Instructions for use

Imegen® PML-RARA Screening

Ref. IMG-130

CE IVD

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

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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 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® PLM-RARA Screening 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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Modifications to the instructions for use (IFU)		
Version 07	AUG 2023	Enzyme reagent renaming in sections 6, 7, and 10.
Version 06	MAR 2023	Enzyme reagent naming change in sections 6, 7, and 10.
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 2020	Change of manufacturer's identification: from Imegen to HEALTH IN CODE, S.L.
Version 03	FEB 2019	Updating the document for CE-IVD marking of the product.

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01 General information

Translocation t(15; 17) (q22; q21) occurs between chromosomes 15 and 17, resulting in the fusion of the retinoic acid receptor alpha (RARA) gene located on chromosome 17 and the *PML* gene located on chromosome 15.

The *PML* gene encodes a protein that acts as a tumor suppressor, whereas the PML–RARA fusion oncogene is unable to block cell proliferation or induce apoptosis and exerts its oncogenic effects by repressing the expression of AR-inducible genes critical for myeloid differentiation.

95% of cases of acute promyelocytic leukemia (APL), a type of acute myeloid leukemia, have a PML–RARα translocation, which is associated with a favorable prognosis.

Given the variability in the breakpoints in *PML* (intron 6, exon 6 or intron 3), three PML–RARα fusion transcripts have been identified: bcr1, bcr2 and bcr3. They represent 55%, 5% and 40% of APL cases, respectively.

It is found in 95% of acute promyelocytic leukemias (APL), where *PML–RARA* exerts its oncogenic effects by repressing the expression of AR-inducible genes critical for myeloid differentiation.

References

› *Leukemia. 2003; Volume 17: 2318–2357. doi:10.1038/sj.leu.2403135*

02 Intended use

Imegen® PML-RARA Screening uses a combination of primers and fluorescent hydrolysis probes validated by real-time PCR to amplify the bcr1, bcr2 and bcr3 variants of the PML-RARα rearrangement resulting from the translocation between chromosome 15 and 17, t(15;17)(q22q21), and the reference *GUS* gene. This analysis will detect the presence or absence of these translocations; however, it cannot state which of them is present in the sample or in what proportion, since it is a qualitative analysis.

The type of sample required for this study is complementary DNA (cDNA). After obtaining the cDNA, the total RNA must be extracted from peripheral blood or bone marrow samples, from which the reverse transcription to cDNA can be performed.

The results will guide the clinician in the diagnosis of the type of leukemia the patient is suffering from.

Imegen® PML-RARA Screening is for *in vitro* diagnostic use only and is intended for professionals in the molecular biology sector.

03 Technical characteristics

Imegen® PML-RARA Screening has been validated using cDNA samples synthesized by total reverse transcription of RNA extracted from the peripheral blood of healthy patients diagnosed with acute promyelocytic leukemia (APL), specifically detecting the fusion products and the *GUS* reference gene set out in Section 2 of this document (Intended use).

The detection limit for both the *PML-RARA* target and *GUS* is 5 absolute copies.

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 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

The kit contains the following reagents necessary to perform 48 real-time PCR reactions for each of the two targets analyzed in this kit:

- ***PML-RARA Screening Master Mix:*** specific oligonucleotides and hydrolysis probe (FAM™) to detect PML-RARA bcr1, bcr2 and bcr3 rearrangements simultaneously.
- ***GUS Master Mix:*** specific oligonucleotides and hydrolysis probe (FAM™) to detect the presence of the *GUS* reference gene.
- ***Positive control:*** a positive control of the bcr1, bcr2, bcr3 and *GUS* translocation.

Reagents	Color	Vials	Storage
PML-RARA Screening Master Mix	Purple pad	3 x 16 reactions	4°C
GUS Master Mix	Yellow pad	3 x 16 reactions	4°C
Positive control	Purple cap	1 vial	4°C

Table 1. Imegen® PML-RARA Screening kit components

**The reagents in this kit are lyophilized. After rehydration, 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
- Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0, ThermoFisher Scientific)

NOTE: This kit does not include the reagents necessary to perform the reverse transcription of RNA to cDNA.

Materials:

- 0.2 mL optical tubes
- 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 the results of the PML-RARα rearrangement screening are positive for any of the samples tested, Health in Code S.L. offers the Imegen® PML-RARA kit **(Ref.: IMG-111)**.

This kit enables the most common PML-RARα fusion transcript to be quantified, bcr1, which accounts for 55% of cases of acute promyelocytic leukemia (APL) with t(15;17)(q22q21).

