



Instructions for use

Imegen[®] Quimera dPCR (Dry)

Ref. IMG-116

CE IVD

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healthincode

Health in Code guarantees that its products are free of defects, in both materials and workmanship. This guarantee remains in force until the expiration date, as long as the conservation practices described in this manual are adhered to.

Our products are intended for *in vitro* diagnostic use. Health in Code provides no guarantee, whether explicit or implicit, that extends beyond the proper functioning of the components of this kit. Health in Code's sole obligation, in relation to the aforementioned guarantees, shall be to either replace the product or reimburse the cost of it, per the client's preference, provided that materials or workmanship prove to be defective. Health in Code is not liable for any cost or expense, direct or indirect, or damage or harm incurred by the customer or user as a result of use of the product by the buyer or user.

All Health in Code products undergo strict quality control. The **Imegen® Quimera Screening Multiplex Plus II** dPCR 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, feel free to contact our Technical Department:

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Imegen® is a trademark registered to Health in Code, Spain.

Modifications to the Instructions for Use (IFU)

Version 04 Format edition

Version 05 Change of manufacturer's identification from Imegen to Health in Code

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01

General information

The analysis of molecular chimerism resulting from allogeneic transplantation has become an established method to follow transplant progression, since it offers accurate and valuable information that allows targeting post-transplant treatments or interventions with the purpose of anticipating any potential risk of relapse, rejection, or graft-versus-host disease. This approach is highly useful not only to determine the risk of relapse, rejection, or graft-versus-host disease, but also to assess the response to different treatment modalities.

The whole **Imegen® Quimera** kit family has been developed in collaboration with the Carlos Haya Regional Hospital (Malaga, Spain), included in the Andalusian regional public healthcare service (Servicio Andaluz de Salud, SAS). As a result of this agreement, Health in Code holds the **exclusive worldwide license** on the *know-how* of the products for their commercial manufacturing and exploitation.

References

- > Jiménez-Velasco A, Barrios M, Román-Gómez J, Navarro G, Buño I, Castillejo J, et al. 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; 1-8.
- > Stahl T, Böhme M, Kröger N, Fehse B. Digital PCR to assess hematopoietic chimerism after allogeneic stem cell transplantation. *Experimental Hematology*. 2015; 43:462-468

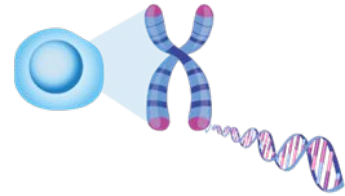
> Procedure for hematopoietic chimerism analysis:

1. EXTRACTION OF GENOMIC DNA

1h

Genomic DNA extracted from peripheral blood or bone marrow samples.

2h30'



2. SCREENING FOR INFORMATIVE POLYMORPHISMS

2h30'

A genotyping assay allows identifying an informative polymorphism suitable for patient follow-up.



3. MARKER SELECTION FOR PATIENT FOLLOW-UP

10'

In hematopoietic stem cell transplant cases, a polymorphism is considered informative when detected in the recipient and not in the donor.

MARKER		RECIPIENT		DONOR		INFORMATIVE
Q116-61	[FAM]	+	-	+	-	X
Q116-31	[VIC]	+	-	+	-	X
Q116-71	[FAM]	+	-	+	-	✓
Q116-12D	[VIC]	+	-	+	-	X

4. QUANTIFICATION OF FOLLOW-UP MARKER

dPCR 4h
qPCR 2h30'

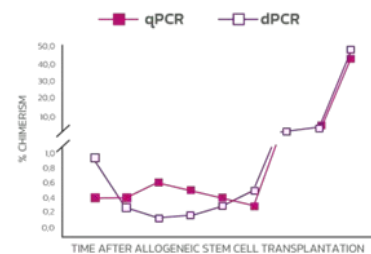
Molecular chimerism is quantified based on the number of copies of the informative marker relative to the number of copies of the reference gene (β -globin).



5. PATIENT FOLLOW-UP FOR HEMATOPOIETIC CHIMERISM

10'

During follow-up, hematopoietic chimerism values are plotted in a graph to study the transplant patient's progression over time.



