

## QXDx™ BCR-ABL %IS Kit



### Measuring Major and Deep Molecular Response Using Droplet Digital™ PCR (ddPCR™) Technology

The best way to assess complete molecular response (CMR) is with a highly sensitive molecular assay. The CE-IVD QXDx BCR-ABL %IS Kit elevates chronic myeloid leukemia (CML) monitoring to a new level of **sensitivity (0.001%IS, MR 5.0 with four wells)**, precision, and reproducibility. Bio-Rad's BCR-ABL assay delivers a scalable, reliable, and robust workflow for monitoring leukemia patients.

### The assay enables:

#### High precision and accuracy

- Increased sensitivity with 2- or 4-well test per patient sample

#### Absolute quantification

- Eliminates the need for the standard curves required with RT-PCR

#### Simplified and scalable workflow

- Flexibility to process 8 to 48 samples per run
- Flexible kit design to meet your laboratory's throughput and workflow needs

#### Standardized interpreted output

- Direct reporting on International Scale (%IS) and molecular response (MR) values

“Bio-Rad’s Droplet Digital PCR System is a powerful platform for monitoring patients being treated for CML. The increased sensitivity and precision of multiplexed BCR-ABL1 measurements, as compared to qPCR, along with absolute quantification in target copies and no standard curves, lends to the rationale for ddPCR use in routine laboratory testing.”

Neils Pallisgaard  
 Department of Pathology  
 Zealand University Hospital, Denmark

## The Need

### Reproducible Quantitative Results

Current practice guidelines from the European LeukemiaNet (ELN) and National Comprehensive Cancer Network (NCCN) for management of patients with CML call for the use of reverse transcription polymerase chain reaction assays during treatment and monitoring of patients for minimal residual disease and for identification of patients at risk of relapse (Baccarani et al. 2013, NCCN Guidelines for CML Leukemia, Version 1, 2016). Globally, the clinical utility of monitoring BCR-ABL mRNA has become the standard of care for managing CML patients. It is essential to minimize or eliminate the variation between and within laboratories. Therefore, reproducible molecular testing is needed to quantify BCR-ABL.

### Overcoming RT-PCR Limitations

Following current practice guidelines, patients are tested every three months and results are reported in International Scale (%IS) units, which standardizes reporting of the molecular response (Branford et al. 2006). However, RT-PCR has inherent limitations with regards to LOD and LOQ (Jennings et al. 2014).

### Scalable Accuracy and Precision

The accuracy and precision of RT-PCR methods, especially at the lower limit of quantification (LOQ) and limit of detection (LOD), may also affect clinical decisions on how CML patients are monitored and therapeutically managed (Jennings et al. 2014). Effective monitoring and treatment require accurate detection at and below MR 4.7 (0.002%IS) precisely.

## Bio-Rad’s Solution

The QXDx BCR-ABL %IS Kit is a digital PCR test that provides **unparalleled reproducibility even for deep molecular responses.**

The digital PCR solution achieves this through absolute quantification of copies of target DNA/RNA. A PCR reaction is partitioned into 20,000 droplets. The droplets containing the target sequence are detected by fluorescence and scored as positive and those without are scored as negative. Poisson statistical analysis of positive and negative droplets yields absolute quantification of the target sequence. Unlike RT-PCR, which relies on a standard curve, sample input is the only thing contributing to minimal variability across dynamic range.

The results are reported on the International Scale (IS) by using an assay-specific conversion factor determined by comparing the assay to an IS reference assay. The results are also reported as molecular response (MR) values.

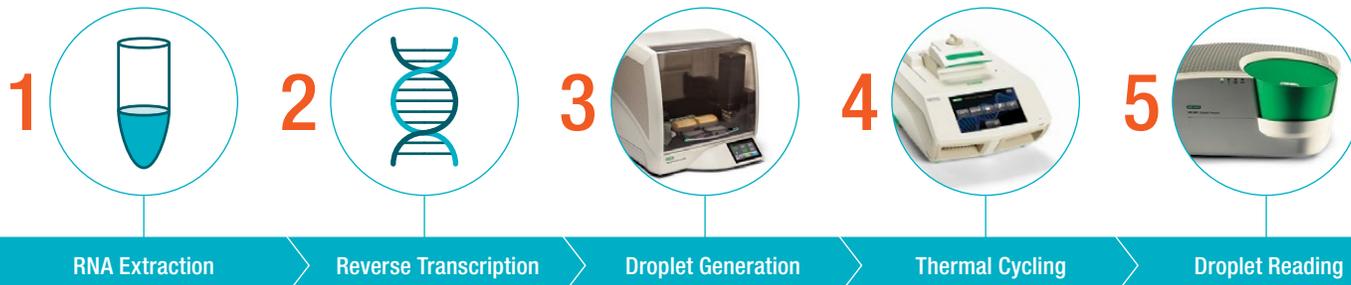
The QXDx BCR-ABL %IS Kit and ddPCR technology have some inherent advantages over conventional RT-PCR. This includes scalable sensitivity with an improved LOD (1 to 2 logs) and less sensitivity to/impact by amplification efficiency compared to RT-PCR.

Hence, independent labs have shown that ddPCR has obvious advantages over RT-PCR for monitoring disease burden (Jennings et al. 2014).

Digital PCR has been shown to be an accurate and highly precise method for detecting the BCR-ABL fusion gene (Cross et al. 2016). It is a lot more precise than traditional RT-PCR since it enables absolute quantification (Cross et al. 2016). The QXDx Droplet Digital PCR Systems are the premier clinical-ready platform enabling precise, sensitive, and scalable quantification of nucleic acids. The systems provide the flexibility to process 8 to 48 samples per run.

