Instructions for use

Imegen® M-BCR-ABL1

Ref. IMG-121

CE IVD

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Code: HIC-PT-KIT 03-F-03 V.03

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Our products are designed for *in vitro* diagnostic use. Health in Code, S.L. provides no other guarantee, whether explicit or implicit, that extends beyond the proper functioning of the components of this kit. Health in Code's sole obligation, in relation to the aforementioned guarantees, shall be to either replace the products or reimburse the price thereof, at the client's choice, provided that, however, materials or workmanship prove to be defective. Health in Code, S.L. shall not be liable for any loss or damage, whether direct or indirect, resulting in economic loss or harm incurred as a result of use of the product by the buyer or user.

All the products marketed by Health in Code, S.L. undergo strict quality control. The Imegen® M-BCR-ABL1 kit has passed all internal validation tests, thus guaranteeing the reliability and reproducibility of each manufactured batch.

If you have any questions about the use of this product or its protocols, please contact our Technical Department:

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Modifications to the Instructions for Use (IFU)		
Version 07	NOV 2023	Contents updated, figures 1 and 2 amended, section 12 "Performance characteristics" added
Version 06	AUG 2023	Enzyme reagent renaming in sections 3, 6, 7, and 10
Version 05	NOV 2022	Change in manufacturer's address: Health in Code S.L., Calle de la Travesía s/n, 15E Base 5, Valencia 46024, España.
Version 04	SEP 2022	Change of manufacturer's identification: from Imegen to HEALTH IN CODE, S.L.
Version 03	AUG 2018	CE-IVD marking update

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01 General information

Translocation between the *BCR* and *ABL1* genes, or the Philadelphia chromosome translocation, is a gene alteration that tends to be found in the majority of patients with chronic myeloid leukemia (CML), and in some patients with acute lymphoblastic leukemia (ALL). When this rearrangement occurs in patients, it is associated with a favorable prognosis.

Fusion between the *BCR*, located on chromosome 22, and the *ABL1* gene, located on chromosome 9, leads to the formation of an oncogene (BCR-ABL) that produces an abnormal protein. This protein results in increased tyrosine kinase activity, which leads to abnormal, uncontrolled lymphocyte growth, triggering leukemia.

Depending on where the breakpoint occurs within the *BCR* gene, different rearrangements are generated. There are three main forms of the chimeric BCR/ABL1 oncogene:

- M-BCR-ABL1: occurs when there is a fusion of b3a2 or b3a3 with b2a2 or b2a3, producing a 210-kDa chimeric tyrosine kinase (p210).
- m-BCR-ABL1: occurs when a fusion of e1a2 or e1a3 occurs, leading to a 190-kDa chimeric tyrosine kinase (p190).
- p230: occurs when there is a fusion of e19a2 or e19a3, producing a 230-kDa chimeric tyrosine kinase.

In addition, two much less frequent rearrangements have been reported in the literature:

- e6a2 or e6a3
- e8a2 or e8a3

References

- *https://crm.jrc.ec.europa.eu/p/q/bcr-abl/ERM-AD623-BCR-ABL-pDNA-CALIBRANT/ERM-AD623*
- *Leukemia. 2003; Volume 17: 2318-2357. doi:10.1038/sj.leu.2403135*

02 Intended use

The Imegen® M-BCR-ABL1 kit uses a combination of oligonucleotides and hydrolysis probes to amplify and quantify the M-BCR-ABL1 (p210) rearrangement by real-time PCR. This kit allows calculating the number of copies of the rearrangement and the number of copies of the endogenous gene *ABL1*, by comparing it with a synthetic plasmid that contains a copy of the two amplification targets. Moreover, this genetic analysis allows the user to detect minimal residual disease (MRD). The level of sensitivity that can be achieved depends on the maximum number of copies of the reference gene *(ABL1)* that can be detected.

To achieve the sensitivity limit required for an assay for minimal residual disease with a clinically significant molecular response, it is important to optimize the extraction process of total RNA from peripheral blood or bone marrow cells and, thereby, the reverse transcription protocols, to maximize the amount of complementary DNA (cDNA) present in each sample.

The standardized calibrant of the Institute of Reference Materials and Measurements (IRMM), Belgium: catalogue number ERM-AD623; **https://web.jrc.ec.europa.eu/rmcatalogue/searchrmcatalogue.do** is used for the calibration of the pIMEGEN plasmid (M-BCR-ABL1:ABL1 with a copy ratio of 1:1), which is used as the quantification standard for the Imegen® M-BCR-ABL1 kit.

Imegen® M-BCR-ABL1 is intended for *in vitro* diagnostic use only and is aimed at professionals in the field of molecular biology.

