Instructions for use

Imegen® BCR-ABL1 Screening

Ref. IMG-108

CE IVD

Manufactured by:

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

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Health in Code, S.L. guarantees that its products are free from defects, both in the used materials and in its manufacturing process. This warranty is extended to the expiration date, as long as the storage conditions specified in this manual are met.

Our products are designed for *in vitro* diagnostics. The user of the product is responsible for validating the usefulness of the protocol proposed by Health in Code, S.L. Health in Code, S.L. does not offer any other warranty, express or implied, which extend beyond the proper functioning of the components of this kit. Health in Code, S.L. sole obligation in respect of the preceding guarantees, will be to replace the product or return the purchase price thereof, as desired by the customer, as long as the existence of a defect in the materials or in the manufacture of its products is identified. Health in Code, S.L. will not be responsible for any damage, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the purchaser or user.

All products sold by Health in Code, S.L. are subjected to rigorous quality control. The Imegen® BCR-ABL1 Screening kit has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.

For any questions about the applications of this product or its protocols, please contact our Technical Department:

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Modifications to the Instructions for Use (IFU)		
Version 08	NOV 2023	Review of document and correction of editing error in section 3. Section 11 "Performance characteristics" added.
Version 07	AUG 2023	Changes in enzyme name in sections 3, 6, 7 and 10.
Version 06	NOV 2022	Change of manufacturer's address: HEALTH IN CODE, S.L., Calle de la Travesía s/n, 15E Base 5, Valencia 46024, España.
Version 05	SEP 2022	Change of manufacturer's identification from Imegen to HEALTH IN CODE, S.L.
Version 04	MAR 2021	Adaptation to the Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices

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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 *BCR* gene, located on chromosome 22 and *ABL1*, located on chromosome 9, produces an oncogene (BCR-ABL1), and consequently, an abnormal protein. This protein results in an increased tyrosine kinase activity that produces the abnormal and uncontrolled growth of lymphocytes, triggering leukemia.

Depending on where the breakpoint is generated within the *BCR* gene, different rearrangements might occur. There are three prominent forms of the chimeric oncogene BCR-ABL1:

- **M-BCR-ABL1 (major region)**: when b3/a2 or a3 and b2/a2 or a3 are fused a chimeric tyrosine kinase of 210 kDa (p210) is generated.
- **m-BCR-ABL1 (minor region)**: when e1/a2 or a3 are fused a chimeric tyrosine kinase of 190 kDa (p190) is generated.
- **p230**: when e19/a2 or a3 are fused a chimeric tyrosine kinase of 230 kDa is generated.

In addition, two other much less frequent rearrangements, which are not studied in this assay, have been described in the literature:

- e6a2 o e6a3
- e8a2 o 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

Imegen® BCR-ABL1 Screening employs a combination of oligonucleotides and fluorescent hydrolysis probes in a diagnostic assay by real-time PCR directed to amplify and detect the most frequent variants of the BCR-ABL1 rearrangement, including M-BCR-ABL1 and m-BCR-ABL1 and the reference gene *ABL1*. This genetic analysis will enable the user to detect the presence or absence of such translocations in a multiplexed reaction, but it does not distinguish between the mayor and the minor translocations as it is a qualitative analysis.

The type of sample required for this analysis is complementary DNA (cDNA). Prior to the synthesis of cDNA, total RNA must be extracted from the peripheral blood cells or a bone marrow sample, from which the retrotranscription to cDNA will take place.

Health in Code, S.L. recommends to consult the local guidelines for the measurement of BCR-ABL1 transcripts for an optimal preparation of the cDNA sample required for the study and the setup of the assay.

The results obtained by this assay will orientate the clinician in the diagnostic of the type of leukaemia suffered by the patient.

Imegen® BCR-ABL1 Screening has been designed for *in vitro* diagnostics and it is directed to professionals from the molecular biology sector.

