Six Carbapenem-resistant Gene Nucleic Acid Test Kit (PCR-Fluorescent Probe Method)

1【Product Name】

Six Carbapenem-resistant Gene Nucleic Acid Test Kit (PCR-Fluorescent Probe Method)

2【Specification】

10 tests/kit (10210108A), 50 tests/kit (10210108B)

3【Intended Use】

This kit is used for in vitro qualitative test of nucleic acid DNA of Carbapenem antibiotic resistant genes (including KPC, NDM, VIM, IMP, OXA-48 and OXA-23 genes) in sputum, bronchoalveolar lavage fluid (BALF) and rectal swab samples. Carbapenems are atypical β-lactam antibiotics with the widest antibacterial spectrum and the strongest antibacterial activity. Because of their stability to β-lactamase and low toxicity, carbapenems have become one of the most important antibacterial drugs for the treatment of serious bacterial infections. The main mechanisms of resistance to carbapenems include the production of carbapenemases, which refers to a class of β-lactamases that can hydrolyze carbapenems. Carbapenemases can be divided into three categories: A, B and D. With the widespread use of antibiotics, the resistance rate to carbapenems has been increasing year by year, posing a serious challenge to clinical anti-infection treatment.

The test results of this product are for clinical reference only and should not be used as the sole criterion for clinical diagnosis. It is recommended to comprehensively analyze the patient's condition by integrating clinical manifestations, other laboratory tests, and relevant diagnostic criteria.

4【Principle of the Procedure】

This kit uses real-time fluorescent PCR technology. This kit employs the TaqMan probe method to detect the amplification curves of KPC, NDM, VIM, OXA-48 and the internal control, and the molecular beacon probe method to analyze the melting curves of IMP and OXA-23. The 5' end of the molecular beacon is labeled with a fluorophore, and the 3' end is labeled with a quenched group. In the free state, the fluorescence emitted by the fluorophore is quenched. When the molecular beacon hybridizes to a target molecule with a completely complementary sequence, the fluorophore and the quencher are separated, and the fluorescence of the beacon molecule is restored. Real-time amplification curves and melting curves are drawn based on the detected signals, enabling the detection of samples. This product is intended for use on the RocGene iFIND® Instrument Systems. RocGene iFIND® Instrument Systems automate and integrate sample purification, nucleic acid amplification, and target sequence determination in single or complex samples. The systems consist of an instrument, a personal computer pre-installed software for running tests and viewing the results. The systems require the use of cartridges. Each cartridge is equipped with ultrasonic buffer, amplification reagents and internal control to enable the integration of sample nucleic acid extraction and detection, and to identify the presence of nucleic acid DNA of Carbapenem antibiotic resistant genes in samples. The cartridge is hermetically sealed and features independently responsive chambers, which effectively prevents cross-contamination.

5【Reagents】

5.1 Materials Provided

The Six Carbapenem-resistant Gene Nucleic Acid Test Kit (Fluorescent PCR Method) kit contains sufficient reagents to process 10 (10210108A) or 50 (10210108B) samples respectively.

Table 1. Components of Kit A

Cartridges	**10 per kit**	**50 per kit**
1. Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge	1 of each per cartridge
2. Wash Reagent	3 mL per cartridge	3 mL per cartridge
3. Sonication Reagent	3 mL per cartridge	3 mL per cartridge
Disposable Transfer Pipettes	10 per kit	50 per kit
Sample Processing Solution	8 mL x 10 tubes	8 mL x 50 tubes
Instructions for Use (Package Insert)	1 per kit	1 per kit

Note: The disposable transfer pipette in kit A is a purchased external component, and its shelf life is specified on the product label.

5.2 Materials Available but Not Provided

Table 2. Contents of Kit B

Positive Control	**Components**
Lyophilized powder of standard strains containing Carbapenem antibiotic resistant genes and internal standard gene fragments	1 test per kit
Negative Control	1 test per kit
Lyophilized powder containing internal standard gene	

fragments	
Diluent	2 tubes per kit
Nuclease-free water 2 mL	

Kit B provides positive and negative controls designed to confirm proper kit function and to assess the testing environment and operator performance. The controls are recommended to be used in the following situations:

1. Initial verification: Run both positive and negative controls when using the kit for the first time.
2. Troubleshooting: If questionable or inconsistent results are obtained, run both controls to evaluate the kit, the environment, and operator handling.
3. Environmental monitoring: If contamination of the testing environment is suspected, run both controls to verify result reliability.

