

A decorative graphic consisting of numerous thin, parallel blue lines that curve and overlap to form a large, smooth, wave-like shape in the upper half of the page.

DiAGSure Staphylococcus aureus Detection Kit

20 Tests

For research use only

Description:

Staphylococcus aureus is a round shaped cocci of Gram positive group of microorganisms. *S. aureus* is a member of the Firmicutes group, which is often found as a natural microorganism present over the human body, skin and upper respiratory tract.

S. aureus causes a variety of diseases from minor skin problems to life-threatening diseases including Staphylococcal scalded skin syndrome, endocarditis, impetigo, cellulitis, food poisoning, septicemia, toxic shock syndrome etc. So, early detection of *S. aureus* is necessary for improving public health. DiAGSure Staphylococcus infection Detection Kit is an in-vitro diagnostic PCR Based detection of *Staphylococcus aureus* in human clinical samples.

Principle:

The DiAGSure Staphylococcus infection Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved *Staphylococcus aureus* specific 267bp region in the *Staphylococcus aureus* genome using gene-specific primers. PCR-based detection is emerging as a highly sensitive diagnostic tool for the detection of pathogen in a wide array of clinical samples. A basic PCR reaction involves three basic steps:

- i. Denaturation, where separation of the two DNA strands occur
- ii. Annealing, where the primers are allowed to anneal to their cognate templates
- iii. Extension, where the actual amplification occurs that is repeated between 25 and 40 cycles in each assay. The PCR

primers have been designed to ensure high specificity and sensitivity.

Features:

- ✓ Fast and simple
- ✓ Rapid detection of *Staphylococcus aureus* in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the *Staphylococcus aureus*
- ✓ Reproducibility of results

Storage and Shelf life:

The provided kit has a shelf-life of 6 months when stored at -20°C. Repeated thawing and freezing of PCR reagents may reduce the sensitivity and therefore should be avoided. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. The degradation of sample DNA specimens may also compromise with the sensitivity of the assay. Usage of the kit after the expiry date stated on pack is not recommended.

Kit contents:

(Storage: -20°C in a frost-free Freezer)

Kit Contents	Volume for 20 Tests
S. aureus Primer mix	45 µL
DiAGPol PCR Master Mix	1.4 mL
DiAGSure DNA ladder	100 µL

Internal control primer mix	25 μ L
Gel loading dye	100 μ L
Nuclease free water	500 μ L

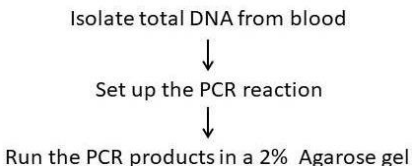
Sample Material Preparation:

The DiAGSure *Staphylococcus aureus* Detection Kit detect the presence of *Staphylococcus aureus* pathogen in human blood samples. Isolate total DNA from blood (which includes bacterial DNA in case of infected samples). Use a specified amount (see below) of this DNA to amplify the *Staphylococcus aureus* specific gene.

Starting volume of blood: 200 μ L

Elution volume: 30 μ L

Basic workflow:



PCR Protocol:

Set up a 20 μ L test PCR reaction by adding the following constituents in PCR tubes:

Template bacterial DNA	1 μ L
DiAGPol PCR Master Mix	18 μ L

***S. aureus* primer mix**

1 μ L

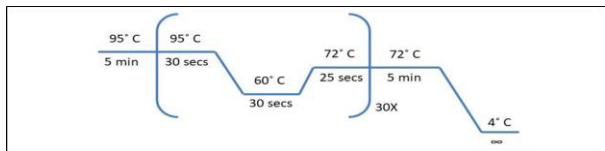
Set up a No Template Control (NTC) reaction with 1 μ L of Nuclease free water in place of template DNA, *S. aureus* primer mix and DiAGPol PCR Master Mix accordingly.

A 20 μ L internal control PCR reaction can also be set up in parallel with 1 μ L of internal control primer mix using 1 μ L of the same template, the other conditions in the PCR mix remaining the same.

Mix vigorously by pipetting up and down and pulse-spin to bring the contents to the bottom of the tube and place the tube in following thermal cycling program.

PCR conditions:

Stage	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	
Annealing	60	30 secs	
Extension	72	25 secs	30
Final extension	72	5 mins	1
Final hold	4	∞	1



Diagrammatic representation of the cycling conditions

Add 1 μ L of the supplied gel-loading dye to the PCR products, mix well and run the PCR products along with 5 μ L of the supplied DiAGSure DNA ladder in a 2% agarose-TAE gel.

Results Interpretation:

The presence of a band of 267-bp with respect to the DiAGSure DNA ladder indicates the presence of the bacterium in the clinical sample. The absence of the 267-bp band in the test sample indicates the absence of the bacterium (See Fig 1).

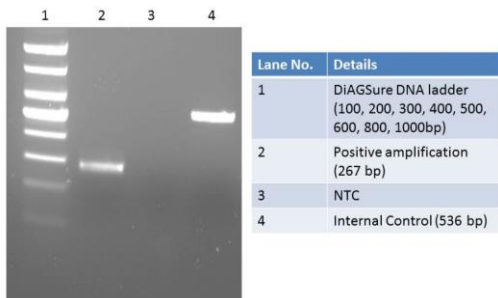


Fig 1. Representative gel image showing amplification of the specific genes. Lane 1: DiAGSure DNA ladder; Lane 2: Positive amplification at 267-bp; Lane 3: Negative; Lane 4: control 536-bp Internal control.

Sensitivity:

The sensitivity of the kit is 0.04 attomoles of *Staphylococcus aureus* genomic DNA.

Quality Control:

All reagents in the DiAGSure *Staphylococcus aureus* Detection Kit are free from endonuclease and exonuclease activities and the kit has been functionally tested for amplification.

Safety information:

The DiAGSure *Staphylococcus aureus* Detection Kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

Technical assistance:

Satisfaction of the customers is our utmost priority. For any kind of technical assistance, always feel free to reach out to us at tech.support@gccbiotech.co.in.