07 Assay protocol

07.1 | Preparation of reagents

All reagents included in the kit are lyophilized. The first step before using any of our kits is to rehydrate the reagents by adding the quantity of nuclease-free water listed in the table below. In order 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.

Reagents	Rehydration
PML-RARA Screening Master Mix	90 µL of water/vial*
GUS Master Mix	90 µL of water/vial*
Positive control	100 µL of water/vial*

Table 2. Rehydration volume of the kit components

(*) If these reagents are not going to be used after rehydration, we recommend storing them at -20°C.

07.2 | Preparation of amplification reactions

The protocol for the preparation of the reactions is given below:

01 Thaw the reagents required for analysis, including:

- *PML-RARA Screening Master Mix / GUS Master Mix*
- *Positive control*
- Undiluted cDNA samples
- Nuclease-free water for PCR control (CPCR)
- *Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0,* ThermoFisher Scientific) (not provided)

02 Vortex each of the reagents and keep cold.

03 Separate master mixes should be prepared for the analysis of the GUS reference gene and the PML-RARA oncogene. For this purpose, separate master mixes are prepared in 1.5 mL tubes according to the following tables:

Master Mix PML-RARA (oncogene)

Reagents	Volume per reaction
PML-RARA Screening Master Mix	5 µL
*TaqMan™ Environmental Master Mix 2.0**	10 µL

(*) See section 6. Equipment, reagents, and materials not included in the kit

- *Master Mix GUS* (endogenous gene)

Reagents	Volume per reaction
GUS Master Mix	5 µL
*TaqMan™ Environmental Master Mix 2.0**	10 µL

(*) See section 6. Equipment, reagents, and materials not included in the kit

The volumes required for each mix should be calculated for the total number of samples to be included in the study. In addition, extra reagents should be calculated to include positive controls and PCR controls (CPCR).

NOTE: It is recommended to add enough reagents to analyze one more reaction or to calculate and add 10% more of each of the reagents when making the calculations.

04 Vortex the PCR mixes and pipette 15 µL into each well.

05 Once the mixes have been dispensed, add the following volumes to the corresponding wells:

- 5 µL of the cDNA sample
- 5 µL of the *PML-RARA* control (positive control)
- 5 µL nuclease-free water (PCR control, CPCR)

PML-RARA Screening Master Mix	
cDNA sample 1	Positive control
cDNA sample 2	CPCR
cDNA sample 3	
cDNA sample 4	

GUS Master Mix	
cDNA sample 1	Positive control
cDNA sample 2	CPCR
cDNA sample 3	
cDNA sample 4	

Figure 1. Example of a possible plate distribution for real-time PCR

07.3 | Real-time PCR program setup

In order to perform real-time PCR, follow the instructions below to set up the amplification program:

- **7500 Fast or StepOne Real-Time PCR system** (ThermoFisher Scientific)

- **Type of experiment**: Quantitation – Standard curve
- **Ramp speed**: Standard
- **Reaction volume**: 20 µL
- **ROX™ baseline reference**: Included
- **Fluorophores of TaqMan® probes**:

Probe	Fluorophore	*Quencher*
PML-RARA	FAM™	TAMRA*
GUS	FAM™	TAMRA*

Table 3. Probe information

(*) This field must be filled in as "None" in the StepOne PCR system (Thermo Fisher Scientific).

- Optimal program:

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

Table 4. Optimal PCR program for the 7500 FAST or StepOne

(*) Fluorescence detection

08 Analysis of results

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

NEGATIVE CONTROLS (CPCR)

- Check that there is no amplification in the **negative controls.** If amplification is detected, it is recommended to repeat the analysis to rule out accidental contamination.

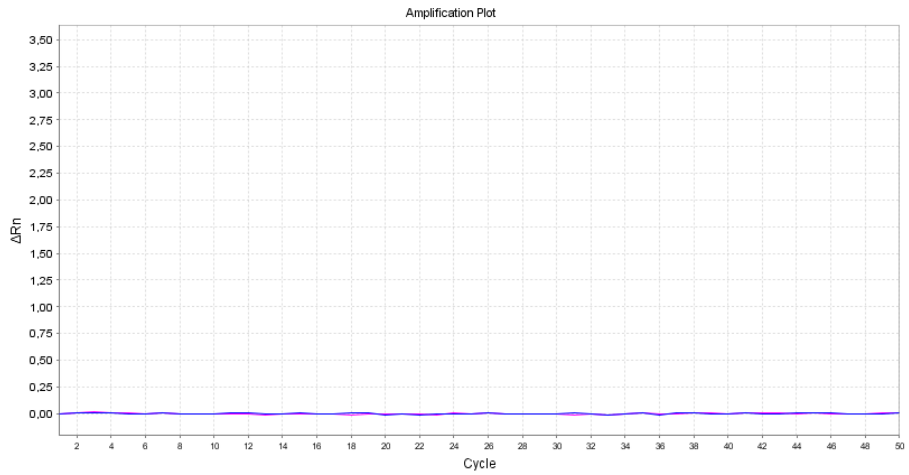

Figure 2. Expected result for the negative control (NTC)

POSITIVE CONTROL

- Check that the **positive control** is amplified in both reactions. If negative, it is recommended to repeat the analysis to rule out any error in the preparation of the reactions.

