Instructions for use

Imegen® Factor V

Ref. IMG-217

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.01

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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* diagnostic use. 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 will be to replace the products or refund the purchase price thereof, 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® Factor V kit has passed all internal validation tests, which guarantee the reliability and reproducibility of each manufactured batch.

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

+34 963 212 340

tech.support@healthincode.com

		Modifications to the instructions for use (IUF)
Version 09	DIC 2022	Modification of the storage and shipping temperature of the GENERAL MASTER MIX reagent (Section 5).
Version 08	NOV 2022	Change of manufacturer's address: Health in Code S.L., Calle de la Travesía s/n, 15E Base 5, Valencia 46024, Spain.
Version 07	SEP 2022	Change of manufacturer's identification: from Imegen to HEALTH IN CODE, S.L.
Version 06	JUL 2019	Inclusion of safety measures in section 4. Safety warnings and precautions. Updating the information regarding the positive control of the kit in section 2. Intended use, paragraph 3. Technical characteristics and section 5. Kit contents and storage conditions
Version 05	DIC 2018	Correction of volumes in section 7. Assay protocol

index

01 General information

The *F5* (Leiden) gene, located on chromosomal region 1q23, encodes coagulation factor V, which is essential in coagulation processes (hemostasis). As a consequence of vascular damage, factor V is activated and interacts with activated factor X, forming a complex that converts prothrombin to thrombin, which in turn converts fibrinogen to fibrin, both of which are associated with the formation of blood clots. Factor V Leiden thrombophilia has a single p. Arg506Gln mutation, causing the factor V to be abnormal, which cannot be inactivated. Therefore, factor V remains activated and cannot stop the coagulation process.

References

› *https://www.omim.org/entry/612309*

02 Intended use

The Imegen® Factor V kit uses a combination of oligonucleotides and fluorescent hydrolysis probes in a validated real-time PCR assay to detect the pathogenic allele that causes variant p. Arg506Gln (NM_000130.4:c.1601G>A) and the normal allele of the FV (Leiden) gene. In addition, it uses heterozygous genomic DNA as a positive control for the mutation under analysis by the kit.

Imegen® Factor V is for *in vitro* diagnostic use only and is intended for professionals in the molecular biology sector.

03 Technical characteristics

This kit has been validated using reference DNA samples obtained via the Coriell Institute for Medical Research, samples previously analyzed by the medical genetics service of Health in Code S.L., and synthetic DNA with a single copy of the target sequences (normal or mutant) of the region under analysis of the FV gene, and specifically detects the expected genotypes.

The material needed for this study is genomic DNA from peripheral blood. The total quantity of DNA needed is 50 ng.

Health in Code S.L. is certified according to **the standard UNE-EN ISO 13485:2018 Medical Devices: Quality Management Systems – Requirements for regulatory purposes** by the AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS (AEMPS, Spanish Agency for Medicinal Products and Medical Devices) for the design, development and production of *in vitro* diagnostic medical devices:

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

04 Safety warnings and precautions

- It is recommended to strictly follow the instructions in this manual, especially regarding the handling and storage conditions of 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 that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Biosafety Level 2 standard for bloodborne pathogens or other relevant biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, 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 responsible 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 in order to make 48 determinations. The list of reagents included in the kit is as follows:

- ***Factor V Master Mix:*** contains oligonucleotides, fluorescent hydrolysis probes (FAM and VIC) and water for amplification and detection of the normal and/or mutant alleles under analysis.
- ***General Master Mix:*** PCR Master Mix with the nucleotides, $MgCl_2$, enzyme and buffer required to perform real-time PCR.
- ***Positive Control:*** Heterozygous genomic DNA for the mutation under analysis, thus allowing simultaneous amplification of the normal allele and the mutant allele under analysis.