03 Technical characteristics

Imegen® BCR-ABL1 Screening has been validated using cDNA samples synthesised from the retrotranscription of total RNA extracted from peripheral blood samples of healthy patients and patients diagnosed with chronic myeloid leukemia, and it reliably detects the fusions products and the reference gene *ABL1* in Section 2 of this manual (Intended Use).

The validation of Imegen® BCR-ABL1 Screening has been carried out using the following reagents not included in this kit:

- **M-MLV RT (Moloney murine leukemia virus reverse transcriptase)**. Retrotranscription performed 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 this assay has been established following the protocol specified on Section 7 (Assay protocol). Hence, the LOD of this assay has been established in 5 total copies for ABL1 and M-BCR using a synthetic DNA vector calibrated with the *IRMM Certified Reference Material* (ERM®-AD623). The LOD for m-BCR has been established in 10 total copies, using a synthetic plasmid containing m-BCR and *ABL1*

04 Safety warnings and precautions

- Strictly follow the instructions of this manual, especially regarding the handling and storage conditions.
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- You must properly protect any skin condition, as well as cuts, abrasions and other skin lesions.
- Avoid discharge of reagents waste to the sink drinking water. Use waste containers established by the legislation and manage their treatment through an authorized waste manager.
- In case of an accidental release of any of the reagents, avoid contact with skin, eyes and mucous membranes and clean with abundant water.
- The materials safety data sheets of all hazardous components contained in this kit are available on request to Health in Code.
- This product requires the handling of samples and materials of human origin. You should consider all human source materials as potentially infectious and handled in accordance with OSHA Biosafety Level 2 of bloodborne pathogens or must use other relevant biosafety practices for materials containing or suspect that they may contain infectious agents.
- The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, corrosive not biological or environmental pollutants.
- This kit has been validated using specific equipment and conditions which might vary from the conditions in other laboratories. Thus, it is recommended that each laboratory performs an internal validation prior to the utilization of the kit.
- The manufacturer is not responsible for the malfunction of the assay when one or more reagents included in the kit are replaced by other reagents not supplied by Health in Code, S.L.
- The manufacturer does not guarantee the reproducibility of the assay when the user employs reagents not validated by Health in Code, S.L., considering them equivalent to those provided in the Kit.

05 Content and storage conditions of the kit

The kit contains the following reagents required to perform 48 real-time PCR reactions for each of the two targets analyzed in this assay:

- **BCR Screening Master Mix**: mixture of specific oligonucleotides and hydrolysis probes (FAM™) that simultaneously detect the M-BCR-ABL1 y m-BCR-ABL1 rearrangements.
- **ABL1 Master Mix**: mixture of specific oligonucleotides and hydrolysis probes (FAM™) to detect the reference gene *ABL1*.
- **Positive Control**: positive control of a BCR-ABL1 transcript and *ABL1*.

Table 1. Imegen® BCR-ABL1 Screening kit contents

Reagents	Color	Vials	Storage
BCR Screening Master Mix	Red pad	2 x 24 reactions	4°C
ABL1 Master Mix	Yellow pad	2 x 24 reactions	4°C
Positive Control	Red cap	1 vial	4°C

(*) The reagents contained in this kit are lyophilized. Once the reagents are rehydrated, should be stored at -20°C

06 Equipment, reagents and materials not included in the kit

Equipment:

- Real-time PCR thermocycler
- Micropipette (10 µL, 20 µL and 200 µL)
- Vortex

Reagents:

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

NOTE: In addition, this kit does not include the reagents required to perform the retrotranscription from RNA to cDNA.

Materials:

- Optical PCR tubes 0.2 mL
- Optical lids for the PCR tubes
- Filter tips (10 µL, 20 µL and 200 µL)
- Sterile tubes 1.5 mL
- Dust-free gloves

Related kits

If the results obtained for the BCR-ABL1 rearrangement is positive in the screening for any of the samples analysed, the quantification assays for the M-BCR-ABL1 transcripts, or the m-BCR-ABL1 transcripts are available as

- IMG-121 Imegen® M-BCR-ABL1
- IMG-122 Imegen® m-BCR-ABL1

In addition, Imegen® M-BCR-ABL1 consists of a precise and sensitive assay calibrated using the **IRMM Reference Material (ERM-AD623a-f)** for the international standardization of the **Molecular Response (MR)**.

