

A decorative graphic consisting of numerous thin, parallel blue lines that curve and overlap to form a large, smooth, wave-like shape in the upper half of the page.

## **DiAGSure Chikungunya Detection Kit**

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**20 Tests**

*For research use only*

## Description:

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Chikungunya is a tropical mosquito-borne disease caused by Chikungunya virus (CHIKV). The symptoms of the disease include fever and acute joint pain along with nausea, rash, headache, and fatigue. The symptoms are often similar to dengue and zika. Over 60,000 cases of Chikungunya were reported in India in 2017. As such, accurate diagnosis of the disease proves helpful for proper treatment of the disease. The chikungunya virus belongs to family *Togaviridae*. 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 CHKV.

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

## Intended Use:

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This kit amplifies a specific 212-bp region in the CHIKV genome. This region is specific for chikungunya virus and is absent in other RNA viruses. This kit also contains a standard marker for size comparison of the amplicon.

## Principle:

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The DiAGSure Chikungunya Detection Kit involves semi-quantitative RT-PCR based detection of a conserved CHIKV 212-bp sequence 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:

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- ✓ Fast and simple
- ✓ Rapid detection of chikungunya virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the ChikV pathogen
- ✓ Reproducibility of results

## Storage and Shelf life:

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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
CHIKV 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

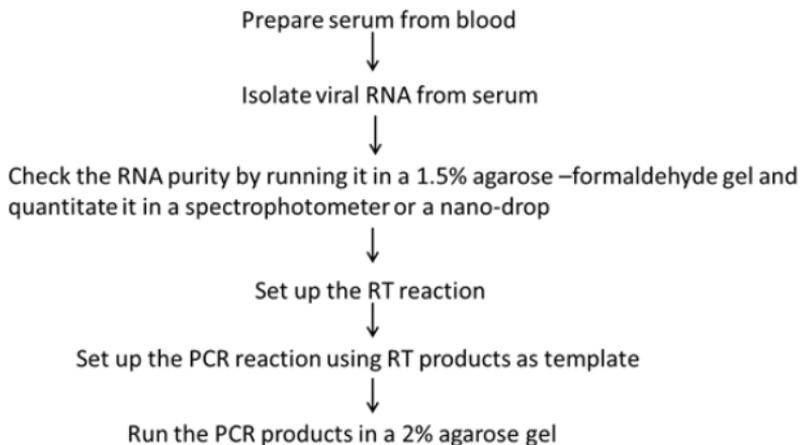
## Sample Material Preparation:

The DiAGSure Chikungunya Detection Kit detects the presence of the Chikungunya 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 212-bp region.

Starting volume of serum = 200µL

Elution volume = 30µL

## Basic workflow:



## RT reaction set up and conditions:

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

<b>Isolated RNA</b>	6 $\mu$ L
<b>dNTP-Primer mix</b>	2 $\mu$ L

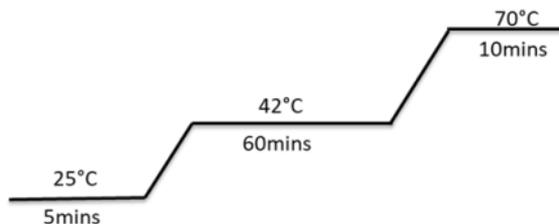
2. Heat the mix at 65 $^{\circ}$ C for 5mins followed by quick-chill on ice for 5mins.

3. Add the following reagents to the tube:

<b>10X RT buffer</b>	1 $\mu$ L
<b>GRTScript Reverse Transcriptase</b>	1 $\mu$ L

4. 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 at the following cycling conditions:

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



Diagrammatic view of RT reaction conditions

**PCR Protocol:**

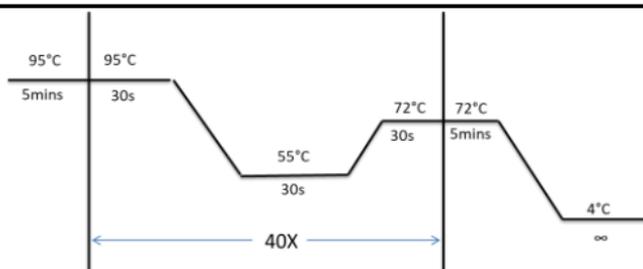
Set up a 25µL PCR reaction by adding the following constituents in a PCR tube:

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

Set up a No Template Control (NTC) reaction, replace cDNA with 10µL of Nuclease free water and add CHIKV 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	
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:

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The presence of a band of 212-bp appearing in between 200bp and 300bp with respect to the standard marker indicates the presence of Chikungunya virus in the clinical sample. The absence of the 212-bp band in the test sample indicates the absence of Chikungunya infection (See Fig 1).

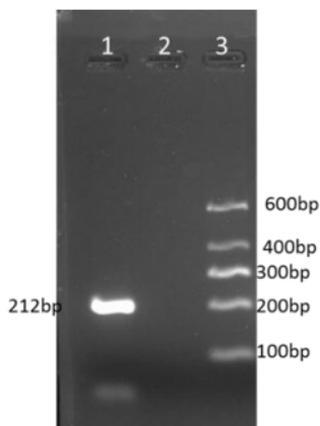


Fig 1. Representative gel image showing amplification of the CHIKV sequence. Lane 1: Positive amplification at CHIKV 212-bp region; Lane 2: Negative control; Lane 3: DiAGSure DNA ladder.

### Sensitivity:

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

### Quality Control:

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

### Precautions:

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- ▲ 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:**

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The DiAGSure Chikungunya Detection Kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

### **Technical assistance:**

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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](mailto:tech.support@gccbiotech.co.in).