



ampli set MTHFR^{CE IVD}

45 tests

cat 1300

detection of C677T polymorphism of the Methylenetetrahydrofolate reductase (MTHFR) gene

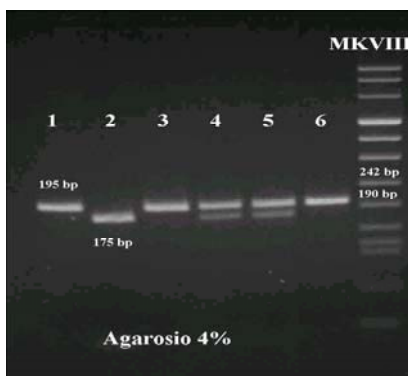
The increase of the level of homocysteine may depend on a metabolic block of the transformation of homocysteine in cystathionine or on the unsuccessful methylation of homocysteine in methionine. The enzyme MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate in 5-methyltetrahydrofolate, the 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. Homozygote 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 HinfI enzyme. The mutation is confirmed by the detection of a restriction cleavage for the HinfI 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.

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)



- 1) Amplification product of a DNA HOMOZYGOTIC RECESSIVE subject
- 2) Restriction cleavage with HINF I of the sample 1
- 3) Amplification product of DNA ETEROZYGOTIC subject
- 4) Restriction cleavage with HINF I of the sample 2
- 5) Restriction cleavage of the Heterozygote control
- 6) Restriction cleavage of Homozygote Normal control
- 7) Marker VIII

References

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