KIT FOR THE DETECTION OF C677T POLYMORPHISM OF THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE

AMPLI-SET-MTHFR C677T Cat. n. 1.300

The increase of the level 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 enzyme MTHFR catalyses the reduction of 5,10-methylentetrahydrofolate in 5-metyltetrahydrofolate, the predominant form of circulating folate and donor of carbon in the process of re-methylation of homocysteine in methionine.
A mutation C-T, which inserts a Valine instead of a Alanine, is associated with a reduced activity and an increased thermolability of this enzyme. Homozigote subjects for the mutation show a significative increase of the plasmatic level of circulating homocysteine, due to the unsuccessful conversion in methionine.
Fasting hyper-homocysteinemia (increased plasmatic level of circulating homocysteine) is associated to an increased risk of vascular cerebral , peripheral and coronary diseases. The detection of the MTHFR (C-T) is carried out starting with an amplification using specific primers of a fragment 198 bp, following by a restriction section due to Hinf I enzyme. The mutation is confirmed by the detection of a restriction cleavage for the Hinf I enzyme. So, the amplification product of the normal allele isn't cut, whereas the one of the mutant allele produces two fragments of 175bp and 23bp.

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.

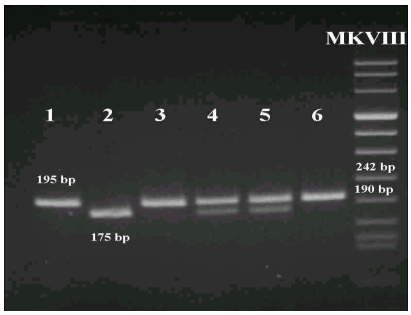
REAGENTS AND STORAGE

AMPLIFICATION and DIGESTION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Hinf I enzyme (10U//µl)	-20°C
Digestion buffer 10X	-20°C
Positive control (Homozygote mutated)	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 198 bp. the PCR fragment containing the mutation is cleaved into two fragments (175 and 23 bp).



Legenda gel:
1) Amplification product of a DNA HOMOZYGOUS ECESSIVE subject
2) Restriction cleavage with HINF I of the sample 1
3) Amplification product from DNA ETEROZYGOSIS subject
4) Restriction cleavage with con HINF I of the sample 2
5) Restriction cleavage on eterozygote control
6) Restriction cleavage on HOMOZYGOUS NORMAL CONTROL.
7)Marker VIII

References:
Frosst P et al. ***Nat Genet*** 1995 May;10(1):111-3.
Margaglione M et al. ***Thromb Haemost*** 1998 May;79(5):907-11.