

# Calculation of minimal residual disease (MRD)

## Normalised Copy Number method (NCN)

Results can be calculated using normalized copy numbers (NCN). This method makes use of standard curves always tested on each PCR plate. In particular, the **QUANT-BCR-ABL p210** kit use three plasmid standard dilutions for the control gene (ABL), and five standard dilutions for the fusion gene (BCR-ABL p210). This methodology has the advantage that degradation of probes can be compensated for, and that data generated on different types of Real time-PCR machines can be compared.

The normalised copy number (NCN) is defined as the copy number (CN) of the fusion gene (FG) per one copy of the control gene (CG) transcript:

mean value of  $\log_{10}(FG_{CN})$  - mean value of  $\log_{10}(CG_{CN})$ .

In the NCN method, the minimal residual disease (MRD) value is a ratio between the CG normalized expression of the FG in follow-up ( $FG_{CN}/CG_{CN}$ )<sub>FUP</sub> and diagnostic samples ( $FG_{CN}/CG_{CN}$ )<sub>DX</sub>.

For calculation of MRD value, Europe Against Cancer (EAC) data (J. Gabert et al. Leukemia 17, 2318-2357 -2003) for the corresponding FG at diagnosis can be used if patient value at diagnosis is not available.

Sensitivity (SENS<sub>v</sub>) is calculated according to the relative expression of the FG at diagnosis ( $FG_{CN}/CG_{CN}$ )<sub>DX</sub> and CG expression ( $CG_{CN,FUP}$ ) in the follow-up sample.

The EAC formula for calculation of MRD value and theoretical sensitivity based on FG and CG Real-Time Quantitative-PCR sets

$$\text{MRD value (MRD}_v) = (FG_{CN} / CG_{CN})_{FUP} / (FG_{CN}/CG_{CN})_{DX}$$

$$\text{Sensitivity (SENS}_v) = CG_{CN,DX} / (CG_{CN,FUP} \times FG_{CN,DX})$$

## References

- 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)



## QUANT-BCR-ABL p210

### Real-Time Quantitative PCR of M-bcr fusion gene transcripts cat 1.002

The Philadelphia chromosome (Ph) is one the most common genetic aberrations detected in leukemias.

The Ph chromosome is found in more than 95% of chronic myeloid leukaemia (CML) cases. In addition, in acute lymphoblastic leukemia (ALL), Ph is detected in 25-30% of adult and 2-5% of childhood cases. Less frequently, it is associated with acute myeloid leukemia (AML).

Ph chromosome 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.

In particular, the breakpoint on chromosome 9 is located in most cases between exons 1 and 2 in the ABL gene. The breakpoints in the BCR gene are clustered within two regions: 1) a sequence of the first intron, called the minor breakpoint cluster region (m-bcr); 2) a region spanning exons 12 to 16, called the major breakpoint cluster region (M-bcr).

In the case of m-bcr breakpoints, the first exon of the BCR gene (e1) is juxtaposed to the second exon of the ABL gene (a2). The resultant fusion transcript (e1-a2) encodes a 190 KDa chimeric protein (p190). This type of fusion transcripts is found in 65 % of adults and 80% of children with Ph positive ALL.

In the case of M-bcr breakpoints the exons of the BCR gene (b2 or b3) are juxtaposed to the second exon of the ABL gene (a2). The resultant fusion transcript (b2-a2 and/or b3-a2) encodes a 210 KDa chimeric protein (p210). This type of fusion transcripts is found in CML and approximately 35% of adults with Ph positive ALL.

Rare cases with e1-a3 transcripts and with b2-a3 and b3-a3 BCR-ABL transcripts can be observed.

The quantification of BCR-ABL transcripts is clinically relevant for minimal residual disease (MRD) monitoring in patients with leukemia undergoing allogeneic hematopoietic stem cell transplantation or treatment with aggressive therapies.

In the **QUANT-BCR-ABL p210** kit the real-time PCR is used in the second step of a two-step reverse transcription-polymerase chain reaction (RT-PCR) protocol. The template, cDNA generated from a reverse transcription reaction, is amplified by PCR using a pair of specific primers and a specific internal double-dye probe (FAM-TAMRA). During the reaction, cleavage of the probe, by the 5' nuclease activity of Taq DNA Polymerase, separates the reporter dye (FAM) and the quencher dye (TAMRA), which results in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. In the **QUANT-BCR-ABL p210** kit, an endogenous control (ABL transcript) is amplified from the sample as well as the fusion transcript of interest. In addition, Standard curves of known amounts of both the endogenous ABL control and the fusion cDNA allow the calculation of the ratio of specific fusion transcript signal to endogenous ABL signal in each sample.

**The QUANT-BCR-ABL p210 allows the quantification of BCR-ABL 210 transcripts in peripheral blood or bone marrow samples of ALL or CML patients according to the Europe Against Cancer studies (J. Gabert et al. Leukemia 2003).**

