



INSTRUCTIONS FOR USE

# Imegen<sup>®</sup> MON89788 & Roundup Ready Soya Event ID Kit

Ref. IMG-200-12

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## Guide overview

The information in this guide is subject to change without notice.

Health in Code, S.L. guarantees that its products are free from defects, both in used materials as in its manufacturing process. This warranty is extended to the expiration date, as long as the storage conditions specified in this manual are met. Our products are designed for research use only. The user of the product is responsible for validating the usefulness of the protocol proposed by Health in Code, S.L. These protocols are considered a guide only. Health in Code, S.L. does not offer any other warranty, express or implied, which extends beyond the proper functioning of the components of this set. Health in Code S.L., sole obligation in respect of the preceding guarantees, will be to replace the product or return the purchase price thereof, as desired by the customer, as long as the existence of a defect in the materials test, or in the manufacture of its products. Health in Code, S.L. will not be responsible for any damage, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the purchaser or user.

All products sold by Health in Code, S.L. are subjected to rigorous quality control (App. A). The **Imegen® MON89788 & Roundup Ready Soya Event ID Kit** has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.

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



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**NOTE:** ImegenAgro® is a trademark of Health in Code, S.L.

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## Instructions for Use (IFU) modifications

<b>V.02</b>	NOV 2023	Contents review. Modification of the storage temperature of the General Master Mix. Change of the manufacturer's identification, going from Imegen to Health in Code, S.L.
<b>V.01</b>	JUN 2022	Contents review

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## 01

# Product information

## 01.1 General description

Genetically modified organisms (GMOs) are widely distributed, with soy and corn being two of the most extensively cultivated crops worldwide. Indeed, Roundup Ready (RR) transgenic soya accounts for some 77% of the soy cultivated worldwide, thus making it the most widespread transgenic event and therefore the one that generates the most problems. Developments in biotechnology and molecular-assisted breeding have enabled Monsanto to generate a second-generation glyphosate-tolerant soybean product, Roundup Ready2Yield™ or MON89788.

Overall, MON89788 accounts for over 7% yield advantage compared to Roundup Ready Soya 40-3-2.

The European Union has established a legal framework to regulate the use, release into the environment and, above all, labelling of foodstuffs containing genetically modified organisms.

Identification of MON89788 & RR DNA presence in food samples is an essential step in order to verify the origin and traceability of the used raw materials, as well as a necessary quality control for handling and cleaning processes of production lines.

## 01.2 Intended use

[Imegen® MON89788 & Roundup Ready Soya Event ID Kit](#) allows detecting MON89788 and Roundup Ready transgenic Soya DNA in a sample.

MON89788 & RR DNA detection is done by real time PCR using hydrolysis probes. One of them, labelled with FAM™ dye, specifically detects MON89788 DNA. The second probe is labelled with VIC™ and specifically detects RR DNA. The third probe is labelled with NED® and detects an Internal Positive Control, which is used to rule out inhibitors in the sample and check the correct functioning of the assay.

## 01.3 Content and storage conditions of the kit

[Imegen® MON89788 & Roundup Ready Soya Event ID Kit](#) contents the necessary reagents to perform 48 reactions:

<b>MON89788 &amp; RR Master Mix</b>	Master Mix with specific oligonucleotides, fluorophore-labelled hydrolysis probes (FAM™ probe for MON89788 detection; VIC™ probe for RR detection and NED® probe for the Internal Positive Control detection, IPC), synthetic plasmid including the specific IPC sequence and nuclease-free water.
<b>General Master Mix</b>	Master Mix of PCR with nucleotides, MgCl <sub>2</sub> , DNA polymerase and buffer needed to carry out RT-PCR.
<b>Positive Control</b>	DNA sample containing 0.1% of <i>Roundup Ready Soya</i> or <i>MON89788</i> specific-DNA.

Table 1. IMG-200-12 Imegen® MON89788 & Roundup Ready Soya Event ID Kit components and description.

<b>Reagents</b>	<b>Color indicator</b>	<b>Quantity</b>	<b>Conservation</b>
MON89788 & RR Master Mix*	Yellow pad	360 µl	-20 °C
General Master Mix*	White pad	600 µl	-20 °C upon receipt. 2 - 8 °C after initial use. Store protected from light
Positive control*	Blue cap	60 µl	-20 °C

(\*) See the expiration date on the box and tubes.

