# Instructions for use

## Imegen® Quimera Screening Multiplex I

Ref. IMG-116-24

CE IVD

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

healthincode.com

Code: HIC-PT-KIT 03-F-03 V.02

healthincode

Health in Code, S.L. guarantees that all its products are free of defects, both in relation to the materials used and the manufacturing process. This guarantee is valid until the expiration date, provided that the storage conditions set out in this manual are followed.

**Our products are designed for *in vitro* diagnostics.** Health in Code, S.L. makes no other express or implied guarantee, which extends beyond the proper operation of the components of this kit. The only obligation of Health in Code, S.L. in relation to the aforementioned guarantees is to replace the products or refund the purchase price, as requested by the customer, provided that the defect in the materials or the manufacture of its products is proven. Health in Code, S.L. shall not be liable for any direct or indirect damages resulting from economic losses or damages that may arise from the use of this product by the purchaser or user.

All the products marketed by Health in Code, S.L. undergo rigorous quality control. The Imegen® Quimera Screening Multiplex I kit for qPCR has passed all internal validation tests, which guarantee the reliability and reproducibility of each assay.

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

+34 963 212 340
tech.support@healthincode.com



| Modifications to the instructions for use (IFU) | | |
|---|---|---|
| Version 05 | AGO 2023 | The enzyme is renamed in sections 6 and 7. |
| Version 04 | MAR 2023 | Incorporation of section 11. Performance characteristics. |
| Version 03 | DEC 2022 | Change of manufacturer's address: Health in Code, S.L., Calle de la Travesía s/n, 15E Base 5, Valencia 46024, Spain. |
| Version 02 | SEP 2022 | Change of manufacturer's identification: from Imegen to HEALTH IN CODE, S.L. |
| Version 01 | MAY 2022 | Adaptation to the requirements of Regulation (EU) 2017/746 of the European Parliament and of the Council of April 5, 2017 on *in vitro* diagnostic medical devices. |

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# 01 General information

The analysis of molecular chimerisms resulting from allogeneic transplantation is now a well-established method for transplant follow-up, as it provides precise, valuable information to orient post-transplant treatment or intervention, with the aim of anticipating possible risks of relapse, rejection or graft-versus-host disease. Likewise, it allows the evaluation of the response to different treatment modalities.

The entire family of Imegen®-Quimera for qPCR kits has been developed in collaboration with the Málaga Regional University Hospital, part of the Andalusian Health Service (SAS). As a result of this agreement, Health in Code, S.L. has an **exclusive, worldwide license** for the know-how of the products for their manufacture and exploitation.

### References


- *Jiménez-Velasco A, Barrios M, Román-Gómez J, Navarro G, Buño I, Castillejo JA, Rodríguez AI, García-Gemar G,Torres A, Heiniger AII. Reliable quantification of hematopoietic chimerism after allogeneic transplantation for acute leukemia using amplification by real-time PCR of null alleles and insertion/deletion polymorphisms. Leukemia. 2005; 19(3):336-43. Doi: 10.1038/sj.leu.2403622. PMID:15674363.*
- *Bustin SA, Benes V, Garson JA, Hellemasn J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009 Apr; 55(4):611-22. Doi: 10.1373/clinchem.2088.112797.Epub 2009 Feb 26. PMID 19246619.*

