

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 *Propionibacterium acnes*
Detection Kit**

20 Tests

For research use only

Description:

DiAGSure *Propionibacterium acnes* Detection Kit is an in-vitro diagnostic PCR Based detection of *Propionibacterium acnes* in human clinical samples. *Propionibacterium acnes* is a gram-positive human skin commensal that prefers anaerobic growth conditions and is involved in the pathogenesis of acne. It is estimated that nearly 20 percent of all visits to dermatologists are related to the treatment of acne. *Propionibacterium acnes* is a Gram-positive bacterium that forms part of the normal flora of the skin, oral cavity, large intestine, the conjunctiva and the external ear canal. Although primarily recognized for its role in acne, *P. acnes* is an opportunistic pathogen, causing a range of postoperative and device-related infections.

Principle:

The DiAGSure *Propionibacterium acnes* Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved *Propionibacterium acnes* specific 334-bp region in the *P. acnes* 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 *Propionibacterium acnes* in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the *Propionibacterium acnes*
- ✓ 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
<i>P. acnes</i> Primer mix	45 µl
DiAGPol PCR Master Mix	1.4 mL
DiAGSure DNA ladder	100 µL
Internal control primer mix	25 µl
Gel loading dye	100 µL
Nuclease free water	500 µL

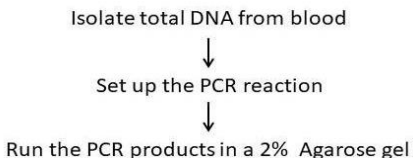
Sample Material Preparation:

The DiAGSure *Propionibacterium acnes* Detection Kit detect the presence of *Propionibacterium acnes* 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 *Propionibacterium acnes* specific gene.

Starting volume of blood: 200µL

Elution volume: 30µL

Basic workflow:



PCR Protocol:

Set up a 20 μ L test PCR reaction and corresponding No Template Control (NTC) by adding the following constituents in PCR tubes:

Template bacterial DNA	1 μL
DiAGPol PCR Master Mix	18 μL
P. acnes primer mix	1 μL

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

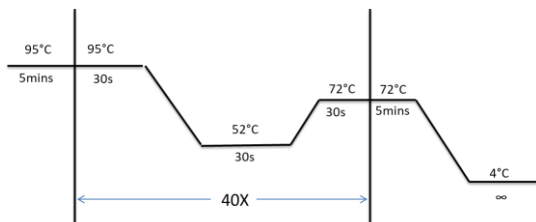
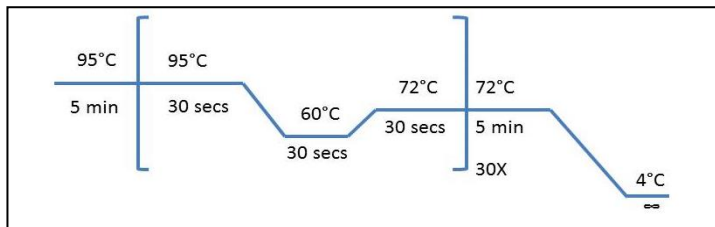
A 20 μ L internal control PCR reaction can also be set up in parallel with 1 μ L of internal control primer mix using 1 μ L of the same template, the other conditions in the PCR mix remaining the same.

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 ($^{\circ}$ C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	
Annealing	60	30 secs	
Extension	72	30 secs	30
Final extension	72	5 mins	1

Final hold	4	∞	1
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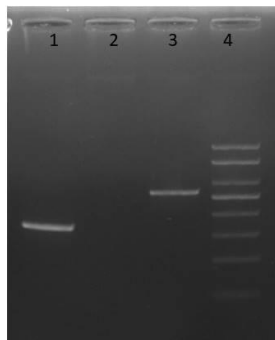


Diagrammatic representation of cycling conditions

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 334-bp with respect to the DiAGSure DNA ladder indicates the presence of the bacterium in the clinical sample. The absence of the 334-bp band in the test sample indicates the absence of the bacterium (See Fig 1).



Lane No.	Details
1	Positive amplification (334bp)
2	NTC
3	Internal Control (536 bp)
4	DiAGSure DNA ladder (100, 200, 300, 400, 500, 600, 800, 1000bp)

Fig 1. Representative gel image showing amplification of the specific genes. Lane 1: Positive amplification at 334-bp; Lane 2: Negative control; Lane 3: 536-bp Internal control; Lane 4: DiAGSure DNA ladder.

Sensitivity:

The sensitivity of the kit is 0.04 attomoles of *Propionibacterium acnes* genomic DNA.

Quality Control:

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

Safety information:

The DiAGSure Propionibacterium acnes 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.