

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 Herpes Simplex Virus Detection Kit

20 Tests

For research use only

Description:

Herpes Simplex Virus (HSV) is dsDNA virus of the Human Herpesviridae family, which causes viral infections in majority of humans. HSV infections could be oral or genital, mainly based on the type of virus, though most oral and genital infections are asymptomatic. Symptomatic cases often include painful blisters or ulcers at the site of infection. The infections mostly involve spreading of skin lesions involving blisters and cutaneous flares. The DiAGSure Herpes Simplex virus Detection Kit has been designed for *in-vitro* use only.

Intended Use:

This kit detects a conserved 92-bp region in the HSV genome specific for this virus. This kit also contains an internal control which is to be set-up in a separate tube and amplifies a 221-bp region from human DNA. This internal control has been included to ensure proper DNA extraction and PCR reaction in the absence of amplification in the target sequence.

Principle:

The DiAGSure Herpes Simplex virus Detection Kit involves semi-quantitative end-point PCR based detection of a conserved 92-bp region in the HSV 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 Herpes Simplex Virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the Herpes Simplex Virus
- ✓ 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
HSV 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 Herpes Simplex Virus Detection Kit detects the presence of Herpes Simplex virus in human blood samples. Isolate total DNA from blood (which includes viral DNA in case of infected samples). Use a specified amount (see below) of this DNA to amplify the 92-bp region of the HSV genome.

Starting volume of blood: 200µL

Elution volume: 30µL

PCR set-up:

Set up a 20 µL test PCR reaction by adding the following constituents in a PCR tube:

Template viral DNA	1 µL
DiAGPol PCR Master Mix	18 µL

HSV primer mix

1 μ L

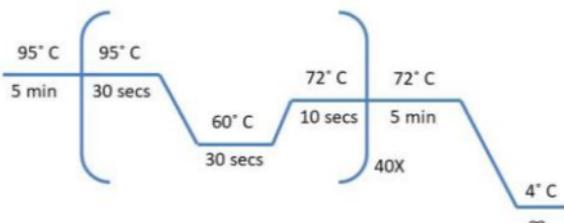
Set up a No Template Control (NTC) reaction with 1 μ L of Nuclease free water in place of template DNA, HSV primer mix and DiAGPol PCR Master Mix accordingly.

A 20 μ L internal control PCR reaction is also 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:

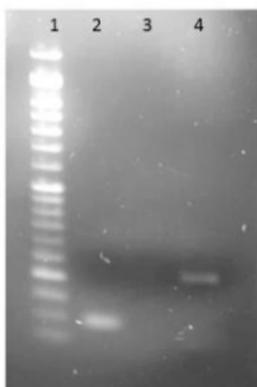
Stage	Temperature ($^{\circ}$ C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	40
Annealing	60	30 secs	
Extension	72	10 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1



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 92-bp band with respect to the DiAGSure DNA ladder indicates the presence of the virus in the clinical sample. The absence of the 92-bp band in the test sample indicates the absence of the virus (See Fig 1).



Lane No.	Details
1	50bp DNA Ladder
2	Positive amplification (92 bp)
3	NTC
4	Internal Control (221 bp)

Fig 1. Representative gel image showing amplification of the HSV gene. Lane 1: 50bp DNA ladder; Lane 2: Positive amplification at 92-bp; Lane 3: Negative control (NTC); Lane 4: 221-bp Internal control amplification.

Quality Control:

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

Safety information:

The DiAGSure Herpes Simplex Virus 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.