Reagents	Color	Quantity	Storage
Factor V Master Mix	Green pad	2 x 180 µL	-20°C
General Master Mix	White pad	600 µl	-20°C*
Positive control	Black cap	100 µl	-20°C

Table 1. Imegen® Factor V kit components

(*) General Master Mix: It is recommended to keep 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
- Centrifuge

Materials:

- Pipette tips with filter (10 µL, 20 µL, 200 µL)
- 1.5 mL sterile tubes
- Optical consumables compatible with the real-time PCR thermal cycler
- Latex gloves

Complementary kits

Health in Code S.L. also offers the following real-time PCR assays for the diagnosis of hematopoietic malignancies:

- Imegen® Cambridge II (ref. IMG-199)
- Imegen® MTHFR (ref. IMG-212)
- Imegen® Factor II (ref. IMG-214)
- Imegen® Factor XII (ref. IMG-215)
- Imegen® MTHFRII (ref. IMG-216)
- Imegen® HFE (ref. IMG-218)

All of them, together with the Imegen® Factor V kit, have been validated using the same real-time PCR program, so they can be analyzed together.

07 Assay protocol

07.1 | Preparation of amplification reactions

In order to estimate the quantity of reagents required, the number of samples and controls to be analyzed simultaneously must be taken into account. We recommend adding one more reaction or increasing the volume of each reagent by 10% when making the calculations.

In order to carry out qualitative analysis, it is recommended to prepare an amplification reaction per sample and to include a negative PCR control to rule out contamination of the reagents, and a positive control.

> IMPORTANT: **A positive and a negative control must be included in the assay if you wish to use the Auto-calling tool in the results analysis to obtain the genotyping of a sample automatically with the analysis software.**

The recommended protocol for the preparation of amplification reactions is shown below:

01 Thaw all kit reagents and DNA from the samples. Vortex each of the reagents and keep cold.

02 Prepare the PCR mix in a 1.5 mL tube by adding the following reagents per sample:

Reagents	Quantity per reaction
Factor V Master Mix	7.5 µL
General Master Mix	12.5 µL

03 Vortex and spin the PCR mix, then dispense 20 µL into the corresponding wells of the optical consumables.

04 Add 5 µL of the diluted samples at a concentration of 10 ng/µL and 5 µL of the positive control, or nuclease-free water (negative control) to the corresponding wells.

05 Place the tubes or plates in the real-time PCR thermal cycler and set up the amplification program as indicated in the following section.

07.2 | Real-time PCR program setup

- **Type of experiment**: Genotyping
- **Ramp speed**: Standard
- **Reaction volume**: 25 µL
- **ROX™ baseline reference**: included

◇ Fluorophores of TaqMan® probes:

Probe	Issuer	Genotyping	Quencher
FV-G-P	VIC™	Normal	MGB
FV-A-P	FAM™	Mutant	MGB

Table 2. Probe information

◇ Optimal program:

Fields	Stage 1 Pre-PCR reading	Stage 2 Enzymatic activation	Stage 3 PCR		Stage 4 Post-PCR reading
No. of cycles	1 cycle	1 cycle	50 cycles		1 cycle
			Denaturation	Oligonucleotide binding/extension	
Temperature	60°C	95°C	95°C	60°C	60°C
Time	1 minute*	10 minutes	15 seconds	1 minute*	1 minute*

Table 3. Optimal PCR program for the 7500 FAST and StepOne (ThermoFisher Scientific).

(*) Fluorescence detection

08 Analysis of results

It is recommended to follow the indications below for the results to be analyzed properly:

- Check that there is no amplification in the negative PCR control, neither in the FAM channel nor in the VIC channel.
- Check that there is an amplification signal in the positive control, both in the FAM channel and the VIC channel.
- In order to analyze the samples, specific software for the real-time PCR thermal cycler employed must be used.