## 01.4 Equipment, reagents and materials required but not supplied

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### Equipment

- ✓ Real-Time PCR Thermal Cycler with channels for detection of FAM™ (520 nm) and VIC™ (550 nm)
- ✓ Micropipettes (10 µL, 20 µL and 200 µL)
- ✓ Tabletop centrifuge with adaptors for 96 well PCR plates and/or 0.2 ml tubes
- ✓ Vortex

### Materials

- ✓ Optical 96-well reaction plates or 0.2 ml optical tubes
- ✓ Optical adhesive film for 96 well plates or optical caps for 0.2 ml tubes
- ✓ Disposable micropipette filter tips (10 µL, 20 µL and 200 µL)
- ✓ 1.5 ml sterile tubes
- ✓ Powder-free latex gloves

### Reagents

- ✓ Nuclease-free water

# 02

## Methods

### 02.1 Preparation of the amplification reactions

[Imegen® MON89788 & Roundup Ready Soya Event ID Kit](#) is designed to determine, in a single PCR reaction, the presence or absence of Maize DNA and the internal positive control.

The PCR Master Mix contains:

- ➔ **MON89788 & RR Master Mix**
- ➔ **General Master Mix (2X)**

The recommended protocol for preparation of amplification reactions is showed below:

- 01.** Thaw the MON89788 and RR Master Mix, the Positive Control vial and samples DNA.
- 02.** Vortex each reagent and keep cold.
- 03.** Add into a 1.5 mL tube (one for each PCR master mix preparation), the following reagents (Table 2). To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples to be simultaneously analyzed, and then considering one more reaction, or increase a 10% the volume of each reagent.

Table 2. Reagents and volumes needed to perform PCR reactions.

Reagents	Amount per reaction
MON89788 & RR Master Mix	7.5 µL
General Master Mix	12.5 µL

- 04.** Vortex and spin the 1.5 mL tube and dispense 20 µl per well or tube of 0.2 ml.

05. Add 5  $\mu\text{L}$  of each DNA sample at 10 ng/ $\mu\text{L}$ , 5  $\mu\text{L}$  of Positive Control and 5  $\mu\text{L}$  of the Negative Controls\* into the appropriate wells.
06. Cover the well plate with optical film or the tubes with optical cover and spin in the centrifuge.

(\*). We strongly recommend using an **extraction negative control** for each run of extractions carried out. This control consists in one tube to which no sample is added, and which is submitted to the same extraction process as the other samples. Likewise, we recommended using a **PCR negative control** for each PCR run; this tube contains no DNA but all PCR reagents.

## 02.2 Settings for the Real-Time PCR program

This kit is compatible with the Real-Time PCR platforms 7500 FAST, StepOne Real-Time PCR System (Thermo Fisher Scientific) and QuantStudio™ 5 Real-Time PCR system.

Table 3. IMG-200-12 Imegen® MON89788 & Roundup Ready Soya Event ID Kit probes and specifications.

Target	Receptor	Quencher
MON89788 DNA	FAM™	MGB
RR DNA	VIC™	MGB
IPC	NED™	MGB

The following instructions should be taken into account in order to setup the amplification program:

- + **Reaction volume:** 25  $\mu\text{L}$ .
- + **Targets:** FAM™ and VIC™.
- + In case the quencher has to be defined, select MGB for all probes. If the real time PCR system does not take into account the quenchers, select only the receptors (FAM™ and VIC™).
- + If the Real-Time PCR system is a 7500 Fast, a StepOne Real-Time PCR system (Thermo Fisher Scientific) or a QuantStudio™ 5 Real-Time PCR system, select Quantitation - Standard curve as a type of experiment and include ROX™ as a reference.
- + **Ramp rate:** standard
- + **Optimal program:**



Table 4. Optimal PCR program.

Fields	Step 1 Enzyme activation	Step 2 PCR	
		36 cycles	
No. of cycles	1 initial cycle	Denaturation	Primers binding/extention
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute *

(\*) Fluorescence detection.

