

BCR-ABL P210 (Mbc) One-Step Detection Kit

Rapid one-step RT-qPCR assay for MRD monitoring with Relative or Absolute Quantification.

- Detects b2a2 and b3a2 transcripts
- One-step RT-qPCR workflow
- Quantitative MRD monitoring
- Relative IS controls are included
- Absolute IS controls available upon request
- Available as RUO (US) / IVD (EU)

INTENDED USE:

The BCR-ABL p210 (Mbc; major breakpoint cluster region) One-Step Detection Kit is intended for the quantitative detection of BCR-ABL p210 b2a2 and b3a2 fusion transcripts in bone marrow or peripheral blood samples of patients who have been diagnosed with Acute Lymphoblastic Leukemia (ALL) or Chronic Myeloid Leukemia (CML). This test can be used to monitor Minimal Residual Disease (MRD) in patients undergoing therapy.

PACKAGE CONTENTS

The BCR210-QRT46 kit contains the following materials sufficient for 46 reactions in duplicate, including all controls and samples. Relative IS controls are included. BCR210 calibrators for Absolute Quantification are not included with kit but can be requested (Cat. #: AQSTD).

Description	Tubes
One-Step QRT-PCR Reaction Mix (5X)	1
Manganese Acetate	1
Molecular Grade Water	1
BCR-ABL p210 Fusion Gene Detection Primer/Probe	1
BCR-ABL P210 Fusion Gene Positive Control	1

This kit does not contain reagents for extraction of total RNA from peripheral blood or bone marrow samples. RNA can be extracted from peripheral blood or bone marrow using commercially available kits.

HANDLING AND STORAGE

This product is shipped on frozen ice packs and may be thawed upon arrival. Do not ship on dry ice. The contents of the shipment should be frozen at -25°C to -15°C immediately upon receipt. Do not store the kit or any of its components below -25°C as it will adversely affect the performance of the assay.

- Store all unopened components in original containers.
- Primer/probe mixes should be protected from light at all times to prevent photo bleaching of the fluorescent dyes.
- Centrifuge the tubes before opening

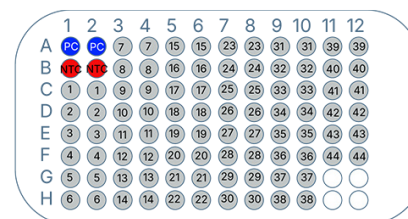
FOR USE WITH:

- Applied Biosystems® - 7500/7500 Fast, StepOne/ StepOne Plus, & 7900HT
- Bio-Rad® - CFX96
- Qiagen Rotor-Gene® Q, & Rotor-Gene® 3000, 6000
- Roche - LightCycler® 480 & Cobas® 4800, Cobas® z480
- Thermo Fisher - QuantStudio™ 5/6

PLATE SETUP

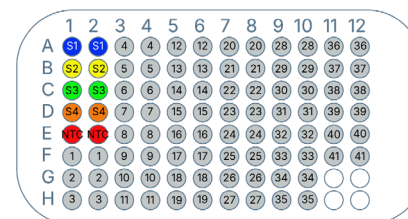
Relative Quantification:

Plate set up: A single 96-well PCR plate can accommodate up to 44 unknown samples, one positive control mix and one no template control (NTC). The relative quantification layout does not require loading the four optional calibrators.



Absolute Quantification:

Plate set up: A single 96-well PCR plate can accommodate up to 41 unknown samples, four calibrators (S1-S4) and one NTC.



EPAS DATA ANALYSIS

EntroGen's PCR Analysis Software (EPAS) provides an automated data interpretation solution for the BCR-ABL P210 (mbcr) One-Step Detection Kit in both Relative and Absolute quantification. Some instruments may require customization. For detailed instructions for use of EPAS download the EPAS IFU.

