SimReal™ GAPDH assay kit

USER MANUAL

INTRODUCTION

GAPDH gene encodes a member of the glyceraldehyde-3-phosphate dehydrogenase protein family. The encoded protein has been identified as a moonlighting protein based on its ability to perform mechanistically distinct functions. The product of this gene catalyzes an important energy-yielding step in carbohydrate metabolism, the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD). The encoded protein has additionally been identified to have uracil DNA glycosylase activity in the nucleus. Also, this protein contains a peptide that has antimicrobial activity against E. coli, P. aeruginosa, and C. albicans. Studies of a similar protein in mouse have assigned a variety of additional functions including nitrosylation of nuclear proteins, the regulation of mRNA stability, and acting as a transferrin receptor on the cell surface of macrophage.

GAPDH is commonly used by biological researchers as a control for qPCR, because the GAPDH gene is often stably and constitutively expressed at high levels in many tissues and cells, which is considered as a housekeeping gene.

SimReal™ GAPDH is an assay kit designed for the GAPDH mRNA quantification on the basis of in-vitro amplification using Real-time PCR technology. The GAPDH expression analysis is based on amplification of the GAPDH specific sequence using corresponding labeled probes. The probes that targeting GAPDH sequence is labeled with HEX/VIC fluorochrome.

The SimReal™ GAPDH assay kit constituents a ready-to-use system for the expression analysis using TaqMan hydrolysis system. The GAPDH cDNA free from inhibitors can be used as template in the amplification reaction to GAPDH mRNA quantification. The provided Master Mix contains reagents and enzymes for the specific amplification of a conserved region of the GAPDH 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 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	HEX/VIC	40X
Annealing*	60˚C	30 s		
Extension	72˚C	30 s		

** Acquire florescent 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= GAPDH specific probe

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

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