07 Assay protocol

07.1 | Preparation of the PCR reagents

All reagents included in the kit are lyophilized. The first step before using any of our kits consists of rehydrating the reagents by adding the amount of nuclease-free water indicated in the following table. To facilitate resuspension of each component, we recommend vortexing well, spinning the tubes and allow them to rest at them at 4°C for one hour before use.

Table 2. Volume of nuclease-free water needed to rehydrate the reagents

Reagents	Rehydration volume
BCR Screening Master Mix	130 µL water/vial *
ABL1 Master Mix	130 µL water/vial *
Positive Control	100 µL water/vial *

(*) If the reagents are not going to be used immediately after rehydration, it is recommended to store them at -20°C.

07.2 | Preparation of the PCR assay

The protocol for preparation of amplification reactions is showed below:

01 Thaw all the reagents needed for the analysis:

- Master Mix BCR Screening / Master-Mix ABL1
- Control BRC-ABL1
- Undiluted cDNA samples
- Nuclease-free water for the negative controls (no template controls, NTC)
- Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0, Thermo Fisher Scientific) (not provided)

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

03 Add the required amounts of the reagents specified below to 1.5 ml tubes. It is recommended to perform the calculations by adding enough reagents to analyze one more reaction, or calculate and add 10% more of each of the reagents.:

- BCR Screening Master Mix (oncogene)

Reagents	Volume per reaction
BCR Screening Master Mix	5 µL
TaqMan™ Environmental Master Mix 2.0 *	10 µL

(*) See section "6. Equipment, reagents and materials not included in the kit".

- ABL1 Master Mix (reference gene)

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

(*) See section "6. Equipment, reagents and materials not included in the kit".

The volumes required of each mix have to be scaled up based on the number of samples that will be analyzed. In addition, extra reagents must be prepared for each mix to enable the inclusion of a negative control (NTC) and a positive control.

04 Vortex the tubes containing the PCR master mixes and dispense 15 µL in each well.

05 Once the master mixes have been dispensed, add the following into the corresponding wells:

- 5 µL of the cDNA sample
- 5 µL of the positive control
- 5 µL of nuclease-free water (NTC)

BCR Screening Master Mix	
cDNA sample 1	Positive control
cDNA sample 2	NTC
cDNA sample 3	
cDNA sample 4	

ABL1 Master Mix	
cDNA sample 1	Positive control
cDNA sample 2	NTC
cDNA sample 3	
cDNA sample 4	

Figure 1. Example of the plate layout for the real-time PCR system

07.3 | Setup of the real-time PCR program

The following instructions must be followed to setup the amplification program:

- **7500 Fast or StepOne Real-Time PCR system** (Thermo Fisher Scientific)

  - **Experiment**: Quantitation- Standard curve
  - **Ramp rate**: Standard
  - **Reaction volume**: 20 µL
  - **Reference ROX™**: Include
  - **TaqMan® probes fluorophores**:

Table 3. Hydrolysis probe information.

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

(*) In StepOne PCR System (ThermoFisher Scientific) this field should be filled as "None"

Optimal PCR program:

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

Fields	Step 1 Enzymatic activation		Step 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

For the correct interpretation of the results, the following recommendations are given:

NEGATIVE CONTROLS

- Confirm that there is not amplification in the **negative controls (NTC)**. The presence of an amplification curve is indicative of accidental contamination, and the assay should be repeated.

