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CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

CE

KIT FOR THE DETECTION OF R-353-Q POLYMORPHISM OF THE FACTOR VII GENE

AMPLI FVII R353Q REAL TIME — Cat. n. 1.312RT

Factor VII coagulation factor is a vitamin-K dependent protease that plays an important role in the extrinsic system of coagulation. It is synthesized by the hepatic cells and it is secreted in the inactivated form as single peptide chain. The activation (FVIIa) consists of a proteolytic snip followed by the disulfide bond of two peptide chains . Genetic and environmental factors can influence the plasmatic levels of Factor VII. Particularly, the third part of the causes of the variations of its plasmatic level may due to genetic polymorphism of the gene encoding FVII. The more common polymorphisms are: 1) the substitution G-A in the exon 8 of the gene, responsible of the substitution R(arginine) – Q(glutamine) in the amino acid 353. 2) The insertion/deletion of 10 nucleotides in position -323 in the promoter region of the gene(-323 P0/P10). Particularly, the homozygosis 353 Arg-Arg is related to the presence of higher plasmatic level of F VII.
The kit allows the detection of the polymorphism R(arginine)-Q (glutamine) of the amino acid 353 by means of a PCR with specific primers and a probe which anneals between primer sites. The probe is labeled with a fluorescent reporter dye bound to the 5' and quencher on the 3' end. During the Taq polymerase extension, the intrinsic Taq polymerase nuclease activity cleaves the reporter dye from the probe generating a fluorescent signal as consequence of releasing of the reporter dye from the quencher proportional to the amplified product (real-time quantitative PCR).
In the kit for the detection of R353Q polymorphism , the probe matching the wild type sequence (allele G) is labeled to the FAM dye reporter, whereas the probe matching the mutation sequence (allele A) is labeled to the JOE dye reporter.

Principle of method: A) extraction of genomic DNA B) amplification C) detection on real-time PCR
Applicability: On extracted and purified genomic DNA from whole blood samples.
Tests: 96.

REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix 2X	+4°C
H_2O sterile	-20°C
Primer-probe mix 20X	-20°C
Positive normal Control (GG)	-20°C
Positive homozygous Control (AA)	-20°C
Positive heterozygous Control	-20°C

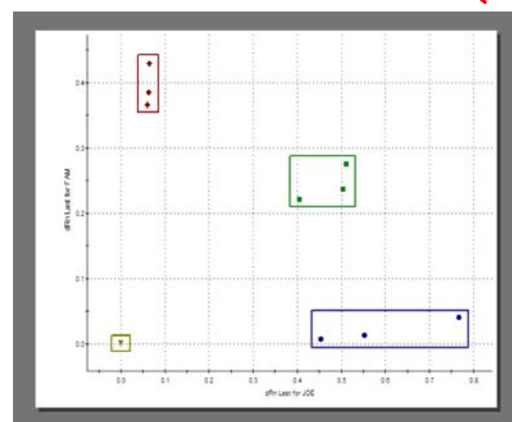
Stability: over 18 months if correctly stored

References:
Arteriosclerosis and Thrombosis 1991, 11, 3:540-6.
Hum Genet 1993, 90:574-576.
Arterioscler Thromb Vasc Biol 1996, 16: 72-76.
Thromb Haemost 1998 : 80: 281-5.
Blood, Vol 93, N. 10, 1999: 3432-3441.

ANALYSIS OF RESULTS

After an AD (ALLELIC DISCRIMINATION) 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 FVII R353Q



Legend:
Red: wt(G)allele
Green: heterozigous (A and G) allele
Blue: mutated (A) allele