## Kit contains and storage

NAME	COLOUR CODE	STORAGE
<b>REVERSE TRANSCRIPTION</b>		
5X First -Strand Buffer	(Green)	-20°C
DTT 100 mM	(Violet)	-20°C
RNase Inhibitor (40U/μl)	(Purple)	-20°C
Random hexamer 100μM	(White)	-20°C
dNTP 20 mM	(Red)	-20°C
Reverse Transcriptase (200U/μl)	(Yellow)	-20°C
H <sub>2</sub> O RNase/DNase-free	(Light Blue)	-20°C
<b>STANDARD DILUTIONS</b>		
<b>St1</b> -ABL-10 <sup>3</sup> copies/5μl	(Green)	-20°C
<b>St2</b> -ABL-10 <sup>4</sup> copies/5μl	(Green)	-20°C
<b>St3</b> -ABL-10 <sup>5</sup> copies/5μl	(Green)	-20°C
<b>St1</b> -BCR-ABL p210 10 <sup>1</sup> copies/5μl (b3-a2 M-bcr)	(Red)	-20°C
<b>St2</b> -BCR-ABL p210 10 <sup>2</sup> copies/5μl (b3-a2 M-bcr)	(Red)	-20°C
<b>St3</b> -BCR-ABL p210 10 <sup>3</sup> copies/5μl (b3-a2 M-bcr)	(Red)	-20°C
<b>St4</b> -BCR-ABL p210 10 <sup>5</sup> copies/5μl (b3-a2 M-bcr)	(Red)	-20°C
<b>St5</b> -BCR-ABL p210 10 <sup>6</sup> copies/5μl (b3-a2 M-bcr)	(Red)	-20°C
<b>REAL-TIME PCR</b>		
10X ABL (Primers/FAM-TAMRA probe)	(Green)	-20°C
10X BCR-ABL p210 (Primers/FAM-TAMRA probe)	(Red)	-20°C
Master Mix 2X	(Purple)	+4°C
H <sub>2</sub> O RNase/DNase-free	(Light Blue)	-20°C

**Principle of assay:** A) extraction of total RNA B) synthesis of cDNA from total RNA C) real-time PCR

**Applicability:** On extracted and purified total RNA from peripheral blood or bone marrow cells.

**Numbers of tests:** 24

**Stability:** over 18 months if correctly stored

**Required materials:** refreshed tube tray; filter sterile tips; PCR tubes DNase and RNase free.

**Reagents not included with the kit:**

- Reagents for total RNA extraction

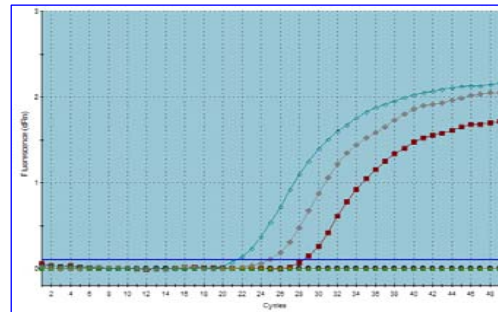
**Required equipment:** Pre-PCR and post-PCR pipettes (0.5 – 20 μl, 10 – 100 μl, 20 – 200 μl, 200 – 1000 μl); Programmable thermal cycler, Class II Bio hazard Biological Safety Cabinet, electrophoresis system with power supply; UV transilluminator; Photodocumentation System; Real-time PCR instrumentation.

## Analysis of results

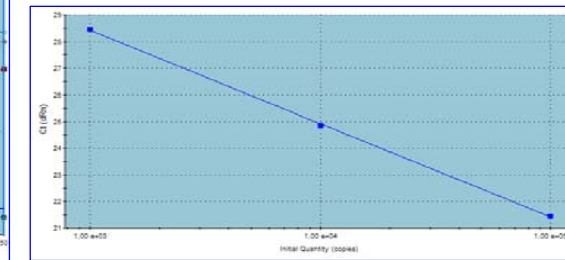
The cycle number at which the reporter dye emission intensities rises above background noise is called the threshold cycle (Ct). The Ct is directly proportional to the copy number of the target template at the beginning of the reaction. (On a TaqMan machine set a threshold at 0.1 and a baseline between cycles 3 and 15). Using Standard curves of known amounts of both the endogenous ABL control and the fusion cDNA allow the calculation of the ratio of specific fusion transcript signal to endogenous ABL signal in each sample. (The theoretical slope of the standard curve is -3,32 for a PCR reaction with a maximum efficiency). The figures below show an example of Amplification plots and Standard curves of ABL and BCR-ABL p210 standard dilutions.

### STANDARD DILUTIONS - Control gene ABL (St.1 - St.2 - St.3)

Amplification Plots



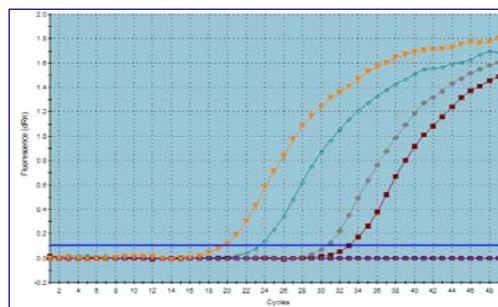
Standard Curve



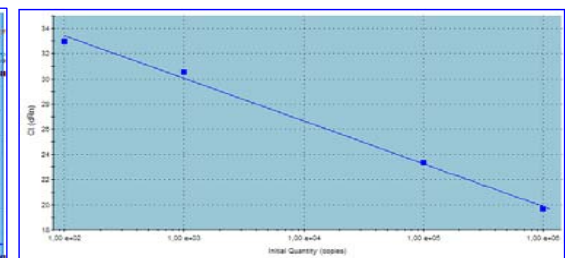
$$Y = -3.507 * \text{LOG}(X) + 38.95, \text{Eff.} = 92.8\%$$

### STANDARD DILUTIONS - Fusion gene BCR-ABL p210 (St.2 - St.3 - St.4 - St.5)

Amplification Plots



Standard Curve



$$Y = -3.378 * \text{LOG}(X) + 40.15, \text{Eff.} = 97.7\%$$