Pineta Grande Hospital
Castel Volturno

dia-chem s.r.l.

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CE

KIT FOR THE DETECTION OF A1691G POLYMORPHISM OF THE FACTOR V GENE

AMPLI-SET-FV Leiden Cat. n. 1.311

A point mutation (substitution A-G) in the position 1691 of the gene encoding for the coagulation factor has been linked to the resistance to the degradation of activated Protein C (1-4). This functional alteration can be correlated to an increased venous thrombotic risk.
The functional assay of rAPC (resistance to activated Protein C) is based on the detection of aPTT in plasma of patient with and without a fixed concentration of activated Protein C. This assay is affected by a lot of factors, as citrate used as anti-coagulative, calcium chloride, the kind of activator, the purity of activated protein C. Reduced levels of F II and X can distort the results (false normal results). It is impossible to perform the functional assay of activated Protein C during the oral anticoagulant therapy (5), therefore only the genetic analysis of DNA allows to detect the mutation.
The resistance to activated Protein C is a dominant autosomal transmission.
The mutation in heterozygosis is linked to an increase of 5-10% of thrombotic risk, whereas homozygosis is joined to an increase of 50%. The detection of mutation G1691a is carried out using the PCR amplification method with specific primers for the exon 10 of a fragment of 287 bp, followed by restriction section due to MnlI enzyme. The loss of a restriction cleavage means the presence of the mutation. **The allele 1691G produces fragments of 37, 93 e 157 bp, whereas the allele 1691A produces two fragments of 130 and 157 bp.**

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

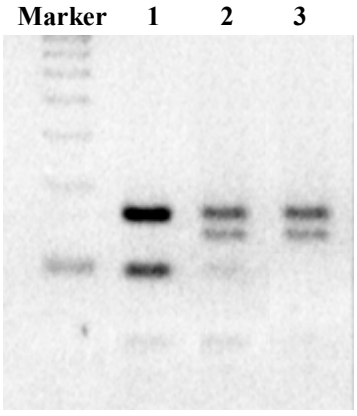
REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Mnl I enzyme (5U//µl)	-20°C
Digestion buffer 10X	-20°C
BSA 100X	-20°C
Positive control heterozigous	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 287 bp. The next restriction section made by the MnlI enzyme can be done the following results:



1	2	3
Absence of mutation Homozygote Normal Patient	**Presence of mutation heterozygote Mutant patient**	**Presence of mutation Homozygous mutant patient**
Presence of 3 lanes	Presence of 4 lanes	Presence of 2 lanes
157 bp	**157 bp**	**157 bp**
93 bp	**130 bp**	**130 bp**
37 bp	**93 bp**	
	37 bp	

References:
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