# DiAGSure® Hepatitis C Viral Load Kit

**Catalog No.: GDQ2042-25R; GDQ2042-50R; GDQ2042-100R**

For use with： Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems［ABI］7500/QuantStudio 5/6, Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

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### Background:

Hepatitis C is an inflammation of the liver caused by the hepatitis C virus. The virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness to a serious, lifelong illness including liver cirrhosis and cancer. The symptoms can include fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine and yellowing of the skin or eyes (jaundice). The hepatitis C virus is a bloodborne virus and most infection occur through exposure to blood from unsafe injection practices, unsafe health care, unscreened blood transfusions, injection drug use and sexual practices that lead to exposure to blood. Globally, an estimated 58 million people have chronic hepatitis C virus infection, with about 1.5 million new infections occurring per year. There are an estimated 3.2 million adolescents and children with chronic hepatitis C infection. There is no vaccine for hepatitis C, but it can be treated with antiviral medications. Early detection and treatment can prevent serious liver damage and improve long-term health. HCV has six genotypes, labeled 1 through 6. HCV genotype 1 may represent a more aggressive strain and one that is less likely to respond to interferon treatment than HCV genotype 2 or 3.

### Test Principle:

The DiAGSure® Hepatitis-C Viral Load Kit 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 Hepatitis C virus RNA in human serum or plasma. A highly conserved region in the viral 5' UTR sequence has been targeted. The kit covers HCV genotypes 1-6. This two-plex PCR includes an RNase-P internal control (IC) in a single- tube reaction.

### Sample types:

Human Plasma, Serum, whole blood, tissue biopsy and other body fluids

### Estimated operating time:

~1 hr 20 mins

### Kit Contents:

| Cap | Contents | Description | GDQ2039-25R | GDQ2039-50R | GDQ2039-100R |
|---|---|---|---|---|---|
| Red | UNG+ OS Master Mix | Enzyme Mix | 120 µL | 240 µL | 500 µL |
| Amber | Primer-Probe Mix | Amplification Reagent | 60 µL | 120 µL | 250 µL |
| Yellow | HCV Standard 1 | Quantification Standard | 50 µL | 100 µL | 200 µL |

| | | | | | |
|---|---|---|---|---|---|
| Yellow | HCV Standard 2 | Quantification Standard | 50 µL | 100 µL | 200 µL |
| Yellow | HCV Standard 3 | Quantification Standard | 50 µL | 100 µL | 200 µL |
| Yellow | HCV Standard 4 | Quantification Standard | 50 µL | 100 µL | 200 µL |
| Blue | Negative Control (NC) | Negative Control | 250 µL | 500 µL | 1 mL |
| White | RNA Dilution Buffer | Tris-based buffer | 250 µL | 500 µL | 1 mL |

**Materials required but not provided**

*Reagents*

- RNA extraction components: Trizin-XP Viral RNA Isolation kit (from plasma/serum/body fluid).
- Nuclease free/sterile PCR grade water

*Consumables*

- Disposable tips containing hydrophobic filters
- Sterile DNase-free 1.5 ml vials (Eppendorf tubes)
- Suitable DNase-free 0.1 or 0.2 ml PCR tubes for use of RGQ instruments
- Suitable multiwell 96 plates (Roche, product code 04 729 692 001) for use of LC480 II instruments
- RNase-free Mic tubes and caps (BMS, ref 60653) for use on the Mic qPCR cycler
- Hard-shell® PCR plates 96-well, thin wall (Bio-Rad, Hsp9655) for use on the CFX96TM
- MicroAmp Fast Optical 96-well reaction plate 0.1 ml (Applied Biosystems, ref 4346906) for use on the QS5

*Equipments*

- Adjustable pipettes: 0.1-2 µl, 2-20 µl, 20-200 µl, 200-1000 µl
- Tube rack for 1.5 ml vials
- Cooling block (1.5 ml) or ice (for placing kit components during reaction set up)
- PCR cooling block or ice for PCR reaction tubes and 96-well plates
- Vortex mixer
- Benchtop centrifuge with a rotor for 1.5 ml tubes
- Centrifuge suitable for PCR plates
- Calibrated real-time PCR instrument
- Automated RNA extraction system

### 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.
- This product can be used for 30 days after opening the vials.
- This product can be used for maximum 10 repeats of freezing and thawing.

