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.

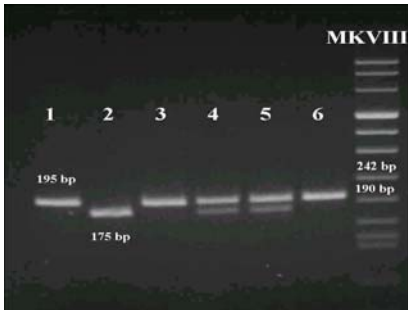
REAGENTS AND STORAGE

EXTRACTION	
Reagent 1 (Separation buffer)	+4°C
Reagent 2 (Lisys buffer)	+4°C
Reagent 3 (Deproteination solution)	+4°C
Reagent 4 (Proteinase k)	-20°C
Reagent 5 (NaCl solution)	+4°C
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
DETECTION	
Agarose gel 4% for electrophoresis in TBE 1X	R.T.
Loading buffer 10 X	R.T.
TBE 10 X	R.T.
Marker ladder 100 bp	-20°C

Stability: over 12 months if correctly stored (Agarose gels, if protected by light, can be stored 1 year at room temperature).
Required Equipment: tubes 1.5 ml screw caps ; tubes 15 ml in polypropylene refreshed tube-tray; plugged (aerosol-barrier) sterile tips; glass Pasteur; tubes NO-OIL
Reagents not included with the kit :bidistilled sterile water, chloroform, Ethanol 70 %, Ethanol 100%.
Required instruments: microcentrifuge with fixed rotor for 2 ml tubes (13-15.000 g); Pre-PCR and Post-PCR Pipettes (0.5 – 20 µl, 10 – 100 µl, 20 – 200 µl, 200 – 1000 µl); Programmable Thermalcycler; Class II Bio Hazard Biological Safety Cabinet; Electrophoresis system with power supply; UV Transilluminator; Photodocumentation System.
All materials requested must be DNase/RNase free, sterile and disposable. It is suggested to operate under safety cabinet and in ice. It is absolutely necessary to use gloves during the handling of samples and reagents.

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 RECESSIVE 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.