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

AMPLI-set FVII R353Q Cat. n. 1.312

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. Particularly, the homozygosis 353 Arg-Arg is related to the presence of higher plasmatic level of F VII 2) The insertion/deletion of 10 nucleotides in position -323 in the promoter region of the gene (-323 P0/P10).
Recently, other polymorphism have been identified in the promoter region of the gene:1) substitution T-C in position -122 (-122 T/C),2) the substitution G-T in position -401 (-401G/T) ;3) the substitution G-A in position -402 (-401 G/A). In 1999 has been demonstrated that the rarest polymorphic variants -401T and 402A are associated respectively to a decreased and to an increased transcriptional activity of the gene. It is evident that the polymorphic form 353Q and -401T may be considered as protective against thrombotic events.
The Ampli Set FVII R-353-Q allows the detection of the polymorphism R(arginine)-Q (glutamine) of the amino acid 353.
The detection is carried out by an amplification with specific primers and an hybridization with a primer able to recognize the internal sequence. The probe is labeled with two different fluorophore (reporter dye and quencher dye). During the amplification the probe will release the quencher increasing the fluorescence, which is directly proportional to the quantity of the recognized amplified product (real-time quantitative PCR).
As the probe detects the wt sequence (allele G) combined with reporter FAM, the probe detecting the polymorphic sequence (allele A) is combined with reporter JOE.

Principle of method: A) extraction of genomic DNA B) amplification C) enzymatic digestion D)detection on agarose gel
Applicability: On extracted and purified genomic DNA from whole blood samples.
Tests: 45

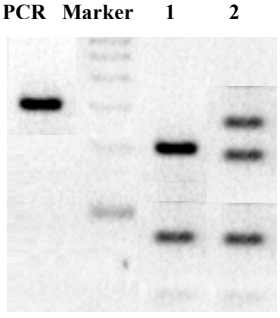
REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Msp I enzyme (10 U/µl)	-20°C
Digestion buffer 10X	-20°C
Positive Control homozygous 353 Arg/Arg	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 312 bp. The next restriction section made by the MnlI enzyme can be done the following results:



1	2	3
Homozigote subject 353 Arg/Arg	**Heterozigote subject 353 Arg/Glu**	**Homozigote subject 353 Glu/Glu**
3 bands	4 bands	2 bands
	272 bp	**272 bp**
205 bp	**205 bp**	
67 bp	**67 bp**	
40 bp	**40 bp**	**40 bp**

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.