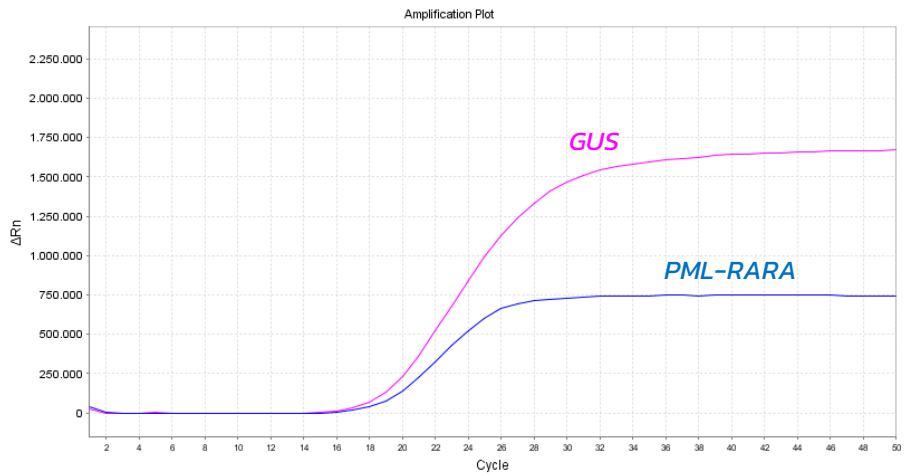

Figure 3. Expected result for the positive control

SAMPLES (cDNA)

GUS Master Mix

- Check that the reference gene is detected in all samples analyzed with the ***GUS Master Mix.*** This reaction makes it possible to verify that the sample contains a sufficient quantity of cDNA of an appropriate quality.

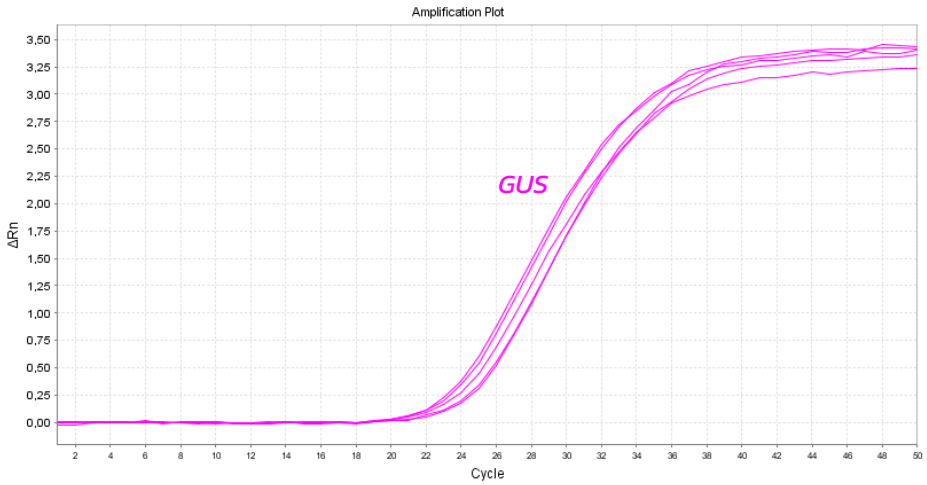

Figure 4. Expected result with the GUS system for a good quality cDNA sample.

PML-RARA Screening Master Mix & GUS Master Mix

- Each sample will be analyzed after the verification of all controls. The sample has the rearrangement if amplification is detected with the ***PML-RARA Screening Master Mix***.

