CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

DETECTION OF POLYMORPHISM CYP3A5*3 in CYP3A5 gene

AMPLI-CYP3A5 Cat. n.2.012

The enzymes of the family of cytochrome P450 3A (CYP3A) are responsible for the metabolism of over 50% of the drugs. The CYP3A5 accounts for 10-30% of the fraction of total cytochrome P450 in Caucasian populations. In particular, since the CYP3A5 isoform is expressed in more than 50% of individuals expressing CYP3A proteins, it has an important role in the variability of drug metabolism mediated by isoenzymes of the 3A family.
The CYP3A5 gene consists of 13 exons and the protein is always composed of a sequence of 502 amino acids. This enzyme is also expressed in extrahepatic tissues such as lung, kidney, breast, prostate and polymorphonuclear leukocytes.
The CYP3A5 gene has several SNPs: 9 in the region between 10 and UTR introns and exons. The main cause for variations in the levels of expression of CYP3A5 in the liver is the presence of the mutation CYP3A5*3 allele.
This allele causes a mutation (6986G> A) in intron 3 that creates a critical cleavage site and causes a premature stop codon, resulting in non-protein expression. The allele *3 is present in different ethnic groups demonstrating an ancient origin, its presence also results in a reduced clearance of some substrates such as lovastatin, the sinvastatina, atorvastatin, tacrolimus, cyclosporine, and midazolam. In clinical terms, the knowledge of the CYP3A5 genotype plays an important role in the choice of drug doses to be administered to the patient.
The kit allows the identification of AMP-CYP3A5, using PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) genetic variant 6986G> A (CYP3A5*3) of the enzyme cytochrome P-450 3A5.

Principle of the method: a) extraction of genomic DNA;
b) amplification;
c) enzymatic digestion;
d) detection on agarose gels.
Applicability: of genomic DNA extracted and purified from whole blood samples.
Number of Tests: 24.

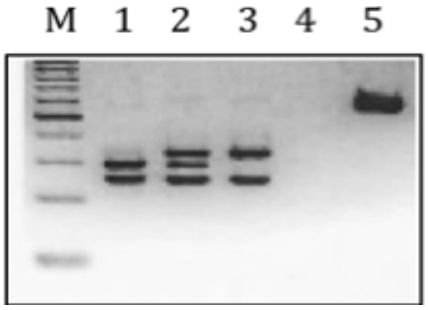
KIT CONTAINS AND STORAGE

AMPLIFICATION	
Mix PCR CYP3A5*3	-20°C
H_2O DNase/RNase-free	-20°C
Taq Polymerase (5U/µl)	-20°C
Controllo DNA	-20°C
ENZYMATIC DIGESTION	
Enzima *Ssp*I (20U/µl)	-20°C
Buffer 10X *Ssp*I	-20°C
H_2O RNase/DNase-free	-20°C

Stability: more than 18 months if properly stored.

ANALYSIS OF RESULTS

The test determines the presence of the variant allele of the gene CYP3A5*3. Based on the results obtained it is possible to evaluate the drug dosage.



Legend:
Lane 1: wild type (allele ***1*** /*1)
Lane 2: heterozigous (allele ***1*** /*3)
Lane 3: mutated homozigous (allele ***3*** /*3)
Lane 4: negative controll
Lane5: PCR product

			Fragments after enzymatic digestion	
Mix PCR	**PCR in bp**	**Restriction enzyme**	**wild type**	**mutato**
CYP3A5*3	**293**	***Ssp*I**	**148** **125** **20**	**168** **125**

References:
Clinical Chemistry (2002) 48:10, *1668-1671.*
Ann Transplant (2009) 14(1), *23-31.*
Pharmacogenetics. (2004) 14(8): *523-5.*
Pharmacogenetics. (2004) 14(3):*147-54.*