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KIT FOR THE DETECTION OF A1298C POLYMORPHISM OF THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE

AMPLI-SET- MTHFR A1298C Cat. n. 1.301

The increase of homocysteine may depend on a metabolic block of the transformation of homocysteine in cystathionine or on the unsuccessful methylation of homocysteine in methionine.The classic form of hyper-homocysteinemia is due to a deficiency of the cystathionine sintetase that catalyzes the production of cystathionine from homocysteine and serine.The enzyme MTHFR catalyses the reduction of 5,10-methylentetraydrofolate in 5- methyltetrahydrofolate, the predominant form of circulating folate and donor of carbon in the process of re-methylation of the homocysteine in methionine. Many mutations are reported in the gene encoding for the MTHFR. The more important are the C677T mutation and the A1298C mutation.
The kit allows to detect the A1298C mutation. This is a point mutation in exon 7, which results in amino acid substitution (glutamate for alanine) in the enzyme. This polymorphism reduces the enzyme. People who are compound heterozygous for A1298c and C677T (people with A1298C/C677T genotype) have increased serum homocysteine levels. Fasting hyper- homocysteinemia is associated to an increased risk of vascular cerebral, peripheral and coronary diseases. The detection of the MTHFR (A-C) is carried out starting with an amplification using specific primers of a fragment of 163 bp, following by a restriction section due to MboII The mutation is confirmed by the lack of a restriction cleavage for the enzyme MboII. So, **the amplification product of the normal allele is cut in 5 fragments, whereas the one of the mutant allele produces 4 fragments ((84, 31, 30, 18 bp).**

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.

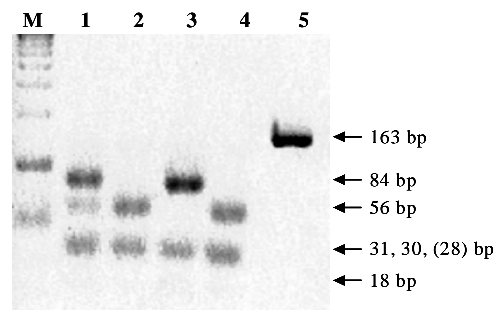
REAGENTS AND STORAGE

AMPLIFICATION and DIGESTION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Mbo II enzyme (10U//µl)	-20°C
Digestion buffer10X	-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 163 bp. After the enzymatic digestion, the product of PCR of the normal allele is cut in 5 fragments (56,31,30,28,18), whereas the mutated allele produces 4 fragments (84,31,30,18)



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

References:
Mol Genet Metab 1998; 64:169-72;
Am J Hum Genet 1998; 62:1044-51