CERTIFIED COMPANY
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# DETECTION OF THE A66G MUTATION OF MTRR GENE

## AMPLI MTRR A66G REAL TIME Cat 1.302RT

The increase of homocysteine may depend on a metabolic block of the transformation of homocysteine in cystathionine or on the unsuccessful methilation of homocysteine in methionine.
The classic form of hyper-homocysteinemia is due to a deficiency of the cystathionine sintetase that catalyzes the production of cystathionine from homocysteine and serine.
The Methionine Synthase Reductase Enzyme (MTRR) is involved in the homocysteine remethylation pathway. Fasting hyper-homocisteinemie is associated to an increased risk of vascular cerebral, peripheral and coronary diseases.
Many mutations have reported in the genes involved in the homocysteine pathway, (C677T MTHFR, A1298C MTHFR). Recently, a polymorphism A66G in the MTRR enzyme was reported, which converts an isoleucin in methionine.
The detection of the polymorphism involves performing a PCR with specific primers and a probe which anneals between primer sites). The probe is labeled 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 A66G polymorphism, the probe matching the wild type sequence (allele A) is labeled to the VIC dye reporter, whereas the probe matching the mutation sequence (allele G) is labeled to the FAM 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.

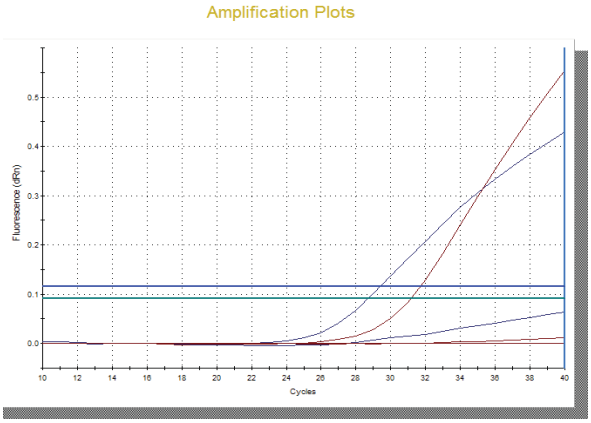
### REAGENTS AND STORAGE

| AMPLIFICATION | |
|---|---|
| PCR mix 2X | +4°C |
| $H_2O$ sterile | -20°C |
| Primer-probe mix 20X | -20°Cin the dark |
| WT Control | -20°C |
| Mutant Control | -20°C |
| **Eterozygosis positive control** | -20°C |

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

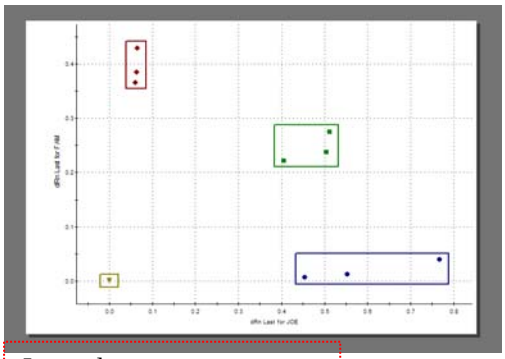
### ANALYSIS OF RESULTS

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.




**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.

### Allelic discrimination MTRR A66G



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

References:

***Nat Genet*** 1995 May;10(1):111-3. Frosst P et al.
***Thromb Haemost*** 1998 May;79(5):907-11. Margaglione M et al.