### 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 below -70 ˚C. Repeated freezing-thawing of samples should be avoided.

### Transportation:

The foam box is sealed with ice for transportation.

### Operation procedure

### A. Sample 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 -20°C and repeated freezing and thawing should be avoided. We recommend a starting sample volume of 200-500 µL and the elution volume to be 50-70 µL. It is advised to start amplification with at RNA of high purity (O.D.260/280 = 1.8-2.0). A starting RNA amount of 100-500 ng 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 qPCR protocol:

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

| Components | Volume per reaction |
|---|---|
| **UNG+ OS Master Mix** | 4 µL |
| **Primer-Probe Mix** | 2 µL |
| **Extracted RNA/ Standards/Negative Control (NC)** | 10 µL |
| **RNA Dilution buffer** | 4 µL |
| **Total Volume** | 20 µL |

## D. Real-time PCR Instrument set up:

Set up the PCR tubes/strips/plate in the real time 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) |
|---|---|---|---|
| HCV 5'-UTR | HEX/VIC (Yellow) | 533 | 559 |
| Reference gene (IC) | Cy5 (Red) | 625 | 660 |

## E. PCR cycling conditions:

| Step | Temperature (°C) | Time | No. of cycles |
|---|---|---|---|
| RT Reaction | 50 | 10 min | **1** |
| Hold stage | 95 | 3 min | 1 |
| PCR Stage | 95 | 10 sec | 45 |
| | 58 | 30 sec* | |

*Acquisition step (Yellow/Red Channel)

## F. Instrument compatibility:

The DiAGSure® Hepatitis C Viral Load Kit is compatible with a broad range of real-time PCR platforms. Performance of the kit has been verified on the following systems:

Agilent, Bio-Rad CFX96, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI] 7500/QuantStudio 5/6, Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

## G. Performance Evaluation:

- **Specificity:**

The target sequences detected in this kit specifically targets the conserved 5'-UTR of HCV covering all 6 genotypes (1-6). The primers used in the assay failed to give any amplification from DNA extracted from the blood of healthy individuals eliminating the possibility of non-specific annealing with control human DNA.

Furthermore, the primers did not cross-react with other related viruses like HAV, HBV, HIV-1, HSV-1/2, CMV, EBV, Adenovirus, Influenza virus A & B, Rhinovirus and COVID-19; as well as the most common bacteria like *E. coli, B. subtilis, Pseudomonas* sp. and pathogens like *Mycobacterium tuberculosis, Streptococcus pneumoniae, Vibrio cholerae, Chlamydia trachomatis*, and *Salmonella typhi*. Hence the specificity of the kit is 100%.

- **Linear range and LoD**

The analytical sensitivity of the assay was obtained using a dilution series of quantified in-vitro transcribed HCV 5'-UTR RNA spiked in negative plasma sample as a template for detection. The

dilution series were calibrated against the 6th WHO International Standard for HCV RNA for NAT (NIBSC Code: 18/184). The linear range of the kit is $4.9X10^1$ IU/mL to $1.2X10^9$ IU/mL. The Limit of Detection (LoD) is 35 IU/mL under in-vitro conditions. The assay was performed in six independent replicates each in triplicate. Linearity is observed in this range.

- **Precision**

Precision was calculated based on intra-assay and inter-assay variability of the lowest copy number kit quantitative standard (HCV Standard 4) for HCV detection (VIC channel) with CV<5%.

Table 1. Intra and Inter-assay variability of HBV standard 4

| | Mean Ct | Std. Dev | Coefficient of variance (%) |
|---|---|---|---|
| **Intra-assay variability : HCV Standard 4** | 33.56 | 0.32 | 1.9 |
| **Inter-assay variability : HCV Standard 4** | 33.54 | 0.58 | 1.8 |

- **Reproducibility**

Reproducibility data permit a regular performance assessment of the DiAGSure® Hepatitis C Viral Load Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

- **Safety information:**

The DiAGSure® Hepatitis C Viral Load 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 |
|---|---|---|
| HCV 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 results 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.

