Instructions for use

Imegen® HLA-B57:01

Ref. IMG-306

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.02

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Health in Code, S.L. guarantees that all its products are free of defects, both in materials and workmanship. This guarantee extends through to the expiry date, so long as the storage conditions described in this manual are observed.

Our products are intended 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.L.'s sole obligation, in relation to the aforementioned guarantees, shall be to either replace the product 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® HLA-B57:01 kit has passed all internal validation tests, thus guaranteeing 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 07	DEC 2023	Review and update of section "3. Technical characteristics".
Version 06	DEC 2022	Modification of the storage and shipping temperature of the GENERAL MASTER MIX reagent (Section 4).
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, Spain.
Version 04	SEP 2022	Change of the manufacturer's identification: from Imegen to Health in Code S.L.
Version 03	MAR 2020	Updated Section 2: Intended Use.
Version 02	DEC 2018	Document update for product's CE-IVD marking

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01 General information

The major histocompatibility complex (MHC), located at 6p21, contains hundreds of human leukocyte antigen (HLA) genes, which encode glycoproteins capable of recognizing exogenous and/or endogenous peptides in cells of the immune system, stimulating cell apoptosis when the peptides are recognized as foreign.

The genes of this complex are categorized into three groups: Class I, Class II, and Class III. In humans, *HLA-B* and two related genes, *HLA-A* and *HLA-C*, are the main MHC Class I genes.

The *HLA-B* gene is a highly variable sequence, which means that there are notable differences between the reaction of an individual's immune system to a wide range of foreign invaders. Hundreds of alleles of the *HLA-B* gene are known, and each is known as a particular number, such as HLA-B*57.

There are different subtypes of the HLA-B*57 allele. Specifically, the HLA-B*57:01 subtype is associated with hypersensitivity to abacavir, a synthetic nucleoside analog reverse transcriptase inhibitor drug used in the treatment of HIV, which causes AIDS. Individuals positive for the HLA-B*57:01 allele have a high risk of suffering a severe reaction to *Abacavir*; therefore, it is not recommended for the treatment of these patients.

References

- *https://ghr.nlm.nih.gov/gene/HLA-B*
- *Robinson J, Halliwell JA, Hayhurst JH, Flicek P, Parham P, Marsh SGE The IPD and IMGT/HLA database: allele variant databases Nucleic Acids Research (2015) 43:D423-431*

02 Intended use

The Imegen® HLA-B57:01 kit is meant for a qualitative analysis using real-time PCR to detect the presence of the HLA-B*57:01 allele. This kit allows identifying hypersensitivity to *Abacavir*, which is a drug used in the treatment of HIV, which in turn causes AIDS.

> IMPORTANT: **The purpose of the Imegen®- HLA-B*57:01 kit is not to determine tissue typing for histocompatibility testing prior to transplantation.**

This assay employs a combination of oligonucleotides and fluorescent hydrolysis probes in a validated assay to detect the presence of the HLA-B*57:01 allele and the endogenous *β-globin* gene, used as an internal positive control for the DNA sample. The results obtained from this test can be used to confirm the patient's diagnosis.

The Imegen® HLA-B57:01 kit analyzes the germline genotype, so the optimal sample type for this analysis is genomic DNA.

The Imegen® HLA-B57:01 kit is intended for *in vitro* diagnostic use only and is aimed at professionals in the field of molecular biology.

03 Technical characteristics

This kit has been validated using samples from patients with confirmed diagnosis, provided by *C.H.U. Insular Materno-Infantil* (*Las Palmas de Gran Canaria*, Spain), and certified synthetic vectors (GenScript) that contain the sequences of interest. This vector is included in the kit as the positive control, to verify the correct functioning of the PCR system. The complete validation gives a robust and specific diagnostic method. As a result of this agreement, Health in Code, S.L. has an exclusive and worldwide license on the know-how of the products for their manufacturing and commercial exploitation.

The type of sample required for this test is genomic DNA from peripheral blood or saliva. The total necessary amount of DNA is 50 ng.

04 Safety warnings and precautions

- Strictly follow the instructions of 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 skin conditions, 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 event of an accidental spill of any of the reagents, avoid contact with the skin, eyes, and mucous membranes and rinse with abundant water.
- Safety data-sheets (MSDS) of all dangerous substances contained in this kit are available on request.
- This product requires the handling of samples and materials of human origin. You should consider all materials of human origin as potentially infectious and handle 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 the malfunction of the assay when the reagents included in the kit are replaced with other reagents not provided by Health in Code, S.L.
- The manufacturer does not guarantee the assay's reproducibility when the user uses reagents that have not been validated by Health in Code, S.L but are considered by the user equivalent to those provided in the kit.