02

Intended use

The **Imegen® Quimera dPCR** kit allows detecting the presence of hematopoietic chimerisms during the post-transplant period by quantification of a molecular marker by digital PCR (dPCR), a real-time PCR method that allows for absolute quantification in a sample that is divided into 20,000 partitions. This assay is based on a previous screening for informative polymorphisms, including INDELs (insertions/deletions) and null alleles, which are used to quantify molecular chimerism either as the absolute amount of the informative marker or as its relative amount with respect to total genomic DNA in the sample (multiplex PCR). To carry out this quantification, it is necessary to test for a reference gene (β -globin), which at the same time works as a positive control for the amplification and an informative polymorphism.

Therefore, it is essential to first select the informative polymorphisms for each transplant case. For this purpose, Health in Code has validated two references: **Imegen® Quimera Screening Multiplex Plus** (IMG-116-26) and **Imegen® Quimera Screening Multiplex Plus II** (IMG-116-25). A polymorphism is considered informative when detected in the transplant recipient but not in the donor.

Once an informative marker has been selected, it can be used for the follow-up of the transplanted patient. For this, a panel with 33 INDELs has been developed; these indels are listed below:

Markers	Insertion (Allele +)	Deletion (Allele -)	Kit reference
SRY	X		IMG-116-27
RhD	X		IMG-116-28
Q116-6I	X		IMG-116-32
Q116-3I	X		IMG-116-29
Q116-7I	X		IMG-116-33
Q116-12D		X	IMG-116-36
Q116-11I	X		IMG-116-34
Q116-5I	X		IMG-116-31
Q116-4I	X		IMG-116-30
Q116-10I	X		IMG-116-35
Q116-23I	X		IMG-116-37
Q116-28I	X		IMG-116-42
Q116-32I	X		IMG-116-46
Q116-31I	X		IMG-116-45
Q116-30D		X	IMG-116-44
Q116-29D		X	IMG-116-43
Q116-27D		X	IMG-116-41
Q116-24I	X		IMG-116-38
Q116-9I	X		IMG-116-48
Q116-20I	X		IMG-116-40
Q116-33I	X		IMG-116-47
Q116-37I	X		IMG-116-49
Q116-38I	X		IMG-116-50
Q116-39I	X		IMG-116-51
Q116-41I	X		IMG-116-52
Q116-42I	X		IMG-116-53
Q116-43I	X		IMG-116-54
Q116-44I	X		IMG-116-55
Q116-45I	X		IMG-116-56
Q116-46I	X		IMG-116-60
Q116-47I	X		IMG-116-57
Q116-49I	X		IMG-116-58
Q116-50I	X		IMG-116-59

Table 1. References of the Imegen® Quimera dPCR kit

These instructions for use are suitable for testing any of the 33 markers included in Table 1, as their operation is optimal under the same PCR settings. Therefore, this technique allows for rapid and effective simultaneous testing for multiple polymorphisms.

The **Imegen® Quimera dPCR** kit can only be used for in vitro diagnosis and is aimed at professionals in molecular biology.

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Technical characteristics

Imegen® Quimera dPCR consists of a test that enables digital PCR quantification of the number of copies of an informative marker for the follow-up of hematopoietic chimerism. This kit uses a combination of specific primers and fluorescent hydrolysis probes to quantify either the absolute amount of an informative marker or its relative amount with respect to the reference gene, β -globin.

The type of sample used to validate the **Imegen® Quimera dPCR** kits is genomic DNA from peripheral blood or bone marrow samples from patients who have undergone allogeneic stem cell transplant. The limit of quantification (LoQ) has been determined to be 0.05%, while the limit of detection (LoD) has been established at 0.01% when genomic DNA samples are used.

- > **Type of sample:** genomic DNA from peripheral blood, bone marrow, and cell subtypes.
- > **DNA amount:** 75 ng (BioRad) and 150 ng (*Thermo Fisher Scientific*)
- > **Limit of quantification:** 0.05%
- > **Number of reactions per sample:** 1
- > **Number of targets:** 2
- > **Set-up and preparation:** 15 min – 1 h 30 min
- > **Duration of the dPCR program:** 1 h 30 min – 3 h
- > **dPCR platforms:**
 - QuantStudio 3D Digital PCR System (*Thermo Fisher Scientific*)
 - QuantStudio Absolute Q PCR System (*Thermo Fisher Scientific*)
 - QX200™ Droplet Digital™ PCR System (*Bio Rad*)

