SA (Staphylococcus Aureus) and Meticillin Resistant Staphylococcus Aureus (MRSA) Nucleic Acid Detection Kit Manual
(Fluorescent PCR Method)

【Product Name】
SA (Staphylococcus Aureus) and Meticillin Resistant Staphylococcus Aureus (MRSA) Nucleic Acid Detection Kit (fluorescent PCR method)

【Packaging Specification】
48 tests/box

【Intended Use】
Staphylococcus aureus (SA) is an important pathogenic bacterium in medicine. It is widely distributed with various infection types and high drug resistance rate. In particular, methicillin-resistant Staphylococcus aureus (MRSA) has multiple drug resistance and high mortality, which has become an aporia in clinical anti-infection treatment. The kit is suitable for detecting staphylococcus aureus and methicillin-resistant strain nucleic acid in sputum samples, and can be used for auxiliary diagnosis and curative effect monitoring of staphylococcus aureus and methicillin-resistant strain infection.

【Detection Principle】
This kit uses polymerase chain reaction (PCR) and fluorescence labeled probes to rapidly detect specific heat resistant nuclease genes (nuc) of SA and Methicillin-resistant genes (mecA) in clinical samples to determine the presence of SA and MRSA strain. The use of uracil -N-glycosylase and dUTP in the kit is to avoid contamination.

【Kit Contents】

Contents	Specification and quantity	Ingredients
10x Concentrated Cleaning Solution A	5mL x 1 vial	NaOH
10x Concentrated Cleaning Solution B	20mL x 1 vial	Tris-HC1, EDTA
Extraction Solids	48 vials	/
SA-PCR Reaction Solution	1.1mL x 2 vials	Buffer, probes, primers,Dntp, $MgCl_2$
mecA-PCR Reaction Solution	1.1mL x 2 vials	Buffer, probes, primers,Dntp, $MgCl_2$
Taq DNA Polymerase (5U/μL)	50μL x 1 vial	/
Uracil N-Glycosylase (UNG) (1U/μL)	20μL x 1 vial	/
Internal Control	1mL x 1 vial	Plasmids containing internal control genes
Negative Control	1mL x 1 vial	Tris-HC1, EDTA
Positive Control	1mL x 1 vial	Plasmids containing target genes

Note: The positive control is plasmid containing the target gene, which is from the original strain of the American Strain Collection Center (ATCC).

【Storage and validity】

Kit should be kept at -20℃ and it is valid for 8 months. Keep at 2-8℃ after opening and use within 1 week. Avoid repeated freezing and thawing (less than 5 times).

Transport the kit at low temperature. Use the ice packs for long distance transportation.

【Compatible Instruments】

ABI7500, STRATAGENE Mx3000p, TIB8600 fluorescent PCR instrument etc..

【Sample Collection, Storage and Transport】

1. Recommended specimens: sputum.
2. If the initial concentration of the sample is low, it is suggested to enrich the initial sample before testing.
3. Specimen collection: 1-3mL sputum coughed from deep lung in the early morning is collected into sterile glass tube and sealed for inspection.

Note: External use of respiratory medicine (ointment, spray, drops) is prohibited within 24 hours before sample collection.

【Procedure】(Please read this operating procedure carefully before use)

1. Reagent Preparation (reagent preparation area)

1.1 Dilute the 10 x concentrated cleaning solution A and 10 x concentrated cleaning solution B with sterilized ultra-pure water at 1:9 volume ratio and store it at 4 ℃.

1.2 Briefly spin down the Tag DNA Polymerase and Uracil N - Glycosylase (UNG) and then store them at -20 ℃.

1.3 Calculate the number of PCR reaction tubes N (N = specimen number + negative control + positive control). Take out the SA-PCR reaction solution and mecA-PCR reaction solution, pipet N x 44.3μL SA-PCR reaction solution/mecA-PCR reaction solution, N x 0.5μL Taq DNA Polymerase, N x 0.2μL Uracil N - Glvcosylase (UNG) together into one centrifuge tube and vortex, then spin down briefly, and aliquot them into N x PCR reaction tubes with 45μL/tube, tightly cover the tubes with covers and put into 4 ℃ refrigerator. (Pay attention to distinguish the SA-PCR reaction tubes and mecA-PCR reaction tubes)

1.4 Transfer the extraction solids, positive control, negative control, internal control to the sample processing area and store at 4 ℃.

