Hepatitis-C [HCV] Real-time PCR Kit

(For Qualitative Detection)

REF 8003

INTENDED USE

HCV Real-time PCR is an *in-vitro* nucleic acid amplification test, based on real-time PCR technology, for the detection and quantification of human hepatitis C virus (HCV) specific RNA (genotypes 1 to 6) in human EDTA plasma.

KIT CONTENTS

Components	Number of vials	Volume Per vials
One step RT-PCR Master Mix	1	200µl
RT-Taq enzyme mix	1	50µl
HCV Primer Probe Mix [HCV PP mix]	1	65µl
Internal control Primer Probe Mix [IC PP Mix]	1	65µl
Internal control template [IC template]	1	125µl
HCV Positive control [QS1]	1	150ml
Water, PCR grade	1	4ml

STORAGE

1. The kit is shipped on Gel ice [Blue ice]. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

PRODUCT DESCRIPTION

HCV Real-time PCR Kit constitutes a ready-to-use system for the detection of HCV specific RNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the HCV genome, and for the direct detection of the specific amplicon in FAM channel. In addition, it contains an internal control amplification system to identify possible PCR inhibition and RNA purification efficiency. External positive control is supplied, which can be used as both qualitative and quantitative to determination the amount of viral load.

SPECIFICITY

HCV primer and probe have been designed for the specific and exclusive *in-vitro* quantification of HCV subtypes 1 to 6. The target sequence (3'UTR) is highly conserved and sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of RNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified HCV specific pDNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.2 copies per micro liter/2copies per reaction.

DNA PURIFICATION

Purified RNA is the starting material for the Real-time PCR assay. The quality of the purified RNA has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for RNA purification is compatible with real-time PCR technology.

INTERNAL CONTROL TEMPLATE

Add 5µl of the internal control template to each test sample. Do not add directly to test sample. Add after adding lysis buffer to the test sample [sample/lysis buffer mix]. Complete purification according to the manufacturer's protocols.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 21 +/- 10.

PREPARATION OF STANDARD CURVE DILUTION SERIES:

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction. Always prepare fresh.
8. 1 copy = 1 IU

Standards	Copies per µl
QS-1	2500000
QS-2	250000
QS-3	25000
QS-4	2500

DETECTION PROTOCOL

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Make sure that internal control template is added during RNA purification. If not, pipette 2.5µl of the internal control template directly into the purified RNA OR 0.5ul per reaction into final master mix [0.5ul volume increase will not affect the performance]
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

PRODUCT USE LIMITATIONS

Components	Volume per reaction
One step RT-PCR Master Mix	8µl
RT-Taq enzyme mix	2µl
HCV PP Mix	2.5µl
IC PP Mix	2.5µl
Purified DNA / Positive Control / Negative Control	10µl
Final reaction volume	25µl

Negative Control setup [NTC]
Add 10µl of PCR grade water.

Qualitative Positive Control setup
Add 10µl of any one of the Positive controls [From QS1 to QS4]

Quantitative Positive controls setup
10µl of all Positive controls prepared from QS1 to QS4.

PROGRAMMING THERMAL CYCLER

Sample volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference	None
Ramping rate	Default

THERMAL PROFILE

Cycles	Step	Time	Temp
1	Reverse transcriptase	20min	50ºC
	Taq enzyme activation / Hold	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	58ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	HCV
HEX	Internal control

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

READING THE GRAPH

Step-1 – Internal control Validation
Select the test samples alone for the internal control analysis. Select HEX dye and view the graph of internal control amplification. A successful amplification Ct value must be within Ct 21 +/- 10.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

Step-2 – FAM - Negative and Positive control validation
Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-3 –FAM - Test Sample status
In FAM channel, select test sample well one by one, analyze the graph/amplification.

QUALITATIVE INTERPRETATION OF RESULTS

Test Sample	Negative Control	Positive Control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	HCV specific RNA detected
Negative	Negative	Positive	Positive	No HCV specific RNA Detected. Sample does not contain detectable amounts of HCV specific RNA.
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

Qualitative

Observation		Inerpretation
FAM-HCV	HEX-IC	
<37	<31	HCV detected

Recommendation:

In FAM [HCV] channel, the Ct value beyond 35 is required careful analysis. The analysis may include that the status of NTC amplification curve, threshold adjustment, linear/log scale view assessment, etc.,

STANDARD CURVE ANALYSIS

- Interpret the values for unknow samples, only if the R>0.98
- Slope of calibrators is between-3.0 to -3.7
- PCR efficiency is between 85% and no amplification in FAM channel of negative control.
- 1 copy is equal to 1 IU

Calculating copies or IU per ml

Input the machine indicated copy number into the following formula

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

Note:

Elution volume: must be typed in micro liter format, example 30µl, 60µl or 100µl. Sample volume: must be typed in milliliter format, example 0.2ml or 0.5ml

Observation	Interpretation
<50 IU/ml	HCV detected, but below limit of detection
50 IU to 3 x 10^9 IU/mL	HCV detected and quantified
<3 x 10^9 IU/mL	HCV detected, but upper limit of quantitation

LIMITATIONS

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

QUALITY CONTROL

In accordance with in house Quality Management System, each lot of HCV Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Index of symbols

Consult instructions for use	Batch code	Tests per kit
For *in vitro* diagnostic use only	Do not re-use	Keep dry
Temperature limit - 25 °C to - 15 °C	Date of manufacture	Biological Risks
Manufacturer	Use-by date	

Marketed by:

Athenese-Dx Pvt. Ltd.

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