## Hematopoietic chimerism analysis procedure:

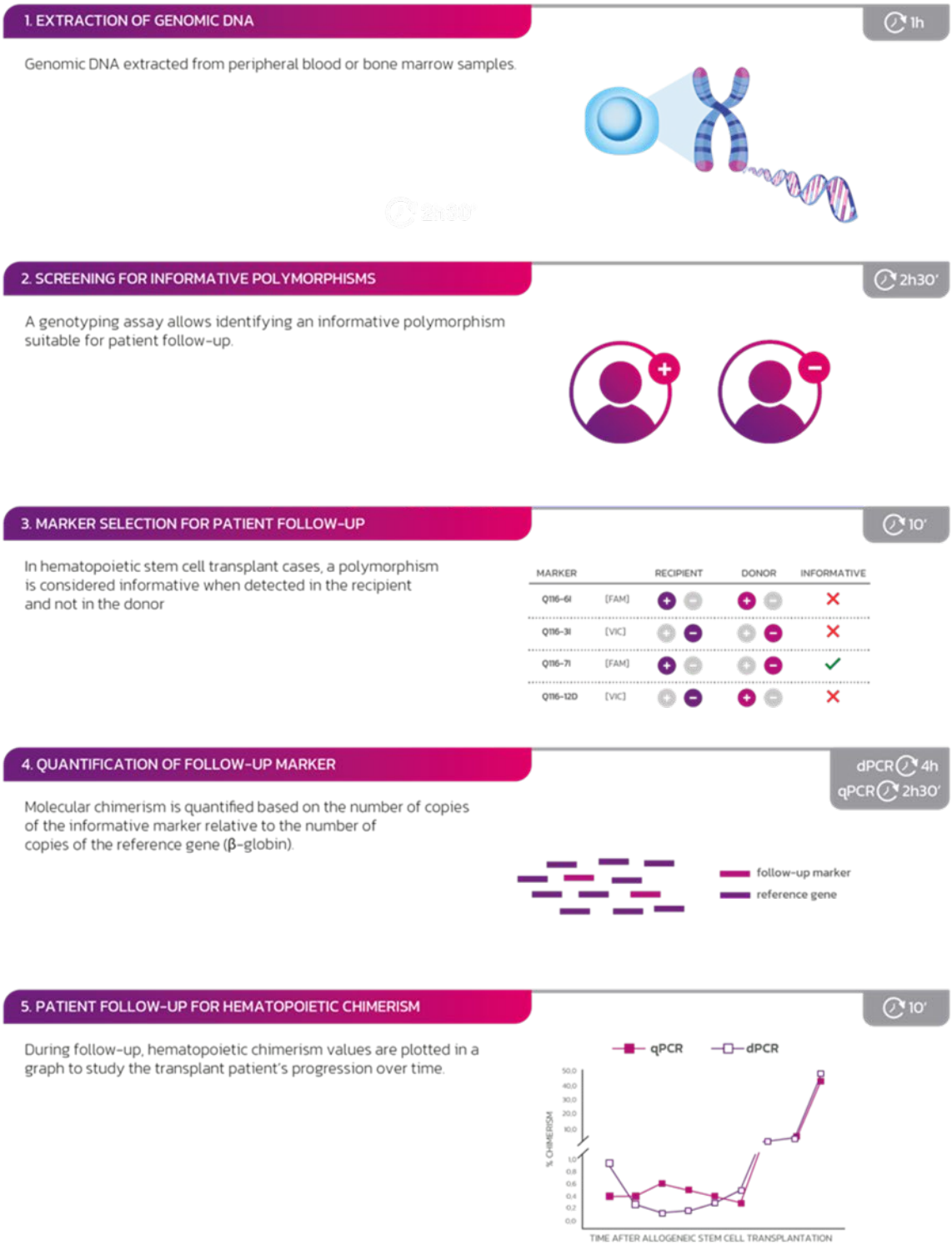



*Figure 1. Hematopoietic chimerism analysis procedure*

# 02 Intended use

The Imegen® Quimera Screening Multiplex I kit allows the selection of informative markers for the follow-up of hematopoietic cell transplant patients through the simultaneous analysis of 16 insertion/deletion polymorphisms (INDEL) in 8 independent real-time multiplex PCR reactions. A polymorphism is considered to be informative when it is detected in the transplant recipient and not in the donor.

To determine the informativity of the SRY and RhD markers it is not necessary to perform a molecular analysis. The SRY marker is informative when the recipient is male and the donor is female and the RhD marker is informative when the recipient has Rh+ blood group and the donor is Rh-.

The Imegen® Quimera Screening Multiplex I kit is for *in vitro* diagnostics and is intended for professionals in the molecular biology sector.

# 03 Technical characteristics

The Imegen® Quimera Screening Multiplex I kit allows performing a genotyping analysis capable of identifying informative markers for the analysis of hematopoietic chimerisms. It employs a combination of specific oligonucleotides and fluorescent hydrolysis probes to detect the presence or absence of 16 polymorphic markers, including INDELs and null allele markers. Together with Imegen® Quimera Screening Multiplex II, the SRY marker, present on the Chr Y, and RhD, includes a total of 34 markers.

