



Instructions for use

Imegen[®] MTHFR II

Ref. IMG-216

CE IVD

Manufactured by:

HEALTH IN CODE, S.L.

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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® MTHFR II** 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:

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Imegen® is a registered trademark of Health in Code, S.L. in Spain.

Modifications to the instructions for use (IUF)		
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 5).
Version 05	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 04	SEP 2022	Change of manufacturer's identification: from Imegen to HEALTH IN CODE, S.L.
Version 03	DEC 2018	Correction of the volumes in section 7.1 Preparation of amplification reactions

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01 General information

The *MTHFR* gene is located on chromosomal region 1p36.22. This gene encodes the enzyme methylenetetrahydrofolate reductase, essential for the processing of vitamin folate (vitamin B9), which in turn is necessary for the synthesis of the amino acid methionine, the building block of proteins.

c.1298A>C polymorphism results in an enzyme variant with reduced activity associated with an increased risk of developmental defects of the brain and spinal cord, including anencephaly, spina bifida and other syndromes such as homocystinuria and a greater risk of developing vascular problems, including heart problems and apoplexy, which are characterized by an increase in blood homocysteine, a cosubstrate of methionine remethylation.

References

> <https://www.omim.org/entry/607093>.

02 Intended use

The **Imegen® MTHFR II** kit uses a combination of oligonucleotides and fluorescent hydrolysis probes in a validated real-time PCR assay to detect the pathogenic allele traditionally called c.1298A>C (NM_005957.4) and the normal allele of the *MTHFR* gene. In addition, as a positive control it uses a v/v mixture of synthetic DNA with a copy of *MTHFR* with the mutated allele and synthetic DNA with a copy of *MTHFR* with the normal or wild-type allele for qualitative analysis.

Imegen® MTHFR II 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 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 *MTHFR* 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.

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.

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:

- **MTHFR 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 nucleotides, MgCl₂, enzyme and buffer to perform real-time PCR.
- **Positive Control:** positive control for the simultaneous amplification of the normal and mutant allele under analysis (simulating a heterozygous sample).

Reagents	Color	Quantity	Storage
MTHFR II Master Mix	Yellow pad	2 x 180 µL	-20°C
General Master Mix	White pad	600 µl	-20°C*
Positive control	Orange cap	100 µl	-20°C

Table 1. Imegen® MTHFR II 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

For the genotyping analysis of targets related to hematological diseases, and specifically to alterations in the coagulation process, Health in Code, S.L. also offers the following kits:

- Imegen® Cambridge II (ref. IMG-199)
- Imegen® MTHFR (ref. IMG-212)
- Imegen® Factor II (ref. IMG-214)
- Imegen® Factor V (ref. IMG-217)
- Imegen® Factor XII (ref. IMG-215)
- Imegen® HFE (ref. IMG-218)

All of them, together with the Imegen® MTHFR^{II} 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:

Reagents	Quantity per reaction
<i>MTHFR11 Master Mix</i>	7.5 μ L
<i>General Master Mix</i>	12.5 μ L

- 03 Vortex and spin the PCR mix, then dispense 20 μ L into the corresponding wells of the optical consumables.
- 04 Add 5 μ L of the diluted samples at a concentration of 10 ng/ μ L and 5 μ L 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 μ L
- ◇ ROX™ baseline reference: included

◇ Fluorophores of TaqMan® probes:

Probe	Issuer	Genotyping	Quencher
MTHFR11-A-P	VIC™	Normal	MGB
MTHFR11-C-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 (Thermo Fisher 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® MTHFR11 kit are shown below:

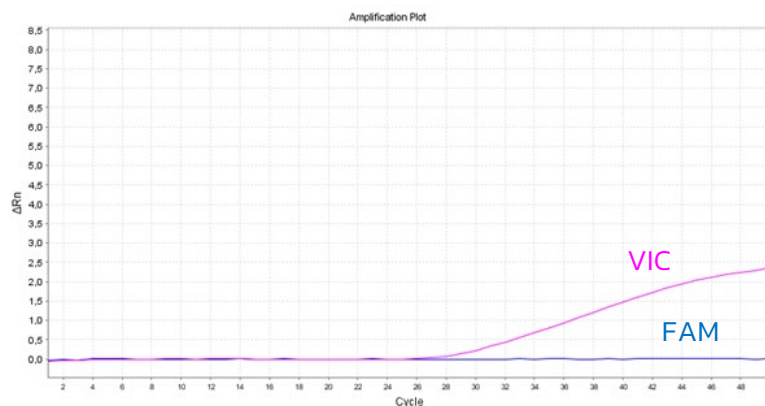


Figure 1. Result obtained from a homozygous mutant sample (A/A). Amplification is only observed in the VIC channel.

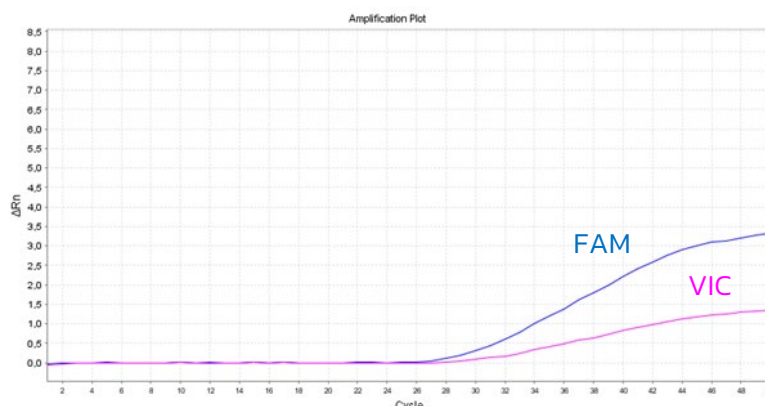


Figure 2. Result obtained from a heterozygous sample (A/C). Signal is observed in both FAM and VIC channels, the fluorescence intensity being higher in the FAM channel.

