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IDENTIFICATION OF t(15; 17) TRANSLOCATION PML-RARA

AMPLI-SET-PML RARA Cat. n. 1.402

The translocation t(15;17) is associated with è acute promomyelocytic leukemia (APL). The two genes involved in the translocation are PML, coding for a putative new transcriptional factor, on chromosome 15, and the gene α receptor of retinoic acid (RARA) on chromosome 17. The breaking sites on chromosome 17are located in a DNA fragment of 15 kb in the gene RARA intron. On the contrary, three regions of PML locus are involved in the translocations: intron 6 (bcr1; 55% of cases), eson 6 (bcr2; 5% of cases) and intron 3 (bcr3; 40% cases). The chimeric transcript PML-RARA and RARA-PML are formed as result of mutual translocation between PML and RARA loci. The presence of different "breakpoint" region in PML locus and the presence of alternative PML splicing are responsible for heterogeneity of PML-RARA junctions observed in patients with APL.
The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) analysis of fusion genes s based on primers' design on on opposite sites of fusion regions so that the PCR product will include the specific fusion sequence.

Principle of assay: **A)** extraction of RNA **B)** retro-transcription **C)** amplification and detection on agarose gel
Applicability: exctracted and purified RNA
Number of tests: 45.

REAGENTS AND STORAGE

RETROTRASCRIZIONE e AMPLIFICAZIONE	
Mix RT	-20°C
Rnase inhibitor (40U/µl)	-20°C
Reverse Transcriptase (10U/µl)	-20°C
Random Primers	-20°C
Mix PCR bcr1-bcr2	-20°C
Mix PCR bcr1-bcr2 nested	-20°C
Mix PCR bcr3	-20°C
Mix PCR bcr3 nested	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Control cDNA bcr3	-20°C
RIVELAZIONE	
Gel precast di agarosio 4% per elettroforesi in TBE 1X	T.A.
Loading buffer 10 X	T.A.
Buffer di corsa 5 X (TBE 5X)	T.A.
Marker di peso molecolare ladder 100 bp	-20°C

Stability: over 18 months if correctly stored (agarose gel have to be kept in the dark, they are stabile fo eone year at room temperature)

ANALYSIS OF RESULTS

The amplified products size varies depending on where the fusion between the two genes take place.

	I PCR	Nested PCR
Amplified product size in bp		
bcr 1	381	214
bcr 2	345	178
bcr 3	376	289

example of agarose in sample with rearrangment bc3.

Nested PCR
M 1

M Marker
1) PCR product og 289 bp = sample bcr 3

References:
Nature **347**:558-561 (1990)
Genes Chromos Cancer **2**: 79-87 (1990)
Blood **80**: 494-497 (1992)
Nature **347**-558-561 (1990)
Genes Chromos Cancer **2**: 79-87 (1990)
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92: 659-664 (1996)