↘ 34 markers on 18 chromosomes

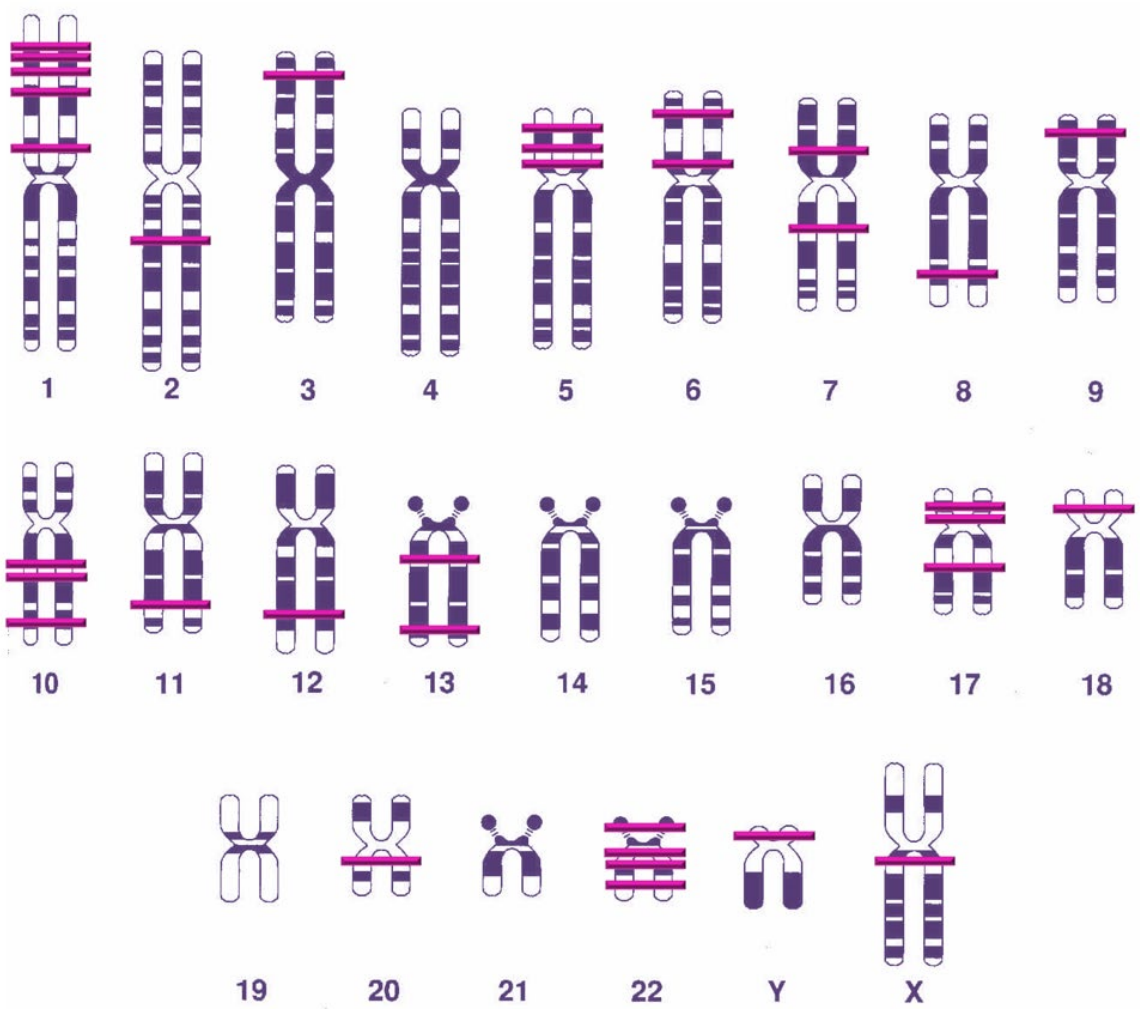



*Figure 2. Chromosomal representation of the biomarkers included in the genotyping and chimerism tracking assays.*

The material needed for this study is genomic DNA from human peripheral blood or bone marrow samples. The total amount of DNA needed is 450 ng of pre-transplant recipient sample and 450 ng of donor sample.

IMG-116-24 Screening Multiplex I

| Biomarker Name | Chromosomal Position |
|---|---|
| 3I | 20q11.22 |
| 6I | 10q26.2 |
| 12D | 5p13.2 |
| 7I | Xq28 |
| 11I | 1p13.3 |
| 5I | 10q21.2 |
| 4I | 17p13.2 |
| 10I | 22q13.32 |
| 23I | 13q34 |
| 9I | 22q11.22 |
| 8I | 22p13 |
| 12I | 5p13.3 |
| 4D | 17p13.2 |
| 5D | 10q21.2 |
| 10D | 22q13.32 |
| 20I | 8q24.22 |

IMG-116-74 Screening Multiplex II

| Biomarker Name | Chromosomal Position |
|---|---|
| 33I | 1p36.13 |
| 37I | 5p15.32 |
| 38I | 6p12.3 |
| 44I | 13q14.11 |
| 43I | 12q24.21 |
| 49I | 2q21.2 |
| 39I | 7p12.3 |
| 50I | 1p36.11 |
| 46I | 9p23 |
| 47I | 11q23.2 |
| 32I | 3p25.3 |
| 31I | 6p21.2 |
| 29D | 17q21.31 |
| 30D | 7q21.3 |
| 27D | 18p11.22 |
| 24I | 1p34.1 |

## ↘ Additional markers

| Biomarker Name | Chromosomal Position |
|---|---|
| SRY | Yp11.2 |
| RhD | 1p36.11 |

*Table 1. Chromosomal position of the biomarkers.*

The clinical performance of this kit has been validated using genomic DNA extracted from peripheral blood or bone marrow of human samples.

The cumulative informativity of this panel, together with the SRY and RhD markers, is 96%. If the panel of markers included in Imegen® Quimera Screening Multiplex II is also analyzed, the cumulative informativity is 99.96%.

