Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus Drug-Resistance Gene Detection Kit (PCR Fluorescence Probe Method)

Igenesis CE IVD *FOR PROFESSIONAL USE ONLY*

REF 106-0141-01

Product Name
Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus Drug-Resistance Gene Detection Kit (PCR Fluorescence Probe Method)

Specification
12 reactions/kit

Intended Use
This kit adopts real-time multiplex fluorescence PCR technology, for in vitro qualitative detection of the *Staphylococcus aureus* spa gene and the methicillin-resistant *Staphylococcus aureus* (MRSA) resistance gene mecA in human sputum, throat swab or nasal swab samples
Staphylococcus aureus (SA) is a Gram-positive coccus, a kind of Staphylococcus, widely distributed, and a variety of animals and humans are susceptible to it. It is a common pathogen clinically. SA is the most common pathogen for suppurative infection, and it may cause local skin and soft tissue infections and even systemic infections such as sepsis. The large-scale use of antibiotics has led to the emergence of drug-resistant *Staphylococcus aureus*, such as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA has multi-drug resistance, and is widely resistant to antibiotics other than glycopeptide antibiotics. MRSA infection mainly occurs in the lungs, is easily transmitted in hospitals, and has a wide range of prevalence and high morbidity. Therefore, rapid detection of SA and MRSA is conducive to providing accurate and effective reference for clinical diagnosis.

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 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.
This kit utilizes multiplex Taqman fluorescence PCR method, with methicillin/oxacillin-resistance gene *mecA* (FAM labeled), staphylococcal protein A gene *spa* (CY5 labeled) as the detection target regions, and specific primers and fluorescence probes designed separately for PCR. Moreover, primers and probes (HEX labeled) for quality control samples are designed, and by testing quality control samples, it can be confirmed whether the target bacteria have been fully processed, and whether PCR inhibitors are present.

Components

Kit Components			Content	Quantity
MRSA iCassette (12 pcs)	Nucleic acid extraction reagent	MRSA lyophilization A	Proteinase K	1pc/iCassette
		MRSA lyophillization B	*Bacillus atrophaeus*	1 pc/iCassette
		Virus lysate solution	Guanidine isothiocyanate	800μL/iCassette
		Virus binding solution	Guanidine isothiocyanate	200μL/iCassette
		Virus washing solution	Sodium chloride	900μL/iCassette
		Virus eluent	Tris-HCl	90μL/iCassette
		Magnetic beads	Magnetic microsphere	11μL/iCassette
	MRSA PCR reagent	MRSA PCR cosolvent solution	PCR Buffer, $MgCl_2$	35μL/iCassette
		MRSA lyophillization	Specific primer probe, dNTP, enzyme	1 pc/tube
Control materials	MRSA positive control		Pseudovirus containing target fragments	1tube(1000μL)
	MRSA negative control		Sterilized purified water	1tube(1000μL)
Pretreatment	Pretreatment solution		NaOH	2 x 28mL/bottle
	Enhancer		/	1 tube (1mL)

Note: Components in different lot numbers are not interchangeable.

Storage conditions and Shelf Life
The kit should be stored at 2-8℃ and the shelf life of this kit is 9 months. The transportation temperature range of the kit should be kept at 2-8℃.
Do not open the iCassette lid before adding the sample. If you open the iCassette lid, it must be used within 30 minutes.

Applicable Instrument
Automated Fully Enclosed qPCR Instrument : Galaxy Nano,Galaxy Neo, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Neo,Galaxy Lite or Galaxy Pro.
- Ultrosonic machine: Galaxy USL III *(not required for Galaxy Neo)*.
- 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.

Sample Requirements
1. Sample types: Sputum, throat swab and nasal swab.
2. Sample collection
2.1 Sputum sample：Use spit cup to take 1-3 mL of sputum coughed up from the deep lungs of the patients and seal it immediately.
2.2 Throat swab sample:Use a sterile swab to collect pharyngeal secretions by wiping the posterior pharyngeal wall and tonsils on both sides with moderate force, and avoid touching the tongue,quickly put the swab into the sampling tube for storage. After being sent for testing in an enclosed form, the sample can be used for testing immediately.
2.3 Nasal swab ample:Use a sterile swab to enter the nostril and gently advance along the floor of the lower nasal passage (as the nasal passage is curved, do not apply excessive force to avoid injury or bleeding). Once the tip of the swab reaches the posterior wall of the nasopharyngeal cavity, rotate it gently for one full turn. If a reflex cough occurs, pause briefly before proceeding. Then, slowly withdraw the swab and immediately place it into the sampling tube for preservation. Seal the tube and send the specimen for testing. The sample can be used for testing immediately.
3. Storage and transportation of samples
3.1 The specimens used for virus isolation and nucleic acid detection should be tested as soon as possible.All collected samples can be tested immediately, or stored at 2–8 °C for 7 days, or stored at ≤ −20 °C for 6 months, avoiding repeated freezing and thawing.
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 the MRSA iCassette
1.1 Process the samples:
A. Samples pretreatment
1)For sputum sample: add 3 times the sample volume of pretreatment liquid, then add the appropriate amount of enhancer in the proportion indicated in Table 1, shake vigorously for 1 minute, and leave 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.