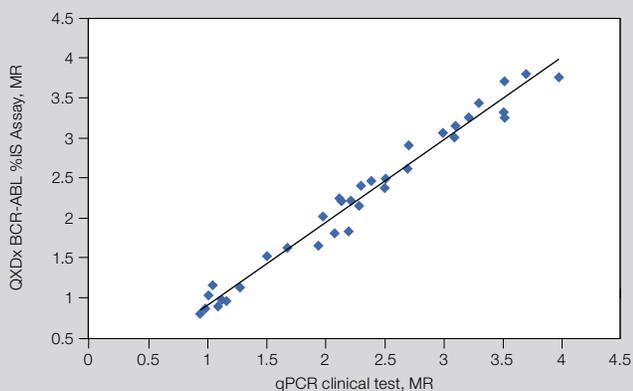
	2-Well Test	4-Well Test
LOQ (%IS)	0.002%IS (MR 4.7)	0.001%IS (MR 5.0)
LOD (%IS)		

# QXDx BCR-ABL %IS Kit Workflow



## Clinical Performance

**Correlation of the QXDx BCR-ABL %IS Kit and RT-PCR Lab Developed Test (LDT).**



Linear regression of the QXDx BCR-ABL Assay vs. qPCR. 34 clinical patient samples.  $y = 1.03322x - 0.0722$ ,  $R^2 = 0.976$ .

## Analytical Data

**Sensitivity and specificity of the QXDx BCR-ABL Assay.**

	2-Well Test	4-Well Test
Limit of blank (LOB)	0	0
Limit of quantitation (LOQ)	0.002%IS (MR 4.7)	0.001%IS (MR 5.0)
Limit of detection (LOD)	0.002%IS (MR 4.7)	0.001%IS (MR 5.0)

Transcript	Samples	Slope	R <sup>2</sup> Value	2nd Order Fit Deviation
e13a2	10 (MR 0.3 to MR 4.7)	1.03	0.996	≤0.09 MR units
e14a2	10 (MR 0.3 to MR 4.7)	1.04	0.996	≤0.09 MR units

## Precision — Minimal Variability across Dynamic Range of %IS and MR Value

**QXDx BCR-ABL %IS Kit precision data — patient and control samples.** Precision: n > 100 samples were verified as SD ≤ 0.25.

Sample ID	Target MR	n	Mean MR Level	MR Total Precision		Target % BCR-ABL	n	Mean %IS Level	% BCR-ABL Total Precision	
				SD	%CV				SD	%CV
MR 1	1	108	1.37	0.035	2.533	10	108	4.28	0.29	6.98
MR 2	2	108	2.44	0.049	2.004	1	108	0.37	0.037	10.3
MR 2.5	2.5	108	2.77	0.05	1.804	0.32	108	0.17	0.019	11.1
MR 3	3	108	3.28	0.081	2.462	0.10	108	0.05	0.009	17.9
MR 3.5	3.5	108	3.60	0.103	2.861	0.03	108	0.026	0.0058	22.3
MR 4	4	108	4.10	0.166	4.044	0.01	108	0.0085	0.008	37.0
Cell line control 1	<1.0	108	0.70	0.007	1.063	>10	108	20.13	0.77	3.82
Cell line contol 2	4.7	108	4.63	0.250	5.420	0	108	0.0025	0.0017	70.7

SD, standard deviation.

## References

- Baccarani M et al. (2013). European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood* 122, 872–884.
- Branford S et al. (2006). Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukaemia. *Leukemia* 20, 1,925–1,930.
- Cross NC et al. (2016). Development and evaluation of a secondary reference panel for BCR-ABL1 quantification on the International Scale. *Leukemia* 30, 1,844–1,852.
- Jennings LJ et al. (2014). Detection and quantification of BCR-ABL1 fusion transcripts by droplet digital PCR. *J Mol Diagn* 16, 174–179.

## Ordering Information

Catalog #	Description
12006134	<b>QXDx BCR-ABL %IS Kit, CE-IVD*</b> ; 192 reactions (96 samples)

### Materials needed but not provided

#### Reagents and Consumables

12001921	<b>ddPCR Dx Consumable Pack*</b> ; 192 reactions
12001922	<b>ddPCR™ Dx AutoDG™ Consumable Pack*</b> ; 480 reactions
12002526	<b>ddPCR Dx Droplet Reader Oil Pack*</b> ; 784 reactions

#### Instruments

17002229	<b>QX200™ AutoDG Droplet Digital PCR Dx System*</b>
17000034	<b>QX200 Droplet Digital PCR Dx System*</b>
12001045	<b>QX200 Droplet Reader, IVD*</b>
12001630	<b>QX200 Automated Droplet Generator, IVD*</b>
12001049	<b>QX200 Droplet Generator, IVD*</b>
10026368	<b>QuantaSoft™ Software*</b>
1814000	<b>PX1™ PCR Plate Sealer</b>
—	<b>Thermal cycler</b> , with the following specifications: <ul style="list-style-type: none"><li>– Accuracy: ± 0.2°C</li><li>– Uniformity: ± 0.4°C well-to-well within 10 sec</li><li>– Adjustable ramp capability with required ramp rate: up to 2°C/sec</li><li>– Temperature range: 0–100°C</li></ul>

\* These products are CE Marked IVD and have not been submitted to U.S. FDA and are not available in the U.S. market.

**Note:** Bio-Rad is in the process of updating IVD product names to include QXDx. Visit [bio-rad.com/web/QXDxNameUpdate](http://bio-rad.com/web/QXDxNameUpdate) for more detailed information about product name updates. Current product is labeled as QX200.

Visit [bio-rad.com/web/CEIVD/BCR-ABL](http://bio-rad.com/web/CEIVD/BCR-ABL) for more information.

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