Instructions for use

Imegen® MPL

Ref. IMG-236

CE IVD

Manufactured by:
HEALTH IN CODE, S.L.
Calle de la Travesía s/n, 15E Base 5, Valencia 46024, España
+34 963 212 340 - info@healthincode.com

healthincode.com

Code: HIC-PT-KIT 03-F-03 V.01

healthincode

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, if the storage conditions specified in this manual are met.

Our products are designed for *in vitro* diagnostic use. 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 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, if 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® MPL kit has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.

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

+34 963 212 340

tech.support@healthincode.com

Instructions for Use (IFU) modifications		
Version 10	DEC 2022	Modification of the storage and shipping temperature of the GENERAL MASTER MIX reagent (Section 4).
Version 09	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 08	SEP 2022	Change in manufacturer's identification: from Imegen to Health in Code S.L.
Version 07	MAY 2021	Positive controls for targets W515L, W515K MPL and β-globin.
Version 06	AUG 2019	Enhanced analytical sensitivity (LOD 0.1%).
Version 05	APR 2019	Input DNA adjustment.

index

01 General information

Myeloproliferative neoplasms (MPNs) are a family of chronic cancer of the myeloid line in which there is an overproduction of blood cell precursors in an advanced estate of maturation.

The thrombopoietin receptor (MPL or CD110, located on 1p34) is mutated in 8-9% of essential thrombocythemias and primary myelofibrosis with no mutations in *JAK2*, the main oncogenic event of this type of cancer1,2. The thrombopoietin receptor is a 635 aa transmembrane protein with 2 extracellular cytokine receptor domains. Upon binding of thrombopoietin MPL forms homodimers, triggering the activation of the JAK-STAT pathway. The correct functioning of this pathway is crucial for the formation of megakaryocytes and platelets.

MPL mutations in MPNs specifically affect codon 515 within exon 10, being W515K and W515L the most frequent mutated forms.

References

- *Beer, P. A. et al. MPL mutations in myeloproliferative disorders: Analysis of the PT-1 cohort. Blood 112, 141–149 (2008).*
- *Pikman, Y. et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 3, 1140–1151 (2006).*

02 Intended use

Imegen® MPL employs a combination of oligonucleotides and fluorescent hydrolysis probes in a qualitative diagnostic assay by real-time PCR directed to amplify and detect the most frequent variants of the MPL gene, including W515L (NM_005373.2:c.1544G>T) and W515K (NM_005373.2:c.1543_1544delTGinsAA). Additionally, each assay multiplexes the analysis of an endogenous gene, β-globin, to ensure the quality and purity of the DNA sample.

This genetic analysis allows the user to confirm with full confidence and reliability the diagnosis of the patient with an analytical sensitivity as low as 1% of mutated MPL gene. This assay is not optimal for the study of minimal residual disease (MRD) in patients of AML.

Imegen® MPL kit has been designed for in vitro diagnostics and it is directed to professionals from the molecular biology sector.

03 Technical characteristics

Imegen® MPL has been validated using gDNA samples from the INCLIVA Biomedical Research Institute and reference material provided by *Hospital Regional Universitario Carlos Haya* (Málaga, España). Additionally, the specificity and sensitivity was tested using wild type samples from the repository of the *Medical Genetics Unit* of Health in Code S.L. and synthetic plasmids (*GenScript*) containing target sequences. These plasmids provide a positive control with the application to confirm the correct functioning of the PCR systems. The validation provides a robust and specific diagnostic method.

The sensitivity and specificity of the *MPL* detection systems was confirmed by in silico studies and empirically tested using wild-type samples and mutated MPL samples previously genotyped using a different technology. The sensitivity of the assay allows for the routine detection of the W515L and W515K MPL exon 10 point mutations at an allele burden of 1%.

The type of samples needed for this assay is genomic DNA extracted from peripheral blood. The total amount of samples required per assay is 100 ng.

