



SIMBIOLAB

SimReal™ LSEctin (CLEC4G) assay kit

USER MANUAL

INTRODUCTION

LSEctin (liver node Sinusoidal Endothelial Cell lectin, CLEC4G) encodes a glycan-binding receptor. LSEctin specifically expressed on LSECs and Kupffer cells. LSEctin is a member of the C-type lectin family which plays a role in the T-cell immune response. This family comprising CD23, DC-SIGN and DC-SIGNR, that possesses a typical carbohydrate recognition domain (CRD) and binds to mannose, N-acetyl glucosamine (GlcNAc) and fucose in a Ca²⁺-dependent manner.

Negative regulation of T cell mediated immunity, negative regulation of T cell proliferation, and regulation of immune response are some of the pathways that LSEctin involves. This protein can act as a receptor for Japanese encephalitis virus, Ebolavirus, SARS coronavirus/SARS-CoV, and Lassa virus and Lymphocytic choriomeningitis virus glycoprotein. SimReal™ LSEctin (CLEC4G) is an assay kit designed for the LSEctin mRNA quantification on the basis of in-vitro amplification using Real-time PCR technology.

The LSEctin expression analysis is based on amplification of the LSEctin specific sequence using corresponding labeled probes. The probes that targeting LSEctin sequence is labeled with HEX/VIC fluorochrome.

The SimReal™ LSEctin (CLEC4G) assay kit constituents a ready-to-use system for the expression analysis using TaqMan hydrolysis system. The LSEctin cDNA free from inhibitors can be used as template in the amplification reaction to LSEctin mRNA quantification. The provided Master Mix contains reagents and enzymes for the specific amplification of a conserved region of the LSEctin cDNA. Amplification can be detected via fluorescent acquisition in yellow channel (HEX/VIC fluorophore).

KIT CONTENTS

Components	Labels	Cap Color	Volume
			500 Tests
Primer and Probes mix	Oligomix	White	500 µl
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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 RNA Preparation Kit).

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

b) cDNA Synthesis

cDNA synthesis must be done using appropriate cDNA synthesis kit (e.g. Sim cDNA Preparation Kit).

c) Preparation of the PCR mix

Prepare the PCR reaction for sample detection as shown in below Table. The recommended amount of the cDNA sample is 2µL. However, a volume between 1 and 5µL of cDNA sample may be used as the 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

d) Real time PCR cyclers programming

Set device as the following profile:

Step	Temp	Time	Data collection	
Initial activation	95°C	15 min		1X
Denaturation	95°C	30 s	HEX/VIC	45X
Annealing*	60°C	30 s		
Extension	72°C	30 s		

* Acquire fluorescent signal in Yellow channel

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 HEX/VIC= LSEctin specific probe

Evaluation mRNA expression of the LSEctin can be performed using the $\Delta\Delta C_t$ method.

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