03 Technical characteristics

The Imegen® M-BCR-ABL1 kit has been validated using samples of cDNA synthesized from total RNA reverse transcription, extracted from peripheral blood of healthy patients and patients diagnosed with chronic myeloid leukemia, specifically detecting both the fusion products and the reference gene *(ABL1)* defined in section 2 of this manual (Intended use).

Validation of the Imegen® M-BCR-ABL1 kit has been performed using the following reagents not included in this kit:

- ***M-MLV RT (Moloney murine leukemia virus reverse transcriptase).*** Reverse transcription using 1 µg of total RNA.
- ***Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0,*** Thermo Fisher Scientific)

The limit of detection (LoD) for M-BCR-ABL1 and ABL1 has been established at 5 total copies, using our synthetic DNA standard (plasmid pIMEGEN) calibrated using IRMM Certified Reference Material (ERM®-AD623).

The limit of quantification (LOQ) is the minimum quantifiable value, set at 50 total copies for both the M-BCR-ABL1 rearrangement and the *ABL1* reference gene. Therefore, the most diluted point in the standard curve corresponds to the LOQ in both cases. This kit can detect molecular response (MR) corresponding to a 4.5 log reduction from the International Randomized Study, defined as:

- Detectable disease ≤0.0032% BCR-ABL1
- Undetectable disease in cDNA with ≥32,000 copies of the reference gene *ABL1*

04 Safety warnings and precautions

- It is recommended to strictly follow the instructions in this manual, especially regarding the handling and storage conditions of the reagents.
- Do not mouth-pipette.
- Do not smoke, eat, drink, or apply cosmetics in areas where kits and samples are handled.
- Any cuts, abrasions, and other skin injuries must be properly protected.
- Do not pour the remains of reagents down the drain. It is recommended to use waste containers established by the legal norm and manage their treatment through an authorized waste management facility.
- In the case of an accidental spill of any of the reagents, avoid contact with the skin, eyes, and mucous membranes and rinse with a large amount of water.
- Safety data-sheets (MSDS) of all dangerous substances contained in this kit are available on request.
- This product requires the manipulation of samples and materials of human origin. It is recommended to consider all materials of human origin as potentially infectious and manipulate them according to level 2 of the OSHA norm on biosafety and bloodborne pathogens or other practices related to biosafety of materials that contain or are suspected to contain infectious agents.
- The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, corrosive, or environmental biological pollutants.
- This kit has been validated with specific equipment and under specific conditions that may vary widely among laboratories. Therefore, it is recommended that each laboratory conduct an internal validation when the kit is to be used for the first time.
- The manufacturer assumes no responsibility for any damage or failure of the assay caused by substituting reagents included in the kit for ones not provided by Health in Code, S.L.

05 Content and storage conditions of the kit

This kit contains the following reagents necessary to carry out the 48 real-time PCR reactions:

- ***M-BCR Master Mix***: mixture of specific oligonucleotides and hydrolysis probes (FAM™) specifically designed to detect M-BCR-ABL1 rearrangement.
- ***ABL1 Master Mix***: mixture of oligonucleotides and hydrolysis probes (FAM™) specifically designed to detect the reference gene *ABL1*.
- ***M-BCR-ABL1 Standard***: plasmid *(pIMEGEN)* at a concentration of 30 x 10^4 copies/µl of M-BCR-ABL1 and ABL1 at a ratio of 1:1

Table 1. Components of the Imegen® M-BCR-ABL1 kit

Reagents	Color	Vials	Storage
M-BCR Master Mix	Green disc	2 x 24 reactions	4°C
ABL1 Master Mix	Yellow disc	2 x 24 reactions	4 °C
M-BCR-ABL1 Standard	Green cap	4 vials	4 °C

(*) The reagents in this kit are freeze-dried. Once rehydrated, the reagents must be stored at -20 °C

06 Equipment, reagents and materials not included in the kit

Equipment:

- Real-time PCR thermal cycler (FAM and VIC channels)
- Micropipettes (10 µL, 20 µL and 200 µL)
- Vortex mixer
- Centrifuge

Reagents:

- *Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0,* ThermoFisher Scientific)
- Nuclease-free water

NOTE: This kit does not include the necessary reagents for RNA reverse transcription to cDNA.

