DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
BIOLOGIA MOLECOLARE

UNI EN ISO 9001
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KIT FOR THE DETECTION OF A66G MUTATION OF THE METHIONINE SYNTHASE REDUCTASE ENZYME (MTRFR) GENE

AMPLI-SET-MTRR Cat. n. 1.302

The increase of homocysteine may depend on a metabolic block of the transformation of homocysteine in cystathionine or on the unsuccessful methilation of homocysteine in methionine.
The Methionine Synthase Reductase Enzyme (MTRR) is involved in the homocysteine re-methylation pathway. Fasting hyper-homocisteinemie is associated to an increased risk of vascular cerebral , peripheral and coronary diseases.
Many mutations have reported in the genes involved in the homocysteine pathway, (C677T MTHFR, A1298C MTHFR). Recently, a polymorphism A66G in the MTRR enzyme was reported, which converts an isoleucin in methionine. The detection of the mutation is carried out starting with amplification using specific primers of a fragment of 256 bp, following by a restriction section due to NdeI enzyme. The mutation is confirmed by the detection of a restriction cleavage due to NdeI enzyme. The amplification product of **the normal allele is cut in 2 fragment (231 and 25 bp), whereas the mutant allele produces 3 fragment (198,33,25 bp)**
The simultaneous occurrence of this mutation and other risk factor (i.e. folic acid status), may affect the homocysteine pathway, increasing its serum level.

Principle of Assay: 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

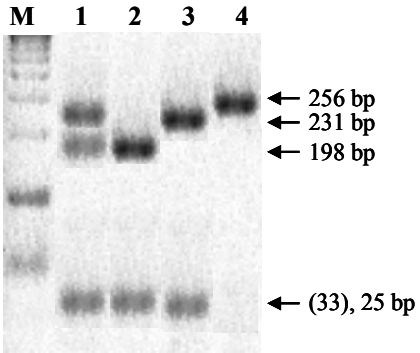
REAGENTS AND STORAGE

AMPLIFICATION	
PCR Mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Nde I Enzyme (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 256 bp. After the enzymatic digestion, the product of PCR of the normal allele is cut in 2 fragments (231 e 25 bp), whereas the mutated allele produces 3 fragments ((198, 33, 25 bp).



Legenda gel:
M)Marker ladder 100 bp.
1) Restriction cleavage with Nde I of a mutant heterozygous
3) Restriction cleavage with Nde I of a mutant homozygous
3) Restriction cleavage with Nde I of a normal homozygous
4) Not digested amplification product.

References:
Gene 240 (1999) 75-88.
Mol Genet Metab. 1999 Aug;67(4):317-23