DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
BIOLOGIA MOLECOLARE

UNI EN ISO 9001
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KIT FOR THE DETECTION OF PLATELET GLYCOPROTEIN RECEPTOR IIb/IIIa POLYMORPHISM

AMPLI-set-GpIIb/IIIa Cat. n.1.340

Platelet membrane glycoprotein IIb/IIIa (GpIIb-IIIa) is platelet membrane receptor and member of the integrin family of adhesive molecules that, when activated, binds fibrinogen and von Willebrand factor, thereby promoting platelet aggregation and clotting. The gene encoding the GpIIIa arm of the integrin molecule is polymorphic (substitution C - T) at exon 2. This single base change results in a leucine/ proline polymorphism at amino acid 33 of mature glycoprotein IIIa. The more common allele encodes a leucine (P1A1), and the less common allele encodes a proline (P1A2).
The GpIIb-IIIa is involved in the pathogenesis of acute coronary syndromes. In different studies the P1A2 allele of GpIIb-IIIa was reported to be an inherited risk factor for acute coronary artery events.
In this Kit the detection of the polymorphism C-T is performed starting with an amplification (PCR) using specific primers of a fragment 266 bp, followed by the digestion with the restriction enzyme *MspI.* The polymorphism C-T (P1A2 allele) is confirmed by the detection of an additional cleavage site for the restriction enzyme MspI.

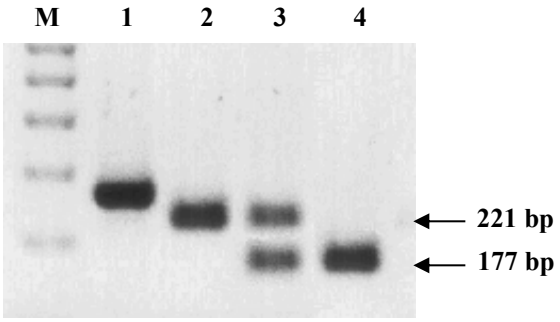
Principle of method: A) extraction of genomic DNA B) amplification C) enzymatic digestion D)detection on agarose gel.
Applicability: On extracted and purified genomic DNA from whole blood samples.
Tests: 45

REAGENTS AND STORAGE

AMPLIFICATION	
Mix PCR GpIIb-IIIa	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Msp I Enzyme (5U//µl)	-20°C
Digestion buffer 10X	-20°C
Positive control	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS



M) Marker 100 bp ladder
1) PCR Product undigested 266 bp
2) Normal subject **P1A1/P1A1**
3) Heterozygote subject **P1A1/P1A2**
4) Homozygote subject **P1A2/P1A2**

Normal subject **P1A1/P1A1** Polimorphism absence	**Heterozigous subject** **P1A1/P1A2** Polimorphism presence on an allele	**Homozigous subject** **P1A2/P1A2** Polimorphism presence on both the alleles
2 fragments	4 fragments	fragment3
221 bp 45 bp	**221 bp** **177 bp** 50 bp 45 bp	**177 bp** 50 bp 45 pb

N.B. The fragments in grey usually are not evident on agarose gel.

References:
The New England Journal of Medicine, **334**(17):1090-1095 (1996)
J. Clin. Invest. **83**:1778-1781 (1989)