SimReal™ Poliovirus receptor (PVR, CD155) assay kit

USER MANUAL

INTRODUCTION

CD155, also known as PVR (poliovirus receptor), Necl-5 (nectin-like molecule-5) and, in rodents, TAGE4 (tumor-associated glycoprotein E4) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. CD155 binds other molecules including Vitronectin, Nectin-3, DNAM-1/CD226, CD96, and TIGIT. The gene is specific to the primate lineage and serves as a cellular receptor for poliovirus in the first step of poliovirus replication.

CD155 is barely or weakly expressed in various normal human tissues but frequently overexpressed in human malignant tumors. CD155 overexpression promotes tumor cell invasion and migration and is associated with tumor progression and poor prognosis. Studies show that CD155 levels were significantly higher in the patients with lung, gastrointestinal, breast, and gynecologic cancers. In addition, the CD155 levels were significantly higher in patients with early stage (stages 1 and 2) gastric cancer and were increased in patients with advanced stage (stages 3 and 4) disease. Moreover, the CD155 levels were significantly decreased after surgical resection of cancers. Thus, CD155 level in serum may be potentially useful as a biomarker for cancer development and progression.

The SimReal™ Poliovirus receptor (PVR, CD155) kit is an assay kit designed for the PVR mRNA quantification on the basis of in-vitro amplification using Real-time PCR technology.

The PVR expression analysis is based on amplification of the PVR specific sequence using corresponding labeled probes. The probes that targeting PVR sequence is labeled with FAM fluorochrome.

The SimReal™ Poliovirus receptor (PVR, CD155) assay kit constituents a ready-to-use system for the expression analysis using TaqMan hydrolysis system. The PVR cDNA free from inhibitors can be used as template in the amplification reaction to PVR mRNA quantification. The provided Master Mix contains reagents and enzymes for the specific amplification of a conserved region of the PVR gene. Amplification can be detected via fluorescent acquisition in Green channel (FAM 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 cycler programming

Set device as the following profile:

Step	Temp	Time	Data collection	
Initial activation	95˚C	15 min		1X
Denaturation	95˚C	30 s	FAM	45X
Annealing*	60˚C	30 s		
Extension	72˚C	30 s		

** Acquire florescent signal in green 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 FAM= PVR specific probe

Evaluation mRNA expression of the PVR can be performed using the ΔΔCt method.

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