KIT FOR THE DETECTION OF I278T POLYMORPHISM OF THE CYSTATHIONINE-B-SYNTHASE (CBS)

AMPLI-SET CBS I278T Cat. n. 1.331

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 heterozygotic , 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 I278T, causing the substitution of isoleucine with a threonine in position 278 due to a transition T→C in position 833
The detection of the mutation is performed with the amplification with specific primers of a fragment of 174 bp, followed by restriction section due to *BsrI* enzyme. The mutation creates a new cleavage site, therefore the PCR fragment containing the mutation is cleaved into two fragments (132 and 42 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

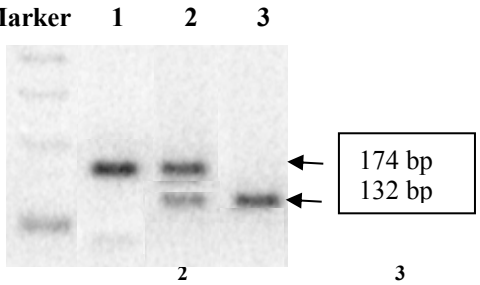
REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Bsr I enzyme (10U//µl)	-20°C
Digestion buffer 10X	-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 174 bp. The next restriction section made by the Bsr *I* enzyme can give the following results:



1	2	3
Absence of mutation normal homozygous	**Presence of mutation mutant heterozygous**	**Presence of mutation Mutant homozygous**
1 fragment	3 fragments	2 fragments
174 bp	**174 bp** **42 bp**	**42 bp**

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