DiAGSure® Hepatitis E Virus TaqMan PCR kit

Catalog No.: GDQ2100- 20R; GDQ2100- 50R; GDQ2100-100R

Color Coding (Caps)	Reagents	Description	GDQ2100-20R (20 tests)	GDQ2100-50R (50 tests)	GDQ2100-100R (100 tests)
Amber	2X one-step Master Mix	Amplification Mix	300 µL	700 µL	1.4 mL
Amber	Primer-Probe Mix	Amplification Reagent	40 µL	100 µL	200 µL
Yellow	HEV Standard 1	Quantification Standard 1	50 µL	100 µL	200 µL
Yellow	HEV Standard 2	Quantification Standard 2	50 µL	100 µL	200 µL
Yellow	HEV Standard 3	Quantification Standard 3	50 µL	100 µL	200 µL
Yellow	HEV Standard 4	Quantification Standard 4	50 µL	100 µL	200 µL
Blue	Negative Control (NC)	Negative Control	200 µL	500 µL	1 mL
White	RNA Dilution Buffer	Tris-based buffer	200 µL	500 µL	1 mL

Intended Use:

Hepatitis E is an infectious disease of the liver caused by Hepatitis E Virus (HEV); it is a type of viral hepatitis. Symptoms of hepatitis E range from Fatigue, sudden nausea and vomiting, abdominal pain or discomfort diarrhea, dark-coloured urine and jaundice (a yellowing of the skin and whites of the eyes). Not everyone who is infected will have all of the symptoms.

The DiAGSure® Hepatitis E Virus TaqMan PCR kit PCR assay can detect HEV and is a much more sensitive and fast detection method for the detection of viral infection. The DiAGSure® Hepatitis E Virus TaqMan PCR kit (Multiplex) is an in vitro diagnostic (IVD) Real-time Reverse Transcriptase-polymerase chain reaction (qRT-PCR) test involving TaqMan chemistry intended for the quantitative detection of viral RNA in human plasma and serum samples. This two-plex PCR detects the HEV capsid gene and includes an endogenous RNase-P internal control (IC) in a single-tube reaction.

Estimated operating time:

~1 hour(s) 20 minutes

Kit Stability:

- Kit is shipped on dry ice and should be stored immediately upon receipt at -20°C in a constant temperature freezer. Please refer to product label for final expiry date.
- Repeated thawing and freezing (>7x) should be avoided, as this may reduce sensitivity.
- If the reagents are to be used only intermittently, they should be frozen in aliquots.

Specimen handling & Storage:

After sample collection, perform the test on the same day. Otherwise, store it in the following condition: 2 to 8˚C for no more than 24 hours. Storage condition is below -20 ˚C for no more than 3 days. Samples can be stored for a long time at -80˚C. Repeated freezing-thawing of samples should be avoided.

Transportation:

The foam box is sealed with ice for transportation.

Operation procedure

A. Sample solution preparation:

Clinical samples are extracted according to the corresponding requirements and steps with the viral RNA extraction kit, and the extracted RNA could be directly used for detection. If the extracted RNA is not immediately tested after extraction, they can also be stored at -80˚C and repeated freezing and thawing should be avoided. It is advised to start amplification with at RNA of high purity (O.D.260/280 = 2.0). A starting RNA concentration of at least 10 ng/ µL should be used. Too high RNA concentration might necessitate template dilution according to requirement.

*Note: Use of freshly extracted RNA is recommended.

B. Preparation of amplification reagent:

Take the reagents out from the -20˚C freezer and thaw them on ice. After thawing, briefly vortex the reagents followed by a short spin. The PCR reagents should be kept on ice.

C. TaqMan qRT-PCR protocol:

Per sample, set up a single reaction according to the following table:

Components	Volume per reaction
2X one-step Master Mix	13 µL
Primer-Probe Mix	2 µL
Extracted RNA/ Quantification Standard/Negative Control (NC)	10 µL
Total Volume	25 µL

D. Real-time PCR Instrument set up:

Set up the PCR tubes/strips/plate in the Real time PCR machine and label the sample slots accordingly. Select the sample type (Unknown/PC/NTC) and select the acquisition channel for each slot as follows:

Gene	Channel	Source wavelength (nm)	Detection wavelength (nm)
HEV capsid gene	HEX/VIC (Yellow)	553	559

Reference gene (IC)	Cy5 (Red)	625	660

E. PCR cycling conditions:

Step	Temperature (°C)	Time	No. of cycles
Hold stage 1(RT-step)	50	10 min	1
Hold stage 2	95	3 min	1
PCR Stage	95	10 sec	45
	58	30 sec*	
***Acquisition step (Yellow/ Red Channel)**			

F. Instrument compatibility:

DiAGSure® Hepatitis E Virus TaqMan PCR kit is compatible with a broad range of real-time PCR platforms. Performance of the kit has been verified on the following systems:

Applied Biosystems® 7500/7500 Fast Real-Time PCR System, QuantStudio 5, BIORAD™ CFX96, QIAGEN® Rotor Gene Q, Roche Light-cycler® 480 and Analytik jena qTOWER^3-G.

