INSTRUCTIONS FOR USE

Imegen® MON89788 Quantification Kit

Ref. IMG-256

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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® TNOS Maize Quantification 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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Instructions for Use (IFU) modifications

V.05	NOV 2024	Transcription error: modification of no of cycles in table 4. Content revision in 2.1. and added in AA2.
V.04	OCT 2023	Contents review. Change of the manufacturer's identification, going from Imegen to Health in Code, S. L., and update of the front page (logo ISO 9001 removed). Modification of the storage temperature of the General Master Mix
V.03	JUL 2019	Contents review

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01

Product information

01.1 General description

Genetically modified organisms (GMOs) are widely distributed, with soya and corn being two of the most extensively cultivated crops worldwide. Indeed, these species and their derivatives (corn starch, soya protein, etc.) are found in more than 60% of the food we eat. 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 Soybean 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 such organisms.

01.2 Intended use

The Imegen® MON89788 Quantification Kit allows the percentage of Roundup Ready 2 or MON89788 Soya in a sample to be determined with respect to total Soya.

This kit uses Real-Time PCR technology and contains all the reagents required to quantify the MON89788 Soya in DNA obtained from any food or feed. Furthermore, the kit contains the plasmid DNA used as a standard with which the samples can be compared to determine the percentage of MON89788 Soya.

The assay is composed of two specific PCR systems containing oligonucleotides and hydrolysis probes labeled with the FAMTM fluorophore that will enable to amplify the transgenic event, MON89788, as well as the amplification of a Soya endogenous gene, Lectin. MON89788 PCR system specifically enables the amplification of a fragment in the integration region where the construct was inserted into the plant genome (5' insert-to-plant junction). This fragment is event-specific, amplifies the binding region between the Soya genome and the construct introduced un MON89788 Soya.

The inclusion of a plasmid system containing each of the targets, MON89788 and lectin in an equimolar proportion enables the construction of a standard curve of known copy number to compute the proportion of each one of the transgenic events.

01.3 Content and storage conditions of the kit

Imegen® MON89788 Quantification Kit contents the necessary reagents to perform 48 reactions.

MON9788 Master Mix	Master Mix with two specific oligonucleotides and a hydrolysis probe labelled with the FAM™ fluorophore to amplify the MON89788 transgenic event DNA in the sample.
Soya Master Mix	Master Mix with two specific oligonucleotides and a hydrolysis probe labelled with the FAM™ fluorophore to amplify the endogenous gene, Lectin, in the sample.
General Master Mix	Master Mix of PCR with nucleotides, $MgCl_2$, DNA polymerase and buffer needed to carry out RT- PCR.
MON89788 Standard	Plasmid containing equimolar quantities of the targets (Lectin:MON89788 in a 1:1 ratio)

Table 1. IMG-256 Imegen® MON89788 Quantification Kit components and description.

Reagents	Color indicator	Quantity	Conservation
MON89788 Master Mix*	Blue pad	360 µl	-20 °C
Maize Master Mix*	Purple pad	360 µl	-20 °C
General Master Mix*	White pad	2 x 600 µl	-20 °C upon receipt. 2 - 8 °C after initial use. Store protected from light.
TNOS standard*	Brown cap	6 x 50 µl	-20 °C

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

01.4 Equipment, reagents and materials required but not supplied

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

Related kits

- Imegen® Food Extraction Kit (IMG-262)
- Imegen® GMO Screening Kit (IMG-273)
- Imegen® Roundup Ready Soya Quantification Kit (IMG-274)
- Imegen® P35S Maize Quantification Kit

02 Methods

02.1 Preparation of the amplification reactions

Preparation of the amplification reactions includes:

- **Specific Master Mixes:**
  - MON89788 Master Mix
  - Soya Master Mix
- **MON89788 Standard (Positive control)**
- **DNA samples (10-25 ng/µl)**
- **Nuclease-free water for the negative controls (PCR and Extraction controls)**
- **General Master Mix**

The recommended protocol for preparation of reactions is showed below:

01. Thaw all reagents needs for the analysis.
02. Vortex and spin each reagent to mix thoroughly and keep on ice.
03. Using the MON897888 Standard, prepare serial dilutions, 1/10, to construct the standard curves that will enable the calculation of copy numbers for each one of the targets.

