DiAGSure Dengue Detection Kit

Description:

Dengue is a tropical mosquito-borne disease caused by dengue virus (DENV) which is transmitted by the mosquito *Aedes aegypti*. The symptoms of the disease include severe pain in muscles and joints, swelling of lymph nodes, headache, fever and rash. Progression of infection may lead to Dengue Hemorrhagic Fever (DHF) – a severe form of the disease involving vascular and hemostatic abnormalities. Dengue affects various countries of the Asia, the Pacific, the Americas, Africa, and the Caribbean. In India, more than 20,000 cases of dengue are reported each year. As such, accurate diagnosis of the disease proves helpful for proper treatment of the disease. The dengue virus belongs to family Flaviviridae which also includes West Nile virus, Japanese Encephalitis virus, Yellow Fever virus and Zika virus. The genome of the virus is a single-stranded (+) RNA (Baltimore class IV). Reverse Transcriptase-Polymerase chain reaction (RT-PCR) has been proven to be extremely useful and a sensitive diagnostic tool for the detection of RNA viruses, including DENV.

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

Intended Use:

This kit amplifies a specific 511-bp sequence in the D1/D2 region in the DENV genome. This region is specific for all serotypes of dengue virus and is absent in other closely-related viruses of Flavivirus genus. This kit also contains a standard marker for size comparison of the amplicon.

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 dengue virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the dengue pathogen
- ✓ 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 RT enzyme and 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 RNA 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:

Table for kit of 20 tests:

Part 1 of 3 (Storage: Room temperature)
GSure Viral RNA Mini kit (Cat. No. XXXXX) – 20preps
Part 2 of 3 (Storage: Room temperature)

Kit Contents	Volume for 20 tests
Agarose	20g
GPure TAE Buffer, 40X	50mL
0.2mL PCR tubes	70 no.s
1.5mL microcentrifuge tubes	20 no.s
Ethidium Bromide Solution (10mg/ml)	100µL

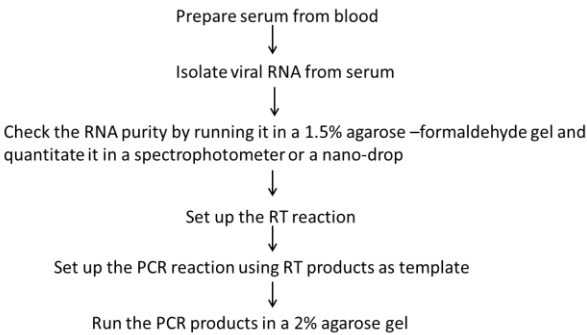
N.B.: On receipt, store the Ethidium bromide solution at 4°C.
Part 3 of 3 (Storage: -20°C)

Kit Contents	Volume for 20 tests
DEPC treated water	1.5mL
10X RT buffer	100µL
GRTScript Reverse Transcriptase enzyme	40µL
DENV RT primer	40µL
10mM dNTP mix	100µL
DENV Primer mix	40µL
DiAGPol PCR Master Mix	1.25mL
DiAGSure DNA ladder	100µL
6X Gel loading dye	1mL
Nuclease free water	1.5mL X 2

Sample Material Preparation:

The DiAGSure Dengue Detection Kit detects the presence of the Dengue virus in human serum samples. Isolate viral RNA from serum using GSure Viral RNA isolation kit provided with this kit. Use a specified amount (see below) of this RNA and prepare cDNA which has to be used as a template for amplification of the 511-bp region.

Basic workflow:



Serum preparation:

Incubate 2mL of whole blood at room temperature for 30 mins followed by mild centrifugation at 3000rpm for 10mins. Use the clear supernatant (serum fraction).

Viral RNA isolation:

Isolate viral RNA from serum using GSure Viral RNA isolation kit (Part 2 of 3 in this kit) as follows:

1. To 140µL of serum taken in a 1.5mL microcentrifuge tube, add 560µL of lysis buffer and vortex mildly for 15secs.
2. Incubate the mix for 10mins at room temperature.

