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Quantiplus® BCR-ABL RT-PCR Kit

(Major, Minor, and Micro Quantitative Kit)

QT-BCRM2mi-25 : 25 rxns
QT-BCRM2mi-50 : 50 rxns

RUO

PI/QTBCRM2mi-01

Introduction & Product Description

BCR-ABL is an activated protein kinase resulting from the reciprocal translocation of the long arms of chromosomes 9 and 22 t (9;22), which is commonly referred to as the ***Philadelphia chromosome*** (Ph+). The Ph chromosome is present in more than 95% of cases of Chronic Myeloid Leukemia (CML). It is also found in 5% of Acute Lymphoblastic Leukemia (ALL) in children and 10 - 25 % of ALL cases in adults. Depending on the precise location of the BCR-ABL fusion the molecular weight of the protein can range from 185 to 230 kDa. Three clinically important variants are the p190(minor), p210(major) and p230(micro) isoforms. p190 is generally associated with acute lymphoblastic leukemia (ALL), while p210 is generally associated with chronic myeloid leukemia but can also be associated with ALL. p230 is usually associated with chronic neutrophilic leukemia.

Kit components

Box 1 of 2 Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-BCRM2mi-25)	50 rxns (QT-BCRM2mi-50)
Green	R Fast Core qPCR Mix (2X)	cDNA/DNA Amplification Mix	4 x 325 µL	4 x 650 µL
Amber	BCR-ABL Major PPM	Target Specific Primer Probe Mix	1 x 50 µL	1 x 100 µL
Amber	BCR-ABL Minor PPM		1 x 50 µL	1 x 100 µL
Amber	BCR-ABL Micro PPM		1 x 50 µL	1 x 100 µL
Amber	ABL PPM		1 x 50 µL	1 x 100 µL
Natural	qPCR Additive	PCR reaction Enhancer	1 x 200 µL	2 x 200 µL
White	Huwel PW	Purified water	1 x 500 µL	1 x 500 µL

Box 2 of 2 Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-BCRM2mi-25)	50 rxns (QT-BCRM2mi-50)
Pink	BCAQS1 (2×10^6 copies/10µL)	BCR-ABL and ABL Quantitation Standards	1 x 50 µL	1 x 100 µL
Pink	BCAQS2 (2×10^5 copies/10µL)		1 x 50 µL	1 x 100 µL
Pink	BCAQS3 (2×10^4 copies/10µL)		1 x 50 µL	1 x 100 µL
Pink	BCAQS4 (2×10^3 copies/10µL)		1 x 50 µL	1 x 100 µL
Pink	BCAQS5 (2×10^2 copies/10µL)		1 x 50 µL	1 x 100 µL
Red	BCR-ABL Minor PC	P190 Positive Control	1 x 50 µL	1 x 100 µL
Red	BCR-ABL PC (RNA PC)	RNA Positive Control	1 x 50 µL	1 x 100 µL
Note: **IS-Calibrator is supplied separately and is not a part of the kit**				
Red	Huwel BCR-ABL IS Calibrator	International scale Calibrator	1 x 10 µL	1 x 10 µL

Quick Reference

Sample Type

K_2EDTA-Blood, Bone Marrow. Heparinized Blood must not be used as they inhibit the PCR reaction

Assay Procedure

RNA Extraction

Name of the Extraction Kit	**Recommended Sample volume *(to be taken for RNA Extraction)***	**Recommended Final Elution volume**
QIAamp Blood RNA Mini Kit (Cat. No. 52304)	1 mL	50 µL
Huwel Blood Genomic RNA Extraction Kit (HL-GRX-100)	1 mL	50 µL

qPCR Protocol

Set up a Real time single step RT qPCR reaction (final volume of 26µL) for BCR-ABL Major, Minor and Micro **Quantitation in Test Samples** and controls (Huwel PW, RNA PC) along with the quantitation standards (BCR-ABL, ABL) as follows:

-	**Reaction for BCR- ABL Major**	**Reaction for BCR- ABL Minor**	**Reaction for BCR- ABL Micro**	**Reaction for ABL**	**Reaction for quantitation standards (ABL)**	**Reaction for quantitation standards (BCR-ABL)**	**Reaction for MinorPC**
Components	Volume per reaction in µL	Volume per reaction in µL	Volume per reaction in µL	Volume per reaction in µL	Volume per reaction in µL	Volume per reaction in µL	Volume per reaction in µL
R Fast Core qPCR Mix (2X)	13.0	13.0	13.0	13.0	13.0	13.0	13.0
BCR-ABL Major PPM	2.0	-	-	-	-	2	-
BCR-ABL Minor PPM	-	2.0	-	-	-		2
BCR-ABL Micro PPM	-	-	2.0	-	-	-	-
ABL PPM	-	-	-	2.0	2		
BCR ABL Minor PC	-	-	-	-	-	-	10
BCAQS1-BCAQS5	-	-	-	-	10	10	-
qPCR Additive	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Test sample RNA/ Huwel PW/ BCR- ABL PC (RNAPC)	10.0 *	10.0	10.0	10.0 *	-	-	-

- **BCR-ABL PC (RNA PC) can be used as control for BCR-ABL major and ABL testing only**
- **Prepare a standard curve for ABL using ABL PPM and quantitative standards (BCAQS1-BCAQS5)**
- **Prepare a standard curve for BCR- ABL using BCR- ABL major PPM and quantitative standards (BCAQS1-**

BCAQS5). Use the standard curve to quantify major, minor and micro transcripts

Cycling conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Reverse Transcription)	1	53	5 min.
2 (Initial denaturation)	1	95	2 min.
3 (PCR cycling)	45	95	30 sec.
		60*	60 sec.
* **Plate Read/Data Acquisition in FAM channel for BCR Transcript Standards and Unknown Samples**			
* **Plate Read/Data Acquisition in YAKIMA YELLOW/HEX/VIC channel for ABL Standards and Unknown Samples**			

- Set the reaction volume as 25 µL (the final volume is 26.0 µL but selecting 25 µL doesn't make any difference to
the final result/sensitivity).

Data Analysis

Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to R_n/Cycle amplification plot.

Result and Interpretation

The values for unknown samples would appear in the result column in copies in FAM Channel and HEX/VIC channel. Samples showing no amplification in FAM channel should show amplification in HEX/VIC channel (≥ 10,000 copies or Ct ≤ 25) to avoid false negative results due to the quality of RNA, and then only results should be considered. The negative control should not show any value in the result column for FAM and HEX channel. Any amplification in the negative control indicates cross contamination.

As standards are tenfold dilution, the theoretical slope of the curve is −3.37. A slope between −3.0 and −3.9 is acceptable as long as R^2 is >0.95. However, a value for R^2 >0.98 is desirable for accurate results. The efficiency of the assay should also be between 0.9 – 1.1 (90% - 110%).

Note: Each user should measure their own reproducibility in their laboratory. BCR-ABL PC is provided as RNA control. It is not necessary to establish standard curve

Normalized copy number (NCN)

The ABL copy numbers (ABL CN) and BCR-ABL copy numbers (BCR-ABL CN) obtained in the test results should be used to calculate the Normalized copy number for samples and IS calibrator.
The ratio of these CN values gives the normalized copy number (NCN):

$$\text{NCN (\%)} = \frac{\text{BCR-ABL CN}}{\text{ABL CN}} \times 100$$

The NCN result obtained for the HUWEL IS Calibrator must be within the interval 0.05–0.2. Otherwise, NCN values cannot be converted to the International Scale.

Conversion of Results to international scale

Use the experimental Huwel IS calibrator NCN result (NCN Cal), and its assigned value (Huwel IS-Cal value) indicated

in the contents table, to calculate the normalized copy number on the international scale for unknown samples (Huwel-IS- NCN sample).

$$\text{IS-NCN sample} = \frac{\text{NCN Sample x Huwel IS Cal value}}{\text{NCN Huwel IS Cal}}$$

- **(*Note* IS calibrator is not part of kit, can be bought separately)**

  IS- NCN can be calculated only for Major transcript and not for minor and micro-Transcript.

Assay Characteristics

Analytical Sensitivity of qPCR assay is **0.5 %** of the fusion BCR-ABL transcript.
Linear Range of the assay is **20 copies/10µL to 2x 10^9 copies/10µL**.

Specificity

The specificity of the Quantiplus® BCR-ABL RT-PCR Kit is ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all published sequences (GenBank) by BLAST analysis.

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