Table 2. Interpretation of result

| Target | HCV 5'-UTR | IC | Interpretation | Results |
|---|---|---|---|---|
| **Fluorophore*** | **VIC/Yellow** | **Cy5/Red** | | |
| Case-1 | Positive | Positive/ Negative | HCV Positive | HCV RNA is detected in the clinical specimen. |
| Case-2 | Negative | Positive | HCV Negative | HCV RNA 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 (VIC/HEX 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 | IU/µL |
|---|---|
| **HCV Standard 1** | $10^4$ |
| **HCV Standard 2** | $10^3$ |
| **HCV Standard 3** | $10^2$ |
| **HCV Standard 4** | $10^1$ |

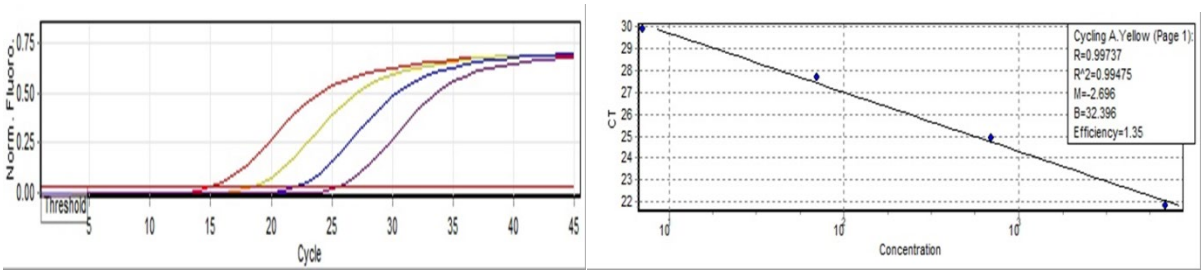


Fig: HCV standard curve plotted using DiAGSure HCV quantitation standards

A plot of Ct value vs DNA IU 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 (IU/ µL) with $R^2$ value>0.99. The efficiency of amplification should be above 80% with the slope of the standard curve in the range -3.1 to -3.8.

The quantitation standards are defined as IU/µL prepared in accordance with 6th WHO International Standard for HCV DNA for NAT (NIBSC Code: 18/184). The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/}\mu\text{l) x Elution Volume (}\mu\text{l)}}{\text{Sample Volume (ml)}}$$

The conversion factor between HCV RNA copies/mL and HCV International Units/mL (IU/mL) is 1 IU = 2.18 copies.

## General precautions

Performing lab activities should always be done in compliance with general safety regulations. For more information on chemicals, consult the appropriate material safety data sheets (MSDS), which is part of the kit insert.

The following precautions should be taken to both avoid contamination and allow optimal performance and reproducibility of the assays:

- The PCR assay should only be performed by qualified laboratory personnel.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Physically separate the workplaces as outlined in table 3.
- Use disposable tips containing hydrophobic filters to prevent cross-contamination.
- Use DNase-free PCR vials.
- Thaw DNA samples always on ice and keep them on ice or on a cooling block.
- Keep enzymes always on ice or on a cooling block when taken out of the freezer. Handle enzymes with care and mix very gently.
- When thawed, spin down the reagents for 5 seconds in a centrifuge and mix by gently pipetting up and down.
- The cycling program should be programmed in the real-time PCR instrument before performing the assay.
- Spin down the PCR mixtures shortly in the PCR plate before transferring to the real- time cycler (not necessary for RGQ).
- Do not open the PCR vials/plates after PCR amplification.

## Reagent storage and handling

Table 3. Handling procedures in different areas

| Location | Handling |
|---|---|
| Area 1 | UNG+ OS-Master Mix, Primer-probe mix<br>Preparation of PCR mix |

| | |
|---|---|
| | Aliquoting of PCR mix in respective wells<br>Addition of Negative Control (NC) |
| Area 2 | Storage of Quantitative Standards<br>RNA extraction from samples<br>Adding RNA extracts to the real-time PCR mix<br>Adding standards to the real-time PCR mix |
| Area 3 | Real-time PCR reaction |

It is recommended to perform all activities in laminar air flow safety cabinet II in order to minimize the chances of cross-contamination.

## 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.

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