DiAGSure Hepatitis B virus Detection Kit

Description:

Hepatitis B virus (HBV) is the causative agent of the liver disease Hepatitis B. The virus infects the liver leading to liver cirrhosis, liver failure and hepatocellular carcinoma. Both acute and chronic cases have been reported. Hepatitis B is the most common serious liver infection in the world and is more common in young adults aged 20-50 years. According to an estimate, about 2 billion people bear serological evidence of past or present HBV infection. India has over 40 million HBV infected patients and has the second highest burden of Hepatitis B infected individuals after China. The HBV is a DNA virus with a double-stranded gapped DNA genome that replicates through an RNA intermediate (Fam. Hepadnaviridae). Among the different means of Hepatitis B diagnosis, polymerase chain reaction (PCR) has been proven to be extremely useful and a sensitive diagnostic tool.

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

Intended Use:

This kit detects a conserved 140-bp region in the HBV genome specific for this virus. This kit also contains an internal control which is set-up in a separate tube and amplifies a 221-bp region from human DNA. This internal control has been included to ensure proper DNA extraction and PCR reaction in the absence of amplification in the target sequence.

DiAGSure DIAGNOSTIC for the people, by the people

GCC Biotech (India) Pvt. Ltd.
tech.support@gccbiotech.co.in| 91-33-24951044/24950004

Principle:

The DiAGSure Hepatitis B virus Detection Kit involves semi-quantitative end-point PCR based detection of a conserved 140-bp region in the HBV 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. 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 Hepatitis B virus in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the Hepatitis B pathogen
- ✓ 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:

Table for kit of 100 tests:

Part 1 of 3 (Storage: Room temperature)

GSure Blood DNA Mini kit (Cat. No. XXXXX) – 100preps

Part 2 of 3 (Storage: Room temperature)

Kit Contents	Catalog No.	Volume for 100 tests
Agarose	G4652	100g
GPure TAE Buffer, 40X	GS1006	220mL
0.2mL PCR tubes		350 no.s
1.5mL microcentrifuge tubes		100 no.s
Ethidium Bromide Solution (10mg/ml)	GCR-28	500µL

N.B.: On receipt, store the Ethidium bromide solution at 4°C.

Part 3 of 3 (Storage: -20°C)

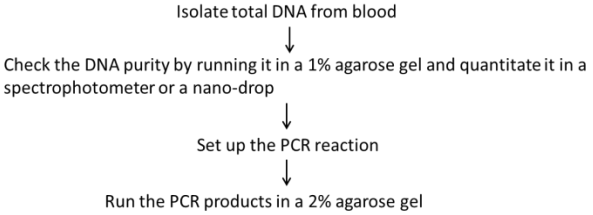
Kit Contents	Catalog No.	Volume for 100 tests
HBV Primer mix	-	330µl
DiAGPol PCR Master Mix	G7117	1.5mL X 2
DiAGSure DNA ladder		500µL

Positive control primer mix		330µl
6X Gel loading dye	GCR-19	1.75mL
Nuclease free water	GCR-30	1.5mL X 3

Sample Material Preparation:

The DiAGSure Hepatitis B virus Detection Kit detects the presence of Hepatitis B virus in human blood samples. Isolate total DNA from blood (which includes viral DNA in case of infected samples) using GSure Blood DNA isolation kit provided as a part of this kit. Use a specified amount (see below) of this DNA to amplify the 140-bp region of the HBV genome.

Basic workflow:



PCR Protocol:

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

Nuclease-free water –	6.5µL
2X G9 Taq PCR Master Mix –	12.5µL
HBV Primer mix –	1µL
Template DNA –	5µL

PCR conditions:

Stage	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	35
Annealing	60	30 secs	
Extension	72	15 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1

Agarose gel electrophoresis and gel visualization:

1. Cast a 2% Agarose gel as follows. Weigh out 1g of Agarose and add it to 50mL of 1X TAE buffer taken in a conical flask and heat it until the Agarose has dissolved completely. Cool the flask slightly and add 2µL of 10mg/mL Ethidium bromide

solution and pour immediately into the gel casting tray. After gel solidification, place the gel in 1X TAE buffer for 15mins for equilibration.

2. Add 1µL of 6X DNA loading dye to the PCR product and load it into appropriate wells of the gel placed in the gel running apparatus containing fresh 1X TAE buffer. Visualize the gel in a UV-trans illuminator or a gel documentation instrument.

Results Interpretation:

The presence of a 140-bp band appearing in between 100bp and 200bp with respect to the standard marker indicates the presence of the virus in the clinical sample. The absence of the 140-bp band in the test sample indicates the absence of the virus (See Fig 1). However, in the absence of the 140-bp band in the test sample, amplification of the 221-bp internal control must be obtained to ensure proper extraction and amplification process.

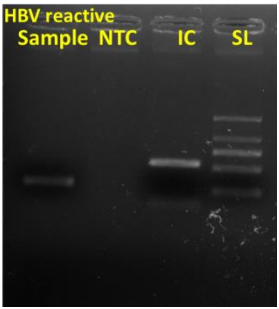


Fig 1. **Representative gel image showing amplification of the HBV gene. Lane**
1: Positive amplification at 140-bp; Lane
2: Negative control; Lane
3: 221-bp Internal control (IC); Lane
4: DiAGSure DNA ladder (SL).

Sensitivity:

The DiAGSure HBV Detection Kit can detect ≥ 6X 1010 copies of the Virus.

Quality Control:

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

Troubleshooting Guide:

1. *No amplification in internal control*
- Ensure that DNA has been properly isolated.
- Use freshly isolated DNA for amplification.
- 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.

2. *Amplification in negative control*
- Indicates that reagents have been contaminated. Repeat the reaction using a fresh aliquot.

3. *Variability among replicates*
- This can be due to manual pipetting error. In case of multiple replicates, prepare a master mix and aliquot it into replicate tubes.

4. *Clear gel with no bands*

- Ensure that EtBr has been added to the gel.
- Ensure mild cooling of the gel post dissolution of the Agarose prior to EtBr addition.

Safety information:

The DiAGSure HBV 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.