ESKAPE Multipathogenic Nucleic Acid Test Kit (Fluorescent PCR Method)

【Product name】

ESKAPE Multipathogenic Nucleic Acid Test Kit (Fluorescent PCR Method)

【Specification】10 tests/ kit (10210106A), 50 tests/ kit (10210106B)

【Intended use】

This kit is used for in vitro qualitative test of nucleic acid DNA of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Enterococcus* in sputum, serum, bronchoalveolar lavage fluid (BALF) and rectal swab samples.

Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and *Enterococcus* are important pathogens that cause lower respiratory tract infections, sepsis and gastrointestinal infections. At present, biochemical indicator identification and pathogen culture are commonly used in clinical to determine the pathogen of infection, but the period is long, isolation and culture are difficult, and the positive rate is low. Molecular technology provides a convenient method for detecting infectious pathogens.

This kit is for clinical reference only and should not be used as the sole criterion for clinical diagnosis. It is recommended to conduct a comprehensive analysis of the patient's condition based on their clinical manifestations and other laboratory tests.

【Test principle】

Based on the principle of fluorescent PCR technology, specific primers and probes are designed for *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus*. Fluorescent PCR equipment is used for nucleic acid detection of these bacteria.

This kit adopts an endogenous internal quality control to monitor the collection, transportation, extraction and amplification procedures of test samples to avoid false negative results.

This product uses the Full-Automatic Nucleic Acid Detection and Analyzer with a Cartridge to achieve automatic sample purification, nucleic acid amplification, and target sequence determination in a sample. The reactions are performed in the Cartridge. The Cartridge is well sealed and each chamber reacts independently, which can effectively prevent contamination.

【Main components】

Table 1 Kit components

Component		Component name	Specification and quantity	
			10 tests/kit	50 tests/kit
Kit A	Cartridge	Cartridge	10 tests	50 tests
	Sample processing solution	Sample processing solution	8 mL × 10 tubes	8 mL × 50 tubes
	Disposable pasteur pipettes	Disposable pasteur pipettes	12 pieces/box	60 pieces/box
Kit B	Controls	Positive control	1 test × 1 tube	
		Negative control	1 test × 1 tube	
		Diluent	2 mL × 2 tubes	

Table 2 Main components of reagents in each chamber of the Cartridge

Chamber	Ingredient	Specification and quantity
Sample chamber	-	-
Clean chamber	Tris-HCl buffer, etc	2 mL/chamber
Waste liquid chamber	-	-
Buffer chamber	Tris-HCl buffer, etc	1mL/chamber
Reaction chamber	Lyophilized beads containing DNA polymerase mix, dNTP, primers & probes	3 lyophilized beads/chamber

Note:

1. The disposable pasteur pipette in kit A is purchased externally, and the validity period shall be based on that indicated on the pipette package.
2. The sample processing solution is different for different samples, which should be purchased separately. The sample processing solution provided with kit A is only applicable to sputum.
3. Kit B is not supplied with the kit and must be purchased separately from the manufacturer.

【Storage conditions and shelf-life】

1. Both kit A and kit B are stored at 2℃~ 28℃ protected from light and avoid freezing. The shelf life is 12 months.
2. Production date and expiration date: see outer packaging box.

【Applicable instrument】

The kit is suitable for Full-Automatic Nucleic Acid Detection and Analyzer (Model: iFIND S2, iFIND S4, iFIND S8) produced by RocGene (Xuzhou) Technology Limited.

【Sample requirements】

1.Sample type

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

2.Sample collection

2.1 Sputum

Using a sterile sputum sample collection box, morning sputum is recommended. Rinse mouth with water first to avoid food residue, breathe deeply for 2 to 3 times, and forcefully cough the deep sputum into a sterile sputum box. Tighten the lid of the box. The amount of sputum should not be less than 1 mL. Qualified sputum samples should be cheesy or mucoid, avoid the use of spit sputum.

2.2 Serum

Using EDTA blood collection tube to collect whole blood, then stay still for at least 30 min or centrifuge it at 4000 rpm for 10 min to collect serum, aspirate serum into a clean tube and save it at 4℃ for later use.

2.3 Bronchoalveolar lavage fluid (BALF)

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

2.4 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 3-4cm 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) in time.

3.Sample preservation

Samples should be tested as soon as possible after collection. Samples are stored at 2~8 ℃ for no more than 10 days, stored at -20±5 °C for no more than 12 months, and stored at room temperature (10~30℃) for no more than 3 days. Frozen specimens should be thoroughly thawed and mixed, and avoid repeated freeze-thaw for more than 3 times.

