

DETECTION OF Y1702C MUTATION IN FV GENE

AMPLI FV Y1702C REAL TIME

Cat 1.316RT

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 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).

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

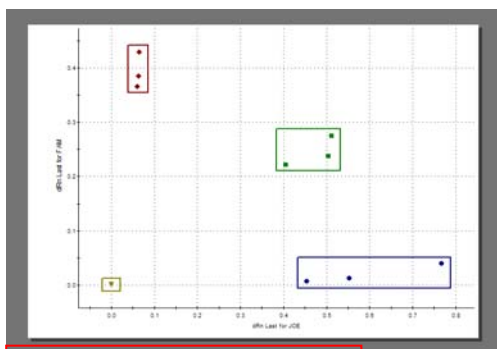
PCR mix 2X	+4°C
H ₂ O sterile	-20°C
Primer-probe mix 20X	-20°C in the dark
WT Control	-20°C
Eterozygosis positive control	-20°C

Stability: over 18 months if correctly stored.

ANALYSIS OF RESULTS

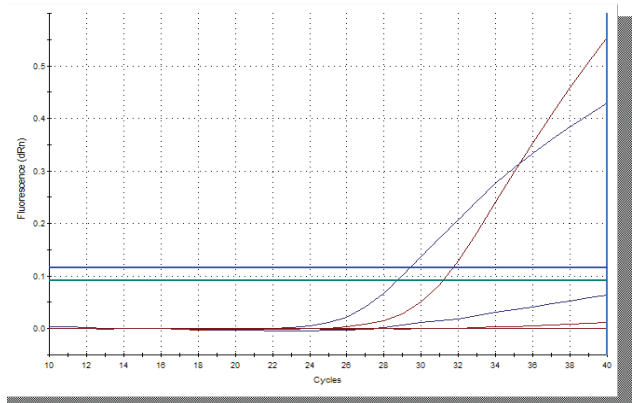
After an AD post-read run, the software analyzes raw data using the AD specific program. Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.

Allelic discrimination FV H1299R



Legend:
Red: allele G
Green: A and G alleles
Blue: allele A

Amplification Plots



Amplification plot: eterozygous 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