SimReal™ HCV Detection and Quantitation Kit

Cat. No: SBL11-1388

USER MANUAL

INTRODUCTION

Hepatitis C virus (HCV) is an enveloped, positive-sense single-stranded RNA virus of the Flaviviridae family. Hepatitis C virus is the cause of hepatitis C and some cancers such as liver cancer (hepatocellular carcinoma, abbreviated HCC) and lymphomas in humans.

The level of HCV RNA in serum and plasma can be used in conjunction with other clinical markers and clinical findings to distinguish between acute and chronic HCV infection and to assess the viral response to antiviral treatment. The detection kit is not intended for screening of blood or blood products for HCV RNA or for confirming of the HCV infection.

PRODUCT DESCRIPTION

The SimReal™ HCV Detection and Quantification Kit is deliberated for detection and quantification of Hepatitis C Virus (HCV) RNA in human plasma or serum samples on the basis of in-vitro amplification using Real-time PCR technology.

The viral genome detection is based on amplification and detection of HCV conserved sequence using corresponding labeled probes. The probes targeting viral sequence and internal control sequence are labeled with FAM and HEX fluorochrome, respectively. Furthermore, viral genome quantitation can be performed using absolute quantitation method.

KIT CONTENTS

Components	Labels	Volume
2x Reaction Mix	Reaction Mix	250 µl
Reverse transcriptase	RTase	25 µl
Oligomix	Oligomix	50 µl
Standard 1 (10^2 copy/µl)	STD 1	30 µl
Standard 2 (10^3 copy/µl)	STD 2	30 µl
Standard 3 (10^4 copy/µl)	STD 3	30 µl
Standard 4 (10^5 copy/µl)	STD 4	30 µl
Standard 5 (10^6 copy/µl)	STD 5	30 µl

TEST PRINCIPLE

SimReal™ HCV PCR Detection and Quantification Kit constituents a ready-to-use system for the detection of HCV using Taq-Man hydrolysis system. The viral cDNA free from inhibitors can be used as the template in a PCR reaction for HCV detection using the provided HCV Master Mix.

This Master Mix contains reagents and enzymes for the specific amplification of a conserved region of the HCV genome and also human GAPDH sequence as internal/isolation control gene. This control gene can be used to identify possible PCR inhibition and/or inefficient cDNA synthesis. The amplification and detection of internal control (IC) do not reduce the detection limit of the analytical HCV PCR. Amplification of HCV cDNA and internal control can be detected via fluorescent acquisition in Green channel (FAM fluorophore) and yellow channel (Hex fluorophore), respectively.

PROTOCOL

a) RNA Extraction

RNA from biologic samples such as serum, whole blood, urine and amniotic fluid must be isolated using appropriate extraction kit (e.g. Simex viral RNA Preparation Kit).

The extracted RNA can be stored for several months at ≤ -70°C.

b) Preparation of the PCR mix

Prepare the PCR reaction for sample detection as shown in Table 1 below. Recommended amount of the RNA sample is 2 µl.

However, a volume between 1 and 4µL of RNA sample may be used as template. Adjust the final volume of the PCR reaction to 20µL by the Nuclease Free Water.

NOTE. For each PCR set, prepare one positive, one negative and one blank control PCR

Component Labels	Volume/reaction
Reaction Mix	10 µl
RTase	1 µl
Oligomix	2 µl

Standard samples are necessary for tittering of viral load by standard curve quantitation method. At least three standards should be used, respectively, in order to providing standard curve, for example 103, 104 ،105 and 106.

You can use one of standards as unknown sample to evaluate reaction accuracy.

c) Real time PCR cycler programming

Refer to the specific handbook of the equipment used but be sure to set the following thermal profile.

Step	Temp	Time	Data collection	Cycle
Pre-cycling	50˚C	30 min		
	95˚C	15 min		
Denaturation	95˚C	30 s	*	45X
Annealing	57˚C	30 s		
Extension	72˚C	30 s		

 # Acquire florescent signal in green channel

Notice: appropriate reaction template files can be found in www.sim-biolab.com

Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis**.**

DATA ANALYSIS

The fluorescence in each channel indicates the hybridization of the probe

Channel 1 for FAM= HCV specific probe

If a sample shows fluorescence in the green channel, the sample is positive for virus genome**.**

For estimating viral titer, absolute quantitation method should be used.

- ✓ You can download absolute quantitation program from *www.sim-biolab.com*

Standard curve design and calculate the HCV cDNA in each sample, automatically. These data are using for the HCV genome concentration estimation in copies/ml according to below equation:

$$\frac{\textbf{Sample concentration}\left(\frac{\textbf{copy}}{\textbf{ul}}\right)\times\textbf{Elution volume (ul)}}{\textbf{Isolation volume (ml)}}=\textbf{HCV}\frac{\textbf{copy}}{\textbf{ml}}$$

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