CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

DETECTION OF MUTATION G20210A POLYMORPHISM IN THE PROTHROMBIN GENE

AMPLI FII G20210A REAL TIME Cat 1.310RT

The development of thrombotic diseases is one of the major cause of morbidity and mortality in the western countries. The alteration of Homeostasis is the central mechanism of thrombosis. The causes of this unbalance can be genetic. This evidence underlines the importance of the interaction between genes and environment in the thrombotic diseases.
The Prothrombin protein, precursor of thrombin in the coagulation process, is encoded by a gene of 21Kb localized on the chromosome 11, position 11p11-q12; organized in 14 exons separated by 13 introns with a region near the end 5' and the end 3' untranslated (UT untranslated), which have an important role in the regulation of the expression of this gene. In 1996 a common polymorphism in the 3' untranslated region has been identified; in this gene an A replaces a G in position 20210 in the untraslated region 3'-UT. This mutation as been identified in the 18% of patients selected because of previous personal events or familiar of venous thrombosis, in the 6.2% of noselected patients with a first event of deep venous thrombosis and in the 2.3% of healthy patients. Carriers of the 20210-A allele have higher plasma prothrombin levels than controls with the normal 20210 GG genotype and have a 2,8-fold increased risk of venous thrombosis
As shown in fig 1, the detection of the polymorphism involves performing a PCR with specific primers and a probe which anneals between primer sites (1). The probe is is labeled with a fluorescent reporter dye bound to the 5' and quencher on the 3' end. (2). Due to the 5' nuclease activity of Taq pol during extension, the cleavage of the probe causes an increase of the reporter dye signal (3), and the fluorescent intensity is proportional to the amount of amplicon produced (4) (real time quantitative PCR).
In the kit for the detection of G20210A polymorphism , the probe matching the wild type sequence is labeled to the FAM dye reporter, whereas the probe matching the mutation sequence is labeled to the VIC dye reporter.

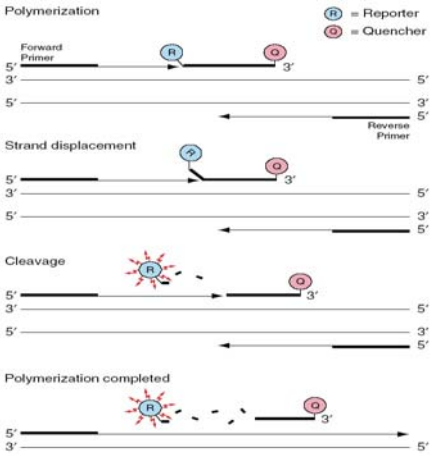


Fig. 1

Principle of method: A) extraction of genomic DNA B) amplification C) detection using real time PCR instrument
Applicability: Genomic DNA extracted and purified by whole blood samples
Number of reactions: 96.

REAGENTS AND STORAGE

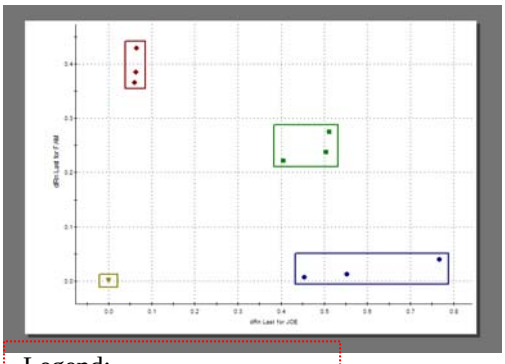
AMPLIFICATION	
PCR mix 2X	+4°C
H_2O sterile	-20°C
Primer-probe mix 20X	-20°C in the dark
WT Control	-20°C
Mutant Control	-20°C
Eterozygosis positive control	-20°C

Stability: over 18 months if correctly stored.

ANALYSIS OF RESULTS

After an AD post-read run, the software analyzes raw data using the AD specific program. Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.

Allelic discrimination FII G20210A



Legend:
Red: allele G
Green: A and G alleles
Blue: allele A

References:
Dahlback B., Thromb. And Hemost. 1995;73:739-42. Roger M., et al. Letter to Nature 1994,369:64-67.