IDENTIFICATION OF POLYMORPHISM
Leu858Arg EGFR GENE

AMPLI-set-Leu858Arg EGFR RT   Cat. 2.003RT

EGFR (epidermal growth factor receptor) is a membrane receptor tyrosine kinase belonging to the family of ErbB receptors. This receptor, once bound its specific ligand EGF (epidermal growth factor) and TGFα (transforming growth factor α), activates multiple signal transduction pathways that regulate various cellular processes: division, apoptosis, motility, adhesion.

EGFR mutations are implicated in about 30% of all epithelial tumors. About 90% of EGFR mutations include a substitution of leucine with arginine at position 858 (L858R) and a deletion in exon 19, which influences the sequence conserved LREA (delE746-A750). These mutations cause constitutive activation of the EGFR tyrosine portion, destabilizing its self-inhibitor conformation, normally maintained in the absence of ligand, these activating mutations confer hypersensitivity to the inhibitors gefitinib and erlotinib tyrosine kinases. Several retrospective studies have shown that EGFR mutations are an independent predictor of response, overall survival (OS) and progression-free survival (PFS) in patients with metastatic non-small cell lung cancer (NSCLC) treated with gefitinib, the most of whom underwent prior chemotherapy.

The kit allows the identification of polymorphism Leu858Arg, using the technique of Real-time PCR. The research of this polymorphism is performed after amplification with specific primers and hybridization with a probe that recognizes an internal sequence. The kit used for detection of polymorphism Leu858Arg, the probe that recognizes the sequence wt (T allele) is conjugated to the FAM reporters, and one that recognizes the sequence polymorphism (G allele) is conjugate to reporters Joe.

Principle of the method: A) extraction of genomic DNA
B) amplification and detection using real-time PCR equipment.
Applicability: On genomic DNA extracted and purified from biological fluids and tissue samples from fresh and paraffin.
Number of Tests: 24.

KIT CONTAINS AND STORAGE

AMPLIFICATION	
PCR mix 2X	+4°C
H$_2$O sterile	-20°C
Primer-probe mix 20X	-20°C
Wild type control (TT)	-20°C
Heterozigous control (TG)	-20°C

Stability: more than 18 months if properly stored.

References:
Science (2004) 304, 1497-1500.
N Engl J Med (2004) 350, 2129-2139.
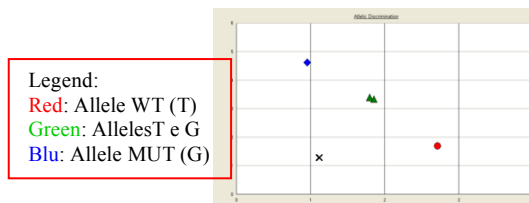Proc Natl Acad Sci U S A (2004) 101, 13306-13311.
L Clin Oncol (2005), 23:2513-2520.

ANALYSIS OF THE RESULTS

The analysis of the results will be made by the specific program (ALLELIC DISCRIMINATION) Real-time PCR instrument previously set. In any case, however, is also useful to analyze the graphs of Amplification PLOT, to ensure that the reaction has taken place correctly.

Allelic discrimination EGFR Leu858Arg

Allelic discrimination EGFR Leu858Arg

Legend:
Red: Allele WT (T)
Green: AllelesT e G
Blu: Allele MUT (G)

Amplification plots EGFR Leu858Arg

Legend:
Blu: Allele WT (T)
Purple: Allele Mut (G)