Health in Code is certified against the norm **UNE-EN ISO 13485:2018 Medical devices: Quality management systems – Requirements for regulatory purposes** by the SPANISH AGENCY OF MEDICINES AND MEDICAL DEVICES 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

1. It is recommended to strictly follow the instructions in this manual, especially regarding the handling and storage conditions of the reagents.
2. Do not mouth-pipette.
3. Do not smoke, eat, drink, or apply cosmetics in areas where kits and samples are handled.
4. Any cuts, abrasions, and other skin injuries must be properly protected.
5. 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.
6. In the event of an accidental spill of any of the reagents, avoid contact with the skin, eyes, and mucous membranes and rinse with a large amount of water.
7. Safety data-sheets (MSDS) of all dangerous substances contained in this kit are available on request.
8. This product requires the manipulation of samples and materials of human origin. It is recommended to consider all materials of human origin as potentially infectious and manipulate 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.
9. The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, corrosive, or environmental biological pollutants.
10. 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.
11. The manufacturer assumes no responsibility for any damage or failure of the assay caused by substituting reagents included in the kit for ones not provided by Health in Code.
12. The manufacturer does not guarantee the assay's reproducibility when the user uses reagents that have not been validated by Health in Code 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 freeze-dried reagents to carry out 12 digital PCR determinations:

- > **Polymorphism Master Mix:** necessary oligonucleotides to amplify the target marker (polymorphism) and the reference gene (β -globin), two labeled probes (one with FAM™ to detect the marker and one with VIC® for the specific detection of β -globin, which acts as active reference for quantification).

Reagents	Quantity	Conservation
Polymorphism Master Mix	2 x 6 rxn	4°C

Table 2. Components of the Imegen® Quimera dPCR kit

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Necessary equipment, reagents, and material not included in the kit

Equipment:

- > Micropipettes (10 μ L, 20 μ L, and 200 μ L)

QuantStudio 3D Digital PCR (ThermoFisher Scientific)

- > QuantStudio 3D Digital PCR (Chip Loader)
- > ProFlex™ 2x Flat PCR System (dPCR thermal cycler)
- > QuantStudio 3D Digital PCR instrument (Chip Reader)

QuantStudio Absolute Q PCR (ThermoFisher Scientific)

- > QuantStudio™ Absolute Q Digital PCR System, desktop (dPCR thermal cycler)

Droplet Digital PCR (Bio-Rad)

- > QX200™ Droplet Digital™ PCR system or QX100™ Droplet Digital™ PCR system
- > PX1™ PCR Plate Sealer
- > C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module

Reagents:

- > Nuclease-free water

QuantStudio 3D Digital PCR (ThermoFisher Scientific)

- > QuantStudio 3D Digital PCR Master Mix v.2
- > Absolute ethanol

QuantStudio Absolute Q PCR (ThermoFisher Scientific)

- > Absolute Q DNA Digital PCR Master Mix (Ref. A52490)
- > Isolation Buffer (Ref. A52730)

Droplet Digital PCR (Bio-Rad)

- > ddPCR™ Supermix for probes (No dUTP)
- > Droplet generation oil for probes

Materials:

- > Sterile 0.2 and 1.5 ml tubes
- > Pipette filter tips (10 µL, 20 µL y 200 µL)
- > Powder-free latex gloves

QuantStudio 3D Digital PCR (ThermoFisher Scientific)

- > QuantStudio® 3D Digital PCR 20K Chip Kit v2 (12 pack) (Ref.A26316)
- > Precision wipes

QuantStudio Absolute Q PCR (ThermoFisher Scientific)

- > MAP16 Plate Kit (Ref. A52865)

Droplet Digital PCR (Bio-Rad)

- > Droplet Generator Cartridges and Gaskets (Ref.1864007)
- > 96-well plates for ddPCR (Ref.12001925)
- > Pierceable foil heat seal (Ref.1814040)

Complementary kits

Before quantifying an informative marker by digital PCR, the previous step consists in determining the informativity of the possible polymorphisms by real-time PCR; for this purpose, Health in Code has developed the following kits:

Kit name	Reference
Imegen® Quimera Screening Multiplex Plus	IMG-116-26
Imegen® Quimera Screening Multiplex Plus II	IMG-116-25

Table 3. Marker screening kit

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Assay protocol

7.1 Reagents preparation

The reagent included in this kit is freeze-dried. Before applying the protocol, the first step is to rehydrate the reagents by adding 10 µL of nuclease-free water per vial*. To enable resuspension, it is recommended to shake and spin the tubes containing the reagents and store them at 4 °C for one hour before their use.