4.Sample pre-processing

4.1 For sputum

4.1.1 Place the sputum sample in a sterile sputum box. Depending on the viscosity of the sputum sample, it is recommended to add 2~5 times the volume of sample processing solution. The amount of sample processing solution can be appropriately increased based on the viscosity of the sputum sample. After tightening the lid, vortex for about 1 minute to homogenize the sample.

4.1.2 Leave at room temperature for 15~30 minutes and mixed once or twice in between until the sample is completely liquefied. (There is no obvious solid material and no pull filament-like when absorbing. Then add sample into Cartridge.)

4.2 For serum, add sample processing solution to serum according to the ratio of 1:1, vortex for about 1 minute to homogenize the sample, then add sample into Cartridge.

4.3 For BALF, add sample to the Cartridge directly.

4.4 For rectal swab, after vortex shaking and mixing, centrifuge at 3000rpm for 1 minute. Using the disposable pasteur pipette provided, aspirate the supernatant sample up to the mark on the pipette (which is approximately 2 mL), and then add sample into Cartridge.

【Test method】

1. Preparation of the kit (sample processing area)

1.1 When testing for the first time or using a new batch of kits, it is necessary to use positive and negative controls for quality control of the operating environment and kit quality. Dissolve the positive control in 2 mL diluent until the liquid is clear and without precipitation.

1.2 Open the lid of the Cartridge and remove the aluminum film slowly. Using the disposable pasteur pipette provided with the kit, aspirate the processed sample just above the indicator line on the pipette, adhere to the wall and slowly add the sample to the sample chamber, be careful not to create bubbles. The remaining samples can be stored at 2 ~ 8°C for 12 hours for

repeating test.
1.3 Cover the lid of the Cartridge slowly, and ensure the lid tightly closed. Avoid splashing of liquid by excessive force.
1.4 When the Cartridge is ready, it should be tested as soon as possible.

2. PCR amplification and detection (amplification area)

2.1 Open the Full-Automatic Nucleic Acid Detection Analyzer, input user name and password, then click “OK” to enter the main interface of the instrument. Click “Running” on the page and select “Unit” of the instrument.
2.2 Click the “Scan” button on the “Unit” interface, use the barcode scanner to scan the barcode of the Cartridge, and enter the necessary information such as the experiment name.
2.3 Click “Open Gate” on the operation interface, and gently put the Cartridge into the groove of the corresponding unit of the Full-Automatic Nucleic Acid Detection and Analyzer, then close the gate.
2.4 Click “Start Run” for testing.
2.5 The green light flashes during the testing process. The green light remains on after the test is completed.
2.6. After the experiment, click the “OK” button according to the prompt box, and then go to the “Record” interface, and query the test result in the “Record” interface.

【Quality control】

Table 3 Detection indicator of each channel

Channel	CY55	ROX	FAM	VIC	ATTO	CY5
Corresponding target	Internal control and *Escherichia coli*	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Enterococcus*

1.Internal control: For negative samples, CY55 channel has the characteristic peak of melting curve, and the Tm value is between 65-68℃. For positive samples, at least one of ROX, ATTO, VIC, CY5 and FAM channels has obvious amplification curves, or CY55 channel has melting curve characteristic peaks between 75-78℃. The CY55 channel may have melting curve characteristic peaks between 65-68℃, or there may be no the characteristic peak of melting curve due to the inhibitory effect of high concentration samples, which is a normal situation.
2.Probe quality control: All probe fluorescence detection backgrounds are within the corresponding peak range.
3.Positive control: ROX, ATTO, VIC, CY5, and FAM channels have amplification curves, while CY55 channel has two melting curve characteristic peaks between 65-68℃ and 75-78℃, and the result is “positive for *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus*”.
4. Negative control: CY55 channel has a melting curve characteristic peak and the Tm value is between 65-68℃. ROX, ATTO, VIC, CY5, and FAM channels do not have amplification curves. The result is "sample negative".

Note: Both conditions 1 and 2 must be met in one experiment. When the operating environment and kit quality need to be controlled for the first testing or using a new batch of

kits, all four conditions must be met simultaneously, otherwise the test results are invalid.