05 Content and storage conditions of the kit

This kit contains sufficient reagents to perform 48 determinations. The reagents included in this kit are listed below:

- ***HLA-B57 Master Mix:*** Specific PCR Master Mix containing the oligonucleotides and fluorescent hydrolysis probes FAM™ and VIC™ used for the amplification and detection of the HLA-B*57:01 allele and the *β-globin* gene.
- ***General Master Mix:*** PCR Master Mix with the nucleotides, $MgCl_2$, enzyme, and buffer necessary for the real-time PCR.
- ***Positive Control:*** positive control for the simultaneous amplification of the HLA-B*57:01 allele and the endogenous gene, *β-globin*.

Reagents	Color indicator	Amount	Storage
HLA-B57 Master Mix	Yellow disc	2 x 180 µl	-20°C
General Master Mix	White disc	600 µl	4°C*
Positive Control	Yellow cap	1 x 100 µl	-20°C

Table 1. Components of the Imegen® HLA-B57:01 kit

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

06 Equipment, reagents and materials not included in the kit

Equipment:

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

Reagents:

- Nuclease-free water

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

For sensitive and specific detection of other HLA alleles with different clinical targets, Health in Code, S.L. has developed Imegen® HLA-B27 **(ref IMG-289)** and Imegen® Coeliac **(ref IMG-307)**.

07 Assay protocol

07.1 | Preparation of amplification reactions

01 Thaw all kit reagents and DNA from the samples.

02 Shake each reagent on a vortex mixer and keep cold.

03 In a 1.5-mL tube, add the following reagents to prepare the PCR mix:

Reagents	Volume per reaction
HLA-B57 Master Mix	7.5 µL
General Master Mix	12.5 µL

NOTE: to estimate the necessary amount of reagents according to the number of samples and controls that will be simultaneously analyzed in each run, we recommend either including one extra reaction in the calculations or increasing the volume of each reagent by 10%.

04 Mix on a vortex mixer, spin the PCR mix and dispense 20 µL into the corresponding wells of the optical consumables.

05 Once the PCR mixes have been dispensed, add the following amounts to the corresponding wells:

- 5 µL of the genomic DNA sample (10 ng/µL).
- 5 µL of the positive control (included in the kit).
- 5 µL of nuclease-free water (negative control for PCR).

NOTE: it is recommended to add one negative PCR control per master mix to rule out reagent contamination, as well as one positive control per master mix to ensure the correct functioning of the PCR reaction.

06 Place the tubes or plates into the real-time PCR thermal cycler and configure settings for the amplification program as indicated in the next section.

07.2 | Settings for the real-time PCR program

- **Type of experiment**: Quantitation – Standard Curve
- **Ramp rate**: Standard
- **Reaction volume**: 25 µL
- **ROX™ baseline reference**: included
- **TaqMan® probes fluorophores**:

Probe	Receptor	Genotyping	Quencher
HLA-B57-P	FAM™	HLA-B*57:01 allele	MGB
β-Globin-P	VIC™	*β-Globin*	MGB

Table 2. Information about probes

◇ Optimal program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR	
No. of cycles	1 initial cycle	36 cycles	
		Denaturation	Primer binding/extension
Temperature	95°C	95°C	64°C
Time	10 minutes	15 seconds	1 minute*

Table 3. Optimal PCR program for 7500 FAST, StepOne (Thermo Fisher Scientific)

(*) Fluorescence detection

08 Analysis of results

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

- The specific software of the real-time PCR thermal cycler employed must be used to analyze the samples.
- Make sure that no amplification occurred in negative PCR controls, either in the FAM or in the VIC channels.
- Make sure that amplification signal is visible in positive PCR controls, both in the FAM and in the VIC channel.
- Make sure that all samples, both positive and negative, have a signal visible in the VIC canal, which comes from the amplification of the endogenous gene *β-globin*.

The possible results obtained using the Imegen® HLA-B57:01 kit are shown below:

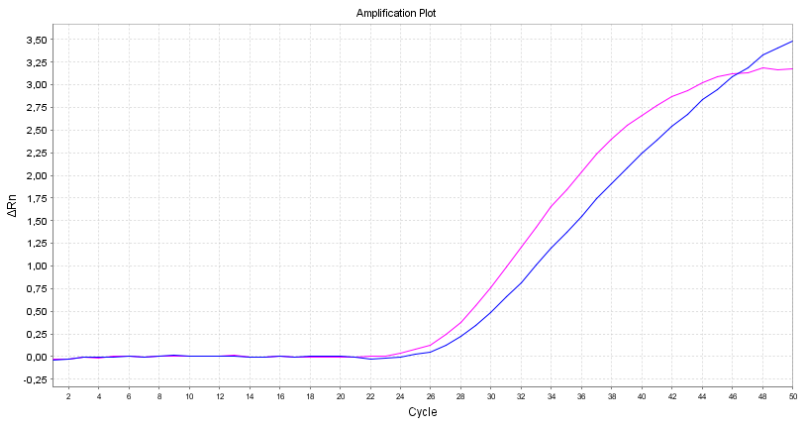

Figure 1. Result obtained from a positive sample. Signal visible in both channels: FAM (blue) and VIC (pink).