# 04 Safety warnings and precautions

- We recommend strictly following the instructions in this manual, especially regarding the handling and storage conditions for the reagents.
- Do not pipette by mouth.
- Do not smoke, eat, drink or apply cosmetics in the areas where kits and samples are handled.
- Any skin conditions, as well as cuts, abrasions and other skin lesions should be properly protected.
- Do not pour reagent residues into the drinking water system. It is recommended to use the waste containers set out by the legal regulations and to manage them via an authorized waste manager.
- In the case of accidental spillage of any of the reagents, avoid contact with skin, eyes and mucous membranes and clean with plenty of water.
- Material safety data sheets (MSDS) for all hazardous components contained in this kit are available upon request.
- This product requires the handling of samples and materials of human origin. It is recommended to consider all materials of human origin as potentially infectious and to handle them in accordance with the EU-OSHA, the European Union's information agency for safety and health at work, on Biosafety level 2, applicable to clinical diagnostic laboratories where broad-spectrum microorganisms of moderate risk and associated with human diseases of varying severity are handled.
- The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic or corrosive and do not cause biological environmental contamination.
- This kit has been validated with specific equipment and under specific conditions that may vary significantly in other laboratories. It is therefore recommended that each laboratory perform an internal validation when using the kit for the first time.
- The manufacturer is not liable for the assay not working properly when the 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 includes reagents not validated by Health in Code, S.L., considering them equivalent to those supplied in the kit.

# 05 Content and storage conditions of the kit

This kit contains sufficient reagents to analyze 10 different genomic DNA samples or 5 recipient/donor cases.

The kit consists of a strip of 8 tubes containing one Screening Master in each tube. Each Master consists of two pairs of oligonucleotides and two TaqMan®-MGB probes with different labeling (FAM™ or VIC™) for the simultaneous analysis of two different polymorphisms (Table 2).

| Tube | Reactions | Markers | Storage | Rehydration |
|---|---|---|---|---|
| 1 | 10 reactions | Q116-6I<br>Q116-3I | 4°C | 33 µL of water/vial* |
| 2 | 10 reactions | Q116-11I<br>Q116-10D | 4°C | 33 µL of water/vial* |
| 3 | 10 reactions | Q116-4I<br>Q116-5D | 4°C | 33 µL of water/vial* |
| 4 | 10 reactions | Q116-5I<br>Q116-4D | 4°C | 33 µL of water/vial* |
| 5 | 10 reactions | Q116-10I<br>Q116-20I | 4°C | 33 µL of water/vial* |
| 6 | 10 reactions | Q116-7I<br>Q116-12D | 4°C | 33 µL of water/vial* |
| 7 | 10 reactions | Q116-12I<br>Q116-9I | 4°C | 33 µL of water/vial* |
| 8 | 10 reactions | Q116-8I<br>Q116-23I | 4°C | 33 µL of water/vial* |

*Table 2. Components of the Imegen® Quimera Screening Multiplex I kit and storage temperature.*

*(*) Once rehydrated, the reagents should be stored at -20°C.*

# 06 Equipment, reagents and materials not included in the kit

**Equipment:**

- Real-time PCR thermal cycler
- Micropipettes (10 µL, 20 µL and 200 µL)
- Vortex

**Reagents:**

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

**Materials:**

- 96-well optical plates or 0.2 mL optical tubes
- Optical film for 96-well plates or optical caps for 0.2 mL tubes
- Pipette tips with filter (10 µL, 20 µL and 200 µL)
- 1.5 mL sterile tubes
- Powder-free latex gloves

## *Complementary kits*

If no informative marker is identified, the use of the Imegen® Quimera Screening Multiplex II kit (IMG-116-74), which offers 16 alternative markers, is recommended.

Once a polymorphism has been identified as informative, it is recommended to acquire its corresponding Imegen®-Quimera qPCR kit (Table 3), in order to carry out patient follow-up, analysis of the transplanted organ and assessment of the risk of relapse. The Imegen®-Quimera qPCR kits allow absolute quantification of the amount of informative marker (chimerism) or relative quantification of the total amount of genomic DNA, using a reference gene (β-globin). The reference gene is analyzed in an independent reaction that serves as a control of the quality and quantity of DNA in the analyzed sample.