    As represent in Figure 1, four seral dilutions should be prepare starting from the undiluted MON89788 Standard containing $2x10^5$ copies. For this, add 5 µl of the MON89788 standard and 45 µl of nuclease-free water, until the preparation of the lowest concentration containing 20 total copies.

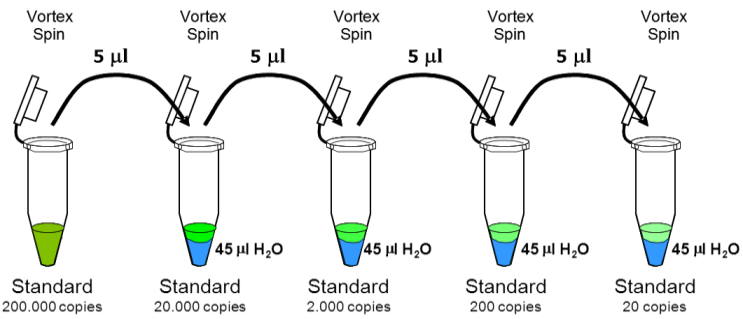

Figure 1. Four serial standard dilutions are made from TNOS Standard to perform two standard curves.

04. Vortex and spin each dilution mix before starting to prepare the next dilution.

05. 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 to make calculations taking into account the number of samples to be simultaneously analyzed, and then considering one more reaction.

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

Reagents	PCR target (Amount per reaction)	
	MON89788	Soya
MON89788 Master Mix	7.5 µl	-
Soya Master Mix	-	7.5 µl
General Master Mix	12.5 µl	12.5 µl

06. Vortex and spin the 1.5 mL tubes and dispense 20 µL per well or tube of 0.2 ml (see the example for three samples in figure 2).

07. Add 5 µl of each DNA sample at 10-25 ng/µl, into the appropriate wells.

08. Add 5 µl of MON89788 Standard, Positive Control and Negative Controls* into the appropriate wells.

09. 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 summited 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-256 Imegen® MON89788 Quantification Kit probes and specifications.

Target	Receptor	Quencher
MON89788	FAM™	MGB
Soya	FAM™	MGB

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

- **Reaction volume:** 25 µL.
- **Targets:** FAM™.
- 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™).
- 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**	
No. of cycles	1 initial cycle	50 cycles	
		Denaturation	Primers binding/extension
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:

- **Negative controls:** No amplification should be detected in either the reaction corresponding to MON89788 or that corresponding to Soya. Amplification in a negative control indicates the presence of contamination and therefore that the assay should be repeated.
- **Standard Curve:** Confirm that the serial dilutions prepared using MON89788 Standard produce suitable standard curves for all the targets when a linear regression is fitted to logarithmic copy numbers:

- The efficiency of the curve should be between 90% and 110%.
- The slope of the curve should be between -3.1 and -3.7.
- The correlation coefficient (R2) should be greater than 0.998.

If no amplification is detected in the MON89788 Standards, see section 03 (Troubleshooting). The highest concentration of the MON89788 Standard corresponds to 200,000 total copies.

