Identification of 3435 C>T polymorphism of ATP-binding cassette 1 (ABCB1) gene using Real Time PCR

AMPLI SET ABCB1 Cat. n. 1.356 RT

The ABCB1 gene, known as multiple resistance gene (MDR1), is the carrier best characterized thanks to its capacity to confer a phenotype MDR to the cancer cells that have developed resistance to drugs.
The MDR1 gene codes for glycoprotein-P (PgP) plays an important role in the developing of the resistance to many anticancer chemotherapy precluding the accumulation at neoplastic cells level in case of super-expression. More generally the PgP is responsible for biliary and kidney excretion of many drugs and can influence the intestinal absorption or the CNS transit. These proteic structures seem to be a sort of intelligent gate thanks to the cells can regulate the income and outcome from the cytoplasm of some particular substances. These structures can be essential factors for the cell life that, for some reasons, would not pass the cell membrane (macromolecules or hydrophobic substances), the metabolism products if not discarded could compromise the cell life and the corresponding organ functionality.
The single nucleotide polymorphism 3435 C→T in the exon 26 of MDR1 gene, has a frequency of 73%-84% in individuals of African origin and a frequency of 34%-59% in European and Asiatic individuals. This polymorphism is associated to drug-kinetic alteration of several drugs such as digoxin. This allelic variant implies a significant increase of digoxin plasmatic concentration (to parity to oral dosage) in homozygous patients (3435TT), in relation to its increased bioavailability due to the decreased PgP expression in the duodenal mucosal.
The AMPLI set ABCB1 Real Time kit allows the detection of this polymorphism using the Real Time PCR discrimination allelic technique.

Principle of method:) genomic DNA extraction B) amplification and revelation by Real-Time PCR instrument
Applicability: on extracted and purified genomic DNA

Number of Test: 25.

Stability: over 12 months if correctly stored.

REAGENTS AND STORAGE

AMPLIFICATION	
PCR Mix 20 X	-20°C
H_2O RNase/DNase FREE	-20°C
Taq Polymerase 2X	-20°C
Wilde Type control	-20°C
Mutated homozygous control	-20°C
Heterozygous control	-20°C

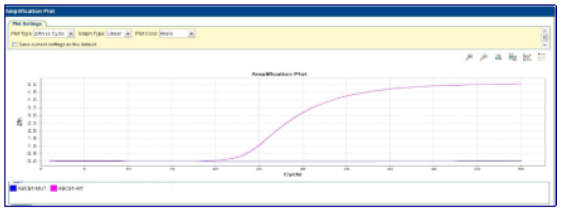
References:

-Haplotypes of ABCB1 1236C >T (rs1128503), 2677G >T/A (rs2032582), and 3435C >T (rs1045642) in patients with bullous pemphigoid. Mariola Rychlik-Sych, et al. Arch Dermatol Res. 2018.

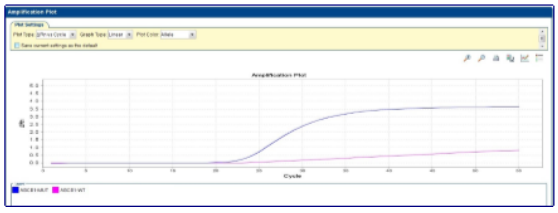
Analysis of the results will be done by specific program (allelic DISCRIMINA TION) Real-time PCR instrument has been set. In any case, however, it is useful to also analyze the graphs AMPLIFICATION PLOT, to ensure that the reaction has correctly taken place.
Following a discrimination allelic graph of an heterozygous sample for the polymorphism, a wild type sample and a mutated homozygous sample using APPLIED BIOSYSTEM instrumentation.

CAMPIONE ETEROZIGOTE

CAMPIONE WILDE TYPE



CAMPIONE OMOZIGOTE MUTATO



REV 001

INTERPRETATION OF RESULTS