G. Performance Evaluation:

- **Specificity:**

  The target sequences detected in this kit specifically targets a conserved region in the capsid gene of HEV. The primers used in the assay failed to give any amplification from RNA extracted from the blood of healthy individuals eliminating the possibility of non-specific annealing with control human RNA.

  Furthermore, the primers did not cross-react with viruses like Japanese encephalitis virus (JEV), West Nile virus, Chikungunya virus, Nipah virus, Hepatitis A virus, Hepatitis C virus, Hepatitis B virus Also, the primers did not cross-react with the most common bacteria like *E. coli*, *B. subtilis*, *Pseudomonas* sp. as well as pathogens like *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Vibrio cholerae*, *Chlamydia trachomatis*, and *Salmonella typhi*, and the protozoa *Plasmodium* sp. and *Giardia lamblia*.

- **Linear range and LoD:**

  The analytical sensitivity of the assay was obtained using a dilution series of quantified in-vitro transcribed HEV RNA spiked in negative plasma sample as a template for detection. The linearity of the kit was observed in the range 10 – 10^8 RNA copies/µL. The Limit of Detection (LoD) of the kit is 25 RNA copies/mL under in-vitro conditions. The assay was performed in six independent replicates each in triplicate.

- **Safety information:**

  The DiAGSure® Hepatitis E Virus TaqMan PCR kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

- **C_t cut-off for each fluorescent channel:**

Target	Cut-off C_t	Interpretation
Viral gene (HEX/VIC)	Ct≤40	Viral RNA positive
IC (Cy5)	Ct≤35	Internal control positive

- **Interpretation of the results:**

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, the patient's result cannot be interpreted.

Refer to the table below for the validity and the interpretation of each specimen result according to the results of each channel.

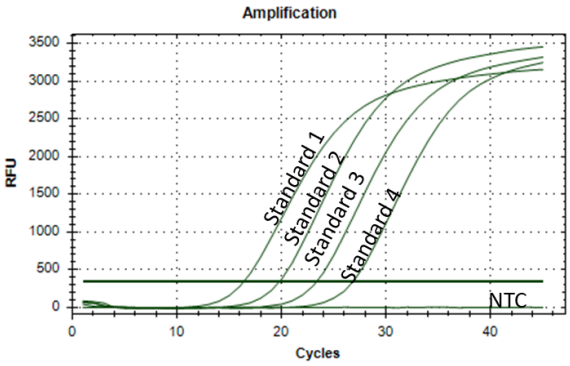
Target	Viral gene	IC	Interpretation	Results
Fluorophore*	HEX/Yellow	Cy5/Red		
Case-1	Positive	Positive/ Negative	HEV Positive	Viral infection is detected in the clinical specimen.
Case-2	Negative	Positive	HEV Negative	Viral infection is not detected in the sample.
Case-3	Negative	Negative	Invalid	Testing should be repeated once from extraction. If a second failure occurs, it is reported to sender as invalid and sample recollection is recommended.

* If the target gene signal (HEX/VIC Channel) is strong, the IC (Cy5 Channel) may be negative.

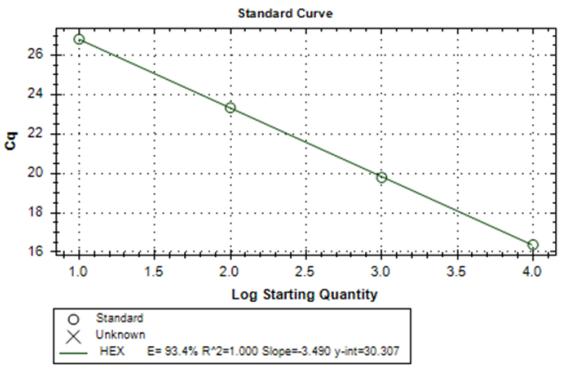
- **Viral Load Estimation:**

There are four quantification standards provided with the kit with viral RNA copies as follows –

Standard	Copies/µL
HEV Standard 1	10^6
HEV Standard 2	10^5
HEV Standard 3	10^4
HEV Standard 4	10^3



Amplification from HEV standards



HEV standard curve

A plot of Ct value vs RNA copies of the standards will fetch an equation wherefrom the viral copy number of an unknown sample can be deduced from its Ct value. The quantification standards exhibit linearity when Ct values are plotted against log (copies/ µL) with R^2 value>0.98. The efficiency of amplification should be above 70% with the slope of the standard curve in the range -3.1 to -3.9.

The quantitation standards are defined as RNA copies/µL. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

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.