Materials:

- Filter pipette tips (10 µL, 20 µL and 200 µL)
- Sterile 1.5-mL tubes
- Optical consumables compatible with the real-time PCR thermal cycler
- Latex gloves

Complementary kits

Health in Code, S.L. provides a real-time PCR screening kit that allows the user to test for the presence of the most common BCR-ABL1 oncogenes, including M-BCR-ABL1 (p210) and m-BCR-ABL1 (p190). If the screening results for BCR-ABL1 rearrangement are positive, quantification of both M-BCR-ABL1 and m-BCR-ABL1 is recommended:

- Imegen® BCR-ABL1 Screening (Ref.: IMG-108)
- Imegen® m-BCR-ABL1 (Ref.: IMG-122)

07 Assay protocol

07.1 | Preparation of the PCR reagents

All the reagents included in this kit are freeze-dried. Before any of our kits is used, the first step is to rehydrate the reagents by adding the amounts of nuclease-free water indicated in the table below. To enable resuspension of each component, it is recommended to shake and spin the tubes containing the reagents and store them at 4 °C for one hour before use.

Table 2. Rehydration volume for the components of the kit

Reagents	Rehydration
M-BCR Master Mix	130 µL water/vial*
ABL1 Master Mix	130 µL water/vial*
M-BCR-ABL1 Standard	50 µL water/vial*

(*) If these reagents are not to be used immediately after rehydration, storage at -20 °C is recommended.

07.2 | Preparation of standard curves

The recommended protocol for the preparation of amplification reactions is the following:

01 Thaw all reagents:
- *m-BCR-ABL1 Standard*
- Nuclease-free water for negative controls (not included in the kit)

02 Vortex and spin all reagents. Keep on ice.

03 Prepare dilutions of the calibrated pIMEGEN standard with the IRMM standard to generate standard curves with known M-BCR-ABL1 and *ABL1* copy numbers.

04 Once the standard has been rehydrated, perform serial dilutions to generate standard curves for both the M-BCR-ABL1 rearrangement and ABL1.

The number of M-BCR-ABL1 standard copies is $30x10^4$ copies/µL (1,500,000 total copies). Preparation of the dilutions just before performing the assay is recommended.

05 Prepare four 1/10 serial dilutions, mixing 5 µL of positive control and 45 µL of nuclease-free water. Then, make a 1/3 dilution to prepare the least concentrated point of the curve, which is equivalent to 50 total copies.

06 Shake and spin each dilution in a vortex mixer before moving on to the preparation of the next dilution with lower concentration.

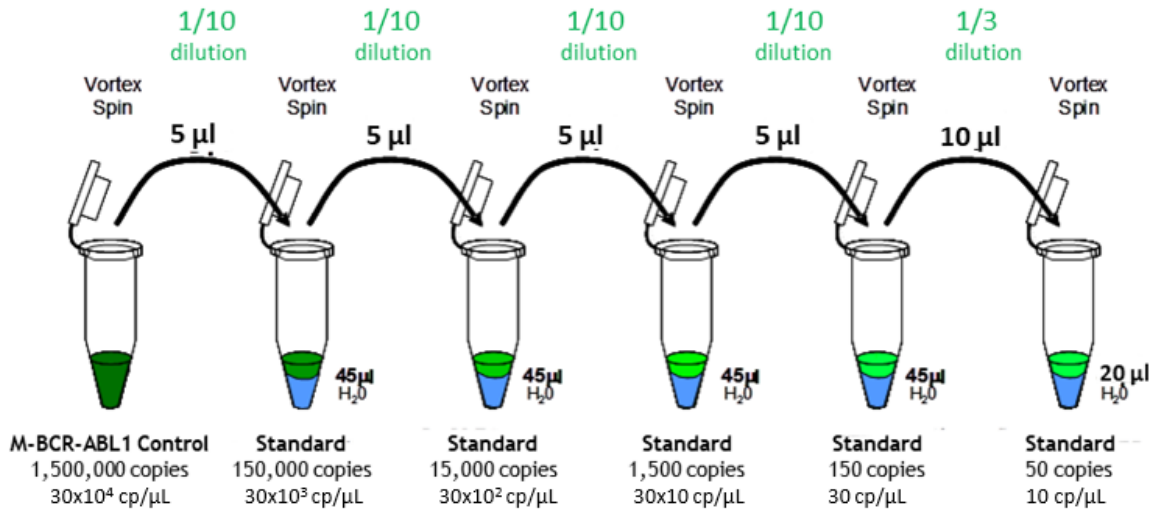

Figure 1. Protocol to prepare the standard curve using M-BCR-ABL1 Standard

07.3 | Preparation of amplification reactions

The protocol for the preparation of the reactions is shown below:

01 Thaw the following reagents:

- *M-BCR Master Mix*
- *ABL1 Master Mix*
- *Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0,* Thermo Fisher Scientific) (not included)

02 Vortex each reagent to mix thoroughly and keep on ice.

03 Add the necessary amounts of the reagents specified below to 1.5-mL tubes. To perform the calculations, it is recommended either to add a sufficient amount of reagents to perform one extra reaction or to add an extra 10% of each reagent.