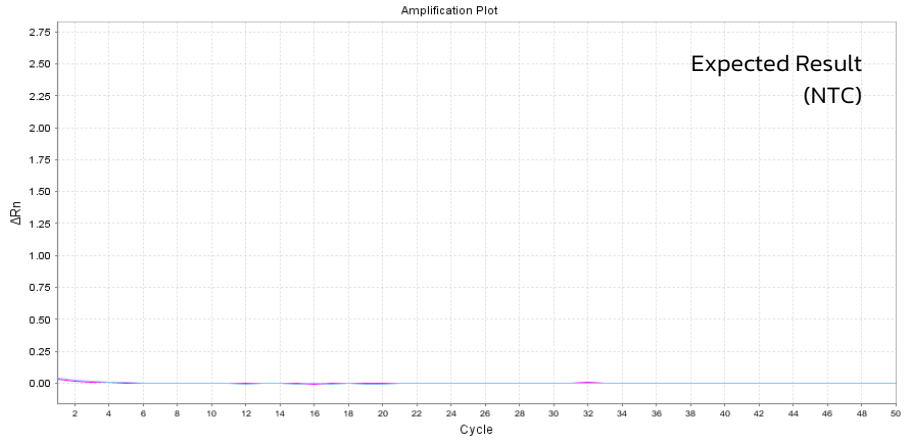

Figure 2. Expected result for the negative control (NTC).

POSITIVE CONTROL

- Confirm that there is amplification for ABL1 and for BCR-ABL1 in the **positive control**. If no amplification is detected in the positive control, see section 9 (Troubleshooting). The positive control should be detected between Ct16 and Ct20.

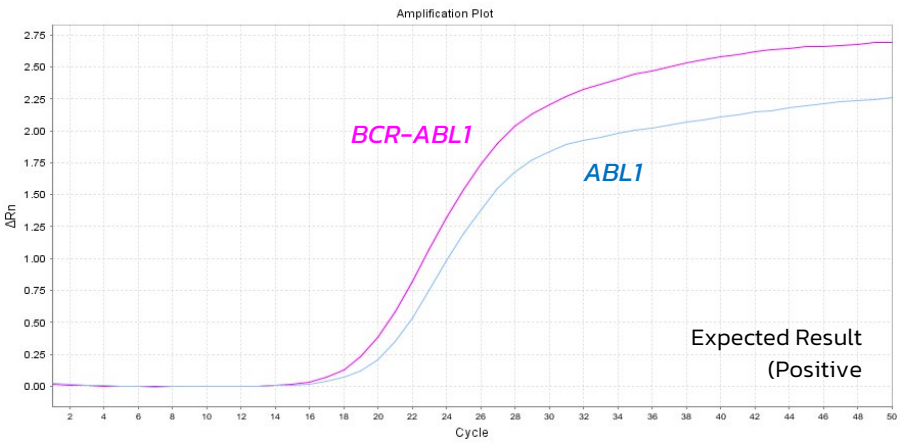

Figure 3. Expected result for the positive control. BCR-ABL1 and ABL1 transcripts are detected. The BCR Screening and the ABL1 systems are set up on different PCR reactions.

cDNA SAMPLES

ABL1 Master Mix

- Confirm that the reference gen (reactions prepared with **ABL1 Master Mix**) is detected in all the cDNA samples. ABL1 is a reference gene constitutively expressed, thus this reaction informs the user of the good quality and integrity of the cDNA sample.