  - Negative sample:

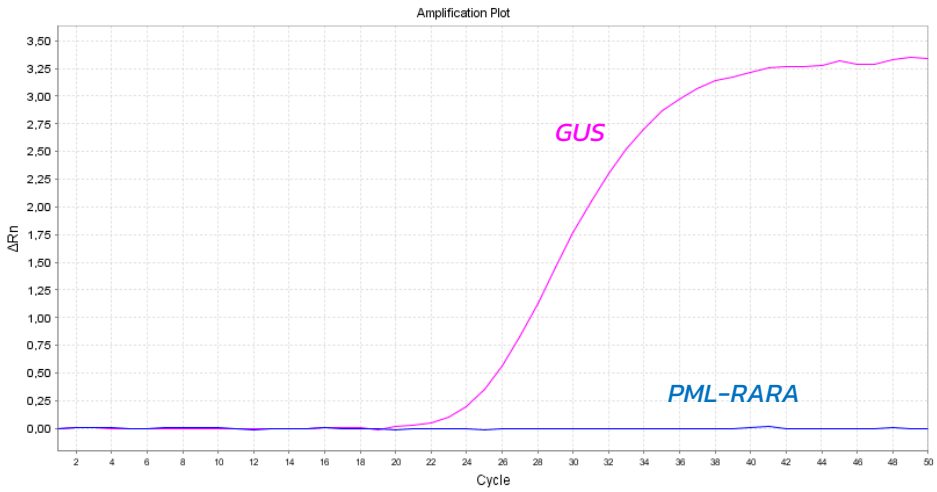

Figure 5. Expected result in non-pathogenic cDNA samples. The GUS system will amplify this gene, but the PML-RARA Screening system will not amplify the PML-RARA oncogene.

- Positive sample:

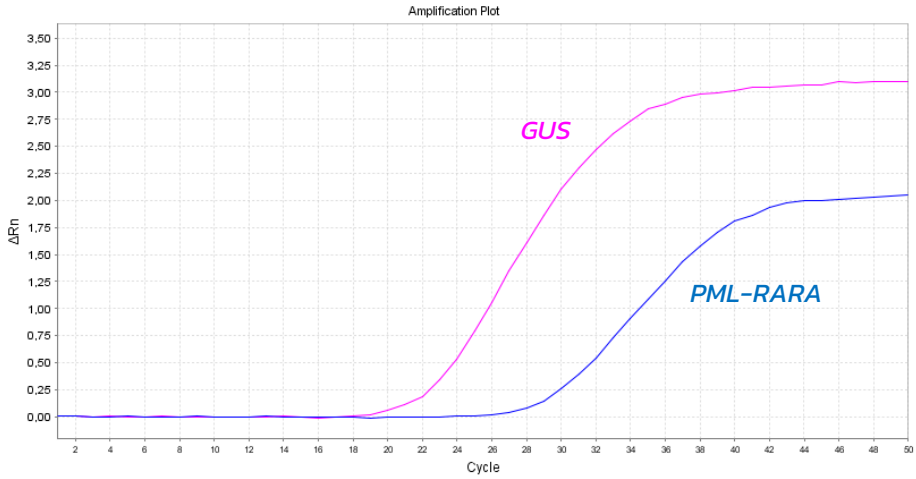

Figure 6. Expected result in pathogenic cDNA samples. Both systems will amplify their targets, both the GUS gene and the PML-RARA oncogene.

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.

Controls and samples	*PML-RARA*	*GUS*	Cause
Positive control	+	+	Expected result
	–	–	Failed PCR amplification[1]
Sample to be analyzed	–	+	Expected result
	+	+	
	–	–	Failed sample setup[2]
PCR negative control	–	–	Expected result
	+	+	Contamination of PCR with human or positive control DNA[3]

Table 5. Interpretation of the possible results of the Imegen® PML-RARA Screening kit

(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 or positive control 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.

10 Limitations

10.1 | Equipment

Imegen® PML-RARA Screening has been validated with the following real-time PCR kits:

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

In principle, this kit is compatible with all real-time PCR platforms that detect FAM fluorescence.

If you use a make or model of thermal cycler other than those mentioned above, you may need to adjust the amplification program. Please contact our technical service for any questions or clarifications.

10.2 | Reagents

The Imegen® PML-RARA Screening kit has been validated with the reagents included in the kit and the following reagents not included in the kit:

- *M-MLV RT* (Moloney murine leukemia virus reverse transcriptase). Reverse transcription carried out using 1 µg of total RNA.
- *Hot Start PCR Master Mix (TaqMan™ Environmental Master Mix 2.0,* ThermoFisher Scientific)

If a PCR Master Mix other than the one included in the kit is used, it is recommended to perform a validation with this new reagent. Please contact our technical service for any questions or clarifications.

In addition, this kit does not include the reagents necessary to perform the reverse transcription of RNA to cDNA. It is recommended to use a protocol starting from 1 µg of RNA to perform reverse transcription.

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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