Health in Code S.L. is certified under **UNE-EN ISO 13485:2018 Medical Devices: Quality Management Systems - Requirements for regulatory purposes** standard by the SPANISH AGENCY OF MEDICINES AND MEDICAL DEVICES (AEMPS) for the Design, development, and production of medical devices for *in vitro* diagnostic use:

- Genetic testing kits
- Software for the bioinformatics analysis of genetic data

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 (MSDS) of all hazardous components contained in this kit are available on request to Health in Code S.L.
- This product requires the handling of samples and materials of human and animal origin. You should consider all human and animal 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.
- 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 master mixes analysed in this assay:

- **Master Mix MPL W515K**: Specific oligonucleotides and probes for the PCR amplification of the W515K system and the endogenous gene, β-globin. The hydrolysis probe directed to the detection of the *MPL* mutant is labelled with the FAM™ fluorophore and the endogenous gene labelled with the VIC™ fluorophore.
- **Master Mix MPL W515L**: Specific oligonucleotides and probes for the PCR amplification of the W515L system and the endogenous gene, β-globin. The hydrolysis probe directed to the detection of the *MPL* mutant is labelled with the FAM™ fluorophore and the endogenous gene labelled with the VIC™ fluorophore.
- **General Master Mix**: Mix of a thermostable DNA polymerase, dNTPs and buffer required for the real-time PCR amplification.
- **MPL W515K Control**: DNA sample with mutation W515K in heterozygosity.
- **MPL W515L Control**: DNA sample with mutation W515K in heterozygosity.

Reagents	Color indicator	Quantity	Conservation
Master Mix MPL W515K	Red Disk	198 µl	-20°C
Master Mix MPL W515L	Yellow Disk	198 µl	-20°C
General Master Mix	White disk	660 µl	-20°C*
Control MPL W515K	Red lid	25 µl	-20°C
Control MPL W515L	Yellow lid	25 µl	-20°C

Table 1. Components of the Imegen® MPL kit

(*) **General Master Mix**: Recommended to be kept frozen until first use, protected from light, and stored between 2- 8 °C after first use.

06 Equipment, reagents and material not included in the kit

Equipment:

- Real-time PCR thermocycler (Channels FAM and VIC (HEX))
- Micropipette (10 µL, 20 µL and 200 µL)
- Vortex
- Micro centrifuge

Reagents:

- Nuclease-free water

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

Complementary kits

Health in Code S.L. has developed the following real-time PCR assays directed to the diagnostics of haematopoietic neoplasies:

- Imegen® NMP1 (ref IMG-235)
- Imegen® BCR-ABL1 Screening (ref IMG-108)
- Imegen® Inv16 (ref IMG-109)
- Imegen® M-BCR-ABL1 (ref IMG-121)
- Imegen® m-BCR-ABL1 (ref IMG-122)
- Imegen® PML-RARA (ref IMG-111)
- Imegen® PML-RARA Screening (ref IMG-130)

In addition, the following assays are also directed to the diagnostics of haematopoietic neoplasies by fragment analysis and capillary electrophoresis:

- Imegen® CALR (ref IMG-237)
- Imegen® FLT3 (ref IMG-238)

07 Assay protocol

07.1 | Preparation of the PCR assay

The protocol for preparation of amplification reactions is showed below:

01 Thaw all the reagents needed for the analysis including the kit reagents, the genomic DNA samples (50ng/µl) and nuclease-free water for the negative controls (no template controls, NTC).

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

03 In fresh 1.5 mL tubes, prepare two PCR mixes according to the following table:

	Volume per reaction	
Reagent	MPL W515L Master Mix	MPL W515K Master Mix
MPL W515L Master Mix	7.5 µL	-
MPL W515K Master Mix	-	7.5 µL
General Master Mix	12.5 µL	12.5 µL

NOTE: To estimate the amount of necessary reagents, we recommend to make the calculations taking into account the volume of reagents needed to analyze all the samples to be included in the same PCR analysis and add 10% extra of each reagent.

04 Vortex the tubes containing the PCR master mixes and dispense 20 µL in each well.

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

- 5 µL of the gDNA sample (10 ng/µL)
- 5 µL of each positive control
- 5 µL of nuclease-free water (NTC)

NOTE: It is recommended to use a negative PCR control in each master mix to ensure the absence of contamination, as well as positive control in each master mix to ensure the correct functioning of the PCR system.

