DiAGSure West Nile Virus (WNV) Detection Kit

20 Tests

For research use only

DiAGSure DIAGNOSTIC
For the people, by the people

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Description:

West Nile Virus (WNV) is a virus belonging to family Flaviviridae and is spread by *Culex* mosquitoes feeding on infected birds. The symptoms of the disease include fever, headache, body aches, skin rash, and swollen lymph nodes. The severe cases may involve stiff neck, sleepiness, disorientation, coma, tremors, convulsions, and paralysis. Initially isolated in Uganda in 1937, the disease has spread worldwide. Japanese encephalitis virus (JEV) is found to dominate in the southern states of India, though it is widespread in Haryana, Assam and Maharashtra. The WNV genome 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 WNV.

DISCLAIMER: The DiAGSure West Nile Virus (WNV) Detection Kit has been designed for *in-vitro* use only.

Intended Use:

This kit amplifies a unique 480-bp sequence specific for WNV and is absent in other closely-related viruses of Flavivirus genus. This kit also contains a standard marker for size comparison of the amplicon.

Principle:

The DiAGSure West Nile Virus (WNV) Detection Kit involves semi-quantitative RT-PCR based detection of a conserved specific 480-bp sequence in the WNV 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 West Nile Virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the West Nile 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 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:

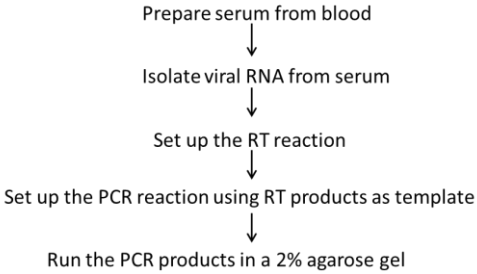
(Storage: -20°C, in a Frost-free freezer):

Kit Contents	Volume for 20 tests
10X RT buffer	50 µL
GRTScript Reverse Transcriptase	25 µL
dNTP-Primer Mix	50 µL
WNV 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

Sample Material Preparation:

The DiAGSure West Nile Virus Detection Kit detects the presence of the West Nile virus in human serum samples. Isolate viral RNA from serum. Use a specified amount (see below) of this RNA and prepare cDNA which has to be used as a template for amplification of the 480-bp region.

Basic workflow:



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

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

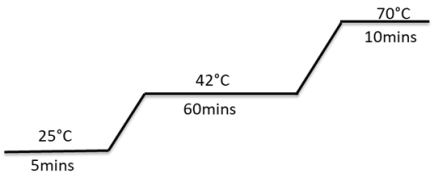
2. Incubate the mix at 65°C for 5mins followed by quick-chill on ice for 2-3mins.

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:

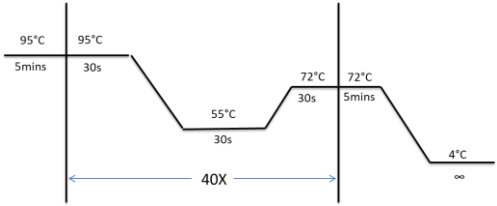
To the RT Reaction tube, add the following constituents:

Template cDNA	10 µL
DiAGPol PCR Master Mix	9 µL
WNV primer mix	1 µL

Set up a No Template Control (NTC) reaction, replace cDNA with 10 µL of Nuclease free water and add WNV 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	30 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 band of 480-bp size appearing close in between 400bp and 500bp with respect to the standard marker indicates the presence of the West Nile Virus in the clinical sample. The absence of the 480-bp band in the test sample indicates the absence of WNV infection (See Fig 1).

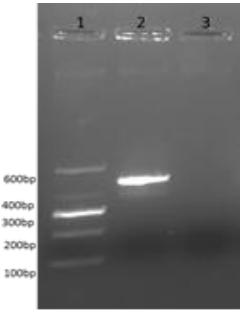


Fig 1. Representative gel image showing amplification of the WNV sequence. Lane 1: DiAGSure DNA ladder; Lane 2: Positive amplification of the WNV amplicon at 480-bp; Lane 3: Negative control.

Sensitivity:

The DiAGSure West Nile Virus (WNV) Detection Kit is highly sensitive and can detect <10 copies of the virus under *in vitro* conditions.

Quality Control:

All reagents in the DiAGSure West Nile Virus (WNV) 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.
- It is advisable to store the reagents in aliquots for multiple uses.

Safety information:

The DiAGSure West Nile Virus (WNV) 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.