**If this reagent is not to be used immediately after rehydration, storage at -20 °C is recommended.*

7.2 Preparation of amplification reactions

The assay must include the following reactions:

- > **Reaction for each sample.**
- > **Recommended:** negative control reaction (a reaction that contains water instead of DNA to confirm the absence of contamination during the process).
- > **Optional:** if you wish to determine the cellularity of the selected informative marker, analyzing the recipient's pre-transplant sample is recommended. This test will allow determining whether the recipient is homo- or heterozygous for the selected marker, thus correcting the allelic load to obtain cellularity.

Each PCR mix must contain:

- Polymorphism Master Mix (Polymorphism Master Mix + β-Globin Master Mix)
- General Master Mix (not included in the kit)

Below you can find the protocol to be followed in order to prepare amplification reactions and perform digital PCR according to the platform used:

1. **Thaw the necessary reagents to perform the analysis:**
 - a. Genomic DNA samples diluted to the optimal concentration (25 ng/µL).
 - b. Polymorphism Master Mix (rehydrated)
 - c. Nuclease-free water for negative controls.
 - d. Digital PCR Master Mix (not included).
2. **Vortex and spin the reagents.**

QuantStudio™ 3D Digital PCR System (ThermoFisher Scientific)

3. Add the indicated amount of each reagent to 1.5 mL tubes according to the total number of reactions. To perform the calculations, it is recommended either to add a sufficient amount of reagents to perform one extra reaction or to add an extra 10% of each reagent.

Reagents	Volume per reaction
Polymorphism Master Mix	1.5 µL
QuantStudio 3D Digital PCR Master Mix v.2	7.5 µL

Table 4. Volume of reagents needed per reaction using the QuantStudio 3D dPCR System

4. Mix the PCR mix by pipetting (avoiding bubble formation) and dispense 9 µL into the corresponding 0.2 mL tubes.
5. Dispense 6 µL of the DNA sample at 25 ng/µL or of nuclease-free water (negative control) to the corresponding tubes.
6. Load 14.5 µL of the PCR reaction into the *QuantStudio 3D Digital PCR Chip Loader* to load the chip, following the manufacturer's instructions.

QuantStudio® Absolute Q PCR system (ThermoFisher Scientific)

3. Add the following reagents to a new 1.5 mL tube:

Reagents	Volume per reaction
Polymorphism Master Mix	1.5 µL
Absolute Q DNA Digital PCR Master Mix (5x)	2 µL

Table 5. Volume of reagents needed per reaction using the QuantStudio Absolute Q dPCR System

4. Mix the PCR Master MIX by pipetting up and down carefully to avoid bubbling (do not use a vortex mixer) and dispense 3.5 µL into new 0,2 ml tubes.
5. Add 6.5 µL of the DNA sample at 25 ng/µL or of nuclease-free water (negative control) to tubes containing the PCR Master Mix.
6. At a 45° angle, load 9 µL of the PCR reagents to the bottom of the well in the MAP16 plate, following the manufacturer's instructions. Pipette the mixture only to the first stop.
7. Afterwards, at a 45° angle, load 15 µL of Isolation Buffer against the side of the well and let it pour on the reagents to prevent mixing and bubble formation.

Droplet Digital™ PCR system (BIO–RAD)

3. Add the indicated amount of each reagent to 1.5 mL tubes according to the total number of reactions. To perform the calculations, it is recommended either to add a sufficient amount of reagents to perform one extra reaction or to add an extra 10% of each reagent.

Reagents	Volume per reaction
Polymorphism Master Mix	1.5 µL
ddPCR™ Supermix for probes (No dUTP)	10 µL
Nuclease-free water	5.5 µL

Table 6. Volume of reagents needed per reaction using the Droplet Digital PCR system

4. Mix the PCR mix by pipetting to prevent bubble formation and dispense 17 µL in a 96-well plate:

Note: If any well in the columns with samples remains empty, add 20 µL of buffer or nuclease-free water.
5. Dispense 3 µL of the DNA sample at 25 ng/µL or of nuclease-free water (negative control) to the corresponding wells.
6. Load 20 µL of the PCR solution with a multi-channel pipette in the corresponding cartridge, according to the manufacturer's recommendations.

