

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 Influenza A (H1N1) Detection Kit**

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**20 Tests**

*For research use only*

## Description:

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Influenza A virus is the most common cause of flu. This Orthomyxoviridae member has haemagglutinin (H) and neuraminidase (N) cell surface glycoproteins. The influenza virus is classified into subtypes based on the structure of the H and N antigens. The 16 H and 9 N types in combination forms the different Influenza A subtypes. The swine flu virus (H1N1) was the cause of influenza outbreak in 2009 and the Spanish flu in 1918. The symptoms include cough, fever, sore throat, stuffy or runny nose, body aches, headache, chills and fatigue and in severe cases, it leads to pneumonia and severe respiratory troubles. The genome of H1N1 comprises of eight segments of single-stranded (-) RNA (Baltimore Class V). Upon entering a cell, the (-) RNA serves as a template for synthesis of the complimentary (+) RNA which can be translated into proteins. Reverse Transcriptase-Polymerase chain reaction (RT-PCR) has been proven to be extremely useful and a sensitive diagnostic tool to detect the presence of RNA viruses, including H1N1.

DISCLAIMER: The DiAGSure Influenza A (H1N1) Detection Kit has been designed for *in-vitro* use only.

## Intended Use:

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This kit amplifies a specific 173-bp sequence specific for Influenza A Virus (H1N1) and is absent in other closely-related Orthomyxoviruses. This kit also contains a standard marker for size comparison of the amplicon.

## Principle:

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The DiAGSure Influenza A (H1N1) Detection Kit involves semi-quantitative RT-PCR based detection of a conserved specific 173-bp sequence in the H1N1 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:

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- ✓ 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:

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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:

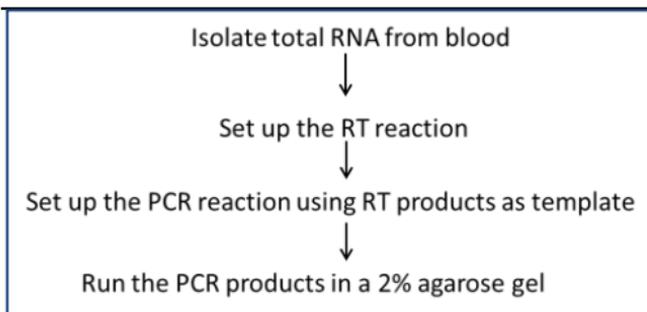
**(Storage: -20°C, in a Frost-free freezer):**

Kit Contents	Volume for 20 tests
10X RT buffer	50 µL
GRTScript Reverse Transcriptase	25 µL
dNTP-Primer Mix	50 µL
H1N1 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 Influenza A (H1N1) Detection Kit detects the presence of the Influenza A Virus (H1N1) in human blood samples. Isolate viral RNA from blood. Use a specified amount (see below) of this RNA and prepare cDNA which has to be used as a template for amplification of the 173-bp region.

## Basic workflow:



Starting volume of blood: 200µL

Elution volume: 30µL

## RT reaction set up and conditions:

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

<b>Isolated RNA</b>	6 µL
<b>dNTP-Primer mix</b>	2 µL

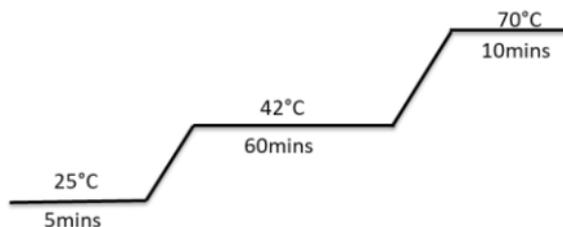
- Incubate the mix at 65°C for 5mins followed by quick-chill on ice for 2-3 mins.
- Add the following reagents to the tube:

<b>10X RT buffer</b>	1 µL
<b>GRTScript Reverse Transcriptase</b>	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:

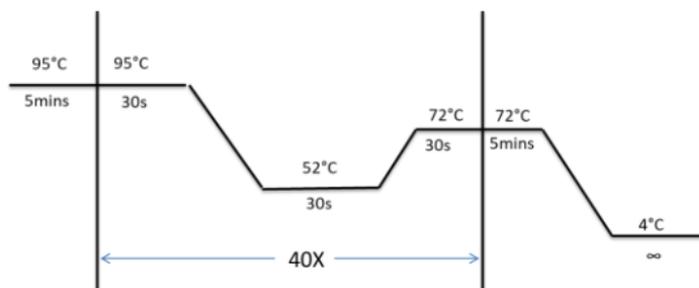
Set up a 25µL PCR reaction by adding the following constituents in a PCR tube:

Template cDNA	10 µL
DiAGPol PCR Master Mix	9 µL
H1N1 primer mix	1 µL

Set up a No Template Control (NTC) reaction, replace cDNA with 10  $\mu$ L of Nuclease free water and add H1N1 primer mix and DiAGPol PCR Master Mix accordingly.

### PCR conditions:

Stage	Temperature ( $^{\circ}$ C)	Time	No. of cycles
<b>Initial denaturation</b>	95	5 mins	1
<b>Denaturation</b>	95	30 secs	40
<b>Annealing</b>	50	30 secs	
<b>Extension</b>	72	30 secs	
<b>Final extension</b>	72	5 mins	
<b>Final hold</b>	4	$\infty$	1



Diagrammatic view of PCR cycling conditions

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

## Results Interpretation:

The presence of a band of 173-bp size appearing close to the 200bp band with respect to the standard marker indicates the presence of the Inf-A virus H1N1 in the clinical sample. The absence of the 173-bp band in the test sample indicates the absence of H1N1 infection (See Fig 1).

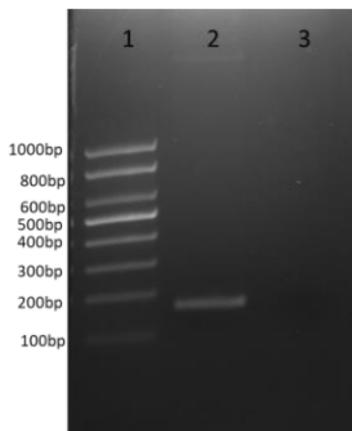


Fig 1. Representative gel image showing amplification of the Inf-A H1N1 sequence. Lane 1: DiAGSure DNA ladder; Lane 2: Positive amplification of the H1N1 amplicon at 173-bp; Lane 3: Negative control.

## Sensitivity:

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The DiAGSure Influenza A (H1N1) Detection Kit is highly sensitive and can detect up to 18.7 attomoles of the virus under *in vitro* conditions.

## Quality Control:

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All reagents in the DiAGSure Influenza A (H1N1) 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 Influenza A (H1N1) 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)