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# DETECTION OF 844ins68 MUTATION IN CYSTATHIONINE-β-SYNTHETASE (CBS) GENE

## AMPLI-set-CBS 844ins68 Real time Cat. n. 1.332RT

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.
Heterozygosis for CBS 844ins68 mutation (present in 7,8% of Caucasian population) isn't a risk factor itself, but it becomes a risk factor when it is associated to mutations of the MTHFR enzyme gene (i.e C677T). In this condition, the risk of occlusive arterial and/or venous pathologies increases of 4 times.
The kit allows the detection of the mutation 844ins68, where there is the insertion of 68 bp in the exon 8. The insertion breaks off the normal protein sequence, causing the abort of the protein.
The detection of the polymorphism is performed carrying out a PCR with specific primers and Sybr Green. This is a DNA-binding agent linking to double-strand DNA, and the emitted fluorescence is proportional to the amount of linked DNA.

**Principle of method:** A) extraction of genomic DNA B) amplification C) detection using real time PCR instrument
**Applicability:** Genomic DNA extracted and purified by whole blood samples
**Number of reactions:** 96.

### REAGENTS AND STORAGE

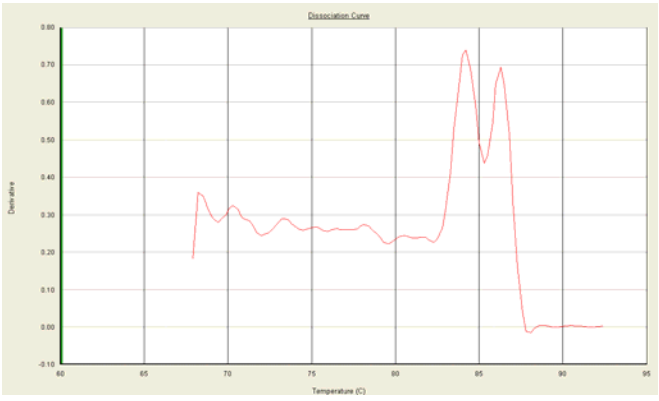
| AMPLIFICATION | |
|---|---|
| PCR mix 2X | +4°C |
| $H_2O$ sterile | -20°C |
| Taq-polymerase 5U/µl | -20°C |
| ROX 1mM | -20°C |
| $MgCl_2$ 50 mM | -20°C |
| Heterozigous Control | -20°C |
| WT Control | -20°C |

**Stability:** over 18 months if correctly stored.

### ANALYSIS OF RESULTS

SYBR Green is a fluorochrome binding double strand DNA. The increase in fluorescence intensity is proportional to the amount of double stranded PCR products produced. Performing a dissociation profile after the PCR reaction, it is possible to relate the emitted fluorescence to the temperature, because every amplicon has its own specific dissociation curve characterized by a specific melting temperature.
The detection of the polymorphism is performed carrying out a PCR with specific primers that produces a product of PCR of 127 bp if the insertion is absent, and of 195 bp if it is present. The analysis of the dissociation protocol allows to detect the WT allele with a melting of 84°C, whereas the mutant allele is characterized by a melting temperature of 86°C .

**Dissociation curve of an heterozigous subjiect**




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