DiAGSure Hepatitis A Detection Kit

20 Tests

For research use only

DiAGSure DIAGNOSTIC
For the people, by the people

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Description:

Hepatitis A, the most acute form of viral hepatitis prevalent throughout the globe, is an infectious disease of the liver caused by Hepatitis A virus (HAV) transmitted through the fecal-oral route. Although many cases of the disease are asymptomatic, the general symptoms of Hepatitis A include fatigue, nausea, vomiting, abdominal discomfort, clay-colored bowel movements, loss of appetite, dark urine, joint pain, jaundice and itching. HAV belongs to family Picornaviridae with non-enveloped ss (+) RNA genome (Baltimore group IV). Only one serotype and seven different genetic groups of this virus have been described. The conventional diagnostic tools for HAV detection involve the detection of HAV-specific IgM antibodies in blood and elevated levels of alanine transferase (ALT) enzyme in blood. RT-PCR based pathogen detection is an emerging tool in the diagnostics regime and highly sensitive at detection of HAV in infected human blood samples.

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

Intended Use:

This kit amplifies a unique 158-bp sequence specific for Hepatitis A virus (HAV) and is absent in other closely-related Picornaviridae members and other viruses causing hepatitis. This kit also contains a standard marker for size comparison of the amplicon.

Principle:

The DiAGSure Hepatitis A Detection Kit involves semi-quantitative RT-PCR based detection of a conserved specific 158-bp sequence in the HAV 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 the virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the 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
HAV Primer mix	45 µL
DiAGPol PCR Master Mix	500 µL
DiAGSure DNA Ladder	100µL
Gel loading dye	100µL
Nuclease free water	500 µL

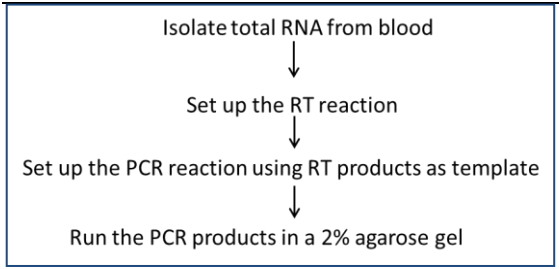
Sample Material Preparation:

The DiAGSure Hepatitis A Detection Kit detects the presence of the Hepatitis A virus (HAV) in human blood samples. Isolate total 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 158-bp region.

Starting volume of blood = 200µL
Elution volume = 30µL

Basic workflow:



RT reaction set up and conditions:

1. For setting up the RT reaction (10µL 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

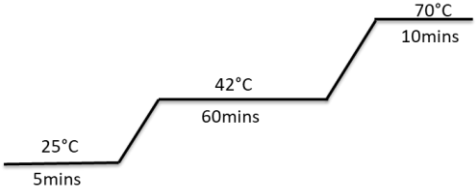
2. Incubate the mix at 65°C for 5mins followed by quick-chill on ice for 2-3 mins.

3. Add the following reagents to the tube:

10X RT buffer	1 µL
GRTScript Reverse Transcriptase	1 µL

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 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:

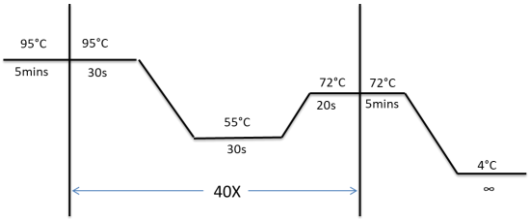
To the RT Reaction tube, add the following constituents:

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

Set up a No Template Control (NTC) reaction, replace cDNA with 10 µL of Nuclease free water and add HAV 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	20secs	
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 158-bp band close to 200-bp size of the standard marker indicates the presence of the Hepatitis A virus (HAV) in the clinical sample. The absence of the 158-bp band in the test sample indicates the absence of HAV infection (See Fig 1).

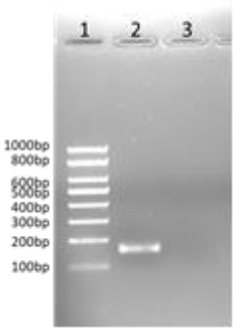


Fig 1. Representative gel image showing amplification of the HAV sequence. Lane 1: DiAGSure DNA ladder; Lane 2:Positive amplification of the HAV amplicon at 158-bp; Lane 3: Negative control.

Sensitivity:

The DiAGSure Hepatitis A Detection Kit is highly sensitive and can detect a minimum of 31 viral RNA copies under *in vitro* conditions.

Quality Control:

All reagents in the DiAGSure Hepatitis A virus (HAV) 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 Hepatitis A virus (HAV) 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.