7.3 Settings for the dPCR program, loading, and reading

QuantStudio™ 3D Digital PCR System (ThermoFisher Scientific)

> Loading of amplification reactions onto the chip

To set up all the necessary devices to load the chip (including consumables) with the prepared amplification reactions, we recommend following the manufacturer's instructions for the QuantStudio 3D Digital PCR Chip Loader (ThermoFisher Scientific). To do this, please download the MAN0007720 QuantStudio™ 3D Digital PCR System User Guide, available at the www.thermofisher.com website, and follow the instructions in Section 3.

> Settings for the PCR program

To arrange the chips on the ProFlex™ 2x Flat PCR System dPCR thermal cycler, please follow the instructions in Section 4 of the MAN0007720 QuantStudio™ 3D Digital PCR System User Guide, available at the www.thermofisher.com website.

- Optimal program:

Fields	Phase 1	Phase 2		Phase 3	
	Enzymatic activation	PCR			
No. of cycles	1 initial cycle (Denaturation)	40 cycles		1 final cycle	Conservation
		Primer binding/ Extension	Denaturation		
Temperature	96°C	56°C	98°C	60°C	10°C
Time	10 minutes	2 minutes	30 seconds	2 minutes	∞

Table 7. Optimal PCR program for Imegen® Quimera dPCR kits on the QuantStudio 3D dPCR system

> Chip reading and generation of results

Once the PCR program is completed, please follow the instructions in Section 5 of the MAN0007720 QuantStudio™ 3D Digital PCR System User Guide, available at the www.thermofisher.com website, to generate the chip reading files.

QuantStudio® Absolute Q PCR system (ThermoFisher Scientific)

> Microfluidic array plate (MAP) loading

Assemble the strips of the MAP plate into the four rows of the wells. Make sure to completely cover the rows, then place the cap strips of the MAP plate on all of them, including unused ones. Please download the MAN0025621 QuantStudio™ Absolute Q™ Digital PCR System User Guide, available at the www.thermofisher.com website, and follow the instructions in Section 2.

> Settings for the dPCR program

Place the MAP plate into the QuantStudio™ Absolute Q™ System, select the protocol from the list, and modify the optimal channels and parameters as required. To facilitate chimerism calculation:

- Experiment settings:

Analysis	Target	Analysis
FAM	Informative marker	CNV
VIC	Beta-globin	CNV Ref

Table 8. Experiment configuration on the QuantStudio Absolute Q dPCR system.

- Optimal PCR program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR	
No. of cycles	1 initial cycle (Denaturation)	40 cycles	
		Denaturation	Annealing and Extension
Temperature	96°C	96°C	60°C
Time	10 minutes	5 seconds	15 seconds

Tabla 9. Optimal PCR program for the QuantStudio Absolute Q dPCR system.

> **Array reading and generation of results**

Once PCR is completed, please follow the instructions in the Digital PCR QuantStudio™ Absolute Q User Guide, available at the www.thermofisher.com website.

Droplet Digital™ PCR system (BIO–RAD)

> **PCR reactions loading**

For the assembly and preparation of all the devices necessary to load the cartridge (consumables not included) with the prepared amplification reactions, we recommend following the manufacturer’s instructions for the QX200™ Droplet Digital™ PCR system or QX100™ Droplet Digital™ PCR system (BIO–RAD). To do this, download the Droplet Digital™ PCR Applications Guide, available at www.bio-rad.com and follow the instructions in ddPCR Experimental Workflow>Droplet Generation in Section 2.

> **Settings for the dPCR program**

Load the 96–well ddPCR plate in [C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module](#):

- Optimal program:

Fields	Phase 1 Enzymatic activation	Phase 2 PCR		Phase 3	
No. of cycles	1 initial cycle (Denat.)	40 cycles		1 final cycle	Conservation
		Desnat.	Primer binding/extension		
Temperature	96°C	94°C	60°C	98°C	4°C
Time	10 minutes	30 seconds	1 minute	10 minutes	∞

Table 10. Optimal PCR program for Imegen® Quimera dPCR kits on the BIO–RAD dPCR platform

> **Fluorescence reading and obtaining the results**

Once the PCR program is finished, follow the instructions in Section 2, specifically, in the Setting Up an Experiment in Quantasoft™ Software and Droplet Reading sections, of the Droplet Digital™ PCR Applications Guide, available at www.bio-rad.com, to obtain the files resulting from the Reading. For experiment type, the RED option must be selected: rare target sequence detection (rare event detection).