The possible results obtained with the Imegen® Factor V kit are shown below:

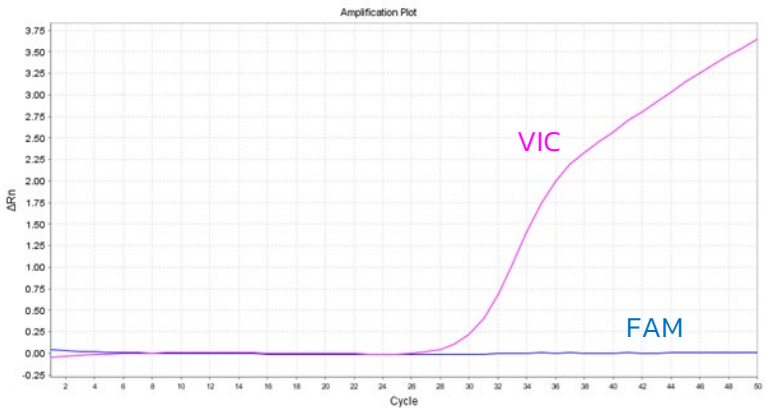

Figure 1. Result obtained from a normal homozygous sample (G/G). Amplification is only observed in the VIC channel.

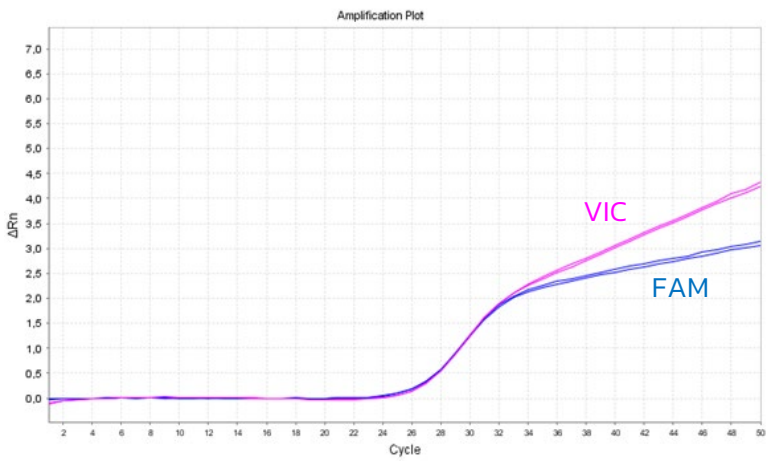

Figure 2 Result obtained from a heterozygous sample (G/A). Signal is observed in both FAM and VIC channels, the fluorescence intensity being higher in the FAM channel.

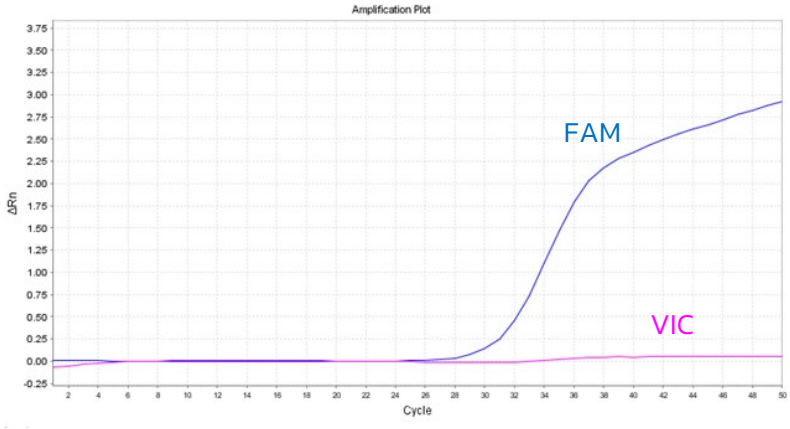

Figure 3. Result obtained from a homozygous mutant sample (A/A). Amplification is only observed in the FAM channel.

