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Quantiplus® HCV FAST RT PCR Kit (Real-Time Quantitative PCR Kit)

QTF-HCV-25 : 25 rxns
QTF-HCV-50 : 50 rxns
QTF-HCV-100 : 100 rxns

IVD

PI/QTFHCV-07

Intended Use

Quantiplus® HCV FAST RT-PCR Kit is a reverse transcription Real-Time PCR based in vitro diagnostic assay for quantitation 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), Standards (HCVFQS1-HCVFQS4), 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. The test is used for HCV Viral load quantitation as it is important to start/ for follow up therapy.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QTF-HCV-25)	50 rxns (QTF-HCV-50)	100 rxns (QTF-HCV-100)
Green	RNA Fast qPCR Mix with UDG/UNG (4X)	PCR Amplification mix	1 x 165 µL	1 x 330 µL	2 x 330µL
Amber	HCV Fast PPM	Target specific Primer Probe Mix	1 x 50 µL	1 x 100 µL	2 x 100µL
Natural	IC-B Mix	Exogenous Internal Control-B mix	1 x 300 µL	1 x 600 µL	2 x 600 µL
Pink	HCVFQS1	1 X 10^4 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HCVFQS2	1 X 10^3 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HCVFQS3	1 X 10^2 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
Pink	HCVFQS4	1 X 10^1 IU/µL	1 x 100 µL	1 x 100 µL	2 x 100 µL
White	MBGPW	Purified water	2 x 500 µL	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 x 10^{-2} IU/µL(3.0 x 10^1 IU/mL or 6.5 x 10^1 copies/mL)
Linear Range	7.3 log IU/mL (2.25 x 10^7 IU/µL) to 1.68 log/mL (9.6 x 10^{-2} IU/µL)

Reporting Units	IU/µL (1 IU = 2.18 copies)
Validated Specimen	Plasma
External Quality Assessment	QCMD EQA Panels
Standards	Calibrated against to HCV NIBSC code: 06/102

Assay Procedure

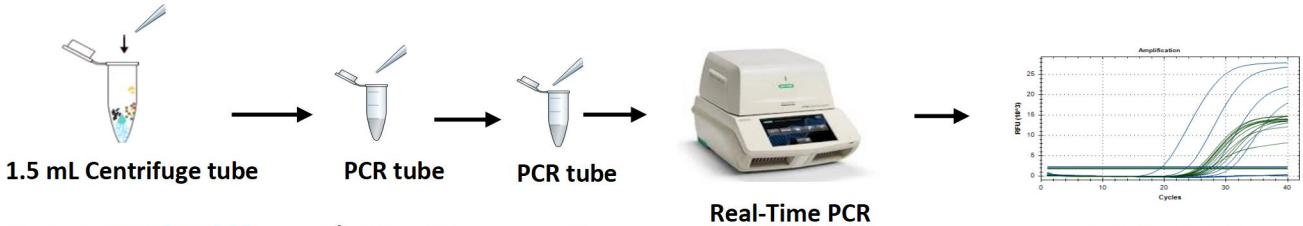
RNA Extraction

Quantiplus® HCV FAST RT PCR Kit (Real-Time Quantitative 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

qPCR Protocol Flow



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/ HCVFQS1- HCVFQS4 / 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 cycler.

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

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 and also ensure that the slope of the standard curve (standards in FAM channel) is between –3.1 to –3.6 (at least three standards should be included) and PCR efficiency is between 90% to 110% (0.9 to 1.1). Interpret the results of 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		
Standards (HCVFQS1 to HCVFQS4)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Standards (HCVFQS1 to HCVFQS4)	√	√
Negative Control (NC)	-	√

Table 2:

S.No	FAM (HCV)	TEXAS RED (IC)	Fluorophore → / Interpretation ↓	Conclusion
1	√	√	HCV RNA detected within quantitation range	Proceed for further Analysis
2	√	-		
3	-	√	HCV RNA below quantitation limit	
4	-	-	Possible inhibition of PCR	Dilute the RNA sample (1:10) and repeat the Assay

Viral load calculation (Conversion of IU/µL to IU/mL)

$$\text{IU/mL} = \frac{\text{Obtained IU/µL X Elution Volume (µL)}}{\text{Sample volume in mL}}$$

For calculating the result of diluted sample (1:10); multiply the observed IU/mL value by dilution factor, 10

$$\text{Result of 1:10 diluted sample (IU/mL)} = \text{Dilution Factor (10)} \times \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

Reporting comments

Results in IU/mL	Comments
Target not detected	HCV RNA not detected in the given sample
<48	HCV RNA detected but below the lower limit of the quantitation range of the assay. The reproducibility of the positive result is not assured
48 to 1.1 X 10^{10}	HCV RNA detected within the linear range of the assay
≥ 1.1 X 10^{10}	HCV RNA detected but above linear range of the assay, dilute the sample for accurate result.

Validated Instruments

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

HLSS Manufacturing Pvt Ltd
Plot No's M14, M15, M16, TSIIC Medical device park
Sultanpur village, Ameenpur Mandal,
SangareddyDist, TS-502319