M-BCR-ABL1 Master Mix (oncogene)

Reagent	Volume per reaction
M-BCR Master Mix	5 µL
TaqMan™ Environmental Master Mix 2.0	10 µL

ABL1 Master Mix (reference gene)

Reagent	Volume per reaction
ABL1 Master Mix	5 µL
TaqMan™ Environmental Master Mix 2.0	10 µL

The required volumes for each mix should be increased based on the number of samples to be tested, as well as the necessary reactions to build the standard curve *M-BCR-ABL1 Standard* and to analyze a negative PCR control (no-template control, NTC).

04 Vortex the tubes containing the PCR master mixes and dispense 15 µl to each well.

05 Once the mixtures have been dispensed, the following reagents must be added to the corresponding wells:

- 5 µL of sample cDNA (in duplicate)
- 5 µL of each *M-BCR-ABL1 Standard (pIMEGEN)*
- 5 µL of nuclease-free water (negative control, NTC)

M-BCR Master Mix	
M-BCR-ABL1 Standard $30x10^4$ cp/µl 1 500 000 total copies	cDNA 1_R1
M-BCR-ABL1 Standard $30x10^3$ cp/µl 150 000 total copies	cDNA 1_R2
M-BCR-ABL1 Standard $30x10^2$ cp/µl 15 000 total copies	cDNA 2_R1
M-BCR-ABL1 Standard 30x10 cp/µl 1 500 total copies	cDNA 2_R2
M-BCR-ABL1 Standard 30 cp/µl 150 total copies	
M-BCR-ABL1 Standard 10 cp/µl 50 total copies	NTC

ABL1 Master mix	
M-BCR-ABL1 Standard $30x10^4$ cp/µl 1 500 000 total copies	cDNA 1_R1
M-BCR-ABL1 Standard $30x10^3$ cp/µl 150 000 total copies	cDNA 1_R2
M-BCR-ABL1 Standard $30x10^2$ cp/µl 15 000 total copies	cDNA 2_R1
M-BCR-ABL1 Standard 30x10 cp/µl 1 500 total copies	cDNA 2_R2
M-BCR-ABL1 Standard 30 cp/µl 150 total copies	
M-BCR-ABL1 Standard 10 cp/µl 50 total copies	NTC

Figure 2. Example of a PCR template. R, replicates; NTC, no-template control; cp/ µl, copias/ µl

07.4 | Settings for the real-time PCR program

To perform real-time PCR, the instructions below must be followed to set up the amplification program:

7500 Fast or StepOne Real-Time PCR system (ThermoFisher Scientific)

- **Type of experiment**: Quantitation – Standard curve
- **Ramp rate**: Standard
- **Reaction volume**: 20 µL
- **Baseline reference ROX™**: included
- **Fluorophores of TaqMan probes**:

Table 3. Information about probes

Probe	Fluorophore	Quencher
M-BCR	FAM™	TAMRA
ABL1	FAM™	TAMRA

(*) On a StepOne PCR System (ThermoFisher Scientific), this field must be indicated as "None"

- Optimal program:

Table 4. Optimal PCR program for the 7500 FAST or StepOne PCR Systems

Fields	Phase 1 Enzyme activation		Phase 2 PCR	
No. of cycles	1 initial cycle	1 initial cycle	50 cycles	
			Denaturation	Annealing / Extension
Temperature	50 °C	95 °C	95 °C	60 °C
Time	2 minutes	10 minutes	15 seconds	1 minute*

(*) Fluorescence detection

08 Analysis of results

The following recommendations should be followed to ensure an adequate analysis of results:

NEGATIVE CONTROLS

- Verify the lack of amplification in **negative controls** (NTC). If amplification is detected, the test should be repeated to rule out accidental contamination.

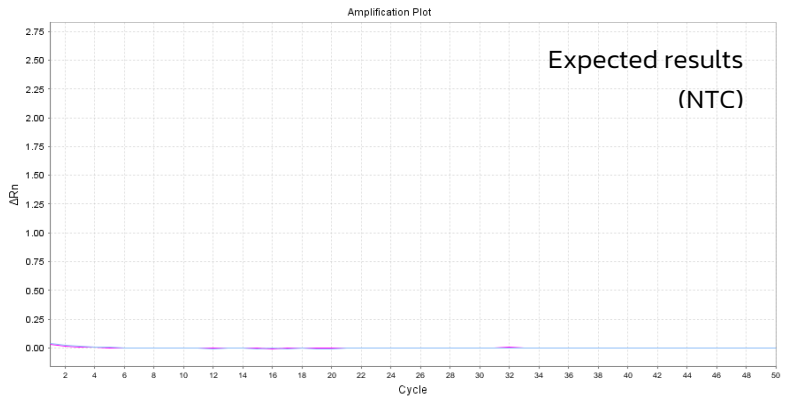

Figure 3. Expected result for the negative control (NTC)