3. Add 560µL of absolute ethanol (not provided with the kit) and mix well by inverting the tubes.
4. Apply the entire solution to GMini Spin Column and centrifuge at 13000rpm for 30secs. Discard the flow-through. (800µL of the sample may be applied to the column at a time; so repeat the centrifugation step until the entire solution passes through the column.
5. Add 500µL of Membrane Wash Buffer 1 and centrifuge at 13000rpm for 30secs. Discard the flow-through.
6. Add 500µL of Membrane Wash Buffer 2 and centrifuge at 13000rpm for another 30secs. Discard the flow-through.
7. Dry the column by centrifugation at 13000rpm for 1min.
8. Add 30µL of nuclease-free water to the column and incubate for 2-5mins. Centrifuge at 13000rpm for 30secs. Collect the eluted RNA and store at -80°C.

PCR Reaction:

1. For setting up a 10µL RT reaction, add the following reagents in a 0.2mL PCR tube and mix by pipetting.

Isolated RNA	**1µL**
10mM dNTP mix	1µL
DENV RT primer	0.5µL

DEPC-treated water	6 µL

2. Heat the mix at 65°C for 5mins followed by quick-chill on ice for 5mins.

3. Add the following reagents to the tube:

GRTScript Reverse Transcriptase	0.5µL
10X RT buffer	1µL

Mix well and pulse spin to bring the contents to the bottom of the tube. Place the tube in a thermal cycler and run the RT reaction at the following cycling conditions:

Stage	Temperature (°C)	Time	No. of cycles
Annealing	25	5mins	1
Extension	42	60mins	
Inactivation	70	10mins	
Final hold	4	∞	

PCR Protocol:

Set up a 25µL PCR reaction by adding the following constituents in a PCR tube:

Nuclease-free water –	1.5µL
2X G9 Taq PCR Master Mix –	12.5µL
DENV pimer mix -	1µL
Template cDNA –	10µL

PCR Protocol:

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

Agarose gel electrophoresis and gel visualization:

1. Cast a 2% agarose gel as follows. Weigh out 1g of agarose and add it to 50mL of 1X TAE buffer taken in a conical flask and heat it until the agarose has dissolved completely. Cool the flask slightly and add 2µL of 10mg/mL Ethidium bromide solution and pour immediately into the gel casting tray. After gel solidification, place the gel in 1X TAE buffer for 15mins for equilibration.
2. Add 1µL of 6X DNA loading dye to the PCR product and load it into appropriate wells of the gel placed in the gel running apparatus containing fresh 1X TAE buffer. Visualize the gel in a uv-transilluminator or a gel documentation instrument.

Results Interpretation:

The presence of a 511-bp band appearing in between 400bp and 500bp with respect to standard marker indicates the presence of the dengue virus in the clinical sample. The absence of the 511-bp band in the test sample indicates the absence of dengue infection (See Fig 1).

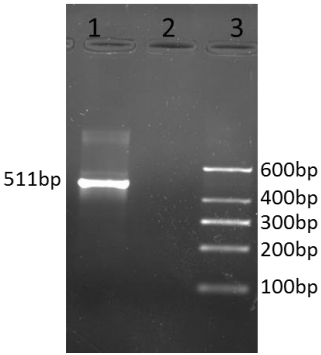


Fig 1. Representative gel image showing amplification of the DENV D1/D2 sequence. Lane 1: Positive amplification at 511-bp; Lane 2: Negative control; Lane 3: DiAGSure DNA ladder.

Sensitivity:

The DiAGSure Dengue Detection Kit can detect <10 copies of the virus.

Quality Control:

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

Troubleshooting Guide:

1. *No amplification in a positive sample*

- Carry out a re-PCR from 1µL of the PCR product using the same set of primers and 2X master mix under the same set of conditions.
- Ensure that RNA has been properly isolated.
- Use freshly isolated RNA for amplification.
- The working desk for RNA isolation should be clean and properly wiped with 70% ethanol.
- Clean the working area and the nozzles of the pipette with RNaseZIP (Cat. No. G7111; Not provided).
- 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.
- It is advisable to store the reagents in aliquots for multiple uses.

2. *Amplification in negative control*

- Indicates that reagents have been contaminated. Repeat the reaction using a fresh aliquot.

3. *Variability among replicates*

- This can be due to manual pipetting error. In case of multiple replicates, prepare a master mix and aliquot it into replicate tubes.

4. *Clear gel with no bands*

- Ensure that EtBr has been added to the gel.
- Ensure mild cooling of the gel post dissolution of the agarose prior to EtBr addition.

Safety information:

The DiAGSure Dengue 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.