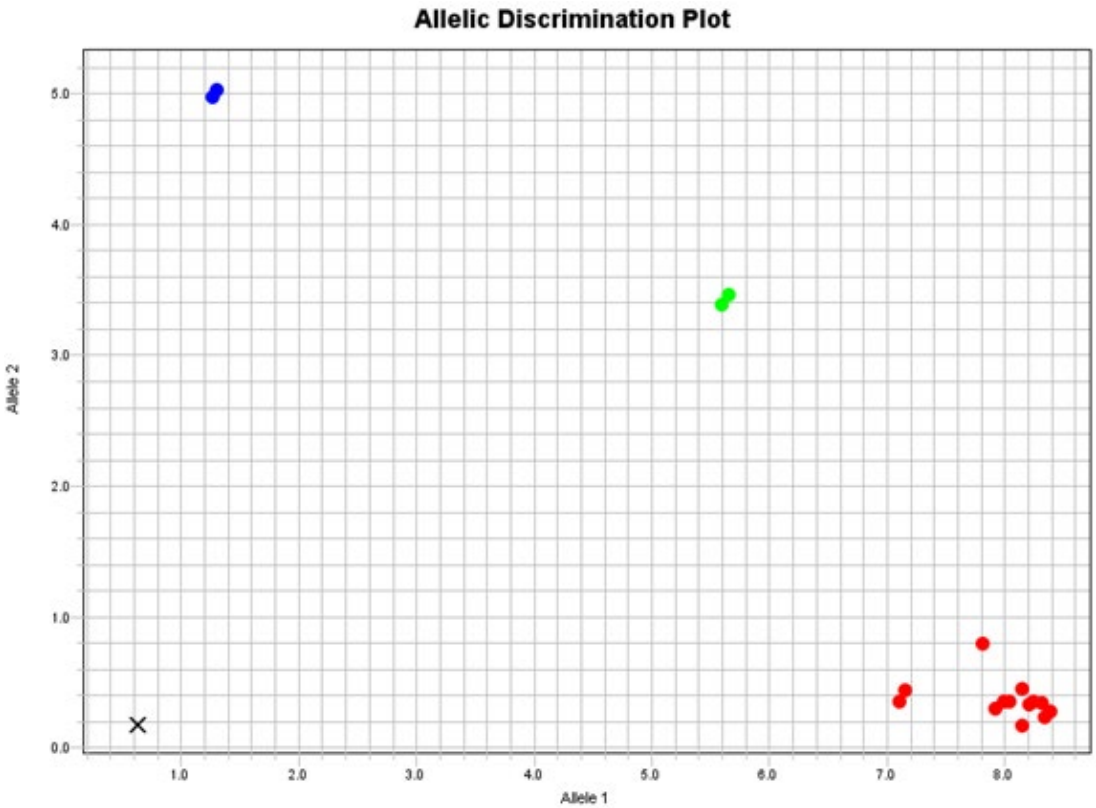

Figure 4. Allelic discrimination plot with the Factor V system.

09 Troubleshooting

The table below shows the results that could be obtained from the analysis of the different controls and a sample in an assay, as well as their interpretation.

Control	Result		Cause
	FAM	VIC	
Positive control	+	+	Expected result
	–	–	Failed PCR setup[1]
Sample	+	+	Expected result
	+	–	
	–	+	
	–	–	Failed sample amplification[2]
PCR negative control	–	–	Expected result
	+	+	Contamination of PCR with human DNA[3]
	+	–	
	–	+	

Table 4. Interpretation of possible results

(1) Failed PCR amplification: Check the amplification program and fluorescence capture settings. Failed amplification may be due to a technical problem in the PC program settings.

(2) Failed sample amplification: Check that the quantification of the sample is as recommended. If so, the specified result may be due to the sample being highly degraded.

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

10 Limitations

10.1 | Equipment

Imegen® Factor V has been validated using the following PCR thermal cyclers:

- *7500 FAST Real-Time PCR System* (ThermoFisher Scientific)
- *StepOne Real-Time PCR System* (ThermoFisher Scientific)
- *StepOne Plus Real-Time PCR System* (ThermoFisher Scientific)

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

10.2 | Reagents

Imegen® Factor V 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 optimum performance of this product is confirmed provided that the recommended storage conditions according to the optimum product date for each production batch are followed.

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

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

MARTIN GONZALEZ MARIA CARMEN - 03448368R
Firmado digitalmente por MARTIN GONZALEZ MARIA CARMEN - 03448368R
Fecha: 2023.05.18 19:54:52 +02'00'

CARLOS| RUIZ| LAFORA
Firmado digitalmente por CARLOS|RUIZ| LAFORA
Fecha: 2023.05.19 16:30:50 +02'00'

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