



KIT FOR THE DETECTION OF Y1702C POLYMORPHISM OF THE FACTOR V GENE

AMPLI-SET-FV Y1702C

Cat. n. 1.316

The heterogeneity of clinic phenotype and the variability of thrombotic events showed by patients with familiarity for thrombotic disease have led to the hypothesis that the predisposition to these type of disorders may be due to many genetic factors. Recently, a complex haplotype of Factor V (HR2), which includes 13 different polymorphisms, has been reported. Among them, 7 cause an amino acid substitution and a functional modification of the protein, leading to an excess of plasmatic isoform FV1 concentration, more thrombogenic.

It isn't clear if haplotype HR2 alone could be a factor of thrombotic risk. It is sure that the risk of clinical thrombotic events in subjects carriers of the F V Leiden mutation is increased.

The detection of the Y1702C polymorphism is carried out starting with an amplification using specific primers of a fragment of 120 bp, following by a restriction section due to *Acc I*. The mutation is confirmed by the loss of a cleavage site for the enzyme *Acc I*.

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

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 120 bp. The next restriction section made by the *Acc I* enzyme can be done the following results:

REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix	-20°C
H ₂ O sterile	-20°C
Taq Polymerase (5U/μl)	-20°C
Acc I enzyme (10 U//μl)	-20°C
Digestion buffer 10X	-20°C
Positive normal control	-20°C

1	2	3
Absence of mutation	Presence of mutation	Presence of mutation
Normal Patient	Eterozigote	Homozigote
Kit control	Mutant patient	mutant patient
2 fragments	3 fragments	1 fragment
	120 bp	120 bp
105 bp	105 bp	
15 bp	15 bp	

Stability: over 12 months if correctly stored.

Usually, the resolution of agarose gel weakens the visualization of the band of 15 bp. The molecular diagnosis is guaranteed from the visualization of other fragments.

References:

- Thrombosis and Haemostasis*, 1996, 75; 45-48.
- Blood*, 1997, 90, 4; 1552-1557.
- Blood*, 2000, 96, 4; 1443-1448.
- Thrombosis and Haemostasis*, 2000, 83; 577-82.
- Haematologica*, 2001, 86, 6; 629-633.
- Blood*, 2001, 98, 2; 358-367.
- Hum. Genet.*, 2002, 111; 59-65.