dia-chem S.R.L.
DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
BIOLOGIA MOLECOLARE

UNI EN ISO 9001
UNI CEI EN ISO 13485

IDENTIFICAZIONE DEI POLIMORPHISMS ASSESSMENT -401 G/T-402 G/A OF CODING GENE OF COAGULATION VII FACTOR

AMPLI-set-FVII -401 G/T-402 G/A Cat. n. 1.313

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 kit allows the detection of– 401 G/T-402 G/A polymorphisms in the gene promoter region. The detection is carried out by an amplification with specific primers of a 475 bp fragment and restriction section made by Bsl I enzyme. The healty sample (-401 GG/-402GG) will give rise to the following enzymatic digestion pattern: 263 bp-120 bp e 93 bp. Only mutated heterozygous and homozygous samples will undergo to II PCR nested and enzymatic digestion with XmnI enzyme.

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 AND DIGESTION	
I PCR mix –401 G/T -402 G/A	-20°C
II PCR mix –401 G/T -402 G/A	-20°C
H_2O RNase/DNase FREE	-20°C
Taq Polymerase (5U/ml)	-20°C
Enzyme Bsl I (10U/ml)	-20°C
Digestion BUFFER 10 X Bsl I	-20°C
Enzyme Xmn I (20U/ml)	-20°C
Digestion BUFFER 10 X Xmn I	-20°C
BSA 100 X	-20°C
Normal positive control (-401GG/-402GG)	-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

The yield of amplification is 476 bp. The next restriction section made by Bsl enzyme can give the following results:

1 **Homozigous subject Normal (-401GG) Normal (-402GG)**	2 **Heterozygous subject**	3 **Mutated homozigous subject**
3 bands 263 bp 120 bp 93 bp	4 bands 356 bp 263 bp 120 bp 93 bp	2 bands 356 bp 120 bp

The first result allows to diagnose the - 401 GG/-402 GG genotype. In other cases the II PCR nested and the Xmn enzymatic digestion are needed allowing the identification of Heterozygous and mutated homozygous.