06 Place the PCR tubes or PCR plate on the real-time thermal cycler and set up the amplification programmed according to the following section.

07.2 | Setup of the real-time PCR programme

The following instructions must be followed in order to setup the amplification programme:

- Hydrolysis probe fluorophores:

Probe	Fluorophore	Quencher
MPL W515K	FAM™	MGB
MPL W515L	FAM™	MGB
β-globin	VIC™	MGB

Table 2. Hydrolysis probe information

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

7500 Fast o StepOne Real-Time PCR system (Applied Biosystems)

- **Experiment**: Quantitation
- **Ramp rate**: Standard
- **Reaction volume**: 25 µL
- **ROX™ baseline reference**: included
- **Optimal PCR programme**:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR	
No. of cycles	1 initial cycle	45 cycles	
		Denaturation	Annealing / Extension
Temperature	95°C	95°C	64°C
Time	10 minutes	15 seconds	1 minute*

Table 3. Optimal PCR programme for 7500 FAST or StepOne PCR Systems

(*) Fluorescence detection

LightCycler 480 Real-time PCR System (Roche)

- **Experiment**: Dual Color Hydrolysis Probe / UPL Probe
- **Reaction volume**: 25 µL
- **Analyses**: Abs Quant / Fit Points
- **Optimal program**:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR		
No. of cycles	1 initial cycle	45 cycles		
		Denaturation	Annealing	Extension
Temperature	95°C	95°C	64°C	72°C
Time	10 minutes	15 seconds	30 seconds*	30 seconds

Table 4. Optimal PCR programme for the LightCycler 480 PCR System

(*) Fluorescence detection

08 Analysis of results

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

- Check that there is no amplification in the negative control. In case of negative control amplification, the assay should be repeated after discarding the source of contamination.
- Check that there is amplification of the *MPL* mutations (FAM channel) in the positive controls and no amplification of β-globin (VIC channel).
- Check that there is amplification of β-globin in all the DNA samples. No amplification of β-globin indicates a highly degraded DNA, an absence of the sample or presence of PCR inhibitors in the sample.
- Proprietary software is needed in order to process the files generated by the Real Time PCR machine. It is recommended to set up the threshold at 0.1 in thermal cyclers from Applied Biosystems, and fix the noise band manually above the residual fluorescent signal on thermal cyclers from Roche.

The following figures are intended to facilitate the interpretation of the results obtained with Imegen® MPL kit:

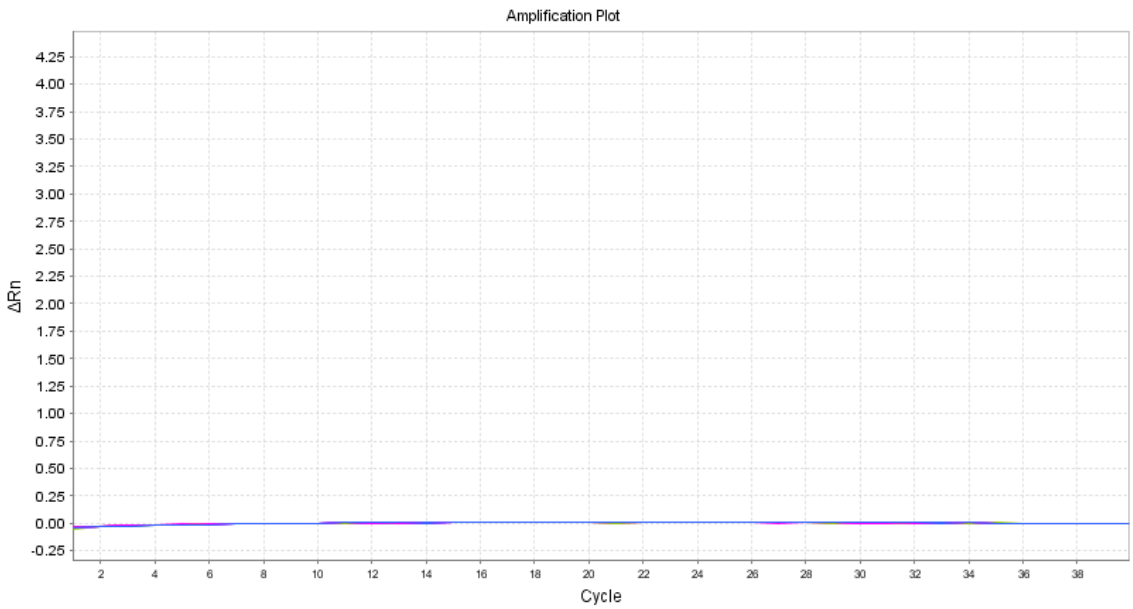

Figure 1. Expected result for the negative control (NTC). No amplification is detected in the FAM or VIC channel.

W515K PCR System

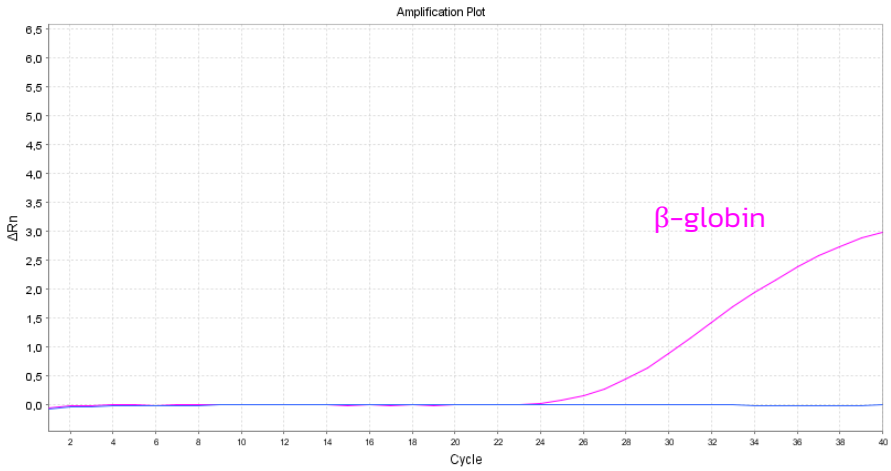

Figure 2. DNA sample without any target mutations in the MPL gene. Amplification is detected only in the VIC (β-globin) channel (here shown in pink)

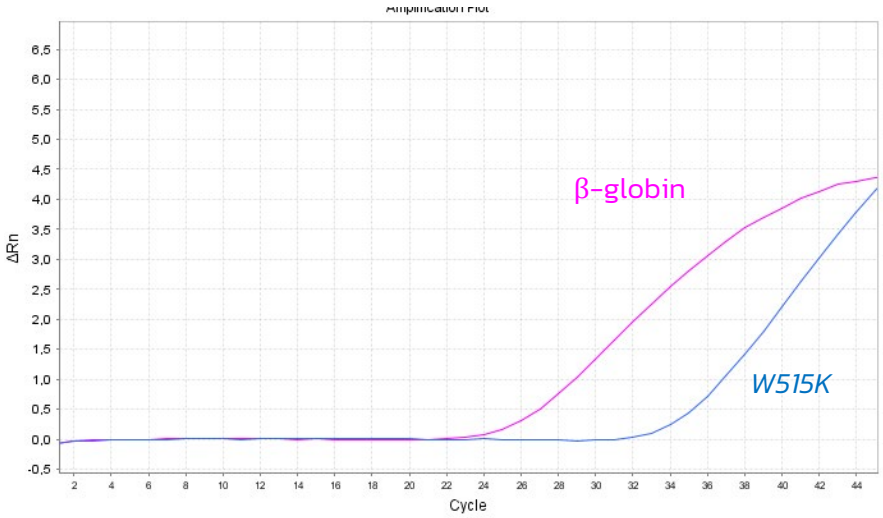

Figure 3. Results obtained from a sample with 1% of the MPL mutated with W515K. Amplification is detected in both fluorescent channels (in this figure, β-globin is shown in pink, and MPL-W515K is shown in blue)