## 02.3 Analysis of results

To analyze the results, it is recommended to establish the Threshold at 0.1 and to keep the default Baseline value to minimize the residual signal in the detection channels.

Ct settings	Threshold	0.1
	Baseline	AUTO

Before analyzing the samples results, it should be checked if obtained results in controls are as expected:

- **Positive control:** the result must always be positive in all amplification reactions, FAM™, VIC™ and NED™ channel.
- **Negative controls:** amplification should only be detected in the NED™ channel. In this channel an internal positive control (IPC) is detected, which determines the absence of inhibition in the sample.

<b>IPC</b>	It must be checked that the IPC (NED™) is positive in all samples, with a Ct similar to the Positive Control. A negative result in the IPC indicates the presence of inhibitors in the sample. It should be noted that IPC result may be negative in samples where a lot of MON89788 DNA (FAM™) or RR (VIC™) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.
<b>MON89788 &amp; RR</b>	Amplification in the FAM™ and VIC™ channel indicates presence of MON89788 or RR DNA in the sample.

In samples where no amplification in the FAM™ and VIC™ channel is seen, we can conclude that no DAS01507 and MON89788 Maize DNA is detected or that their amount in the sample is below than the detection limit.

Establish the positive cut-off value for the test samples and assign results:

$$Ct_{(cut-off)} = Ct_{(Positive\ Control)} + 3.32$$

Table 5. Cut-off values.

Sample Ct value	Sample result
$Ct > Ct_{(cut-off)}$	Negative
$Ct \leq Ct_{(cut-off)}$	Positive

The following table shows graphically the results that may be obtained from one sample analysis, as well as the interpretation that should be done from the obtained result:

Table 6. Results interpretation.

DAS01507 & MON89788 Master Mix			Interpretation
MON89788 (FAM™ channel)	RR (VIC™ channel)	IPC (NED™ channel)	
+	+	+	Transgenic material containing MON89788 and RR events detected
+	-	+	Transgenic material containing MON89788 detected
-	+	+	Transgenic material containing RR detected
-	-	-	PCR inhibitors present in sample*
-	-	+	No transgenic material containing MON89788, and RR events detected. DNA in sample
+	+	-	Sample contains large amount of MON89788 and/or RR DNA
+	-	-	
-	+	-	

(\*) If presence of inhibitors in the sample is detected, we recommend checking whether there has been an excess of DNA in the reaction (the recommended maximum is 250 ng). If the amount of DNA is right, we recommend repeating DNA extraction. If the problem persists, please contact our technical department.

## 03

# Troubleshooting

The following table shows graphically the results that may be obtained from the analysis of different assay controls, as well as the interpretation that should be done from the obtained result:

Table 7. Possible results and their interpretation.

Controls	MON89788 & RR Master Mix		Interpretation
	MON89788/ RR	IPC	
Positive control	+	+	Expected result
	-	-	<sup>1</sup> PCR Amplification Failure
Extraction Negative Control	-	+	Expected result
	+	+	<sup>2</sup> Contamination in the DNA extraction procedure
PCR Negative Control	-	+	Expected result
	+	+	<sup>3</sup> PCR contamination with MON89788/RR DNA

**(1) PCR Amplification Failure:** Check amplification program and configuration of fluorescence capture. Amplification failure may be due to a setup technical problem.

**(2) Contamination in the Wheat DNA extraction procedure:** Contamination may be due to some error made in the process of sample handling, reagents contamination, or environmental contamination. Check DNA extraction protocol, wipe the laboratory where DNA extraction process was performed and take care to avoid any contamination during sample homogenization. If necessary, use new aliquots of the reagents used in DNA extraction.

**(3) PCR contaminations with Wheat DNA:** Contamination of PCR reactions may be due to an error made in the process of sample handling, contamination of the reagents or environmental contamination. Thoroughly clean the laboratory where the PCR process was performed, as well as equipment. If necessary, use new aliquots of the reagents used in the PCR. Prepare the PCR reaction containing the Positive Control last to avoid cross contamination.