6【Storage, Transport and Handling】

1. Kit A should be stored and transported at 2℃ to 28℃, protected from light, and free from freezing. The kits have a shelf life of 12 months when stored under the recommended storage conditions.
2. Production date and expiration date: refer to the outer packaging.

7【Applicable Instrument】

The Kit is compatible with the Full-Automatic Nucleic Acid Detection and Analyzer (Models: iFIND S2, iFIND S4, iFIND S8; Medical Device Registration Certificate No.: 20233221927) manufactured by RocGene (Xuzhou) Technology Co., Ltd.

8【Specimen Collection, Handling and Storage】

8.1 Specimen Type

Sputum, bronchoalveolar lavage fluid (BALF) and rectal swab samples.

8.2 Specimen Collection

8.2.1 Sputum

Follow your institution's guidelines for collecting sputum samples or using the following instructions:
The patient should rinse the mouth with water first to avoid food residue, take 2-3 deep breaths, and forcefully expectorate deep sputum into a sterile sputum container. Morning sputum is recommended. Tighten the container lid. The sputum volume should be at least 1 mL. Qualified sputum samples should be purulent or mucoid, avoid the use of spit sputum.

8.2.2 Bronchoalveolar lavage fluid (BALF)

Collecting it according to hospital standard procedure and save it at 4℃ for later use.

8.2.3 Rectal swab

Take the lateral decubitus position or the knee-thoracic position. Soak a cotton swab with normal saline, insert it into the rectum about 3-4 cm against the anal wall, gently rotate it 2 times and gently twist it out. After that, put the cotton swab in the preservation solution (Normal saline, Cary-blair, or an eSwab system can be used; not provided in the kit).

8.3 Specimen Storage

1. Maintain recommended storage conditions to preserve specimen integrity.
2. Test specimens as soon as possible after collection.
3. Storage conditions:
    - Room temperature (10–30 °C): 6 hours
    - Refrigerated (2–8 °C): 12 hours
    - Frozen (−20 ± 5 °C): 6 months
4. Frozen specimens must be completely thawed and mixed before use.
5. Avoid more than three freeze–thaw cycles.

9【Assay Procedure】

9.1 Specimen Pre-processing

9.1.1 For sputum

1. Pour or pipette the provided Sample Processing Solution into the collected sputum based on sputum viscosity:
    - **Watery sputum (thin, clear or slightly colored mucus):** use a 2:1 ratio (Sample Processing Solution: sputum).
    - **Thick sputum (viscous, dense, often yellow or white):** use a 4:1 ratio (Sample Processing Solution: sputum).
    - **Highly viscous sputum:** increase the volume of Sample Processing Solution as needed until the sample can be homogenized.
2. Secure the lid and vortex for 1 minute to homogenize.
3. Incubate the sample at room temperature for approximately 15 minutes. Vortex vigorously for 30 seconds. If the sample is not completely liquefied (i.e., still contains visible clumps or forms threads when pipetted), repeat vortexing once or twice as needed.

9.1.2 For BALF

Add sample into the cartridge directly.

9.1.3 For rectal swab

Mix the sample thoroughly by shaking the tube about 20 times or vortexing for approximately 10 seconds. If the fecal content is excessive, dilute the sample with the preservation solution (Normal saline, Cary-blair, or an eSwab system can be used) until at least 0.75 mL of clear supernatant is available for pipetting before testing.

9.2 Cartridge Preparation

1. Open the cartridge lid and remove the aluminum seal.
2. Using the provided pipette, transfer 0.75 mL of the liquefied sample into the sample chamber (large opening) of the cartridge

Note: Dispense the sample slowly along the chamber wall to avoid bubbles.

3. Close the cartridge lid firmly.

Note: If repeat testing is needed, store the remaining sample at 2–8 °C for up to 12 hours.

10【Run the Test】

1. Power on the RocGene iFIND® System.
2. Log in with your username and password.
3. Wait for the system to complete the self-check. Once finished, click **"OK"** to enter the **"Running"** window and select the desired instrument unit.
4. Scan or manually enter the **Cartridge ID**; When the Cartridge ID is entered correctly, the corresponding test project will be selected automatically.
5. Scan or manually enter the **Sample ID.** The Sample ID will be inserted into the middle of the default Experiment Name.

