SimReal™ MTHFR C677T Genotyping Kit

Cat. No: SBL11-196/25

USER MANUAL

INTRODUCTION

Methylenetetrahydrofolate reductase (MTHFR), encoded by the MTHFR gene, is the rate-limiting enzyme in the methyl cycle. MTHFR catalyzes the conversion of the 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a substrate for homocysteine remethylation to methionine. Some DNA variants of MTHFR have been reported to influence susceptibility to several health problems such as occlusive vascular disease, neural tube defects, deep vein thrombosis and recurrent pregnancy loss.

Two of the most investigated MTHFR DNA variants are C677T (rs1801133) and A1298C (rs1801131).

Genotyping of MTHFR may help to individually optimize medication and avoid recommending medications known to have worse side effects in people with a weak MTHFR enzyme. Furthermore, MTHFR genotyping can help doctors to predict the risk of Deep Vein Thrombosis and also recurrent pregnancy loss.

PRODUCT DESCRIPTION

SimReal™ MTHFR C677T Genotyping Kit is an in-vitro diagnostic kit designed to determine the genotype of MTHFR gene C677T SNP using Real-time PCR technology.

KIT CONTENTS

Components	Labels	Volume 25 Tests
2x Reaction Mix	Reaction Mix	250 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 C677T genotyping by the Real-Time PCR is based on the amplification of a fragment of the human MTHFR gene using special primers and detection of amplified alleles using TaqMan probes labeled by distinct fluorophore (FAM, HEX/VIC).

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 (HET, HOMO WT), 1 reaction blank and n+1 samples.

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

Component Labels	Volume/reaction
Reaction Mix	10 µl
Oligomix	2 µl

Aliquot 12µl 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*	59 ˚C	30 s		
Extension	72˚C	30 s		

** Acquire florescent signal in green and yellow channels*

Notice: appropriate reaction template files can be found in *www.sim-biolab.com*

DATA ANALYSIS

Presence of amplification curve in each channel indicates counterpart allele type.

FAM Channel = C allele (Wild type)
HEX/VIC Channel = T allele (Mutated)

Notice: genotyping assay can be used in Real-time Device software. Appropriate assay files can be found in *www.sim-biolab.com*

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