

A decorative graphic consisting of numerous thin, parallel blue lines that curve upwards and then downwards, creating a wave-like shape that frames the top and sides of the page.

DiAGSure Cytomegalovirus (CMV) Detection Kit

20 Tests

For research use only

Description:

Cytomegalovirus (CMV) is a very common virus of genus Cytomegalovirus which, in turn, is a member of the viral family known as Herpesviridae or herpesviruses, present naturally in most people. Though it is particularly harmful to newborns and pregnant women, it mostly affects people with low immunity such as AIDS patients. CMV spreads through various body fluids like semen, blood, vaginal fluid, breast milk, etc. DiAGSure CMV Detection Kit is an *in vitro* diagnostic PCR Based detection of Cytomegalovirus in human clinical samples.

Principle:

The DiAGSure CMV Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved Cytomegalovirus specific 143 bp region in the Cytomegalovirus 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 CMV pathogen in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the Cytomegalovirus
- ✓ 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
CMV 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

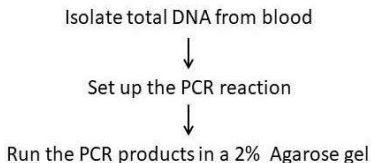
Sample Material Preparation:

The DiAGSure CMV Detection Kit detects the presence of Cytomegalovirus pathogen 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 Cytomegalovirus 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 a PCR tube:

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

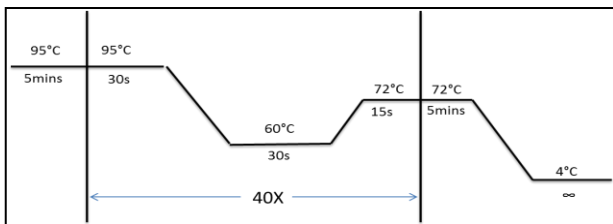
Set up a No Template Control (NTC) reaction with 1 μ L of Nuclease free water in place of template DNA, CMV primer mix and DiAGPol PCR Master Mix accordingly.

A 25 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 (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	
Annealing	60	30 secs	40
Extension	72	15 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1



Diagrammatic view of PCR 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 143-bp with respect to the DiAGSure DNA ladder indicates the presence of the virus in the clinical sample. The absence of the 143-bp band in the test sample indicates the absence of the virus (See Fig 1).

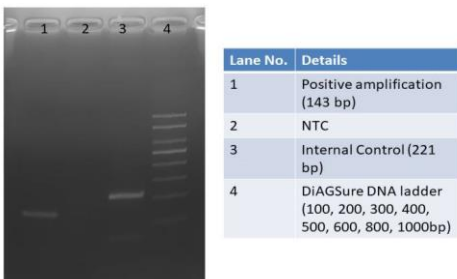


Fig 1. Representative gel image showing amplification of the CMV gene. Lane 1: Positive amplification at 143-bp; Lane 2: No Template Control (NTC); Lane 3: 221-bp internal control amplification; Lane 4: DiAGSure DNA ladder.

Quality Control:

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

Safety information:

The DiAGSure Cytomegalovirus 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.