

A decorative graphic consisting of numerous thin, light blue lines that curve and overlap to form a large, soft-edged arch shape across the upper half of the page.

DiAGSure Human Papilloma Virus Detection Kit

20 Tests

For research use only

Description:

Human Papilloma Virus (HPV) is a very common virus of the papillomavirus family and one of the most common sexually transmitted infections globally. It is a DNA virus with over 170 types and several high-risk serotypes prevalent all over the world. HPV generally causes warts at infected places of the body, including genitals for genital warts. Strains of HPV that do not cause warts usually cause cervical cancer.

DiAGSure HPV Detection Kit is an in-vitro diagnostic PCR based detection of Human Papilloma Virus in human clinical samples. This kit also contains primer sets of 3 high-risk serotypes of HPV, namely HPV 16, HPV 18, and HPV 31. On getting a positive result for HPV, the customer may detect the specific serotype among these three with further PCR amplifications using the specific primer mixes provided.

Principle:

The DiAGSure HPV Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved Human Papilloma Virus specific 138bp region in the Human Papilloma Virus 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 HPV in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the Human Papilloma Virus and high risk serotypes.
- ✓ 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
HPV Primer mix	45 µL
HPV 16 Primer Mix	25 µL

HPV 18 Primer Mix	25 µL
HPV 31 Primer Mix	25 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

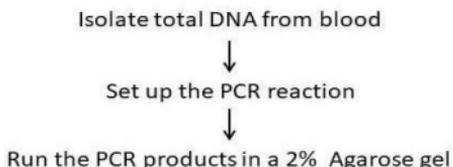
Sample Material Preparation:

The DiAGSure Human Papilloma Virus Detection Kit detects the presence of Human Papilloma 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 Human Papilloma Virus specific gene.

Starting volume of blood: 200µL

Elution volume: 30µL

Basic workflow:



PCR Protocol:

Set up a 20 µL test PCR reaction by adding the following constituents in a 0.2 mL PCR tube, preferably with barrier tips:

Template viral DNA	1 μ L
DiAGPol PCR Master Mix	18 μ L
Parvo B19 primer mix	1 μ L

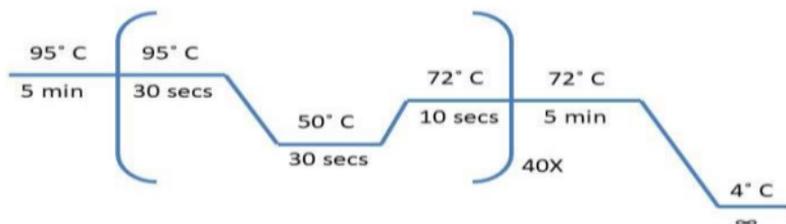
Set up a No Template Control (NTC) reaction with 1 μ L of Nuclease free water in place of template DNA, HPV primer mix and DiAGPol PCR Master Mix accordingly.

A 20 μ L internal control PCR reaction is also to 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	40
Annealing	50	30 secs	
Extension	72	10 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1



Diagrammatic view of the PCR cycling conditions

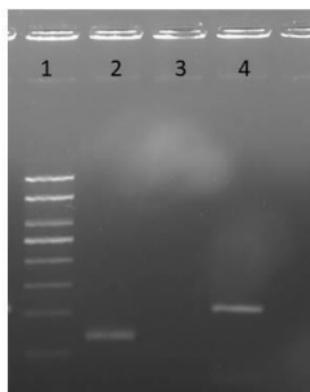
For positive and specific amplifications, the HPV 16, 18 and 31 serotype specific primers (provided with the kit) may also be used to identify the specific serotype of the concerned sample with presence of bands of sizes 93bp, 91bp and 164bp respectively.

In that case, use 1 μ L of the corresponding primer mix and set up the reaction following the abovementioned protocol.

- 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 138-bp with respect to the DiAGSure DNA ladder indicates the presence of the virus in the clinical sample. The absence of the 138-bp band in the test sample indicates the absence of the virus (See Fig 1).



Lane No.	Details
1	DiAGSure DNA Ladder (100, 200, 300, 400, 500, 600, 800, 1000bp)
2	Positive amplification (138bp)
3	Negative Control
4	Internal Control (221bp)

Fig 1. Representative gel image showing amplification of the HPV gene. Lane 1: DiAGSure DNA ladder; Lane 2: Positive amplification at 138-bp; Lane 3: No Template Control; Lane 4: 221-bp Internal control amplification.

Quality Control:

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

Safety information:

The DiAGSure Human Papilloma 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.