SimReal™ Factor V Leiden Genotyping Kit

Cat. No: SBL11-360/25

USER MANUAL

INTRODUCTION

Factor V Leiden thrombophilia is an inherited disorder of blood clotting. Factor V Leiden is the name of a specific gene mutation that results in thrombophilia, which is an increased tendency to form abnormal blood clots that can block blood vessels.

People with factor V Leiden thrombophilia have a higher than average risk of developing a type of blood clot called a deep venous thrombosis (DVT). DVTs occur most often in the legs, although they can also occur in other parts of the body, including the brain, eyes, liver, and kidneys. Factor V Leiden thrombophilia also increases the risk that clots will break away from their original site and travel through the bloodstream. These clots can lodge in the lungs, where they are known as pulmonary emboli. Although factor V Leiden thrombophilia increases the risk of blood clots, only about 10 percent of individuals with the factor V Leiden mutation ever develop abnormal clots.

Genotyping may help to individually optimize medication 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 hyperhomocysteinemia at an early stage.

PRODUCT DESCRIPTION

SimReal™ Factor V Leiden G1691A Genotyping Kit is an in-vitro diagnostic kit designed to determine the genotype of Factor V Leiden gene G1691A SNP related to hyperhomocysteinemia 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 (G1691) and mutant (A1691) alleles are labeled with FAM and HEX flourochrome, respectively.

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 B	20 µl
Mutant homozygote	Control C	20 µl

TEST PRINCIPLE

SimReal™ Factor V Leiden G1691A genotyping Kit employs multiplex PCR. A 233 bp fragment of the human Factor V Leiden gene, whether wild type or polymorphic, is amplified in a single reaction, using sequence-specific primers against mutant and wild-type alleles.

In Taqman real-time PCR the amplified product is detected via fluorescent dyes. Wild type Factor V Leiden allele is amplified and fluorescence detection is accomplished using the HEX channel. Allele with Factor V Leiden polymorphism is amplified and fluorescence detection is accomplished using the FAM channel. Main advantages of the Real time PCR technique, compared to the conventional amplification techniques, are for example the possibility to execute a semi-automated analysis in which the time needed for the visualization of the amplicons is eliminated; and the absence of the post amplification sample manipulation that reduces the possible contamination phenomena.

PROTOCOL

a) Genomic DNA Extraction

DNA-preparation from patients' blood according to standard procedures. We recommend our DNA Extraction kit (SBL15-2005).

The DNA extracted can be stored for several months at ≤ -18°

b) Preparation of the PCR mix

For each experiment, prepare a master mix of an appropriate volume for: 2 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	
Initial activation	95˚C	15 min		1X
Denaturation	95˚C	30 s	FAM	40X
Annealing*	60˚C	40 s	+	
Extension	72˚C	20 s	HEX	

** 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 G (Wild type)
HEX / VIC Channel = Allele A (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.

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