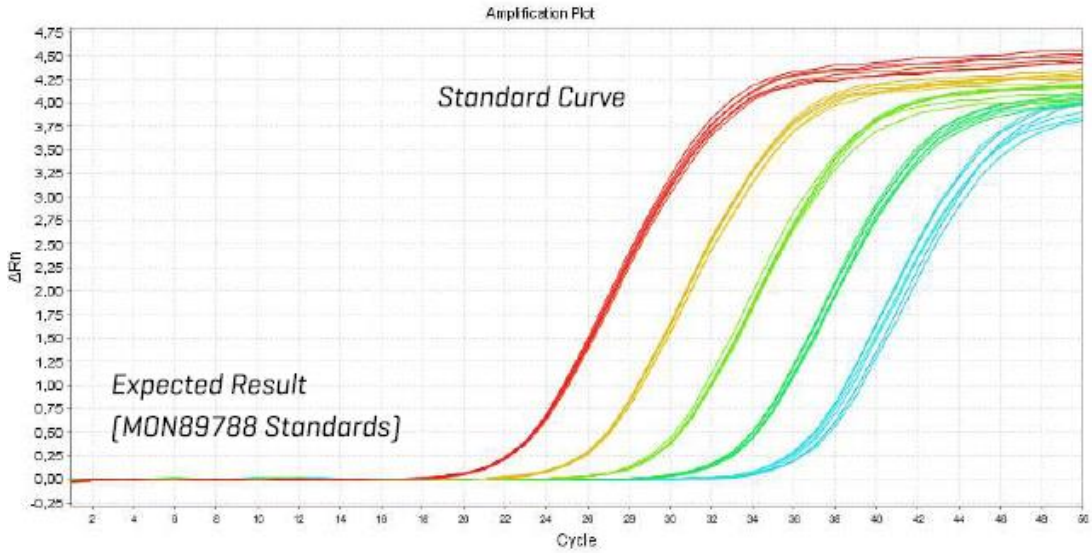

Figure 2. Standard curve amplification.

Once the controls have been verified, the results obtained with the samples can be analysed. If duplicated have been performed, the results for both replicates should be similar.

Three results are possible for each amplification reaction of both Maize and TNOS:

- **Not detected:** No amplification in the sample. The amplification curve is flat.
- **Not quantifiable:** Amplification is detected in the sample but to an extent lower than the last point on the curve. When the Ct for the sample is greater than the Ct for the 20-copy standard, it can be concluded that the analyte is present in the sample but is not quantifiable.
- **Quantifiable:** Amplification is detected in the sample to an extent greater than the last point on the curve. When the amplification Ct for the sample is interpolated between the values for the standard points, the quantitative result can be considered to be reliable and can be used to calculate the percentage of MON89788 Soya.

The following formula should be used to calculate the percentage of MON89788 with respect to total Soya present in the sample:

$$\%MON89788 = \frac{Nº\ of\ copies\ of\ MON89788}{Nº\ of\ Soya\ copies}\ x100$$

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 5. Possible results and their interpretation.

Soya	MON89788	Interpretation
Quantifiable	Not detected	No MON89788 Soya is detected in the sample
Quantifiable	Not quantifiable	The amount of MON89788 Soya detected in the sample is lower than the limit of quantification
Quantifiable	Quantifiable	The amount of MON89788 Soya with respect to total
Not quantifiable	Not detected	No MON89788 Soya is detected in the sample, the amount of Soya present in the sample is lower than the limit of quantification
Not quantifiable	Not quantifiable	The amounts of Soya or MON89788 Soya detected in the sample are lower than the limit of quantification
Not detected	Not detected	No Soya or MON89788 detected in the sample

- **Master Mix Soya:** Confirm that the reference gen (reactions prepared with Soya Master Mix) is detected in all the DNA samples. Lectin is a reference gene constitutively expressed; thus, this reaction informs the user of the good quality and integrity of the Soya DNA sample.

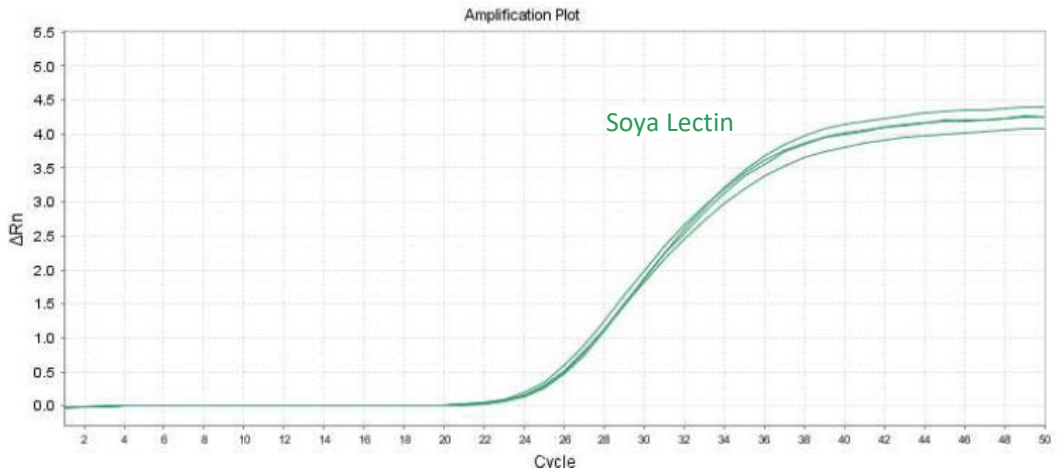

Figure 3. Soya Lectin amplification.

