Instructions for use

Imegen® HLA-B57:01

Ref. IMG-306

CE IVD

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

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

Our products are designed for *in vitro* diagnostic use. 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. only obligation in respect to the previous guarantees, will be to replace the product or return the purchase price, when desired by the customer, if the existence of a defect in the materials or in the manufacturing of its products is proven.

Health in Code S.L. is not liable for any damage, direct or indirect, resulting in economic loss or resulting from the use of this product by the purchaser or user.

All products sold by the Health in Code S.L. are subjected to rigorous quality control. The Imegen® HLA-B57:01 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:

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Instructions for Use (IFU) modifications		
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 in manufacturer's identification: from Imegen to Health in Code S.L.
Version 03	MAR 2020	Section 2. Intended Use.
Version 02	DEC 2018	Update by certified CE/IVD

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01 General information

The major histocompatibility complex (MHC) region, located on 6p21, contains hundreds of human leukocyte antigen (HLA) genes that encode glycoproteins that present exogenous and/or endogenous peptides to the immune cells stimulating apoptosis when the peptide is recognized as foreign.

Genes in this complex are categorized into three basic groups: Class I, Class II, and Class III. In humans, the HLA-B gene and two related genes, HLA-A and HLA-C, are the main genes in MHC class I.

The HLA-B gene has many possible variations, allowing each person's immune system to react to a wide range of foreign invaders. Hundreds of versions (alleles) of the HLA-B gene are known, each of which is given a particular number (such as HLA-B*57).

There are different HLA*B57 allele subtypes. Specifically, the HLA-B*57:01 allotype is associated with hypersensitivity to Abacavir, a highly effective nucleoside compound used to treat HIV infection and AIDS. Individuals who are positive for the HLA-B*57:01 allele are at an increased risk for *Abacavir* hypersensitivity and it is not recommended for use in treating these individuals.

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

Imegen® HLA-B57:01 consists of a qualitative real-time PCR assay aimed at detecting the presence of the HLA-B*57 :01 allele. This kit serves to determine the hypersensitivity to *Abacavir*, a highly effective nucleoside compound used to treat HIV infection and AIDS.

> IMPORTANT: **The purpose of Imegen® HLA-B57:01 kit is not the tissue typing for histocompatibility test prior to transplantation.**

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

The assay studies the genotype in the germline, thus the optimal type of sample required for this analysis is genomic DNA (gDNA).

Imegen® HLA-B57:01 has been designed for *in vitro* diagnostics and it is directed to professionals from the molecular biology sector.

03 Technical characteristics

This kit has been validated using diagnostic samples were kindly provided by *C.H.U. Insular Materno-Infantil* (Las Palmas de Gran Canaria, Spain) as well as certified synthetic vectors (*GenScript*) containing the sequences of interest. This vector is provided as a positive control to ensure the correct functioning of the PCR system. The thorough validation provides a robust and specific diagnostic method. The result of this agreement, Health in Code S.L. has an exclusive worldwide license on the know-how of products for manufacturing and commercial exploitation of them.

The type of sample required for this analysis is genomic DNA extracted from peripheral blood or saliva, a total quantity of 50 ng will be necessary.

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 blood borne pathogens or must use other relevant biosafety practices for materials containing or suspect that they may contain infectious agents.
- Reagents included in this kit are non-toxic, neither explosive, infectious, radioactive, magnetic, corrosive nor environmental polluters.
- This kit has been validated with specific equipment under certain conditions, which could be different in other laboratories. It is recommended that each laboratory performs an internal validation when the kit is used for the first time.
- 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 includes the following reagents, enough to analyze 48 reactions:

- **HLA-B57 Master Mix**: *PCR Master Mix* containing the primers to perform the amplification of the target region and two probes labelled with FAM™ and VIC™ fluorophores which allow the differentiation between HLA-B*57:01 allele and β-Globin gene.
- **General Master Mix**: *PCR Master Mix* containing $MgCl_2$, nucleotides and *Buffer* solution, required for the PCR reaction.
- **Positive control**: Positive control for the HLA-B*57:01 target allele and the endogenous gene, β-Globin.

Reagents	Color indicator	Quantity	Conservation
HLA-B57 Master Mix	Yellow pad	2 x 180 µl	-20°C
General Master Mix	White pad	600 µl	-20°C*
Positive Control	Yellow lid	1 x 100 µl	-20°C

Table 1. Components of the Imegen® HLA-B57:01 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 (FAM and VIC channels)
- Micropipette (10 µL, 20 µL and 200 µL)
- Vortex
- 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

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 | PCR reactions preparation

The recommended protocol for preparation of amplification reactions is showed below:

01 Thaw the *HLA-B57 Master Mix*, the *Positive Control* and the genomic DNA samples.

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

03 Add into a 1.5 mL tube, the following reagents:

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

NOTE: In order to estimate the number of necessary reagents, we recommend making calculations taking into account the number of samples to be included in the assay, and add additionally 10% of the volume of each reagent.

04 Vortex the *PCR Master Mix* tube and dispense 20 µL in the corresponding wells.

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

- 5 µL of the genomic DNA sample (10 ng/µL).
- 5 µL of the positive control (included).
- 5 µL of nuclease-free water (no template control, NTC).

NOTE: It is recommended to add a PCR negative control (NTC) to rule out PCR contamination, and positive controls to ensure the correct set up and functioning of the PCR reaction.

