphylococcus Aureus (MRSA)	Methicillin Sensitive Staphylococcus Aureus (MSSA)
	-	Non-MRSA Methicillin-Resistant Staphylococcus Aureus (MRCNS, etc)	Staphylococcus aureus is not detected; Methicillin-resistant staphylococcus aureus is not detected.

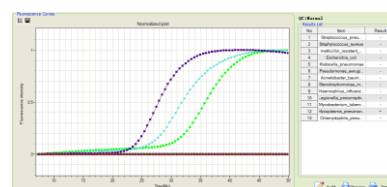


Figure 2 Normal Amplification Curve

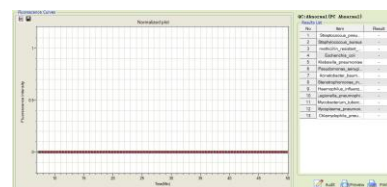


Figure 3 Abnormal Amplification Curve

Figure 3 shows an abnormal amplification result. The testing result of quality control: ‘Abnormal tests (Abnormal positive, normal negative)’, it means that the testing result of the sample is invalid, it need to re-collect the sample to test.

【Limitations】

The kit is only used for detection of nucleic acid extracted from the secretions of the lower respiratory tract. The testing result is only for reference of clinical diagnosis, and clinical diagnosis and treatment to the patients should be combined with signs/symptoms, medical history, laboratory examination and treatment, etc.

The detection objects of the kit are common lower respiratory tract pathogens, a total of 13 species which don't cover all lower respiratory tract pathogens and include virus and fungi associated with lower respiratory tract infections. Therefore, the pathogens in the testing report are only limited to the detectable pathogens of the kit, the negative result in the testing report doesn't mean that the tested sample doesn't have other pathogens.

【Performance Characteristics】

1. Accuracy: The testing results of 26 pathogens enterprise references were correct; The testing results of 15 Mycobacterium tuberculosis national references were correct.
2. Specificity: The testing results of 31 pathogens enterprise references don't exist cross reactions. Among the testing results of 15 Mycobacterium tuberculosis national references, the testing results of 2 references covered by the kit were positive, the testing results of other 13 negative references were negative.
3. Sensitivity: The minimum detection of 12 pathogens enterprise references is 5×10^2 copies/reaction; The minimum detection of Mycobacterium tuberculosis national references is 1×10^2 bacteria/mL.
4. Precision: The testing of 13 pathogens enterprise references were repeated 10 times, the results remain the same and correct; The testing of 10 Mycobacterium tuberculosis national references were repeated 10 times, the results remain the same and correct.

【Precautions】

1. The kit is only used for in vitro diagnostics.
2. Pay attention to the safety protection. The operation of pathogenic bacteria DNA should be carried out in the laboratory. Operators should wear protective clothing, disposable gloves and masks. All materials which have direct contact with bacteria should be destroyed or re-used after strict sterilization.
3. Relevant laboratory management standards must be strictly in accordance with the laboratory management standards of gene amplification detection issued by the industry administrative department.
4. Operators must be trained and qualified.
5. All materials which have contact with nucleic acid amplification reagents should be clean and sterile.
6. Chips and parafilm are disposable, please do not use if the aluminium foil bag is damaged before unpacking the package of chips.
7. Each component of different LOTs of kits can not be interchanged for use, please do not use expired reagents.
8. The room temperature is $10^{\circ}\text{C} \sim 30^{\circ}\text{C}$.

【Label】

The  logo on the label represents in vitro diagnostic medical device.

【Bibliography】

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2. Kang Y, et al. Etiologic diagnosis of lower respiratory tract bacterial infections using sputum samples and quantitative loop-mediated isothermal amplification. *PLoS ONE*, 2012, 7(6): e38743 1-12.
3. Huang GL, et al. Sample high sensitivity rapid measurement method and portable system research, *Journal of Optics*, 2012, 32 (2): 1-8.
4. Chen YS, et al. Application of loop mediated isothermal amplification technology in detection of common pathogens of lower respiratory tract, *Chinese Journal of Tuberculosis and Respiratory*, 2014, 37 (4): 270-273.

【Basic Information】

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【Manufacturing License】

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National Medical Device Registration Approval No. 20163400327