| Kit name | Reference |
|---|---|
| Imegen® Quimera SRY | IMG-116-2 |
| Imegen® Quimera RhD | IMG-116-18 |
| Imegen® Quimera Q116-3I | IMG-116-3 |
| Imegen® Quimera Q116-4I | IMG-116-4 |
| Imegen® Quimera Q116-5I | IMG-116-5 |
| Imegen® Quimera Q116-6I | IMG-116-6 |
| Imegen® Quimera Q116-7I | IMG-116-7 |
| Imegen® Quimera Q116-8I | IMG-116-8 |
| Imegen® Quimera Q116-9I | IMG-116-9 |
| Imegen® Quimera Q116-10I | IMG-116-10 |
| Imegen® Quimera Q116-11I | IMG-116-11 |
| Imegen® Quimera Q116-12I | IMG-116-12 |
| Imegen® Quimera Q116-4D | IMG-116-13 |
| Imegen® Quimera Q116-5D | IMG-116-14 |
| Imegen® Quimera Q116-10D | IMG-116-17 |
| Imegen® Quimera Q116-23I | IMG-116-23 |
| Imegen® Quimera Q116-20I | IMG-116-20 |
| Imegen® Quimera Q116-12D | IMG-116-21 |
| Imegen® Quimera Q116-33I | IMG-116-16 |
| Imegen® Quimera Q116-37I | IMG-116-75 |
| Imegen® Quimera Q116-38I | IMG-116-76 |
| Imegen® Quimera Q116-44I | IMG-116-77 |
| Imegen® Quimera Q116-43I | IMG-116-78 |
| Imegen® Quimera Q116-49I | IMG-116-79 |
| Imegen® Quimera Q116-39I | IMG-116-80 |
| Imegen® Quimera Q116-50I | IMG-116-70 |
| Imegen® Quimera Q116-46I | IMG-116-66 |
| Imegen® Quimera Q116-47I | IMG-116-81 |
| Imegen® Quimera Q116-32I | IMG-116-82 |
| Imegen® Quimera Q116-31I | IMG-116-83 |
| Imegen® Quimera Q116-30D | IMG-116-84 |
| Imegen® Quimera Q116-29D | IMG-116-73 |
| Imegen® Quimera Q116-27D | IMG-116-85 |
| Imegen® Quimera Q116-24I | IMG-116-87 |

*Table 3. Imegen®-Quimera kits for real-time PCR follow-up*

# 07 Assay protocol

## 07.1 | Preparation of reagents

All reagents included in the kit are lyophilized. The first step consists in rehydrating the reagents that make up a kit by adding 33 µL of nuclease-free water/vial*. To facilitate the 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.

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

## 07.2 | Preparation of amplification reactions

The assay should include the following reactions:

- Reactions with the recipient sample.
- Reactions with the donor sample.
- Reaction using the negative control (reaction containing nuclease-free water instead of DNA, to ensure the absence of possible contamination during the process).

Simultaneous analysis of the 16 markers with the Imegen® Quimera Screening Multiplex I kit requires the preparation of eight different PCR mixes. Each PCR mix consists of:

- **Screening Multiplex I Master Mix**
- **PCR Hot Start Master Mix (TaqMan™ Environmental Master Mix 2.0, ThermoFisher Scientific)**

The recommended protocol for preparing the amplification reactions is specified below:

01 Thaw the 8-tube strip containing the Screening Masters. Thaw the recipient and donor DNA.

02 Shake each of the reagents with vortex and keep cold.

03 Add 45 µL of PCR Hot Start Master Mix and 18 µL of recipient DNA at 25 ng/µL into a 1.5 mL tube.

04 Add 45 µL of PCR Hot Start Master Mix and 18 µL of donor DNA at 25 ng/µL into a 1.5 mL tube.

05 Vortex and pipette 7 µL of the *Master Mix* with the recipient DNA into 8 wells and 7 µL of the Master Mix with the donor DNA into another 8 wells.

06 Add 3 µL of Screening Master Mix to each well (with recipient DNA and with donor DNA).

NOTE: 50 ng is the recommended total DNA concentration per well.

# 07.3 | Real-time PCR program setup

Depending on the equipment to be used to perform the real-time PCR, the following instructions should be followed to set up the amplification program.

| Tube | Markers | Insertion (Allele +) | Deletion (Allele -) | Labeling | Emitter or Quencher |
|---|---|---|---|---|---|
| 1 | Q116-6I | X | | FAM™ | MGB |
| | Q116-3I | X | | VIC™ | |
| 2 | Q116-11I | X | | FAM™ | |
| | Q116-10D | | X | VIC™ | |
| 3 | Q116-4I | X | | FAM™ | |
| | Q116-5D | | X | VIC™ | |
| 4 | Q116-5I | X | | VIC™ | |
| | Q116-4D | | X | FAM™ | |
| 5 | Q116-10I | X | | VIC™ | |
| | Q116-20I | X | | FAM™ | |
| 6 | Q116-7I | X | | FAM™ | |
| | Q116-12D | | X | VIC™ | |
| 7 | Q116-12I | X | | FAM™ | |
| | Q116-9I | X | | VIC™ | |
| 8 | Q116-8I | X | | VIC™ | |
| | Q116-23I | X | | FAM™ | |

*Table 4. Information on the probes included in the Imegen® Quimera Screening Multiplex I kit*

