SimReal™ HBV Detection & Quantitation Kit

Cat. No: SBL11-1330

USER MANUAL

INTRODUCTION

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. Hepatitis B virus (HBV) is a member of the hepadnavirus family. The genome of HBV is made of circular DNA.

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins.

The genotype differences affect the disease severity, course, and likelihood of complications, and response to treatment and possibly vaccination.

PRODUCT DESCRIPTION

SimReal™ HBV Detection & Quantitation Kit is a diagnostic kit designed to specific detection of HBV infection on the basis of in-vitro amplification using Real-time PCR technology.

Viral genome detection is based on amplification and detection of HBV conserved sequence using corresponding labeled probes. The probes which targeting the viral sequence and the internal control sequence are labeled with FAM and CY5 flourochrome, respectively.

KIT CONTENTS

Components	Labels	Cap Color	Volume 25 Tests
2x Reaction Mix	Reaction Mix	Black	250 µl
Primer and Probes mix	Oligomix	White	50 µl
Standard 1 (10^2 copy/µl)	STD 1	Green	20 µl
Standard 2 (10^3 copy/µl)	STD 2	Yellow	20 µl
Standard 3 (10^4 copy/µl)	STD 3	Red	20 µl
Standard 4 (10^5 copy/µl)	STD 4	Violet	20 µl
Standard 5 (10^6 copy/µl)	STD 5	Blue	20 µl

TEST PRINCIPLE

SimReal™ HBV Detection & Quantitation Kit constituents a ready-to-use system for the detection of HBV using Taq-Man hydrolysis system. The viral DNA free from inhibitors can be used as the template in the PCR reaction for HBV detection using the provided HBV Master Mix. This Master Mix contains reagents and enzymes for the specific amplification of a conserved region of the HBV 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 DNA extraction. The amplification and detection of internal control (IC) do not reduce the detection limit of the analytical HBV PCR. Amplification of the HBV genome and internal control can be detected via fluorescent acquisition in Green channel (FAM fluorophore) and RED channel (CY5 fluorophore), respectively.

PROTOCOL

a) DNA Extraction

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

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

b) Preparation of the reaction mix

Prepare the PCR reaction for sample detection as shown in Table 1 below. Recommended amount of the DNA sample is 2µL. However, a volume between 1 and 4µL of DNA 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
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 10^3, 10^4, 10^5 and 10^6

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
Initial activation	95˚C	15 min		1 X
Denaturation	95˚C	30 s	*	45 X
Annealing	59˚C	40 s		
Extension	72˚C	30 s		

**Acquire florescent signal in green & RED 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= HBV specific probe
Channel 2 for CY5= internal control probe

If a sample shows fluorescence in 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 calculating of HBV DNA in each sample are automatically performed. These data are used for estimation of HBV DNA concentration in copies/ml according to below equation:

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

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