CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

DETECTION OF I278T MUTATION IN CYSTATHIONINE-β-SYNTHETASE (CBS) GENE

AMPLI-set-CBS I278T Real time Cat. n. 1.331RT

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 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 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 polymorphism involves performing a PCR with specific primers and a probe which anneals between primer sites). The probe is is labelled with a fluorescent reporter dye bound to the 5' and quencher on the 3' end.. Due to the 5' nucleAse activity of Taq pol during extension , the cleavage of the probe causes an increase of the reporter dye signal and the fluorescent intensity is proportional to the amount of amplicon produced (real time quantitative PCR).
In the kit for the detection of I278T polymorphism, the probe matching the wild type sequence (allele C) is labelled to the FAM dye reporter, whereas the probe matching the mutation sequence (allele T is labelled to the VIC dye reporter).

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

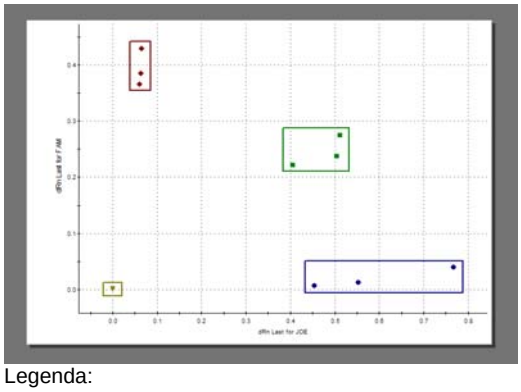
AMPLIFICATION	
PCR mix 2X	+4°C
H_2O sterile	-20°C
Primer-probe mix 20X	-20°C
Controllo positivo ETEROZIGOTE (alleli TC)	-20°C
Controllo positivo OMOZIGOTE (alleli CC	-20°C
Controllo positivo OMOZIGOTE (alleli TT)	-20°C

Stability: over 18 months if correctly stored.

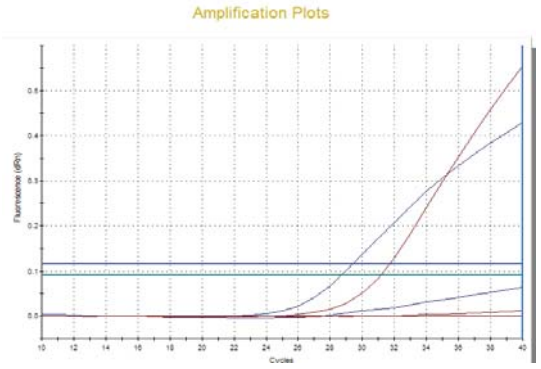
ANALYSIS OF RESULTS

After an AD post-read run, the software analyzes raw data using the AD specific program . Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.

Allelic discrimination CBS I278T



Legenda:
Red: T allele
Green: T and C alleles
Blue: C allele



Amplification plot: eterozigosis sample, both probes indicate a product of PCR.
Red: probe labelled with VIC fluorescent reporter dye.
Blue: probe labelled with FAM fluorescent reporter dye.

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