2. Sample Processing (sample processing area)

2.1 Pipet sputum sample 200μL - 1.5mL into centrifuge tube and add 4 times the volume of 1 x cleaning solution A. Vortex fully and leave at room temperature for 15-30min to liquefy.

2.2 Centrifuge the liquefied specimen at 13000r/min for 5min.

2.3 Discard the supernatant, add 1mL of cleaning solution B to the precipitate and mix evenly, and centrifuge at 13000r/min for 5min.

2.4 Repeat the 2.3 step once.

2.5 Discard the supernatant. Add 100μL of cleaning solution B into the precipitate for later use.

3. DNA extraction (sample processing area)

3.1 Add one tube of extraction solid to each of the above-mentioned processed sample tubes (flick the bottom of the tube to pour the solids out as much as possible), use a powerful vibrator (e.g. Vortex-Genie, USA) to perform high-speed vortex oscillation for 5min minutes. Spin down and add 20μL of internal control.

3.2 Preparation of negative control sample: centrifuge for several seconds at 8000r/min and pipet 100μL - 1.5mL into sterilized centrifuge tube. Add 20μL internal control. Prepare the positive

control same as negative control.

3.3 Spin down the samples, positive control and negative control briefly then 95 ℃ dry bath for 2 min. Ice bath immediately for 2-5 min and centrifuge for 1 min at 13000r/min. Keep the supernatant for PCR amplification.

4. PCR Amplification

4.1 Sample adding (sample processing area or sample adding area)

Add 5μL supernatant of processed sample, negative control, and positive control to the prepared PCR reaction tubes respectively. Spin down immediately after covering the tube tightly.

4.2 PCR amplification (Detection area)

Place the PCR tubes into the PCR instrument, edit the sample information and amplify as per below sequence:

Cycle Parameters: Stage 1: 37° C ---- 2 min Stage 2: 94° C ---- 2 min Stage 3 (10 cycles): 94° C ---- 15 sec 55° C ----45 sec Stage 4 (30 cycles): 94° C ---- 15 sec 55° C ---- 45 sec	SA detection fluorescein: FAM mecA detection fluorescein: HEX Internal control fluorescein: Texas Red Reaction Volume : 50μL Fluorescent signal collection: Stage 4, 55° C ---- 45 sec

SA-PCR reaction system includes SA detection and internal control.

mecA-PCR reaction system includes mecA detection and internal control.

【Reference Value】

Using the instrument matching software to implement automatically analysis, and obtain Ct values for all the samples.

1	SA-FAM or mecA-HEX Ct value ≤ 23 with nice log amplification curve	SA or MRSA Positive	
2	SA-FAM or mecA-HEX Ct value = 30, or "No Ct" (Mx3000P) or "Undet" (ABI 7500), Internal Control (Texas Red) Ct value ＜ 30 with nice log amplification curve.	SA or MRSA Negative (concentration of SA or MRSA DNA is below LOQ)	
3	23 ＜Ct value ＜ 30	Vague result area, should be tested two more times	Retest twice, Ct value=30, SA or MRSA negative
			Retest twice, at least one Ct value ＜ 30 with nice log amplification curve, suspected positive, collect the specimen and test one more time or other detection methods suggested

【Results Interpretation】

1. Result analysis condition setting

1.1 ABI 7500 baseline setting: take the fluorescent signal line between cycle 2 and the sample cycle number 3 cycles before threshold is reached as the baseline. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 30 or "Undet"

1.2 STRATAGENE Mx3000P baseline setting: select the fluorescence signal when "Adaptive baseline" is set. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 30 or "No Ct"

2. Quality control standards

Positive and negative control should meet the following standards at the same time, otherwise the test is invalid.