   ***Example*:** *If the default name is 20250907-165208 and Sample ID 888888 is entered, the name becomes 20250907-888888-165741.*

6. By default, the Experiment Name is generated from the date and time the system was powered on. Edit if needed; the name will be linked to the test results in the **"Analyze"** window.
7. Click **"Open Door"** to open the module.
8. Insert the cartridge with the amplification tube facing the inside of the module.
9. Close the door.
10. Click **"Start Run"** to initiate the assay.
11. During the run, the green indicator flashes. When complete, it remains solid.
12. After the run finishes, click **"OK"** in the prompt box.
13. Navigate to the **Analyze** window to review and confirm test results.

11【Quality Control】

Table 3. Fluorescent Channels and Targets

Channel	CY55	VIC	FAM	ATTO	ROX	CY5
Corresponding target	Internal Control	OXA-48	VIM	KPC	NDM	IMP/OXA-23

Each test includes an internal quality control (IC), which verifies that the cartridge and test process are functioning properly. If the quality control criteria are not met, the test results shouldn't be interpreted.

Internal Control (IC):

The internal control confirms that:

- the sample was added correctly,
- reagents are functioning as intended,
- the procedure was performed correctly, and
- no interfering substances prevented amplification.

External Controls:

External quality controls (see Section 5.2) are available but not provided. They may be used in accordance with local, state, or federal regulations, as applicable

12【Interpretation of Results】

When the instrument is operating normally and the internal control test results meet all requirements, test sample results are analyzed and interpreted. Results are automatically generated by the instrument.

Table 4. Interpretation of Results

Result	Interpretation of results & action
Positive for NDM	There is NDM gene in the sample.
Positive for VIM	There is VIM gene in the sample.
Positive for OXA-48	There is OXA-48 gene in the sample.
Positive for KPC	There is KPC gene in the sample.
Positive for IMP	There is IMP gene in the sample.
Positive for OXA-23	There is OXA-23 gene in the sample.
Negative	There is no KPC, NDM, VIM, IMP, OXA-48 and OXA-23 genes or the content is below the detection limit in the sample.
Invalid	Repeat test using the original sample. If the repeat result remains invalid, consider collecting a new specimen.

13【Limitations】

1. The test results of this product are for clinical reference only and should not be used as the sole criterion for clinical diagnosis.
2. The primer or probe binding region is a conserved region of the pathogen, and mutations rarely occur, but genetic mutations in the epidemic process of the pathogen cannot be ruled out, resulting in false negatives.
3. Inappropriate sample collection, transportation, handling, experimental procedures, or experimental conditions, may all lead to false negative and/or false positive results.
4. Only common mutations have been validated in clinical trials, Rare or novel strains have not been clinically validated.
5. Contamination by microorganisms and amplification products may cause erroneous results. Strict attention should be paid to laboratory division and the PCR standard laboratory operation method should be followed.

14【Analytical Performance Characteristics】

1. **Limit of Detection (LoD)**
   200 CFU/ml.
2. **Analytical specificity**
   No cross reactivity to other pathogens related to respiratory tract and digestive tract.

15【Warnings and Precautions】

1. For in vitro diagnostic use only. Please read the product

manual carefully before operation.

2. All samples for detection should be handled as potentially infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of samples and waste must meet relevant requirements outlined in local, state and national regulations.
3. This kit does not contain active substances of human or animal origin.
4. Product performance is verified only for sputum sample and the sample collection and processing method described in section 8【Specimen Collection, Handling and Storage】. If other sample types or sample collection and processing methods are used, product performance cannot be guaranteed.
5. The kit still has a certain amount of biological load and pollutants after use, handle the reagent with appropriate precautions and good laboratory practice.
6. Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized. Please strictly follow the instructions.

16【Key to symbols】

Symbols	Title	Symbols	Title
CE	CE Marking	IVD	In vitro diagnostic medical device
2°C 28°C	Temperature Limitation		Keep away from sunlight
	Do not use if package is damaged	2	Do not re-use
	Consult Instruction for Use		Caution
	Manufacturer	EC REP	Authorized representative in the European Community/ European Union
REF	Reference	/	/

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