DiAGSure Japanese Encephalitis Virus Detection Kit

20 Tests

For research use only

DiAGSure DIAGNOSTIC
For the people, by the people

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Description:

Japanese Encephalitis Virus (JEV) is an arbovirus of the family Flaviviridae. It propagates in a zoonotic cycle involving pigs, ardeid birds and Culex mosquitoes. Humans are accidental/dead end hosts of JEV infection because they cannot sustain high viral titers, though 1 in 250 JEV infections develop into encephalitis. As the latent period or asymptomatic phase of the infection ranges in days, specific and fast diagnosis of JEV infection is necessary.

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 JEV.

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

Principle:

The DiAGSure JEV Detection Kit involves semi-quantitative RT-PCR based detection of a conserved JEV 488-bp sequence in the JEV 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. Reverse transcriptase converts the viral RNA to cDNA which serves as a template for PCR. 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 JEV pathogen in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the JEV
- ✓ 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:

Kit Contents	Volume for 20 Tests
10X RT buffer	50 µL
GRTScript Reverse Transcriptase	25 µL
dNTP-Primer Mix	50 µL
JEV Primer mix	45 µL
DiAGPol PCR Master Mix	500 µL
DiAGSure DNA Ladder	100 µL
Gel loading dye	100 µL
Nuclease-free water	500 µL

PROTOCOL :

Sample Material Preparation:

The DiAGSure JEV Detection Kit detects the presence of the JEV 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 488-bp region.

Basic workflow:

Prepare serum from blood
↓
Isolate viral RNA from serum
↓
Set up the RT reaction
↓
Set up the PCR reaction using RT products as template
↓
Run the PCR products in a 2% agarose gel

RT reaction set up and conditions:

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

Isolated RNA	6 µL
dNTP-Primer mix	2 µL

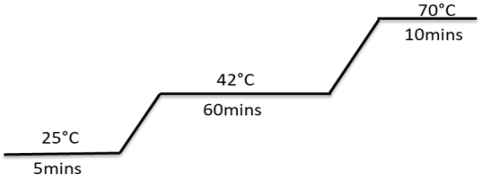
2. Incubate the mix at 65°C for 5mins followed by quick-chill on ice for 2-3 mins.

3. Add the following reagents to the tube:

10X RT buffer	1 µL
GRTScript Reverse Transcriptase	1 µL

Mix vigorously 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 for a single cycle under the following cycling conditions:

Stage	Temperature (°C)	Time
Annealing	25	5mins
Extension	42	60mins
Inactivation	70	10mins
Final hold	4	∞



Diagrammatic view of RT reaction conditions

PCR Protocol

Set up a 20 µL PCR reaction by adding the following reagents in a 0.2mL PCR tube:

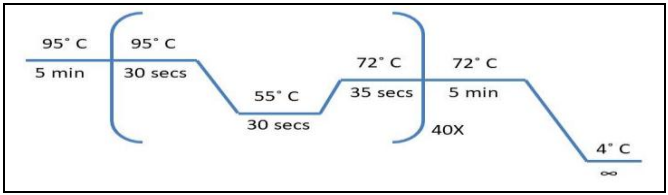
Template cDNA	4 µL
DiAGPol PCR Master Mix	10 µL

H1N1 primer mix	1 µL
Nuclease-free water	5 µL

Set up a No Template Control (NTC) reaction, replace cDNA with 10 µL of Nuclease free water (not provided) and add JEV primer mix and DiAGPol PCR Master Mix accordingly.

PCR conditions:

Stage	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	40
Annealing	55	30 secs	
Extension	72	35 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1

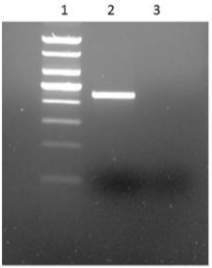


Diagrammatic view of PCR cycling conditions

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 488-bp band appearing in between 400bp and 500bp with respect to DiAGSure DNA Ladder indicates the presence of the JEV in the clinical sample. The absence of the 488-bp band in the test sample indicates the absence of JEV infection (See Fig 1).



Lane No.	Details
1	DiAGSure DNA ladder (100, 200, 300, 400, 500, 600, 800, 1000bp)
2	Positive amplification (488 bp)
3	NTC

Fig 1. Representative gel image of positive amplification from clinical samples. Lane 1:.DiAGSure DNA ladder; Lane 2: JEV amplification (488bp); Lane 3: Negative control.

Sensitivity:

The DiAGSure JEV Detection Kit can detect 0.31 amol of the virus RNA.

Quality Control:

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

Precautions:

- 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.
- For long-term storage, it is advisable to store the reagents (especially the enzymes) in aliquots.

Safety information:

The DiAGSure JEV 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.