

IDENTIFICATION OF POLYMORPHISM ELREA (delE746-A750) OF GENE EGFR

AMPLI set ELREA EGFR Real Time

Cat. 2.002RT

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 TGF α (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 tumours. About 90% of EGFR mutations include a substitution of leucine with arginine at position 858 (L858R) in exon 21, and a deletion of 5 amino acids in exon 19, which affects the conserved sequence ELREA (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 to identify deletions in exon 19 of the EGFR gene (epidermal growth factor receptor) using technology Cold Fast Real-Time PCR. The search is performed by amplification with specific primers, used in a quantitative PCR, and a mix containing SYBR GREEN fluorescent molecule that intercalates into the double helix of DNA. The control of specificity occurs after amplification by melting curve analysis.

Principle of the method: a) extraction of genomic DNA;
b) Fast Cold Real Time PCR

Applicability of genomic DNA extracted and purified from biological samples, fresh tissue and paraffin., and fcDNA

Number of Tests: 50.

Stability: more than 12 months if properly stored.

INTERPRETATION OF RESULTS

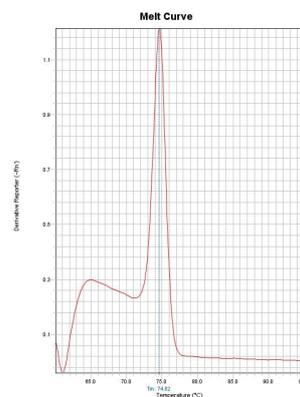
The analysis of the results will be made by specific program of instrumentation previously set. In any case, however, it is also useful to analyze the graphs dell'Amplification Plot, to ensure that the reaction has taken place correctly.

Below is illustrated a graph of Melt Curve analysis performed on Applied Biosystems instruments STEP ONE Real Time PCR System.

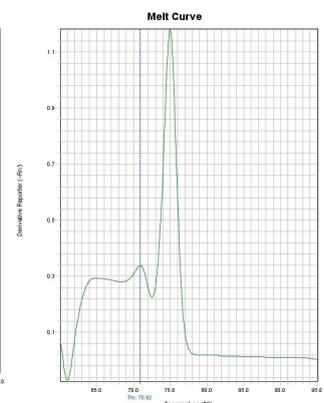
KIT CONTAINS AND STORAGE

<u>AMPLIFICATION</u>	
PCR mix 2X	-20°C
Primers mix 20X	-20°C
H ₂ O DNase/RNase-free	-20°C
Wild type control	-20°C
Heterozigous control	-20°C

Legend:
Red: wt
Green: eterozigote



T_m=74,8 ± 0,38 WT



T_m=74,71 ± 0,44 wt
T_m=70,64 ± 0,24 delezione

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.