2.1 Negative control: in SA-PCR system, SA-FAM Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30 with nice log amplification curve. In mecA-PCR system, mecA-HEX Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30 with nice log amplification curve.

2.2 Positive control: in SA-PCR system, SA-FAM Ct value ≤ 23 with nice log amplification curve, internal control (Texas Red) Ct value ≤ 30. In mecA-PCR system, mecA-HEX Ct value ≤ 23 with nice log amplification curve, internal control (Texas Red) Ct value ≤ 30.

【Results Interpretation】

1. SA test result interpretation (SA-PCR system)

1.1 SA negative: SA-FAM Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30 with nice log amplification curve.

1.2 SA positive: SA-FAM Ct value ≤23 with nice log amplification curve, internal control (Texas Red) Ct value ≤30.

1.3 Invalid results, SA-FAM Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500).

2. MRSA test result interpretation (mecA-PCR system), judge as per below info when the SA was judged positive.

2.1 Methicillin sensitive: mecA-HEX Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30 with nice log amplification curve.

2.2 Methicillin resistant: mecA-HEX Ct value ≤23 with nice log amplification curve, internal control (Texas Red) Ct value ≤30.

2.3 Invalid results, mecA-HEX Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500).

【Detection Limitation】

This kit is suitable for clinical specimen’s detection, but the results are affected by the instruments and operation. Therefore, the results are for reference of clinical diagnosis only, and not the only criterion to confirm or exclude disease cases.

【Kit Performance】

The detection lower limit for this kit is 1.0×103copies/mL, with a linear range between

1.0×108copies/mL and 1.0×103copies/mL

Experiments prove that the kit does not have cross reaction with other common clinical pathogens (staphylococcus epidermidis, enterococcus faecalis, streptococcus pneumoniae, micrococcus luteus, micrococcus, Bacillus cereus, streptococcus pyogenes, Escherichia coli, streptococcus bovis, Pseudomonas aeruginosa, candida tropicalis, Klebsiella pneumoniae, salmonella enteritidis, salmonella, Pseudomonas aeruginosa, saprophyticus, staphylococcus albicans, staphylococcus citricola and staphylococcus pyogenes).

The CV value within same batch and between different batches are both lower than 10%.

In the clinical specimen detection of Staphylococcus aureus, the coincidence rate of this kit compared with latex agglutination test is above 95%. In the detection of methicillin-resistant Staphylococcus aureus, the coincidence rate of this kit compared with drug sensitive paper test results is also above 95%, which meets the clinical application requirements.

【Warnings and Precaution】

1. For in vitro diagnosis use only.
2. Read this manual in detail before the assay, and the assay should be carried out by skilled personnel.
3. Use latex gloves or thin film gloves when handling the PCR tubes.
4. Avoid unnecessary repeated freezing and thawing and keep the PCR solution away from lights since there are enzyme and fluorescent probes in PCR reaction solution.
5. Thoroughly thaw the reagents and spin them down briefly before using.
6. Sterilize centrifuge tubes and pipet tips in high temperature and high pressure before being used.
7. Processing and handling of clinical specimens should be carried out in a biosafety cabinet.
8. After being spin down, the PCR tubes should avoid vortex when being loaded on the PCR instrument.
9. Avoid touching the precipitation when aspirate the template.
10. Paraffin is suggested for sealing and cover the tube caps tightly after sample adding.
11. Dispose the PCR tubes in sealing airtight plastic bags as biohazard waste after the PCR instrument cool down at room temperature.
12. Dispose the pipet tips into the 10% sodium hypochlorite wasted solution vat and sterilize with other wastes.
13. Sterilize the biohazard safety cabinet by UV lights. After the experiment, clean the biohazard cabinet and pipets with 10% pasteurization, then use 75% ethyl alcohol for cleaning after 10 min.
14. Do not mix-use the reagents from different batches. Use this kit before its expiration.

【Manufacturer】

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