STANDARD CURVE (pIMEGEN, M-BCR-ABL1 STANDARD)

- Confirm that serial dilutions prepared using the pIMEGEN plasmid produce suitable **standard curves** for both *ABL1* and M-BCR-ABL1 when linear regression is adjusted to logarithmic copy numbers:
  - Slope: range from -3.1 to -3.7
  - Coefficient of determination: R^2 >0.980

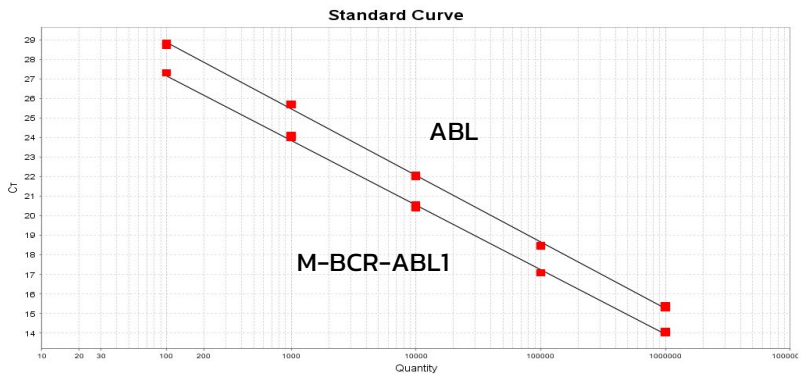

Figure 4. Linear regression of standard curves for ABL1 and M-BCR-ABL1

- If no amplification is detected in M-BCR-ABL1 Standard, see Section 9 (Troubleshooting). The highest concentration of the standard is $30x10^4$ copies per µL (1,500,000 total copies) and the lowest is 10 copies per µL (50 total copies).

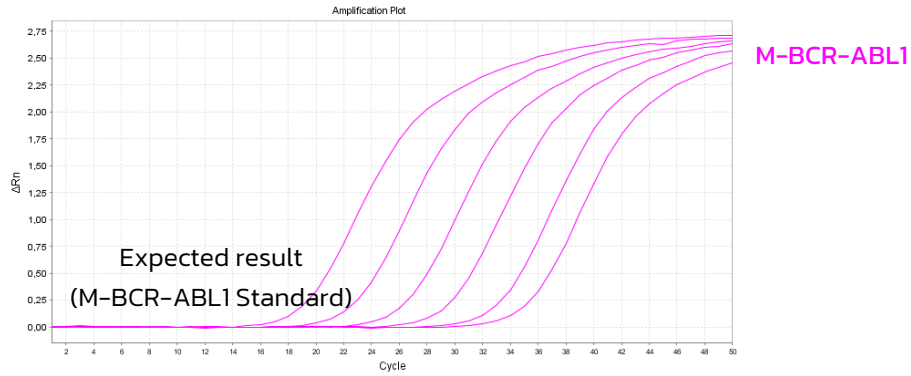

Figure 5. Serial dilutions used to build the standard curve for the PCR M-BCR-ABL1 system

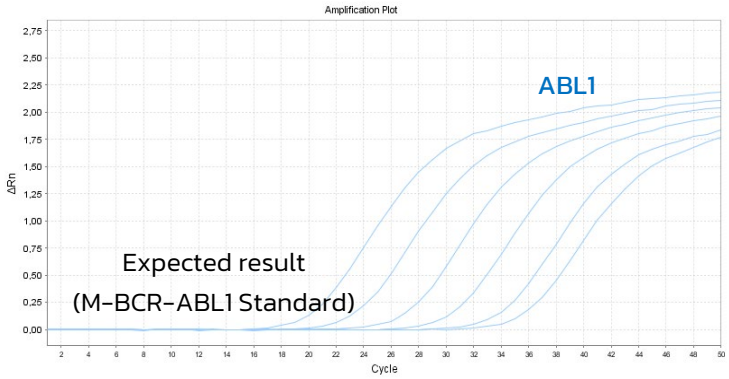

Figure 6. Serial dilutions used to build the standard curve for the PCR ABL1 system

cDNA SAMPLES

ABL1 Master Mix

- Verify that the reference gene *(ABL1)* is detected in all samples in reactions prepared using *ABL1 Master Mix*. *ABL1* is a constitutively expressed gene; therefore, amplification of the endogenous gene allows verifying that the sample contains enough cDNA of sufficient quality.