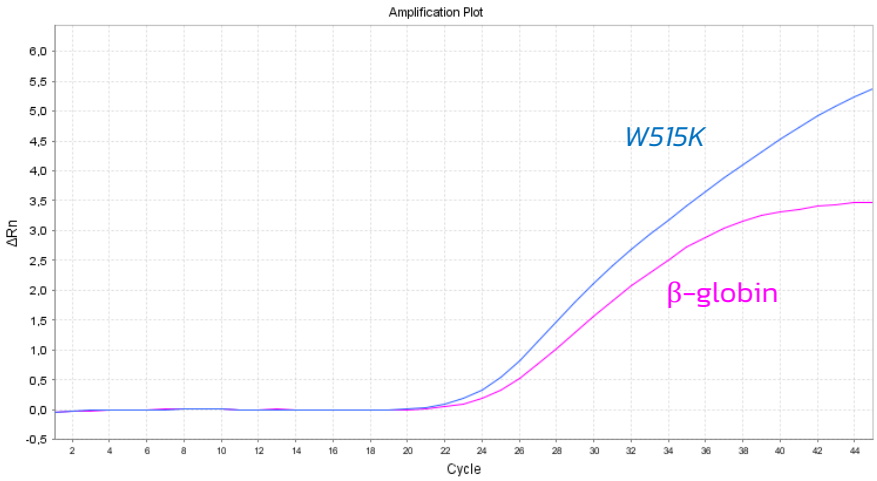

Figure 4. Expected results for the positive control, MPL-W515K mutation. Amplification is detected only in the FAM (MPL) channel (here shown in blue)

W515L PCR System

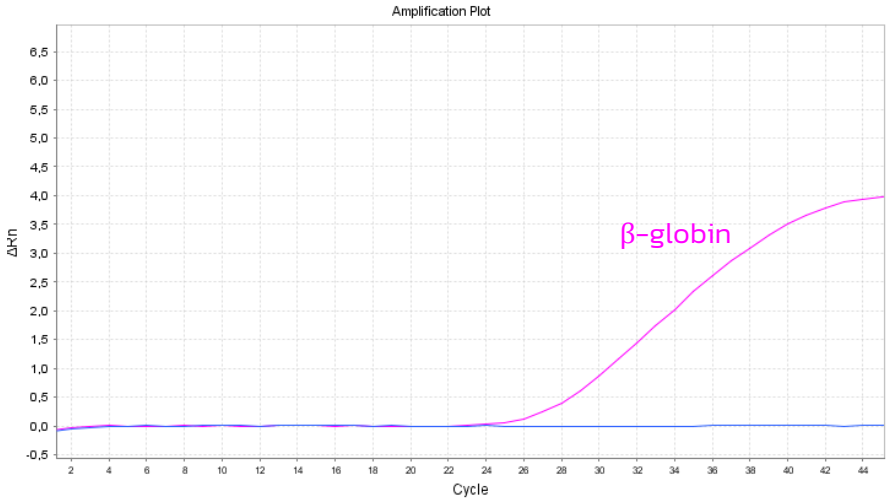

Figure 5. DNA sample without any target mutations in the MPL gene. Amplification is detected only in the VIC (β-globin) channel (here shown in pink)

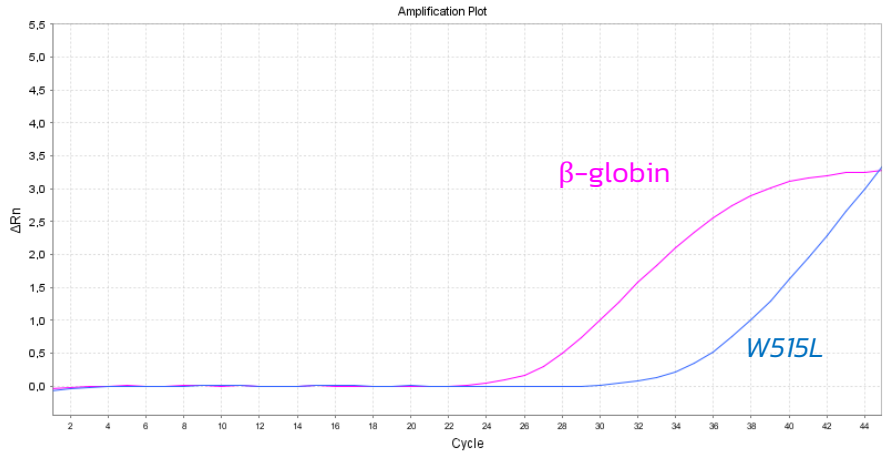

Figure 6. Results obtained from a sample with 1% of the MPL mutated with W515L. Amplification is detected in both fluorescent channels (in this figure, β-globin is shown in pink, and MPL-W515L is shown in blue)