【Positive judgment value】

Based on the clinical sample test results, the ROC curve method was used to determine the positive judgment values of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus*. Ct≤32.5 in ROX channel indicates a positive result for *Acinetobacter baumannii*; Ct≤32.5 in FAM channel indicates a positive result for *Pseudomonas aeruginosa*; Ct≤34 in VIC channel indicates a positive result for *Staphylococcus aureus*; Ct≤34 in ATTO channel indicates a positive result for *Klebsiella pneumoniae*; Ct≤31 in CY5 channel indicates a positive result for *Enterococcus.* Tm value of the characteristic peak of melting curve detected by CY55 channel is 75-78℃, indicating that *Escherichia coli* is positive.

【Interpretation of test results】

When the instrument is normal and the results of internal control and probe quality control meet the experimental validity judgment, the analysis and interpretation of the test results of the test sample are carried out, and the result is automatically provided by the instrument.

Table 4 Interpretation of test results

Result determination	Interpretation of test results
Positive for *Escherichia coli*	1.There is *Escherichia coli* in the sample; 2.Internal control: Due to competition between the amplification of positive samples and the internal control, there is no requirement for the internal control signal; 3. The probe quality control is normal.
Positive for *Staphylococcus aureus*	1.There is *Staphylococcus aureus* in the sample; 2.Internal control: Due to competition between the amplification of positive samples and the internal control, there is no requirement for the internal control signal; 3.The probe quality control is normal.
Positive for *Klebsiella pneumoniae*	1.There is *Klebsiella pneumoniae* in the sample; 2.Internal control: Due to competition between the amplification of positive samples and the internal control, there is no requirement for the internal control signal; 3.The probe quality control is normal.
Positive for *Pseudomonas aeruginosa*	1.There is *Pseudomonas aeruginosa* in the sample; 2.Internal control: Due to competition between the amplification of positive samples and the internal control, there is no requirement for the internal control signal; 3.The probe quality control is normal.
Positive for *Acinetobacter baumannii*	1.There is Acinetobacter baumannii in the sample; 2.Internal control: Due to competition between the amplification of positive samples and the internal control, there is no requirement for the internal control signal; 3.The probe quality control is normal.
Positive for *Enterococcus*	1.There is *Enterococcus* in the sample; 2.Internal control: Due to competition between the amplification of positive samples and the internal control, there

	is no requirement for the internal control signal; 3. The probe quality control is normal.
Sample negative	1.There are no *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus* in the sample, or the content is below the detection limit; 2.Internal control: amplification is normal, and experimental process is normal; 3.The probe quality control is normal.
The result needs to be repeated	1.Unable to determine the presence of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus*; 2. Internal control: amplification is abnormal, and experimental process is abnormal; 3. Probe quality control is abnormal.

【Limitations】

1. Improper sample collection, transportation, and storage, as well as improper reagent transportation, storage, and preparation, may affect experimental results and even lead to false negative results.
2. If there is laboratory contamination, reagent contamination, or sample cross contamination, false positive results may occur.

【Performance characteristics】

1. Limit of Detection (LoD): 200 CFU/mL for *Pseudomonas aeruginosa;* 500 CFU/mL for *Klebsiella pneumoniae*、*Escherichia coli;* 1000 CFU/mL for *Acinetobacter baumannii;* 1500 CFU/mL for *Enterococcus*、*Staphylococcus aureus*.
2. Specificity: No cross reactivity to other related pathogens.

【Warnings and precautions】

1. This kit is an in vitro diagnostic reagent. Operators should receive professional training and carefully read this manual before use.
2. During the storage, transportation, and usage, various factors may cause performance changes of the kit, such as improper storage and transportation, non-standard sample collection, processing, and testing procedures. Please strictly follow the instructions for operation.
3. Clinical laboratories should strictly follow the management standards of molecular biology laboratories and clinical gene amplification laboratories.
4. Process quality control for each experiment. Each experiment should have a negative control and a positive control.
5. The workbench and various experimental items are often disinfected with 10% sodium hypochlorite, 75% alcohol, and ultraviolet lamps.
6. The test samples involved in this kit should be considered as infectious substances, and the operation and treatment must comply with the relevant requirements of related guidelines.

【Key to symbols】

Symbols	Title	Symbols	Title

Symbols	Title	Symbols	Title
	Caution		Keep away from sunlight
	Manufacturer		Batch code
	Consult Instruction for Use		Do not re-use
	CE Marking		Use-by date
	Do not use if package is damaged		In vitro diagnostic medical device
	Date of manufactured		Temperature Limitation
	Authorized representative in the European Community/ European Union		Contains sufficient for <n> tests

【Basic information】

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