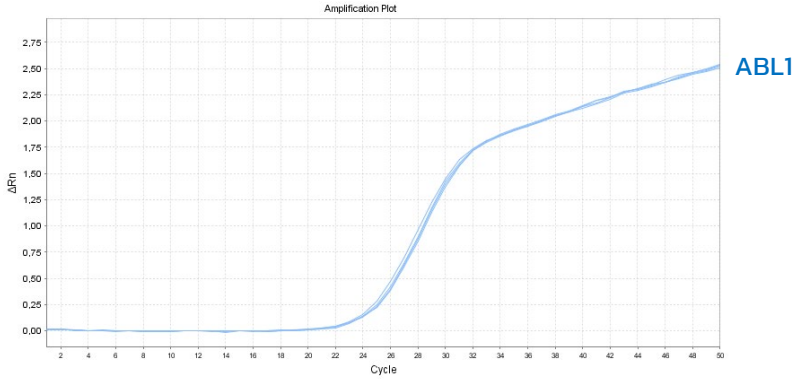

Figure 7. Expected result for a good-quality cDNA sample with the ABL1 system.

M-BCR Master Mix & ABL1 Master Mix

- After verifying all controls, cDNA samples are analyzed. The analyzed sample shows BCR-ABL1 translocation if amplification is detected in reactions using *M-BCR Master Mix*, as shown below:

  - Negative sample

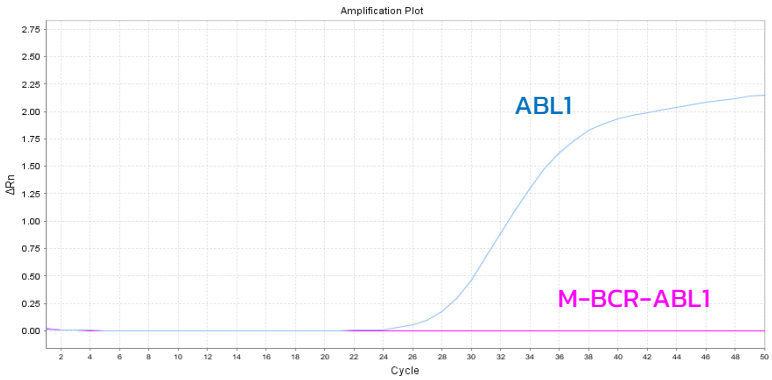

Figure 8. Expected result in non-pathogenic cDNA samples. The ABL1 system amplifies the gene, but BCR Screening does not amplify the M-BCR-ABL1 oncogene.

  - Positive sample:

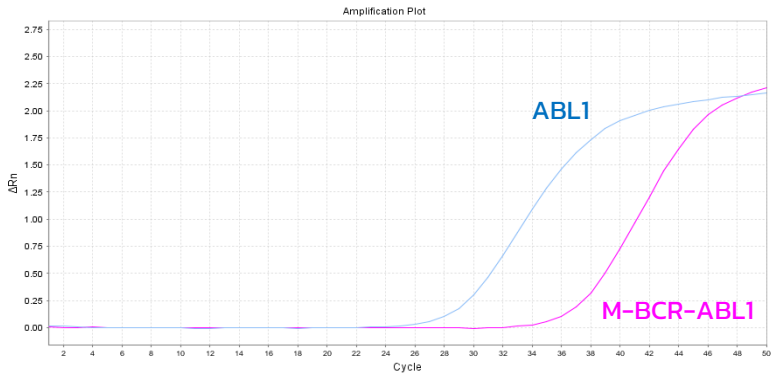

Figure 9. Expected result in pathogenic cDNA samples. Both systems amplify their targets: the ABL1 gene and the M-BCR-ABL1 oncogene

- The samples have the M-BCR-ABL1 rearrangement if amplification is detected in reactions using *M-BCR Master Mix*. To calculate the normalized copy number (NCN), calculate the number of copies of the *M-BCR-ABL1* rearrangement and of the reference gene *(ABL1)*. The rearrangement is quantified (NCN) by the following formula:

$$\mathrm{NCN} = \frac{\mathrm{M-BCR-ABL1_{CN}}}{\mathrm{ABL1_{CN}}} \mathrm{xCF}$$

NCN = Normalized copy number
CF = Conversion factor

To compare results, an International Scale (IS) was developed for reporting of M-BCR-ABL1. This scale is based on the implementation of specific conversion factors (CFs) and the use of kits that are calibrated according to this international scale. To comply with the

recommendation, the pIMEGEN plasmid *(M-BCR-ABL1 Standard)* is calibrated with the standard plasmid ERM AD623 BCR-ABL1 for multiplexed digital PCR (dPCR; QuantStudio 3D Digital PCR System).

