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DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
BIOLOGIA MOLECOLARE

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CE

KIT FOR THE DETECTION OF G20210A POLYMORPHISM OF THE PROTHROMBIN GENE

AMPLI-SET-FII   Cat. n. 1.310

In 1996 a single mutation in the 3'-untranslated region of the prothrombin gene was reported, resulting in a G-to-A substitution. This polymorphism is joined to an increased level of plasmatic prothrombin. This polymorphism changes the efficiency of the "processing" and the stability of his own RNA. The 20210A allele has been found in 18% of a population of patients selected for individual or familiar venous thrombotic events, in the 6,2% f patients with a first event of deep venous thrombosis and in 2,3% of health control subjects. The risk of thrombotic event associated to this polymorphism is 2,8 greater than associated to the 20210G allele.

The detection of mutation G20210A is carried out using the amplification with specific primers of a fragment of 223 bp, followed by restriction section by Hind III enzyme. The gain of a restriction cleavage shows the presence of the mutation. The normal allele produces a fragment of 223 bp, whereas **the mutated allele produces fragments of 197 bp and 26 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$_2$O sterile	-20°C
Taq Polymerase (5U/μl)	-20°C
Enzima Hind III (10U/μl)	-20°C
Digestion buffer 10X	-20°C
Positive heterozygous control	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 223 bp. The wild type allele produces a fragment of 223 bp, whereas the mutated allele produces fragments of 197 bp and 26 bp.
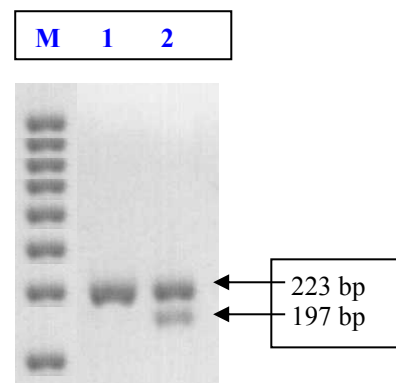
M = Marker 100 bp ladder

1) **Restriction cleavage with Hind III of a normal sample.**
2) **Restriction cleavage with Hind III of a heterozygous sample.**

References:
Dahlback B., *Thromb. And Hemost. 1995;73:739-42.*
Roger M., et al. *Letter to Nature 1994,369:64-67.*