

BCR-ABL p190/p210 t(9-22)

Real-Time RT PCR of Transcripts of the M-bcr fusion gene

AMPLI set BCR-ABL p190/p210

Cat. 1.002/One RT

The Philadelphia chromosome (Ph) is one of the most common genetic aberrations found in leukemia. The Ph chromosome is present in more than 95% of chronic myeloid leukemias (CML). Furthermore, in acute lymphoblastic leukemia (ALL), Ph is present in 25-30% of adult cases and in 2-5% of childhood cases. Less frequently, it is associated with acute myeloid leukemia (AML). The Ph chromosome is the result of the conjunction of the 3' sequence of the proto-oncogene tyrosine - kinase c-ABL on chromosome 9 to the 5' sequence of the BCR gene on chromosome 22. In particular, the breakpoint on chromosome 9 is located in most cases between exons 1 and 2 in the ABL gene. Breakpoints in the BCR gene are grouped into two regions: 1) a sequence of the first intron, called the minor breakpoint cluster region (m-bcr); 2) a region comprising exons 12 to 16, called major breakpoint cluster region (M-bcr). In the case of m-bcr, the first exon of the BCR gene (c1) is flanked by the second exon of the ABL gene (a2). The resulting fusion transcript (c1-a2) encodes a 190 KDa chimeric protein (p190). This type of fusion transcript is present in 65% of adults and 80% of ALL Ph positive children. In the case of M-bcr breakpoints, the exons of the BCR gene (b2 or b3) are placed side by side with the second exon of the ABL gene (a2). The resulting fusion transcript (b2-a2 and / or b3-a2) encodes a chimeric protein of 210 KDa (p210). This type of fusion transcript is present in CMLs and approximately 35% of ALL Ph positive adults. Rare cases of transcripts (c1-a3, b2-a3 and b3-a3) have been observed;. Quantification of BCR-ABL transcripts is clinically relevant for monitoring minimal residual disease in leukemia patients undergoing allogeneic stem cell transplantation or treatment with aggressive therapies.

The -BCR-ABL p190/p210 kit in a single step performs the reverse transcription and specific amplification of the p190/p210 transcript with a pair of specific primers and an internal probe labeled with FAM fluorophore. In the BCR-ABL p190/p210 kit, an endogenous control (ABL transcript) is retro-transcribed and amplified in the sample as well as the fusion transcript of interest. Furthermore, standard curves of known quantities of both the ABL endogenous control and the fusion transcript allow the calculation of the ratio between the signal of the specific fusion transcript and the signal of the endogenous ABL in each sample.

The -BCR-ABL p190/p210 allows the quantification of BCR-ABL 190/210 transcripts in peripheral blood or bone marrow samples from ALL or CML patients in accordance with Europe Against Cancer studies (J. Gabert et al. Leukemia 2003).

Principle of the method: RNA extraction, reverse transcription and amplification by real-time RT PCR OneStep.

Applicability: On genomic and medullary RNA.

Number of tests: 24.

Stability: over 12 months if properly store.

Interpretation of the Results

The number of cycles where the emission intensity of the reporter dye exceeds the background noise is called the threshold cycle Ct (threshold cycle). The Ct is directly proportional to the number of copies of the target template at the start of the reaction (on a TaqMan instrument set the Ct to 0.1 and the baseline between cycles 3 and 15)

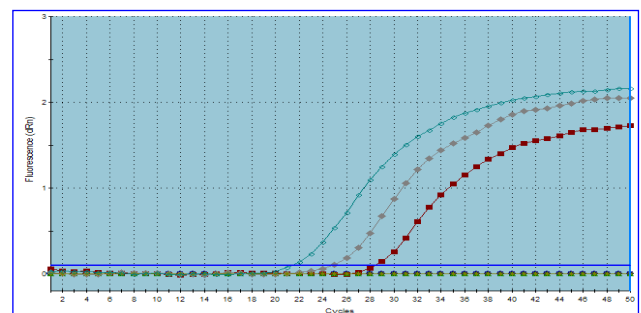
The figures above show an example of Amplification plots of samples positive for the fusion transcript p190 with clear signal in the FAM channel.

CONTENTS OF THE KIT AND ITS STORAGE

AMPLIFICAZIONE

| | |
|--------------------------|-------|
| PCR mix p190 | -20°C |
| PCR mix p210 | -20°C |
| Taq Rt One Step | -20°C |
| Positive Control p190 | -20°C |
| Positive Control p210 | -20°C |
| Positive Control Abelson | -20°C |
| H ₂ O sterile | -20°C |

Amplification Plots



Bibliography :

- 1) VHJ van der Velden et al. Leukemia 17, 1013-1034 (2003)
- 2) E. Beillard et al. Leukemia 17, 2474-2486 (2003)
- 3) J. Gabert et al. Leukemia 17, 2318-2357 (2003)