KIT FOR THE DETECTION OF ITD (INTERNAL TANDEM DUPLICATION) MUTATION OF THE GENE FLT3

AMPLI Set Y FLT3 ITD Cat. n.1.406

FLT3(FMS-like receptor tyrosine kinase) is a member of the receptor tyrosine kinase class III. It is preferentially expressed on the surface of many acute myeloid leukaemia (AML) and B-lineage acute lymphocytic leukaemia (ALL) cells in addition to hematopoietic stem cells, brain, placenta and liver . An interaction of FLt3 and its ligand has been shown to play an important role in the survival, proliferation and differentiation both normal hemopoietic cells and leukaemia cells. The FLt3 gene may present mutations that cause constitutive activation of the receptor independently of the interaction with the ligand.

An Internal tandem Duplication (ITD) of the juxtamembrane (IM) domain-coding sequence of the FLt3 (FLt3/ITD) and a point mutation D835-Mt in the tyrosin-kinase domain –coding sequence of the receptor are frequently present.

The ITD mutation has been identified in 20% of patients with acute myeloid leukaemia (AML) and in 3% of myelodysplastic syndrome. The detection of the mutations of the FLt3 gene allows a new therapeutic approach., using specific inhibitor of the tyrosine-kinase receptor.

In the ITD mutation , a fragment of the JM domain –coding sequence (exons 11 and 12) is duplicated and the size of the duplication ranges from 21 and 174 bp. The analysis of the mutation is carried out starting with PCR using specific oligonucleotides following by detection on 4% agarose gel.

Principle of method: A) extraction of genomic DNA B) amplification C)detection on agarose gel.
Applicability: On extracted and purified genomic DNA from whole blood samples or tissue.
Tests: 45

REAGENTS AND STORAGE

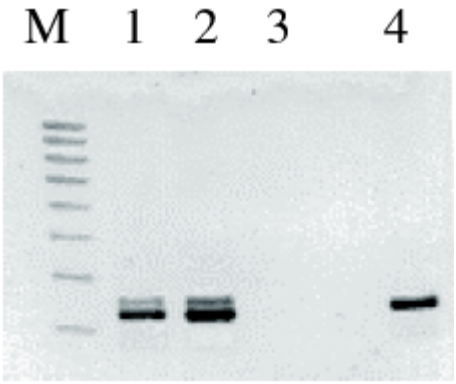
AMPLIFICATION	
PCR MIX	-20°C
sterile H_2O	-20°C
Taq Polymerase (5U/µl)	-20°C
Heterozygosis DNA control	-20°C

Stability: over 12 months if correctly stored.

References:
Blood, 2001, 97, 1, 89-94.
Blood, 2001, 97, 8, 2434-2439.
Oncogene, 2002, 21, 16, 2555-2563.
Leukemia, 2003, 17, 1, 120-4.
Cancer, 2002, 94, 12, 3292-3298.
Blood, 2001, 98, 3, 885-887.
Leukemia and Lymphoma, 2002, 43, 8, 1541-1547

ANALYSIS OF RESULTS

The size of the insertion ranges from 21 and 174 bp. The PCR product is a fragment of 328 bp. The presence of the mutation produces a fragment of 349-502 bp.



M)Marker 100 bp ladder
1) Insertion of 21 bp
2) Insertion of 21 bp
3) H_2O
4) Normal sample 328 bp