SimReal™ MTHFR A1298C Genotyping Kit

Cat. No: SBL11-798/25

USER MANUAL

INTRODUCTION

Methylene tetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme in the methyl cycle, and it is encoded by the MTHFR gene Methylene tetrahydrofolate reductase catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a substrate for homocysteine remethylation to methionine.

Natural variation in this gene is common in healthy people. Although some variants have been reported to influence susceptibility to occlusive vascular disease, neural tube defects, Alzheimer's disease and other forms of dementia, colon cancer, and acute leukemia, findings from small early studies have not been reproduced. Some mutations in this gene are associated with methylene tetrahydrofolate reductase deficiency in 2000 a report brought the number of polymorphisms up to 24. Two of the most investigated are C677T (rs1801133) and A1298C (rs1801131) single nucleotide polymorphisms (SNP).

And to lower therapy costs (prolonged stay in hospital, etc.) arising Due to undesired side-effects as well as to recognize a genetically based risk for hyperhomocystinemia at an early stage.

PRODUCT DESCRIPTION

SimReal™ MTHFR A1298C Genotyping Kit is an in-vitro diagnostic kit designed to determine the genotype of MTHFRgene A1298C SNP related to hyperhomocystinemia on the basis of in-vitro DNA amplification using Real-time PCR technology**.**

Mutation detection is based on amplification and detection of distinct alleles using corresponding labeled probes. The probes targeting normal (A1298) and mutant (C1298) alleles are labeled with FAM and HEX/VIC flourochrome, respectively.

KIT CONTENTS

Components	Labels	Volume
		25 Tests
Reaction Mix	Reaction Mix	288 µl
Primer and Probes mix	Oligomix	50 µl
Wild type homozygote	Control A	20 µl
Heterozygote control	Control B	20 µl
Mutant homozygote	Control C	20 µl

TEST PRINCIPLE

SimReal™ MTHFR A1298C genotyping Kit employs polymerase chain reaction (PCR) to amplifying a fragment of the human MTHFR gene, containing polymorphic nucleotide number 1298, either wild type or polymorphic allele.

Amplified alleles are detected via fluorescent probes. Wild type and mutant alleles can be detected using the FAM and HEX/VIC channels, respectively.

PROTOCOL

a) Genomic DNA Extraction

Nucleic acid isolation should be performed by isolation kits available at the markets according to protocols for the particular clinical material isolation. We recommend our DNA Extraction kit (SBL15-2005). The extracted DNA can be stored for several months at ≤ -18°C.

b) Preparation of the PCR mix

For each experiment, prepare a master mix of an appropriate volume for: 3 controls (A, B, C), 1 reaction blank and n+1 samples.

The reagents of the mix have to mix under this ratio:

Component Labels	Volume/reaction
Reaction Mix	11.5 µl
Oligomix	2 µl

Aliquot 1**3**.5µl of Master Mix in each tube and add 2-4µl of extracted DNA or control DNA into individual tubes, then, add DW up to 20µl; spin tubes shortly and place them in your Real-time PCR device.

c) Real time PCR cycler programming

Set device as the following profile:

Step	Temp	Time	Data collection	
PCR initial heat activation	95˚C	15 min		1X
Denaturation	95˚C	30 s	FAM + HEX	40X
Annealing*	58˚C	30 s		
Extension	72˚C	30 s		

** Acquire florescent signal in green and yellow channels*

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

FAM Channel = Allele A (Wild Type)
HEX / VIC Channel = Allele C (Mutated)

If a sample shows fluorescence only in FAM channel, the sample is homozygous wild type.

If a sample shows fluorescence only in HEX/VIC channel, the sample is homozygous mutated.

If a sample shows fluorescence in both channels (FAM and HEX/VIC), the sample is heterozygous.

Genetics Department, School of Medicine, MUMS Mashhad, IRAN.
Mob: +98 915 383 14 07
Tel: +98 513 72 55 495
Web address: www.sim-biolab.com
E-mail: info@sim-biolab.com