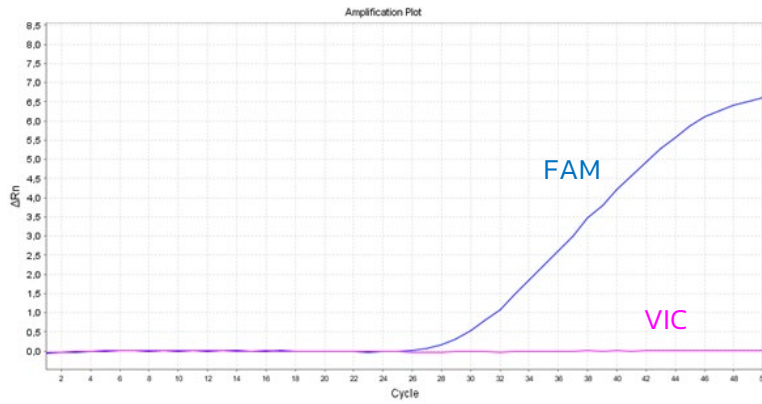


Figure 3. Result obtained from a homozygous mutant sample (C/C). Amplification is only observed in the FAM channel.

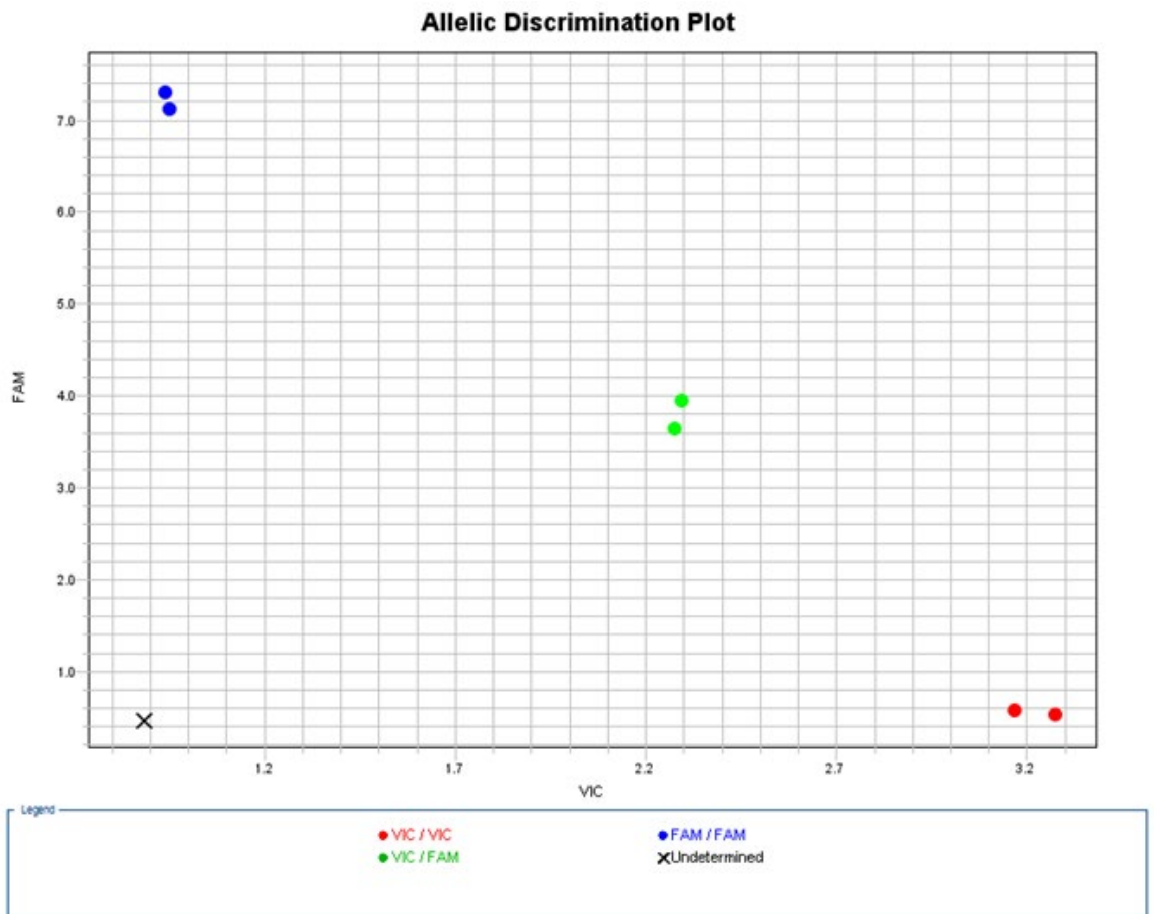


Figure 4. Allelic discrimination plot with the MTHFR11 system.

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 PCR 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® MTHFR^{II} has been validated using the following PCR thermal cyclers:

- + 7500 FAST Real-Time PCR System (Thermo Fisher Scientific)
- + StepOne Real-Time PCR System (Thermo Fisher Scientific)
- + StepOne Plus Real-Time PCR System (Thermo Fisher 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® MTHFR^{II} 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:

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Find out about all
our **diagnostic kits**