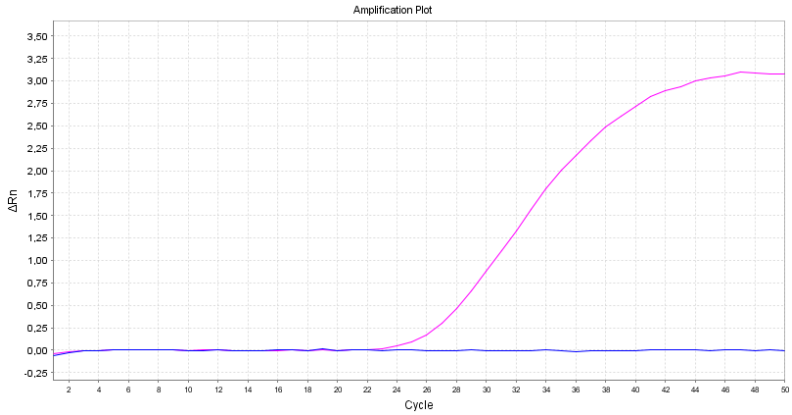

Figure 2. Result obtained from a negative sample. Amplification signal visible only in the VIC channel (pink), resulting from the endogenous gene β-globin

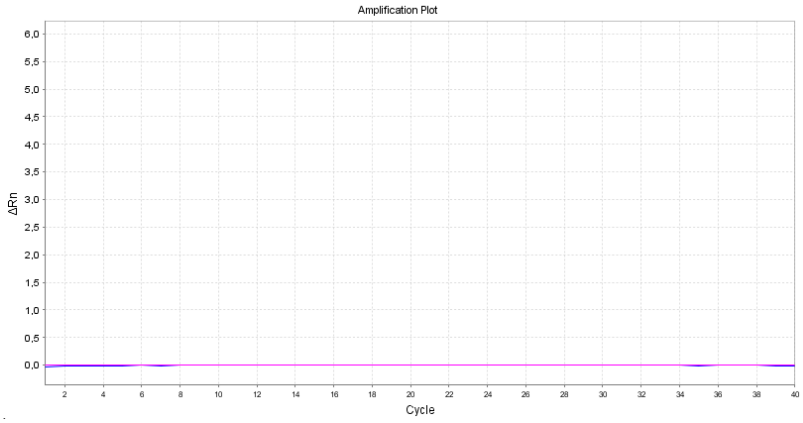

Figure 3. Result obtained from the negative PCR control. No amplification signal is observed in any channel.

09 Troubleshooting

The table below summarizes the possible test results that can be obtained from the analysis of the different controls and one sample in one run, along with their interpretation.

Control	HLA-B*57:01 (FAM)	*β-Globin* (VIC)	Cause
Positive Control	+	+	Expected result
	–	–	Failure of PCR amplification [1]
	+	–	
	–	+	
Sample	+	+	Expected result
	–	+	
	+	–	Failure of PCR amplification [1]
	–	–	Failure of sample amplification [2]
Negative PCR control	–	–	Expected result
	+	+	PCR contamination with human DNA [3]

Table 4. Interpretation of the possible results obtained using Imegen® HLA-B57:01

(1) Failure of PCR amplification: make sure the amplification program and fluorescence detection settings are correct. An amplification error may be due to a technical issue during PCR program setup.

(2) Failure of sample amplification: verify that sample quantification meets the recommendations; if so, the specified result may be due to a highly degraded sample.

(3) PCR contamination with human DNA: PCR contamination may be due to mishandling of the sample, the use of contaminated reagents or environmental contamination. Thoroughly clean the laboratory where the PCR was prepared, as well as the equipment and material used. If necessary, use fresh aliquots of the PCR reagents. Prepare the PCR reaction containing the positive control last, in order to avoid cross-contamination. In this case, it is recommended that the assay be repeated.

10 Limitations

10.1 | Equipment

Imegen® HLA-B57:01 has been validated for use with the following PCR thermal cyclers:

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

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® HLA-B57:01 has been validated using the reagents included in the kit and those recommended in section 6 of this document (Equipment, reagents and materials not included in the kit).

10.3 | Product stability

The optimal performance of this product is achieved provided that the specified recommended storage conditions are applied, within the optimal product expiration date associated with each production batch.

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

tech.support@healthincode.com

+34 963 212 340

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