DiAGSure Parvovirus B19 Detection Kit

20 Tests

For research use only

DiAGSure DIAGNOSTIC
For the people, by the people

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Description:

Parvovirus, the causative organism of erythema infectiosum (or the fifth disease) in children, is a non-enveloped single-stranded DNA virus of family Parvoviridae (Baltimore group II). It has a genome size of about 5.6kb and is the smallest animal virus with DNA genome. The virus spreads through respiratory droplets from infected individuals. The infection can be easily detected in children by the appearance of red rash on the cheeks, often called 'slapped-cheeked' rash which eventually spreads to the other regions of the skin. Adults infected by the disease may remain asymptomatic or have similar rash with fever, joint pain and swelling especially on the hands, wrist and knees. On recovery, the subject develops immunity to future infection. The incidence of erythema infectiosum shows up a seasonal variation which peaks mostly during late winter and early spring. PCR-based detection of Parvovirus B19 in serum and respiratory fluids is highly sensitive and aids early diagnosis of the ailment.

DISCLAIMER: The DiAGSure Parvovirus B19 Detection Kit has been designed for *in-vitro* use only.

Intended Use:

This kit amplifies a unique 202-bp sequence specific for Parvovirus B19 and is absent in other closely-related viruses. This kit also contains a standard marker for size comparison of the amplicon.

Principle:

The DiAGSure Parvovirus B19 Detection Kit involves semi-quantitative PCR based detection of a conserved specific 202-bp sequence in the Parvovirus B19 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 the virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the virus
- ✓ Reproducibility of results

Storage and Shelf life:

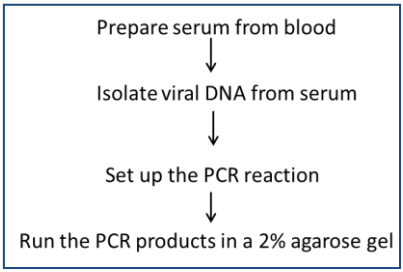
The provided kit has a shelf-life of 6 months when stored at -20°C. Repeated thawing and freezing of the PCR reagents may reduce the sensitivity and therefore should be avoided. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. The 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:

Kit Contents	Volume for 20 Tests
Parvo B19 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

Basic workflow:



Starting volume of serum: 200µL
Elution volume: 30µL

PROCEDURE:

Set up a 20µL PCR reaction with the following constituents:

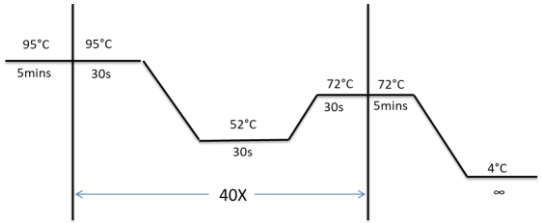
Template viral DNA	1 µL
DiAGPol PCR Master Mix	18 µL
Parvo B19 primer mix	1 µL

Set up a No Template Control (NTC) reaction, replace DNA with 1 µL of Nuclease free water and add B19 primer mix and DiAGPol PCR Master Mix accordingly. A 20 µL internal control PCR reaction can also be set up in parallel with 1 µL of internal control primer mix using 1 µL of the same template.

N.B.: If required, the volume of template may be increased accordingly.

PCR conditions:

Stage	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	40
Annealing	52	30 secs	
Extension	72	30 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1



PCR cycling conditions

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

Results Interpretation:

The presence of a 202-bp band close to 200-bp size of the standard marker indicates the presence of the Parvovirus B19 in the clinical sample. The absence of the 202-bp band in the test sample indicates the absence of Parvovirus B19 infection (See Fig 1).

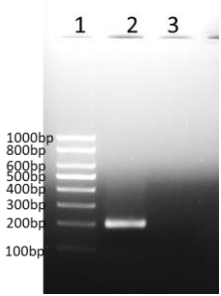


Fig 1. Representative gel image showing amplification of the B19 sequence. Lane 1: DiAGSure DNA ladder; Lane 2:Positive amplification of the B19 amplicon at 202-bp; Lane 3: Negative control.

Sensitivity:

The DiAGSure Parvovirus B19 Detection Kit is highly sensitive and can detect a minimum of 0.763 attomoles of the virus under *in vitro* conditions.

Quality Control:

All reagents in the DiAGSure Parvovirus B19 Detection Kit are free from endonuclease and exonuclease activities and the kit has been functionally tested for amplification.

Precautions:

- Ensure that DNA has been properly isolated.
- Use freshly isolated DNA for amplification.
- The working desk for DNA isolation should be clean and properly wiped with 70% ethanol.

- All microcentrifuge tubes and Pipetman tips should be double-autoclaved.
- The RT reaction should be set up meticulously on ice and carried out under conditions as indicated.
- Verify that all reagents are added to the PCR reaction in indicated amounts and proper PCR conditions have been maintained.
- For long-term storage, it is advisable to store the reagents (especially the enzymes) in aliquots.

Safety information:

The DiAGSure Parvovirus B19 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.