## 7500 Fast o StepOne Plus Real-Time PCR system (ThermoFisher Scientific)

- **Type of experiment**: Quantitation - Standard curve
- **Ramp speed**: standard
- **Reaction volume**: 10 µL
- **ROX™ baseline reference**: included
- **Fluorophores of TaqMan® probes**:
- **Optimal program**:

| Fields | Stage 1 Enzymatic activation | Stage 2 PCR | |
|---|---|---|---|
| **No. of cycles** | 1 initial cycle | 50 cycles | |
| | | Denaturation | Primer binding/extension |
| **Temperature** | 95°C | 95°C | 60°C |
| **Time** | 10 minutes | 15 seconds | 1 minute* |

*Table 5. Optimal PCR program for the 7500 FAST or StepOne Plus*

*(*) Fluorescence detection*

## LightCycler® 480 Real-Time PCR system (Roche)

- Optimal program:

| Fields | Stage 1 Enzymatic activation | Stage 2 PCR | | | Stage 3 |
|---|---|---|---|---|---|
| **No. of cycles** | 1 initial cycle | 50 cycles | | | 1 final cycle |
| | | Denat. | Primer binding | Extension | |
| **Temperature** | 95°C | 95°C | 60°C | 72°C | 40°C |
| **Time** | 10 minutes | 5 seconds | 10 seconds | 15 seconds* | 20 seconds |

*Table 6. Optimal PCR program for LightCycler® 480.*

*(*) Fluorescence detection*

- **Analysis**: Fit points for all samples

# 08 Analysis of results

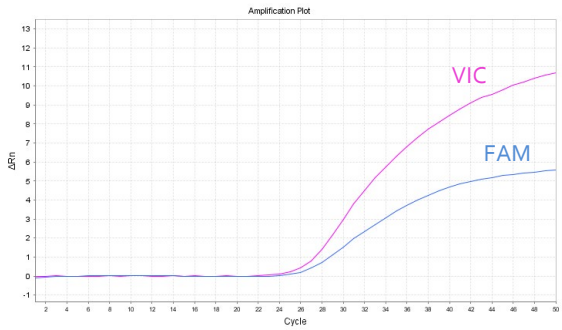
The analysis of the results is based on the detection of an informative polymorphism, i.e. detected in the recipient and not detected in the donor.

Table 7 shows the possible results that can be obtained for a marker evaluated. Figure 3 graphically represents the result of two multiplexed markers. The marker detectable with VIC is informative in case of bone marrow transplantation, but not in solid organ transplantation (Table 7).

| Reagents | Results | | Informativity | |
|---|---|---|---|---|
| | Recipient | Donor | Bone Marrow | Solid organs |
| Polymorphism | + | + | Not informative | Not informative |
| Polymorphism | + | – | Informative | Not informative |
| Polymorphism | – | – | Not informative | Not informative |
| Polymorphism | – | + | Not informative | Informative |

*Table 7. Interpretation of possible results obtained with Imegen® Quimera Screening Multiplex I*

Recipient




Donor

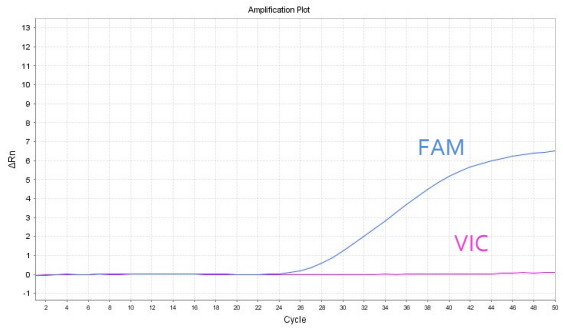



*Figure 3. Results obtained on 7500 FAST Real-time PCR System for recipient and donor samples. Two genetic markers are multiplexed in each PCR reaction. The blue amplification curves represent the amplification signal in the FAM channel and the pink amplification curves represent the amplification signal in the VIC channel.*

For the assay to be considered valid, the following conditions must be met:

- There is no amplification signal from the negative PCR control, neither in the FAM™ channel nor in the VIC™ channel. If amplification is detected, it is recommendable to repeat the assay to rule out possible contamination.
- Considering that the evaluation of biomarkers is performed under the same PCR conditions, similar Ct values are expected. In case any of the markers amplifies with ± 5 Ct difference, it is recommended to repeat the assay.
- Biomarkers that amplify at a Ct > 30 during screening are considered NEGATIVE, as

long as NEGATIVE, provided that the instructions for preparation, type and concentration of starting sample recommended in this manual have been followed.

- In case of obtaining results different from the above, it is recommended to consult section 9 of this manual (Troubleshooting).

If no informative marker is obtained, please contact the technical department: tech.support@healthincode.com.

