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CE

KIT FOR THE DETECTION OF A114V POLYMORPHISM OF THE CYSTATHIONINE-β-SYNTHASE (CBS)

AMPLI-SET-CBS A114V Cat. n. 1.330

The deficit of Cystathionine Beta-Synthase enzyme is an inherited autosomal recessive disorder .The enzyme catalyzes the production of Cystathionine from homocysteine and serine. The deficit causes homocystinuria and the related diseases are dislocated optical lenses, central nervous system involvement ,skeletal abnormalities and vascular disease with severe thromboembolic complications. Two clinical forms can be distinguished on the basis of patient's responsiveness to the treatment with the coenzyme precursor piridoxine.
The mutations of CBS gene may be heterozygote , causing a mild homocystinuria and may be a risk factor for cardiovascular pathologies.
The more frequent mutations in Europe are I278T and A114V .Moreover, in Italian families , is frequent the 844ins68 mutation.
The kit allows the detection of the mutation A114V , changing an alanine in valine in the position 114.
The detection of the mutation is performed with the amplification with specific primers of a fragment of 141 bp, followed by restriction section due to *HpyCH4-IV* enzyme. The mutation creates a new cleavage site, therefore the PCR fragment(141 bp) containing the mutation is cleaved into two fragments (119 and 22 bp)

Principle of method: 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

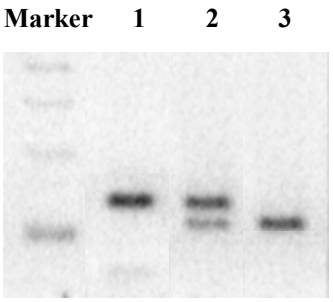
REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Hpy CH4-IV enzyme (10 U/µl)	-20°C
Digestion buffer 10 X	-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 141 bp. The next restriction section made by the *HpyCH4-IV* enzyme can give the following results:



1	2	3
Absence of mutation Normal homozygous pazient	**Presence of mutation Mutant heterozygous pazient**	**Presence of mutation Mutant homozygous pazient**
1 fragment	3 fragments	2 fragments
141 bp	**141 bp** **119 bp** **22 bp**	**119 bp** **22 bp**

References:
Hum. Mol. Genet. 1993; 2:1633-8.
Am. J. Hum. Genet. 1995; 56:1324-1333
Thromb Haemost 2000; 84 (4); 576-82.