In addition, each produced batch undergoes strict quality control and is provided together with a conversion factor (CF) to calculate the normalized copy number (NCN) based on a corrected copy number value for both the M-BCR-ABL1 (p210) rearrangement and the *ABL1* reference gene.

09 Troubleshooting

The table below shows the results that could be obtained from the analysis of the different controls and a sample in an assay, as well as their interpretation:

Table 5. Interpretation of possible results from Imegen®-M-BCR-ABL1

Control	M-BCR-ABL1	ABL1	Result / Interpretation
pIMEGEN (*M-BCR-ABL1 Standard*)	+	+	Expected result
	–	–	Incorrect PCR settings [1]
cDNA sample	–	+	Expected result
	+	+	
	–	–	cDNA samples failed to amplify [2]
Negative control (NTC)	–	–	Expected result
	+	+	Contamination with human cDNA or the standard [3]

(1) Incorrect PCR settings: an amplification error may be due to a technical issue during PCR configuration. Make sure the amplification program and fluorescence detection settings are correct.

(2) cDNA sample failed to amplify: failure to amplify the reference gene in the cDNA sample might suggest that the quantity or quality of the cDNA sample is compromised. In this case, it is advised to perform a new test, RNA extraction, or synthesis of new cDNA samples before interpreting the results.

(3) Contamination with human cDNA or the positive control (standard): PCR contamination could be caused by improper sample handling, the use of contaminated reagents, or environmental contamination. To fix this issue, deep cleaning of the laboratory where PCRs are prepared is advised, including the equipment and material used. If necessary, use new aliquots from PCR reagents and finally prepare the PCR reactions containing the positive controls to avoid any cross-contamination.

10 Limitations

10.1 | Equipment

Imegen® M-BCR-ABL1 has been validated for use with the following real-time PCR thermal cyclers:

- *StepOnePlus™ Real-Time PCR System* (Thermo Fisher Scientific)
- *7500 FAST Real-Time PCR System* (Thermo Fisher Scientific)

Technically, the kit is compatible with any real-time PCR equipment that allows detecting fluorescence emitted by the fluorophore FAM™.

If a different brand or model of thermal cycler is used, the amplification program may need to be adjusted. Should you need further information or advice, please contact our technical support service.

10.2 | Reagents

Imegen® M-BCR-ABL1 has been validated using the reagents included in the kit and the DNA polymerases recommended by the manufacturer of the real-time PCR thermal cyclers used for validation:

- *M-MLV RT (Moloney murine leukemia virus reverse transcriptase)*
- *Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0,* Thermo Fisher Scientific)

If a different PCR enzyme other than DNA polymerase is used for test validation, a previous validation step should be performed using the new reagents. Please contact our Technical Support team for additional information.

Moreover, this kit does not include the necessary reagents for RNA extraction or reverse transcription of RNA to cDNA. The use of a protocol starting from 1 µg of RNA is recommended for performing reverse transcription.

10.3 | Product stability

The optimal performance of this product is achieved provided that the recommended storage conditions specified in section 5 (Content and storage conditions of the kit) of this manual are applied.

11 IRMM calibration

According to the European LeukemiaNet (ELN), for treatment of patients with chronic myeloid leukemia an International Scale for M-BCR-ABL1 has been implemented to express results according to a standardized and traceable calibrant from the Institute for Reference Materials and Measurements (IRMM), Belgium: catalogue number ERM-AD623.

To meet this recommendation, Health in Code, S.L. has conducted internal calibration of the pIMEGEN plasmid with the ERM AD623 BCR-ABL1 plasmid. Both contain M-BCR-ABL1 and ABL1 in a 1:1 ratio. The calibration was based on the absolute copy number of M-BCR-ABL1 and ABL1 and done via multiplexed digital PCR (dPCR; *QuantStudio 3D Digital PCR System*). In addition, each produced batch undergoes strict quality control and is provided together with a conversion factor (CF) to determine the normalized copy number (NCN) based on a corrected copy number value for both the M-BCR-ABL1 (p210) rearrangement and the *ABL1* reference gene.