ASSAY PERFORMANCE

False-Positive and False-Negative Rates

To assess the false-positive and false-negative rates of the assay, RNA isolated from known positive and negative samples were analyzed in duplicate over 5 days. The positive samples comprised the WHO primary standards at 0.01% dilutions, as well as 12 patient samples that were previously tested with an alternative method and were determined to be positive (between 0.2-0.005% BCR-ABL/ABL). The negative samples comprised RNA from HL60 cell line as well as RNA isolated from healthy donor lymphocytes. The table below summarizes the results of this analysis.

False Positivity		False Negativity	
HL60	Healthy Donors	0.01%	Pt samples
0% (0/10)	0% (0/40)	0% (0/10)	0% (0/60)

Cross Reactivity

Synthetic fragment corresponding to the p190 and the p230 fusion variants of the BCR-ABL oncogene were diluted to 50% in ABL background. The controls were run in duplicate on the QuantStudio™ 5 instrument with 3 lots of reagents at 2 max input (VIC = 20).

	Channel	Average CT	SD Ct	Average IS ratio Relative Quant (%)	SD IS Ratio Relative Quant
P 190 50%	VIC	20.28	0.26	0.21	0.33
	FAM	30.43	1.22		
P 230 50%	VIC	20.25	0.14	118.89	13.8
	FAM	20.31	0.12		

Limit of Quantification

To assess the limit of quantification of the assay, synthetic WHO secondary standards of 10% p210 (b2a2 and b3a2) were serially diluted down to 10^{-4.5}. The serial dilutions were run in duplicate on the QuantStudio™ 5 instrument with minimum 3 lots of reagents. Ct values and calculated IS ratios with both Relative and Absolute Quantification are summarized in the table below for the lowest input tested allowing a 100% detection.

The 10^{-4.5} b2a2 and b3a2 standards were run using at 2 different inputs based on the VIC Ct (Ct ~21 and ~22). The IS ratios for the 2 different inputs was calculated using the relative quantification method. Ct values and calculated IS ratios for the 10^{-4.5} standards at both inputs are summarized in the table below.

	Channel	Average CT	SD Ct	Average IS Ratio Relative Quant (%)	SD IS Ratio Relative Quant	Average IS Ratio Absolute Quant (%)	SD IS Ratio Absolute Quant
b2a2 10%	VIC	21.59	0.25	11.27	2.28	8.71	2.33
	FAM	25.06	0.22				
b2a2 1%	VIC	21.51	0.18	0.71	0.13	0.64	0.12
	FAM	28.77	0.18				
b2a2 0.1%	VIC	21.87	0.12	0.080	0.02	0.08	0.02
	FAM	32.12	0.44				
b2a2 0.01%	VIC	21.57	0.20	0.005	0.001	0.006	0.002
	FAM	35.62	0.43				
b2a2 0.0032%*	VIC	22.05	0.18	0.004	0.0029	0.0023	0.0015
	FAM	37.85	2.41				
b2a2 0.0032%*	VIC	21.32	0.34	0.003	0.0011	N/A	N/A
	FAM	36.41	0.46				
b3a2 10%	VIC	21.64	0.32	11.89	2.33	7.33	1.72
	FAM	24.87	0.27				
b3a2 1%	VIC	21.91	0.35	1.12	0.12	0.94	0.13
	FAM	28.60	0.16				
b3a2 0.1%	VIC	21.98	0.31	0.11	0.02	0.12	0.02
	FAM	31.93	0.19				
b3a2 0.01%	VIC	22.02	0.27	0.01	0.002	0.017	0.008
	FAM	35.15	0.33				
b3a2 0.0032%*	VIC	21.87	0.14	0.003	0.0018	0.0041	0.0024
	FAM	37.22	1.63				
b3a2 0.0032%*	VIC	21.23	0.25	0.002	0.001	N/A	N/A
	FAM	36.79	0.70				

CONTACT

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For research use (RUO) in the U.S
 For in vitro diagnostic use (IVD) in the European Union