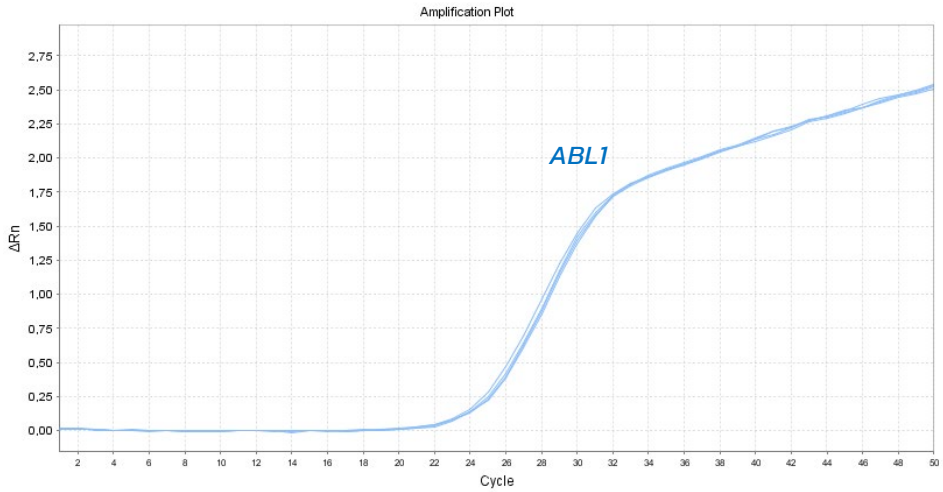

Figure 4. Expected result when a good quality cDNA sample is amplified with the ABL1 Master Mix.

BCR Screening Master Mix & ABL1 Master Mix

- After verifying all the controls included in the analysis are correct, the cDNA samples are analyzed. The sample analysed presents a BCR-ABL1 translocation if amplification is detected with the Master Mix BCR-ABL1 as indicated below.
  - Negative sample:

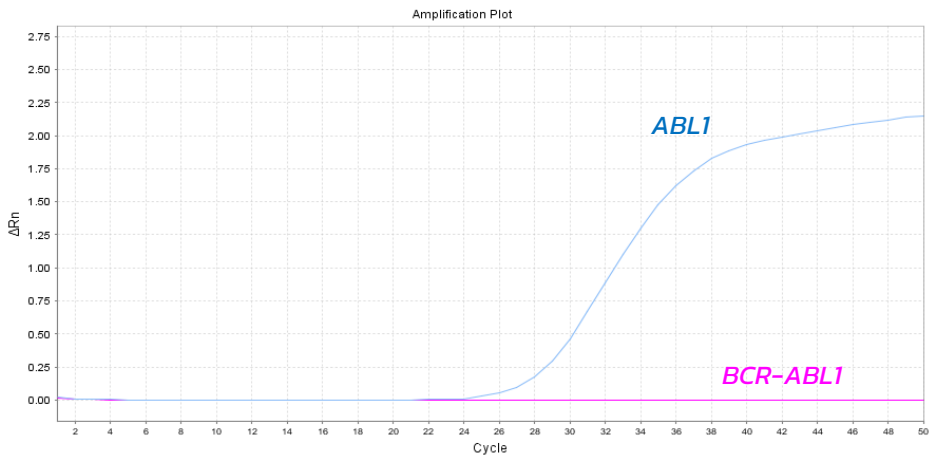

Figure 5. Expected result when a non-pathogenic cDNA sample is analyzed. The ABL1 master mix detects the presence of the ABL1 wild type, but the BCR Screening master mix does not amplify the BCR-ABL1 oncogenes.

- Positive sample:

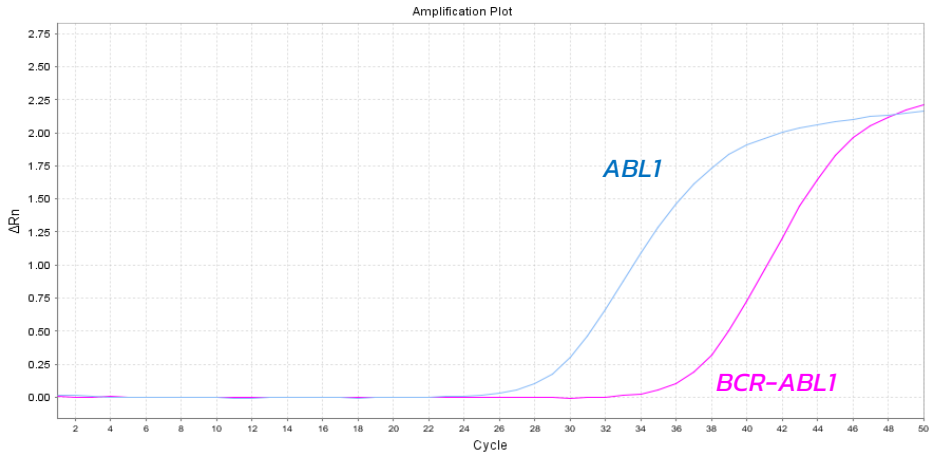

Figure 6. Expected result when a pathogenic cDNA sample is analysed. The ABL1 master mix detects the presence of the ABL1 wild type, and the BCR Screening master mix does amplify the BCR-ABL1 oncogenes.

