

Quantiplus® HCV FAST Detection Kit (Real-Time Qualitative PCR Kit)



QLF-HCV-25 : 25 rxns
 QLF-HCV-50 : 50 rxns
 QLF-HCV-100 : 100 rxns



PI/QLFHCV-05

Intended Use

Quantiplus® HCV FAST Detection Kit is a reverse transcription Real-Time PCR based in vitro diagnostic assay for detection of Hepatitis C Virus (1-6 genotypes) in human plasma. The kit contains Single tube qPCR mix with Reverse Transcriptase and UDG/UNG, Primer Probe Mix (PPM), positive control (HCVFPC), and Internal Control (IC-B mix). This advanced formulation enables performance of fast PCR in shorter run time (≤ 60 min), and UDG/UNG helps in controlling PCR carryover contamination.

This kit is not to be used for screening of blood/blood products from blood donors.

Background Information

Hepatitis C virus causes liver disease which can lead to both acute and chronic hepatitis. It can cause mild illness lasting a few weeks to a serious, lifelong illness. A significant number of chronically infected individuals develop cirrhosis or liver cancer.

Kit Components

| Color Coding (Caps) | Contents | Description | 25 rxns (QLF-HCV-25) | 50 rxns (QLF-HCV-50) | 100 rxns (QLF-HCV-100) |
|---------------------|-------------------------------------|----------------------------------|----------------------|----------------------|------------------------|
| Green | RNA Fast qPCR Mix with UDG/UNG (4X) | PCR Amplification mix | 1 x 165 μ L | 1 x 330 μ L | 2 x 330 μ L |
| Amber | HCV Fast PPM | Target specific Primer Probe Mix | 1 x 50 μ L | 1 x 100 μ L | 2 x 100 μ L |
| Natural | IC-B Mix | Exogenous Internal Control-B mix | 1 x 300 μ L | 1 x 600 μ L | 2 x 600 μ L |
| Red | HCVFPC | Positive Control | 1 x 100 μ L | 1 x 100 μ L | 2 x 100 μ L |
| White | MBGPW | Purified water | 2 x 500 μ L | 2 x 1 mL | 2 x 1 mL |

Note: Please pay attention to the cap color coding and the tube contents.

MBGPW: Molecular Biology Grade Purified Water

Storage and Transportation Conditions

The kits should be transported at temperature below -20 °C. The kit is stable until the expiry date printed on the package, if the storage temperature is within -20 ± 5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical specifications

| | |
|-----------------------------|--|
| Target Sequence | Conserved region of 5' UTR |
| Specificity | 1-6 genotypes with 100% specificity |
| Sensitivity | 6.0×10^{-2} IU/ μ L (3.0×10^1 IU/mL or 6.5×10^1 copies/mL) |
| Validated Specimen | Plasma |
| External Quality Assessment | QCMD EQA Panels |

Assay Procedure

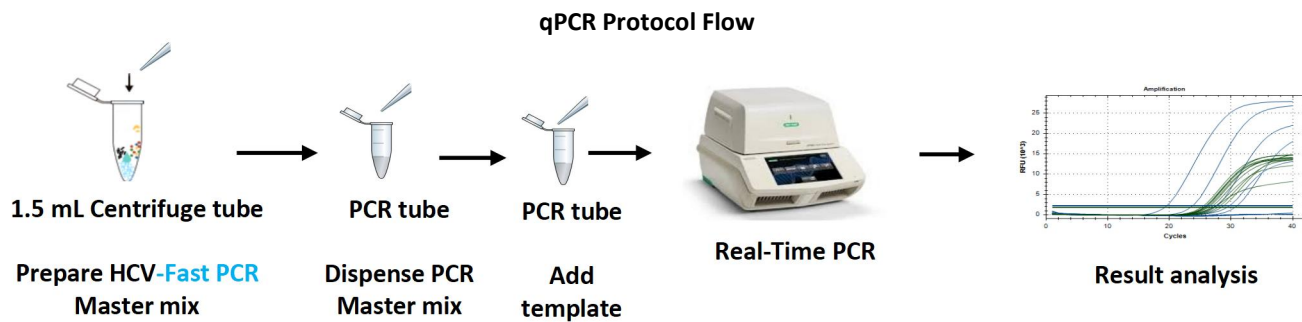
RNA Extraction

Quantiplus® HCV FAST Detection Kit (Real-Time Qualitative PCR Kit) has been validated using the Viral RNA extraction kits mentioned below. Recommended sample volume for extraction and elution are as follows:

| S. No. | Name of the Extraction Kit | Recommended Sample volume for Extraction | Recommended Final Elution volume |
|--------|---|--|----------------------------------|
| 1. | Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100) | 200 µL | 100 µL |
| 2. | QIAamp® Viral RNA Mini Kit (Cat. No. 52904) | 140 µL | 60 µL |

Note: Customer can also validate their own extraction process using other Viral RNA extraction Kits.

IC-B mix can be added at the extraction step or while setting up the PCR



Preparation of Reaction Master mix

| Components | Volume per reaction (for 25µL) |
|--|--------------------------------|
| RNA Fast qPCR Mix with UDG/UNG (4X) | 6.5 |
| HCV Fast PPM | 2.0 |
| IC-B Mix (if not added at extraction step) | 1.0 |
| MBGPW | 5.5 |
| Extracted RNA/ HCVFPC/ MBGPW | 10.0 |

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycle.

Cycling Conditions

| Steps | No. of cycles | Temperature (°C) | Time |
|--------------------------|---------------|------------------|---------|
| 1(Reverse Transcription) | 1 | 53 | 5 min. |
| 2 (Initial denaturation) | 1 | 95 | 1 min. |
| 3 (PCR cycling) | 45 | 95 | 10 sec. |
| | | 60* | 10 sec. |

***Plate read/Data acquisition in FAM and TEXAS RED channels in Bio-Rad™ CFX 96. For Thermo QS5 Real-Time PCR System, use FAM and ROX channels. For Rotor-Gene Q 5 plex, use Green and Orange channels.**

Sample analysis and Interpretation

For unknown sample analysis the cutoff Ct for HCV RNA (FAM) and IC (TEXAS RED) are ≤ 40 and ≤ 30 respectively. The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below. Interpret the results for unknown samples as mentioned in Table 2.

Table 1

| Control | FAM (HCV) | TEXAS RED (IC) |
|---|--------------|----------------|
| If Internal Control (IC-B Mix) is added during extraction | | |
| Positive Control (PC) | √ | - |
| Negative Control (NC) | - | - |
| If Internal Control (IC-B Mix) is added during preparation of reaction master mix | | |
| Positive Control (PC) | √ | √ |
| Negative Control (NC) | - | √ |

Table 2

| S.No | FAM (HCV) | TEXAS RED (IC) | Interpretation | Conclusion |
|------|--------------|-------------------|----------------------------|---|
| 1 | √ | √ | HCV RNA detected | Proceed for further Analysis |
| 2 | √ | - | | |
| 3 | - | √ | HCV RNA not detected | Dilute the RNA sample (1:10) and repeat the Assay |
| 4 | - | - | Possible inhibition of PCR | |

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96
- Rotor-Gene Q 5 plex



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