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DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
BIOLOGIA MOLECOLARE
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UNI EN ISO 9001
UNI CEI EN ISO 13485

KIT FOR THE DETECTION OF TRANSLOCATION T(9; 22) BCR-ABL

AMPLI-set-BCR-ABL Cat. n. 1.403

PCR analysis of fusion genes is based on the design of oligonucleotide primers at opposite sides of the breakpoint fusion regions so that the PCR product contains the tumor specific fusion sequences. The fusion gene is transcribed into fusion mRNA, which can serve as the PCR target after reverse-transcription (RT) into cDNA.
The Philadelphia chromosome (Ph), besides being the hallmark of CML, also occurs in approximately 2-10% of childhood ALL and in 20-50% of adult ALL with an incidence progressively increasing with age. Ph translocation always results in the joining of 3' sequences of the tyrosine kinase c-ABL proto-oncogene on chromosome 9 to the 5' sequences of the BCR gene on chromosome 22. The result of this translocation is the formation of hybrid c-abl mRNA and of a hybrid protein which amino acid sequence overlaps a part of the bcr gene in the N- terminus and a part of the abl gene in the C-terminus. In CML the fusion transcipts encode a BCR-ABL protein of 210 Kda, called p210BCR/ABL. Approximately 40% of Ph+ ALL show the same molecular rearrangements as in CML; the remaining 60% of Ph+ ALL show a BCR/ABL protein of 190Kda, called p190BCR/ABL. Polymerase Chain Reaction (RT-PCR) of fusion genes is based on the design of oligonucleotide primers at opposite sides of the breakpoint fusion regions so that the PCR product contains the tumour specific fusion sequences.

Principle of method: A) extraction of genomic DNA B) reverse transcription C) amplification C)detection on agarose gel
Applicability: On extracted and purified RNA.
Test n: 45

REAGENTS AND STORAGE

REVERSE TRANSCRIPTION AMPLIFICATION	
RT Mix	-20°C
Rnase inhibitor (40U/µl)	-20°C
Reverse transcriptase (10U/ µl)	-20°C
Random Primers	-20°C
p210 PCR Mix	-20°C
p210 nested PCR mix	-20°C
p190 PCR mix	-20°C
p109 nested PCR mix	- 20°C
sterile H_2O	-20°C
Taq Polymerase (5U/µl)	-20°C
p210 b3-a2 c DNA control	-20°C

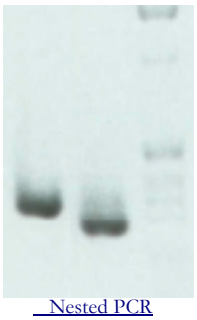
Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The sizes of PCR products are variable due to the different types of breakpoints.

Products PCR Size in bp	I PCR	Nested PCR
BCR-ABL p210		
p210 b3-a2	417	360
p210 b2-a2	342	285
p210 b3-a3	243	186
p210 b2-a3	168	111
BCR-ABL p190		
p190 e1-a2	521	381
p190 e1-a3	347	207

Examplificative agarose gel of a sample with riagreement BCR-ABL p210



Nested PCR
1 2 M

1) PCR product of 360bp = sample p210 b3-a2.
2) PCR product of 285 bp = sample p210 b2-a2.
M Marker

References:
Leukemia **13**:1901-38 (1999)
Leukemia **5**:448-51 (1991)