09 Troubleshooting

The table below represents the results that could be obtained using the positive and negative controls and the cDNA samples. In case an unexpected result is obtained, the interpretation of the result and the cause most likely reason for such result is given in the table below.

Table 5. Interpretation of the possible results obtained using Imegen® BCR-ABL1 Screening

Control	BCR-ABL1	ABL1	Result / Interpretation
Positive control	+	+	Expected result
	–	–	Fail in the PCR setup [1]
cDNA sample	–	+	Expected result
	+	+	
	–	–	Fail to amplify the cDNA sample [2]
Negative control (NTC)	–	–	Expected result
	+	+	Contamination with human DNA or with the positive control [3]

(1) Fail in the PCR setup: an amplification error may be due to a technical problem during configuration of the PCR. Check the amplification program and fluorescence detection settings.

(2) Fail to amplify the cDNA sample: an amplification error of the reference gene in the cDNA sample could suggest that the quantity or quality of the cDNA sample is compromised. In this situation, a second analysis, RNA extraction, or synthesis of new cDNA samples would be recommended before interpretation of the results.

(3) Contamination with human DNA or with the positive control: PCR contamination could be caused by mishandling of the sample, the use of contaminated reagents, or environmental contamination. To resolve this problem, a thorough cleaning of the laboratory where the PCRs are prepared, including the equipment and material used, is recommended. If necessary, use new aliquots of PCR reagents and prepare the PCR reactions that contain the positive controls last to avoid any cross-contamination.

10 Limitations

10.1 | Equipment

Imegen® BCR-ABL1 Screening has been validated using the following real-time PCR systems:

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

Technically, this kit is compatible with any real-time PCR systems that enable the detection of the fluorescence emitted by FAM™ fluorophore.

If a real-time PCR cycler different from the systems described in this section is going to be used for the screening of BCR-ABL1 with this kit, it is possible that the PCR program might need to be readjusted. In this case, please contact our Technical Support Team for more details.

10.2 | Reagents

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

- 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 is used for the analysis, other than the DNA polymerase used in the validation, a prior validation using the new reagents is recommended. Please, contact our Technical Support Team if you request any further information.

In addition, this kit does not include the reagents for the RNA extraction or the retrotranscription of RNA to cDNA. It is recommended that a protocol that uses 1 µg of RNA be used for 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 Performance characteristics

11.1 | Validation samples

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

The assays have been validated with two samples of synthetic DNA (plasmids) and 13 samples of cDNA, with genotypes that had been analyzed beforehand to identify the most common variants of the BCR-ABL1 rearrangement (M-BCR-ABL1 or m-BCR-ABL1).

11.2 | Limit of detection (LOD)

The detection limit of the Imegen® BCR-ABL1 Screening kit has been assessed by preparing serial dilutions of the *pMajor* and *pMinor* synthetic plasmids until 10 and 5 total copies were obtained.

The coefficient of variation (CV) is assessed as the acceptance criterion. It should be <25% among the 11 replicas with *pMinor* at 10 total copies and with *pMajor* at 5 total copies.

11.3 | Reproducibility and repeatability

To assess reproducibility and repeatability, *pMajor* and *pMinor* plasmids are prepared at the limit of detection (LOD = 5 for *pMajor* and LOD = 10 for *pMinor*) 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. The results obtained demonstrate a CV of under 25%, using two different qPCR kits on different days, on 11 replicas from a single assay.

For any questions about the applications of this product or its protocols, please contact our Technical Department:

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