Table 1 Proportion of Added Enhancer

Sputum specimen	Pretreatment liquid	Total volume	Enhancer
1mL	3 mL	4mL	40μL
2mL	6 mL	8mL	80μL

Note: If the sputum is not fully liquefied after being treated in the above manner, 1-2 mL of pretreatment solution can be appropriately added to fully liquefy the sample.
2) For throat swab and nasal swab sample: no need pretreatment.

B. Ultrasonic Crushing:
Open MRSA iCassette package,observe whether the lyophilization in the tube are intact. Pipette 1000 μL of the liquefied sample and slowly add it to the MRSA 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)
C. Place the sonicated iCassette into the instrument.

1.2 Process negative and positive controls
A. Pipette 1000 μL MRSA positive control or negative control into MRSA 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 sonicated iCassette into the instrument.

Note: For the Galaxy Neo, the ultrasound functionality is already built-in, so simply add the sample to be tested or controls into the iCassette and insert it into the machine.

Figure 1. MRSA 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: When the sample test result is negative, the internal control HEX channel must be positive;
Probe quality control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.
Test results of positive control: FAM channel, HEX channel and CY5 channel are all positive.
Test results of negative control: FAM channel and CY5 channel are all negative,HEX channel is positive.

Reference Interval
Based on the analysis of clinical sample test results, using the ROC curve method, the final determined Ct positive threshold value for this kit is 37.

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Interpretation of Test Results

The sample to be tested is judged according to the standards in the table below：

FAM Channel	CY5 Channel	HEX Channel	Result
√	×	√	Within the detection range of this kit, the sample is positive for the mecA gene
×	√	√	Within the detection range of this kit, the sample is positive for the spa gene
√	√	√	1. Positive control test results 2. Within the detection range of this kit, the sample is positive for mecA and spa
×	×	√	1. Negative control test results 2. If the concentration of the sample added is lower than the detection sensitivity of the kit and within the detection range of the kit, the sample is determined as negative.
×	×	×	No internal standard gene is detected, so the result is invalid. The sample is not treated sufficiently or the PCR is inhibited, and a retesting should be performed using a new kit.

Note: "√" indicates the result "shows a significant logarithmic amplification curve, and Ct ≤ 37"; "×" indicates the result "shows no logarithmic amplification curve or Ct > 37". The HEX channel is an internal standard channel. Due to the specific competition with the sample, when the test sample is positive, it may be tested negative.

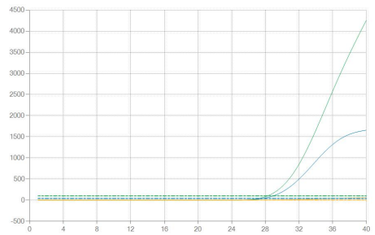

Figure 2. mecA positive

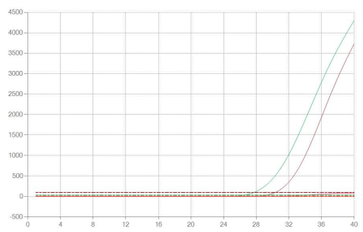

Figure 3. spa positive

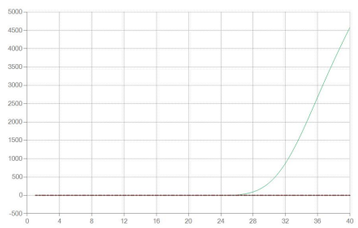

Figure 4. Negative samples

Limitations of Test Method

1. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests, and treatment response.
2. Improper sample collection, handling, transportation, and storage conditions, as well as a low viral titer in the sample, may lead to false-negative results.
3. Unverified cross-reacting substances may cause false-positive results.
4. Other unverified interferences, such as endogenous or exogenous substances introduced into the sample, may result in false-negative results.
5. Cross-contamination between samples may lead to false-positive results.
6. This test kit detects only the mecA resistance gene and cannot identify resistance associated with other resistance genes.
7. This method is intended only for the detection of sputum, throat swab and nasal swab samples. The results cannot distinguish between colonizing bacteria, contaminating bacteria, or pathogenic bacteria.

Product performance Index

1. The LoD of this kit: The LoD by number of plasmid copies is 10^3 copies/mL, while that determined by bacterial culture method is 10^3 CFU/mL.
2. This test kit has no cross-reactivity with *Haemophilus influenzae, Enterobacter cloacae, Acinetobacter baumannii, Neisseria meningitidis, Streptococcus pneumoniae, Enterococcus faecium, Enterococcus faecalis, methicillin-sensitive Staphylococcus epidermidis* (MSSE), *methicillin-sensitive Staphylococcus aureus (MSSA), Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Burkholderia cepacia, Pseudomonas aeruginosa,* and *Candida albicans.*
3. The intra-batch precision coefficient of variation (CV%) is ≤5%.

Precautions

1.The kit must be used within the shelf life.
2. Do not open the iCassette lid before use. If you open it,you must use it within 30 minutes.
3. If you find that the iCassette is oscillated and leaked after adding the sample, do not continue to use it.
4. In order to prevent contamination of the sample, the experimenter should take good care of it and wear disposable gloves and masks.
5. Each iCassette is single-use, please do not reuse it.
6. In order to avoid any potential biological hazards in the sample, the test sample should be regarded as an infectious substance and avoid contact with the skin and mucous membranes; The operation and processing of sample must meet the requirements of local relevant laws and regulations.

References

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Instruction Version

Version: A/1
Date of Issue: May,2022
Last revised: May, 2025

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
	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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