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SIMREAL™ PROTHROMBIN GENOTYPING KIT

INTRODUCTION

Prothrombin 20210 mutation (G20210A) is a genetic variant that approximately doubles or triples the risk of forming blood clots in the veins. The variant is commonly associated with the disease venous thromboembolism (VTE), which includes both deep vein thrombosis and pulmonary embolism. Most carriers, though, never develop VTE in their lifetime. Behind nonO blood type and factor V Leiden, prothrombin G20210A is one of the most common genetic risk factors for VTE. The polymorphism causes elevated plasma prothrombin levels (hyperprothrombinemia), possibly due to increased pre-mRNA stability. Prothrombin is the precursor to thrombin, which plays a key role in causing blood to clot (blood coagulation). G20210A can thus contribute to a state of hypercoagulability, but not particularly with arterial thrombosis. In a recommendation statement on VTE, genetic testing for G20210A in adults that developed unprovoked VTE was misadvised, as was testing in asymptomatic family members related to G20210A carriers who developed VTE. In those who develop VTE, the results of thrombophilia tests (wherein the variant can be detected) rarely play a role in the length of treatment.

PRODUCT DESCRIPTION

SimReal™ Prothrombin Genotyping Kit is an in-vitro diagnostic kit designed to determine the genotype of prothrombin gene G20210A Mutation related to thrombophilia 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 (G20210) and mutant (A20210) alleles are labeled with FAM and HEX fluorochrome, 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	Control B	20 µl
Mutant homozygote	Control C	20 µl

TEST PRINCIPLE

SimReal™ Prothrombin genotyping Kit employs multiplex PCR. A fragment of the human Prothrombin gene, whether wild-type or polymorphic, is amplified in a single reaction, using sequence-specific primers against mutant and wild-type alleles. In the TaqMan Real-time PCR the amplified product is detected via fluorescent dyes. Wild-type allele is amplified and fluorescence detection is accomplished using the FAM channel. Allele with G20210A polymorphism is amplified and fluorescence detection is accomplished using the HEX 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

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: 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

Refer to the specific handbook of the equipment used but be sure to set the following thermal profile

Step	Temp	Time	Data Collection	Cycle
Initial Activation	95 ˚C	15 min		1 X
Denaturation	95 ˚C	30 s	FAM + HEX	40 X
Annealing*	60 ˚C	40 s		
Extension	72 ˚C	20 s		

* Acquire florescent signal in green and yellow channels

Notice:

Appropriate reaction template files can be found in: www.sim-biolab.com

DATA ANALYSIS

The fluorescence in each channel indicates the hybridization of the probe

Channel 1 for FAM= G allele (Wild Type)
Channel 2 for HEX= A allele (Mutated)

If a sample shows fluorescence in channel 1, the sample is the homozygous wild-type.
If a sample shows fluorescence in channel 2, the sample is homozygous mutated.
If a sample shows fluorescence in all channels (1 and 2), the sample is heterozygous.

Notice:

Genotyping assay can be used in Real-time Device software. Appropriate assay files can be found in www.sim-biolab.com