08 Analysis of results

To analyze the results, it is important to know the labels of the probe that detect each of the two kit targets:

Probe	Marking
β-globin	VIC®
Informative polymorphism	FAM™

Tabla 11. Used probe label

Please bear in mind the following recommendations for correct results analysis:

- **Negative controls** (NTC). Verify the absence of amplification signals for any target (FAM and VIC). If amplification is observed, process should be repeated to rule out the possibility of accidental contamination.
- Quantification of hematopoietic chimerism is expressed as a measure of the number of copies of the informative marker relative to the amount of the reference gene, beta-globin. Molecular chimerism percentage is calculated the following way:

$$\text{Chimerism \%} = \frac{\text{Informative marker} \frac{\text{Copies}}{\mu\text{L}}}{\beta - \text{globin} \frac{\text{Copies}}{\mu\text{L}}} \times 100$$

QuantStudio™ 3D Digital PCR System (ThermoFisher Scientific)

QuantStudio™ 3D AnalysisSuite™ Software by ThermoFisher Scientific is used for results analysis. Please follow the instructions in Section 5 of the MAN0007720 QuantStudio™ 3D Digital PCR System User Guide, available at www.thermofisher.com. This manual also contains a Troubleshooting section.

Moreover, the following recommendations should be followed to ensure an adequate analysis of the result:

- Verify that no amplification is found in negative controls. If amplification is detected, it is recommended to repeat the test to rule out accidental contamination.
- If necessary, review and edit the result of the chip reading in the "Review data" section of and ultimately use the copies/μL (FAM) and copies/μL (VIC) data from the "See results" tab of the analysis software:

QuantStudio™ Absolute Q™ dPCR system (Thermo Fisher Scientific)

The QuantStudio Absolute Q Digital PCR software by ThermoFisher Scientific is used for results analysis. To facilitate data interpretation, follow the instructions in Section 3 'Data analysis'. An additional section on troubleshooting is available in the User Guide. The MAN0025621 QuantStudio™ Absolute Q™ Digital PCR System User Guide is available at www.thermofisher.com

- It is recommended to review and, where appropriate, manually edit the results obtained for each sample in the "Analysis" tab.
- For results analysis, access the "Results" tab to review and export the results as a CSV file.

Droplet Digital™ PCR system (BIO-RAD)

QuantaSoft™ Software by BIO-RAD by is used for results analysis. Please follow the instructions in Section 2, in particular the part on Data analysis, of the Droplet Digital™ PCR Applications Guide manual, available at www.bio-rad.com. This manual also contains a Troubleshooting section.

- If necessary, review and edit the result and ultimately use the "concentration (copies/μl)" for the marker (FAM) and the "concentration (copies/μl)" for the reference (VIC) values from the "analyze>Concentration" tab of the analysis software.

Imegen® Quimera Software (patient follow-up application)

Health in Code has designed and developed a user-friendly application that allows creating a patient database, as well as recording screening results for informative polymorphisms, their quantification in the different follow-up samples from a patient, and the medical actions taken in regard to said patient during follow-up. Moreover, the user can plot all the medical actions and the patient's progression and can export the results.

A video tutorial about how to use our **Imegen® Quimera** application is available at the following link: <https://youtu.be/K38cV3hacm8>