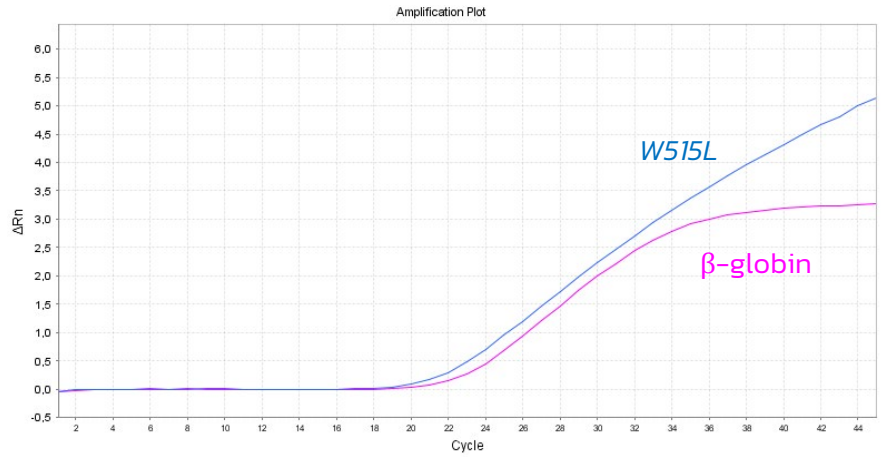

Figure 7. Expected results for the positive control, MPL-W515L mutation. Amplification is detected only in the FAM (MPL) channel (here shown in blue).

09 Troubleshooting

The table below represents the results that could be obtained using the positive control, negative controls and the DNA 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.

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

Table 4. Interpretation of the possible results obtained using Imegen® MPL

(1) Fail in the PCR setup: An error in the amplification might be due to a technical issue during the configuration of the PCR system. Check the amplification programme and the setup of the fluorescence detection.

(2) Fail to amplify the DNA sample: An error to amplify the reference gene in the DNA sample might suggest the quantity or the quality of the DNA sample is compromised. In this situation, a second analysis, DNA quantification and/or DNA extraction from a fresh sample would be recommended before an interpretation of the results is made.

(3) Contamination with human DNA or with the positive control: PCR contamination might be caused by an inappropriate handling of the sample, the use of contaminated reagents or caused by an environmental contamination. To solve this issue, a thorough cleanse of the laboratory where the PCRs are prepared, including the equipment and material used is recommended. If necessary, use fresh aliquots of the PCR reagents and prepare last, the PCR reactions containing the positive controls in order to avoid any cross contamination.

10 Limitations

10.1 | Equipment

Imegen® MPL has been validated using the following real-time PCR system:

- *7500 FAST Real-Time PCR System* (ThermoFisher Scientific)
- *StepOne Real-Time PCR System* (ThermoFisher Scientific)
- *LightCycler 480 Instrument II* (Roche Life Science)

If a real-time PCR cycler different from the systems described in this section is going to be used for the genetic diagnostic of coeliac disease with this kit, it is possible that the PCR programme might need to be readjusted. In this case, please contact our Technical Support team for more details.

10.2 | Reagents

Imegen® MPL has been validated using the reagents included in the kit and the ones recommended in the section 6 of this manual (Equipment and materials not included in the kit).

10.3 | Product stability

The optimal analytical functioning of this product is confirmed as long as the recommended storage conditions are applied as specified on Section 5 (Contents and storage conditions of the kit) from the reception of the kit until the expiry date assigned to each production batch.

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

tech.support@healthincode.com

+34 963 212 340

healthincode