## 04

## Limitations

### 04.1 Equipment

[Imegen® MON89788 & Roundup Ready Soya Event ID Kit](#) has been validated using the following Real-Time PCR systems:

- 7500 FAST Real-Time PCR System (Thermo Fisher Scientific)
- StepOne™ Plus Real-Time PCR System (Thermo Fisher Scientific)
- QuantStudio5™ Real-Time PCR System (Thermo Fisher Scientific)

Technically, this kit is compatible with any Real-Time PCR systems that enable the detection of the fluorescence emitted by FAM™ and VIC™ fluorophores.

If a PCR system different from the systems described in this section is going to be used, it is possible that the PCR program might need to be readjusted. In this case, please contact our Technical Support Team for more details.

### 04.2 Reagents

[Imegen® MON89788 & Roundup Ready Soya Event ID Kit](#) has been validated using the reagents included in the kit and the DNA polymerase recommended by the supplier of the Real-Time PCR systems used in the validation as follows:

- TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific)

If a PCR master mix (DNA polymerase) different from the DNA polymerase used in the validation is going to be used to perform the analysis, a validation with the new reagents is recommended beforehand. Please, contact our Technical Support Team if you request any further information.

## 04.3 Product Stability

The optimal analytical functioning of this product is confirmed as long as the recommended storage conditions are applied as specified on Section 01.3 (Contents and Storage Conditions) from the reception of the kit until the expiry date assigned to each production batch.



# Supplemental information

## AA.1 Sensitivity and specificity

The specificity of the kit was tested through comparison with the NCBI sequence database and was also experimentally tested with success on a collection of reference DNAs. See the results in the table below:

## AA.2 Detection limit

[Imegen® MON89788 & Roundup Ready Soya Event ID Kit](#) can detect a percentage above 0.01% (w/w) of MON89788 & RR DNA. The limit of detection in processed samples varies depending on the composition and food processing.

To ensure the representativeness of the results, we recommend the use of a DNA extraction method that allows you to process a large amount of sample (10-20 g). If you do not have a procedure with these features, we recommend the use of [Imegen® Food Extraction Kit](#) (Ref No.: IMG-262).

## AA.3 Quality certifications

- ✓ Health in Code, S.L. is certified against the standard UNE-EN ISO 9001:2015 "Quality management systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.
- ✓ Health in Code, S.L. is certified against the standard UNE-EN ISO 14001:2015 "Environmental Management Systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.

Appendix  
B

# Safety warnings and precautions



Strictly follow the instructions of this manual, especially regarding the handling and storage conditions.



Do not pipette by mouth.



Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.



You must properly protect any skin condition, as well as cuts, abrasions and other skin lesions.



Send down the drain only those materials found on the safe list. Compounds not listed are not suitable for drain disposal. Use waste containers according to the local legislation and manage their treatment through an authorised waste manager.



In case of an accidental release of any of the reagents, avoid contact with skin, eyes and mucous membranes and clean with abundant water.



The materials safety data sheets of all hazardous components contained in this kit are available on request to Health in Code, S.L.



This product could require the handling of samples and materials of human and animal origin. You should consider all human and animal source materials as potentially infectious and handled in accordance with OSHA Biosafety Level 2 of bloodborne pathogens or must use other relevant biosafety practices for materials containing or suspect that they may contain infectious agents.



Reagents included in this kit are non-toxic, neither explosive, infectious, radioactive, magnetic, corrosive nor environmental pollutants.



This kit has been validated with specific equipment under certain conditions, which could be different in other laboratories. It is recommended that each laboratory performs an internal validation when the kit is used for the first time.



The manufacturer is not responsible for the malfunction of the assay when one or more 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 employs reagents not validated by Health in Code, S.L., considering them equivalent to those provided in the kit.





# Documentation and support

## AC.1 Food safety support

Please, visit our website for the latest services, orders and support information:

 [imegenagro.es](https://imegenagro.es)

Health in Code certificates of analysis and other product documentation:

 [portal.imegen.es/en/certificate-of-analysis/](https://portal.imegen.es/en/certificate-of-analysis/)

## AC.2 Customer and technical support

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

 **+34 963 212 340**

 **[tech.support@healthincode.com](mailto:tech.support@healthincode.com)**

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**NOTE:** For SDSs for reagents and chemicals from other manufacturers, please contact the appropriate manufacturer.

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