## Imegen®-Quimera Software, by Health in Code, S.L.

Health in Code, S.L. has designed and developed an application available to the user through the technical department (tech.support@healthincode.com) that allows them to create a patient database, as well as to record the results of the informative polymorphism screening, the quantifications of the informative polymorphism of the different samples of the patient's follow-up and the medical actions applied to the patient during their follow-up. In addition, the user can visualize all medical actions, the patient's evolution on a graph, and export the results.

For more information, see the video tutorial on the use of the Imegen®-Quimera app at the following link: youtu.be/K38cV3hacm8

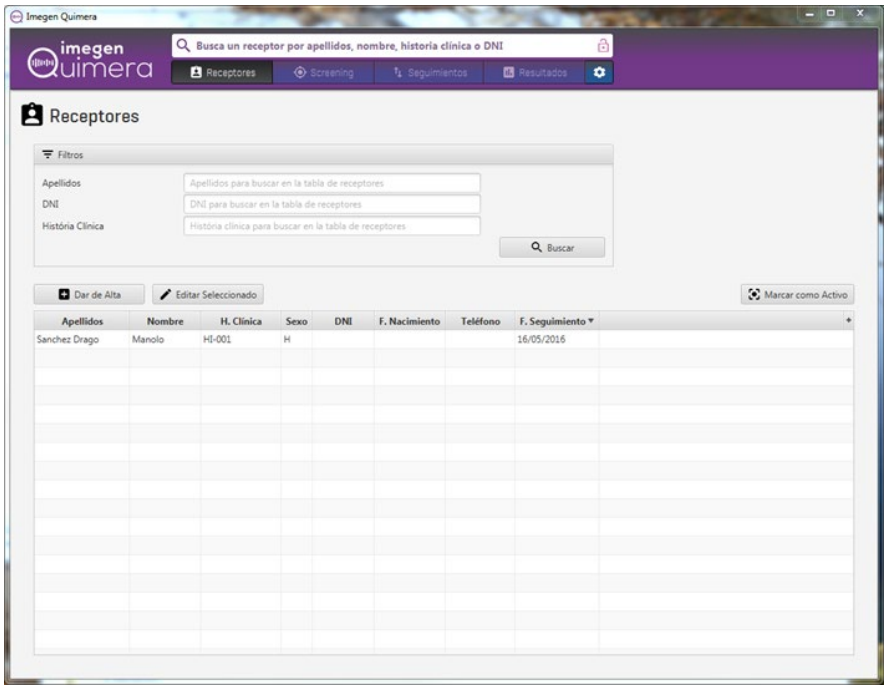



*Figure 4. View of the patient follow-up app developed by Health in Code, S.L.*

NOTE: The Imegen®-Quimera app is not designed for use as a laboratory information management system (LIMS).

# 09 Troubleshooting

Table 8 below graphically indicates the results that can be obtained from the analysis of different positive and negative controls and a genomic DNA sample in an assay, as well as their interpretation:

| Control | $C_T$* Polymorphism | Result | Cause |
|---|---|---|---|
| Analyzed sample | Detected < 30 | + | Expected result |
| | Detected > 30 | + | PCR contamination with human DNA [1], or sample concentration lower than specified in protocol [2]. |
| | Not detected | – | Expected result |
| PCR negative control | Not detected | – | Expected result |
| | Detected | + | Contamination of PCR with human DNA[1] |

*Table 8. Possible results of controls and samples.*

*(*) Cycle threshold ct (abbreviation of cycle threshold).*

**(1) PCR contamination with DNA:** contamination of PCR reactions may be due to a sample handling error, reagent contamination or environmental contamination. It is recommended to thoroughly clean the laboratory where the PCR process was performed, as well as the equipment. If necessary, use new aliquots of the reagents used in the PCR and repeat the assay.

**(2) Inadequate sample concentration:** late amplification of markers may be due to the DNA concentration used in the assay being lower than specified in the protocol. In this case, it is recommended to quantify the sample again by absorbance or fluorescence.

# 10 Limitations

## 10.1 | Equipment

Imegen® Quimera Screening Multiplex I has been validated using the following real-time PCR kits:

- **7500 FAST Real-Time PCR System** (ThermoFisher Scientific)
- **StepOne Plus Real-Time PCR System** (ThermoFisher Scientific)
- **LightCycler® 480 Real-Time PCR System** (Roche)

If you use another make or model of thermal cycler, you may need to adjust the amplification program. Please contact the technical department for any questions or clarifications.

## 10.2 | Reagents

Imegen® Quimera Screening Multiplex I has been validated using the reagents included in the kit. It is advisable to use the PCR reagents recommended by the supplier of the thermal cycler to be used for real-time PCR assays, not provided by the kit manufacturer, as indicated in section 06 of this manual (Required equipment and materials not supplied). If in doubt, please contact the technical department.