06 Place the samples in the *Real Time PCR thermal cycler* and set the PCR programme according to the following section.

07.2 | Setup of the real-time PCR programme

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

- **Recommended experiment for *7500 FAST* or *StepOne***: Quantitation-Standard Curve
- **Reference ROX™ for *7500 FAST* or *StepOne***: included
- **Ramp rate**: Standard

- Reaction volume: 25 µL
- TaqMan® probes fluorophores:

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

Table 2. Hydrolysis probe information

- Optimal real-time PCR programme:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR	
No. of cycles	1 initial cycle	36 cycles	
		Denaturation	Annealing & Extension
Temperature	95°C	95°C	64°C
Time	10 minutes	15 seconds	1 minute*

Table 3. Optimal PCR programme for the 7500 FAST, StepOne (Thermo Scientific)

(*) Fluorescence detection

08 Analysis of results

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

- To analyze the samples, the specific software of the *Real-Time PCR thermal cycler* has to be used.
- Confirm that there is not amplification in the no template control. If amplification signal is detected, the analysis should be repeated to rule out accidental contamination.
- Confirm that the positive controls produced an amplification signal in both the FAM and VIC channels.
- Ensure that every sample, positive or negative, shows a positive signal in VIC proceeding from the amplification of the endogenous gene, β-globin.

In the following represent expected results obtained with the Imegen® HLA-B57:01 kit:

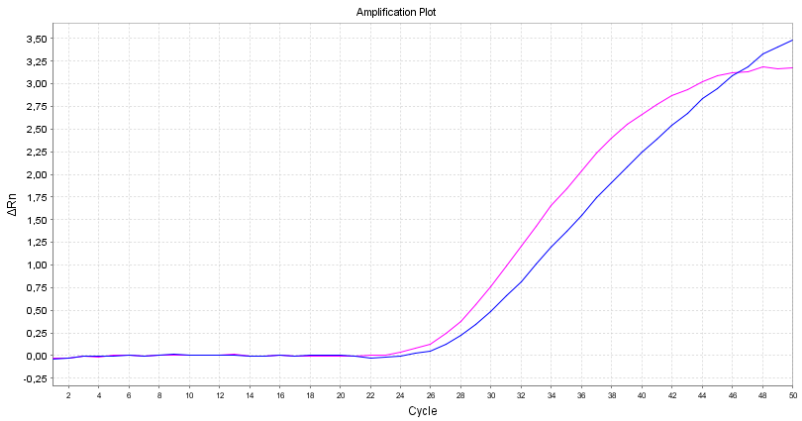

Figure 1. Fluorescent signal resulting from the correct amplification of the positive control. Amplification in FAM (blue) and VIC (pink) channel.

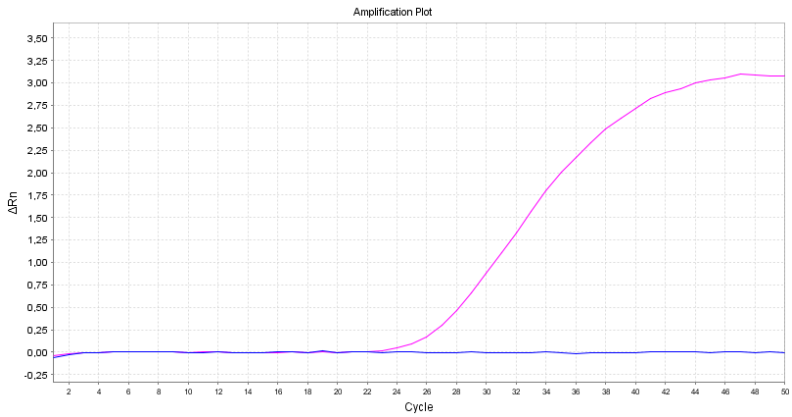

Figure 2. Fluorescent signal resulting from the correct amplification of a DNA samples lacking the HLA-B57:01 allele. Amplification is detected in VIC channel (pink) from β-globin gene

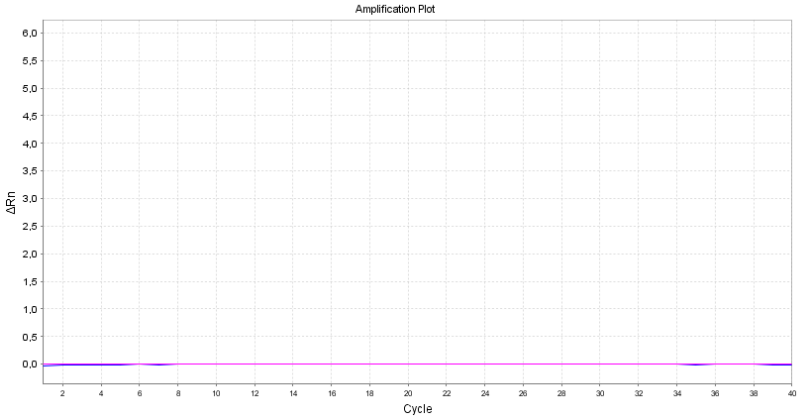

Figure 3. Fluorescent signal resulting from the correct amplification of the NTC. There is no amplification signal.

09 Troubleshooting

The table below represents the results that could be obtained using the positive and negative controls and the 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	HLA-B*57:01 (FAM)	β-Globin (VIC)	Result / Interpretation
Positive control	+	+	Expected result
	–	–	Fail in the PCR setup [1]
	+	–	
	–	+	
Sample	+	+	Expected result
	–	+	
	–	–	Fail to amplify the sample [2]
	+	–	Fail in the PCR setup [1]
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® HLA-B57:01

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

(2) Fail to amplify the sample: An error to amplify might suggest the quantity or the quality of the sample is compromised. In this situation, a second analysis or new extraction of DNA 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 to avoid any cross contamination.

10 Limitations

10.1 | Equipment

Imegen® HLA-B57:01 has been validated using the following real-time PCR systems:

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

If a real-time PCR cycler different from the systems described in this section is going to be used, 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® HLA-B57:01 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 if 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:

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