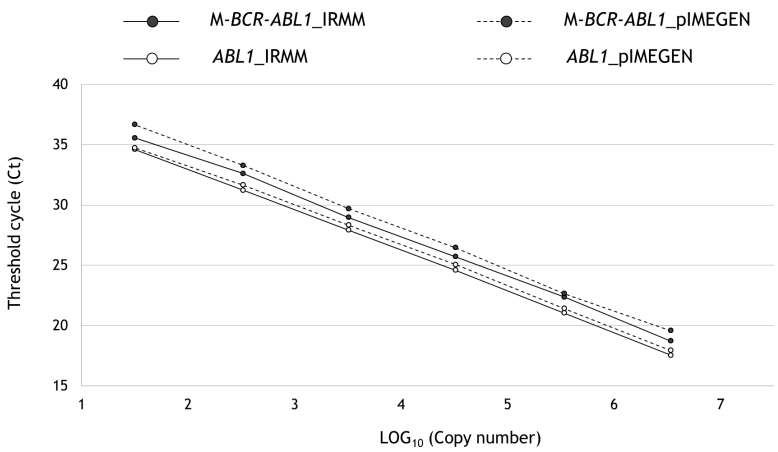

Figure 9. Functional details of the pIMEGEN and IRMM standards

M-*BCR-ABL1*_IRMM	Slope: -3.389, Y-Inter: 38,09, R^2: 0.999
*ABL1*_IRMM	Slope: -3.357, Y-Inter: 39.14, R^2: 0.999
M-*BCR-ABL1*_pIMEGEN	Slope: -3.372, Y-Inter: 38.41, R^2: 0.999
*ABL1*_pIMEGEN	Slope: -3.391, Y-Inter: 39.95, R^2: 0.999

The functional comparison of the IRMM standard and the pIMEGEN standard was analyzed by real-time PCR, evaluating the slope of the linear regression, the Y-intercept (Y-Inter) and the coefficient of determination (R2) of each system. These variables highly depend on the oligonucleotides and hydrolysis probes used.

Reference

› *Cross, N. C. P. et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia 29, 999–1003 (2015).*

12 Performance characteristics

12.1 | Validation samples

The Imegen® M-BCR-ABL1 qPCR kit is designed for analysis of complementary DNA (cDNA) from peripheral blood or from bone marrow.

The assays have been validated with one sample of synthetic DNA (plasmid) and 11 samples of cDNA, with genotypes that had been analyzed beforehand to identify the most common variants of the M-BCR-ABL1 rearrangement.

12.2 | Linearity and efficiency

The degree of linearity and efficiency of the Imegen® M-BCR-ABL1 qPCR kit has been established using a standard curve from serial dilutions (1:10) of an internally calibrated plasmid (pIMEGEN) with a known concentration for M-BCR-ABL1 and *ABL1*.

To determine linearity, the Ct values obtained for each point of the standard curve were plotted against the logarithm at base 10 of the DNA concentration. The points on the curve were fit to a straight line using linear regression, where the value of the correlation coefficient (R^2) is assessed to determine the quality of the system to replicate the results.

Three replicas were produced for each of the six points on the standard curve, and the parameter values for each of the straight lines are shown below:

Table 6. Linearity of the M-BCR-ABL1 assay.

	M-BCR					
	Straight line 1	Straight line 2	Straight line 3	Mean	Standard deviation	CV
Slope	-3,298	-3,362	-3,645	-3,435	0,184	5,375
R^2	0,999	0,995	0,995	0,996	0,002	0,231

	ABL1					
	Straight line 1	Straight line 2	Straight line 3	Mean	Standard deviation	CV
Slope	-3,338	-3,421	-3,419	-3,392	0,047	1,395
R^2	0,978	0,986	0,985	0,983	0,004	0,443

The slope of the regression line is related to the amplification efficiency (E) by means of the following formula:

$$\% E = (10^{-1/\text{pendiente}} - 1) \times 100$$

Efficiency values of between 90% and 110% are considered acceptable. Table 7 shows the efficiency of the amplification reactions in the linear range of the assay.

Table 7. Efficiency of the M-BCR-ABL1 assay.

M-BCR-ABL1			*ABL1*		
Slope	R^2	E (%)	Slope	R^2	E (%)
-3,435	0,996	95,5	-3,392	0,988	97,2

The results demonstrate suitable efficiency and linearity for quantification of the M-BCR-ABL1 rearrangement.

12.3 | Limit of quantification (LOQ)

To establish LOQ, the plasmid (pIMEGEN) calibrated internally at a concentration of 50 total copies of M-BCR-ABL1 and *ABL1* is used. The coefficient of variation (CV) is assessed. This should be <25% among 11 replicas to ensure correct quantification of the analyte.

12.4 | Reproducibility and repeatability

To assess reproducibility and repeatability, plasmids are prepared at a concentration equal to the limit of quantification (LOQ = 50), and repeatability assays are performed by loading 11 replicas into two different qPCR equipment (*7500 FAST Real-Time PCR System* and *StepOnePlus™ Real-Time PCR System*, from Thermo Fisher Scientific).

The reproducibility and repeatability acceptance criterion is for the CV to be below 25% in both assays. Repeatability and reproducibility is over 90%, with 11 replicas loaded in the same assay and in different qPCR kits.

Contact our Technical Department for any questions about the applications of this product or its protocols:

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