## 10.3 | Product stability

The optimum performance of this product is confirmed provided that the recommended storage conditions specified in this manual are applied and within the optimum product date associated with each production batch.

# 11 Performance characteristics

## 11.1 | Validation samples

The Imegen® Quimera Screening Multiplex I kit is designed for the analysis of total genomic DNA (gDNA). The qPCR systems for each marker in the kit have been fine-tuned with 7 synthetic DNA samples (plasmid). In addition, these results have been validated with 31 germline reference samples that present the polymorphism in homozygosis or heterozygosis, analyzing the presence or absence of the polymorphism.

For a sample to be considered positive, a threshold cycle or Ct <30 has been established, provided that the instructions for sample preparation, type and starting sample concentration recommended in this manual have been followed.

## 11.2 | Linearity and efficiency

The linearity of the Imegen® Quimera Screening Multiplex I kit is established by means of a standard curve based on serial dilutions of a synthetic calibrator (plasmid) of known concentration for each of the genetic markers. The results obtained during validation with plasmids (Table 9) confirm adequate efficiency and linearity ($R^2 > 0.99$) for the quantification of each of the markers evaluated. These parameters have been assessed according to the MIQE guidelines, *minimum information for publication of quantitative real-time PCR experiments* and ISO 20395:2019 standard *Biotechnology-Requirements for evaluating the performance of quantification methods for nucleic acid target sequences-qPCR and dPCR*.

The efficiency value ($\overline{E}$) of each marker is calculated using the following formula:

$$\overline{E} = 10^{-1/\text{pending}} - 1$$

| Name marker | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Marker | | | β-globin | | |
| | Pending | $R^2$ | $\bar{E}$ | Pending | $R^2$ | $\bar{E}$ |
| Q116-8I | -3.25 | 0.99 | 1.93 | -3.32 | 0.99 | 2.00 |
| Q116-6I | -3.35 | 0.99 | 1.87 | -3.32 | 0.99 | 2.00 |
| Q116-11I | -3.51 | 0.99 | 1.92 | -3.32 | 0.99 | 2.00 |
| Q116-4I | -3.25 | 0.99 | 1.96 | -3.32 | 0.99 | 2.00 |
| Q116-5I | -3.15 | 0.99 | 1.99 | -3.32 | 0.99 | 2.00 |
| Q116-10I | -3.32 | 0.99 | 2.00 | -3.32 | 0.99 | 2.00 |
| Q116-7I | -3.35 | 0.99 | 1.94 | -3.32 | 0.99 | 2.00 |
| Q116-12I | -3.30 | 0.99 | 2.01 | -3.32 | 0.99 | 2.00 |
| Q116-3I | -3.32 | 0.99 | 2.01 | -3.21 | 0.99 | 2.05 |
| Q116-5D | -3.52 | 0.99 | 1.89 | -3.21 | 0.99 | 2.05 |
| Q116-4D | -3.60 | 0.99 | 1.98 | -3.21 | 0.99 | 2.05 |
| Q116-20I | -3.11 | 0.99 | 2.10 | -3.21 | 0.99 | 2.05 |
| Q116-12D | -3.35 | 0.99 | 2.04 | -3.21 | 0.99 | 2.05 |
| Q116-9I | -3.16 | 0.99 | 2.08 | -3.21 | 0.99 | 2.05 |
| Q116-23I | -3.25 | 0.99 | 1.96 | -3.21 | 0.99 | 2.05 |
| Q116-10D | -3.31 | 0.99 | 2.00 | -3.21 | 0.99 | 2.05 |

*Table 9. Results of linearity and efficiency parameters obtained with the synthetic calibrators for each qPCR system. R2: correlation coefficient.*

$\bar{E}$ *average qPCR efficiency for each marker, each assay has been repeated 3 times.*

## 11.3 | Limit of detection (LOD)

To study the limit of detection of the Imegen® Quimera Screening Multiplex I kit, the performance of the qPCR assay for each marker was evaluated by varying the starting gDNA concentration. For this purpose, a heterozygous reference sample was selected for the biomarker under analysis and decreasing amounts of gDNA were used, including the optimal amount recommended for the assay (50 ng, 5 ng and 1 ng). Three replicates of each concentration were made and evaluated in the compatible kits specified in section 10 of this manual (Limitations).

In all cases and concentrations tested, the established coefficient of variation (CV) criterion was met (CV < 25%). The detection limit set is 1 ng total genomic DNA.

## 11.4 | Repeatability and reproducibility

The reproducibility and repeatability of the Imegen® Quimera Screening Multiplex I kit have been evaluated, testing 11 times 0.1% of each marker under the recommended assay conditions. The assays have been validated by independent operators on compatible equipment with a total of 33 measurements. The assays are considered repeatable and reproducible with a coefficient of variation of less than 25%.

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

tech.support@healthincode.com

+34 963 212 340

healthincode