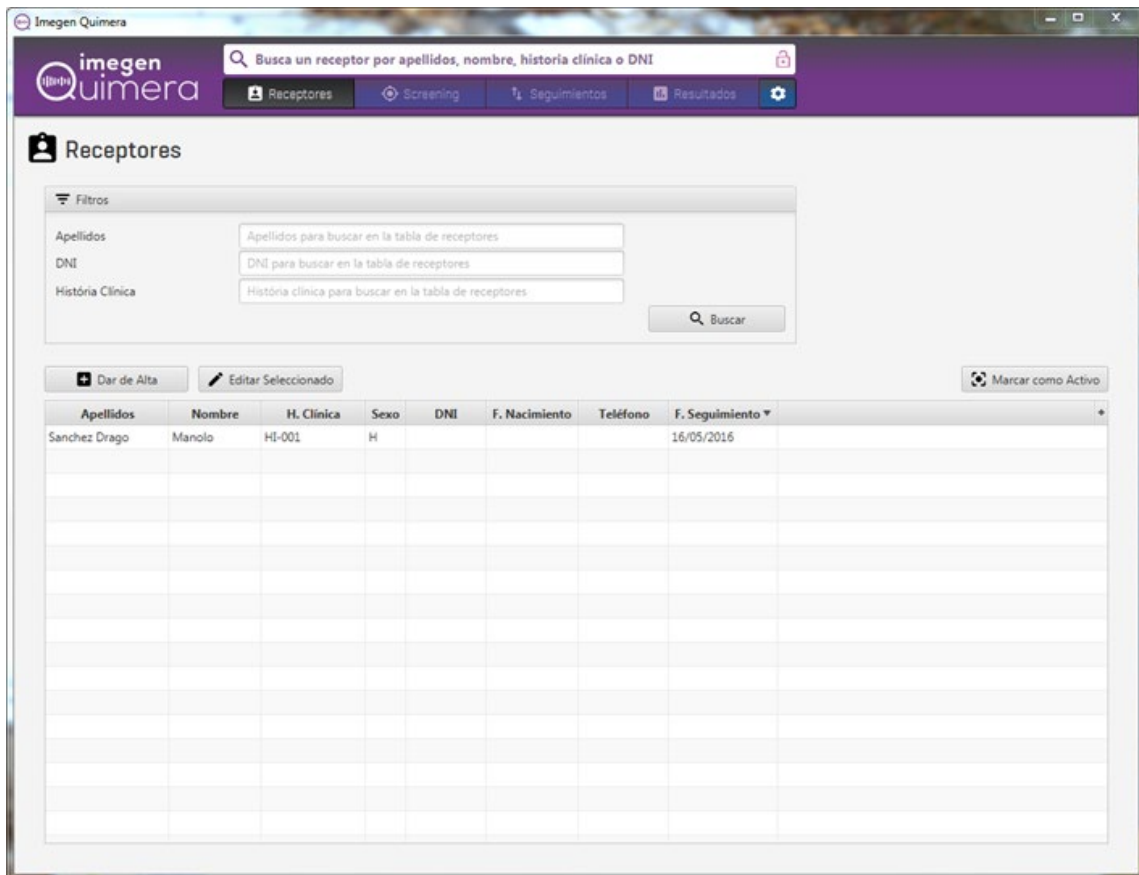


Image 1. View of the patient follow-up application developed by Health in Code.

09

Troubleshooting

The table below graphically summarizes the possible test results that can be obtained for the different positive and negative controls and from one DNA sample in one run, along with their interpretation and the most likely reasons for each possible result:

Control	Polymorphism	β -Globin	Cause
DNA sample	+	+	Expected result
	-	-	Failure of sample amplification ¹
Negative PCR control	-	-	Expected result
	+	+	Human DNA contamination ²

Table 12. Interpretation of the possible results obtained using Imegen® Quimera dPCR

¹ **Failure of sample amplification:** Verify that DNA concentration and quality meet the requirements; if so, the result may be caused by a highly degraded sample. In this case, it is advised to perform a new analysis or DNA extraction before interpreting the results.

² **PCR contamination by 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..

10

Limitations

10.1. Equipment

Imegen® Quimera dPCR has been validated using the following digital PCR platforms:

- > QuantStudio® 3D Digital PCR system (ThermoFisher Scientific)
- > QuantStudio® Absolute Q Digital PCR system (ThermoFisher Scientific)
- > QX200™ Droplet Digital™ PCR system y QX100™ Droplet Digital™ PCR system (BIO-RAD)

If a different brand or model of a digital PCR instrument is used for the quantification of hematopoietic chimerism, 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® Quimera dPCR kits have been validated using the reagents included in the kit and those recommended in section 6 of this manual (Necessary equipment and materials not included in the kit).

We recommend using the PCR reagents indicated by the manufacturer of the thermal cycler to be used for digital PCR assays. Should you have any questions, please contact our technical support team.

10.3. Product stability

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.