NOTE: If a sample different from Soya is being analyzed, food or feedstuff free from any Soya traces, no amplification will be expected on this screen.

- **MON89788 Master Mix:** After verifying all the controls included in the analysis are correct, the DNA samples are analysed. The sample analysed contains

transgenic MON89788 if amplification is detected with MON89788 Master Mix as indicated below.

- Negative sample:

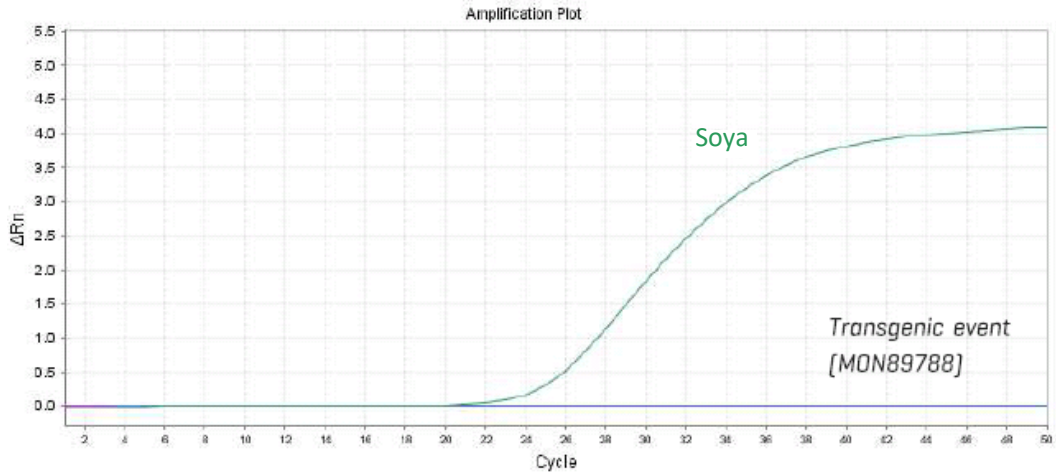

Figure 4. Amplification of a negative sample.

- Positive sample:

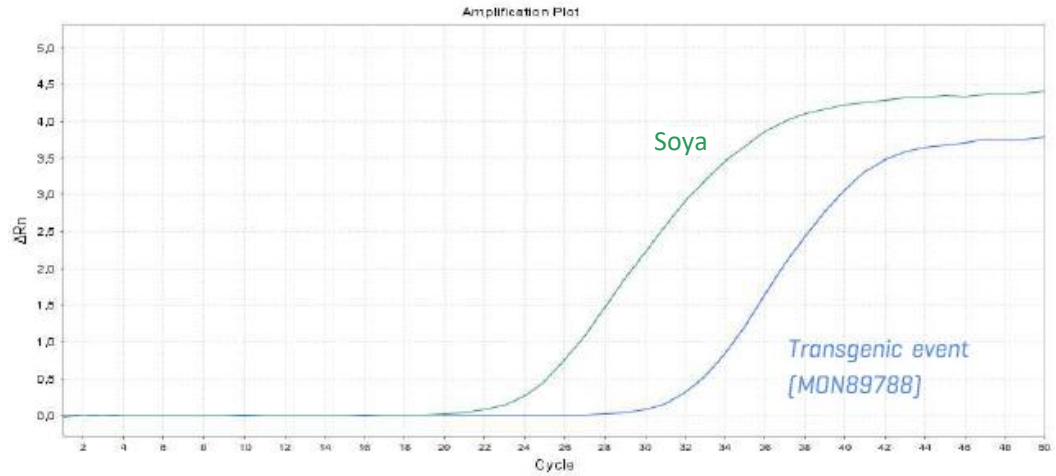

Figure 5. Amplification of a positive sample.

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 6. Possible results and their interpretation.

Control	Soya	MON89788	Result/Interpretation
Positive Control	+	+	Expected result
	-	-	[1]Fail in the PCR setup
DNA sample	+	-	Expected result
	+	+	
	-	-	[2]Fail to amplify the DNA sample
Negative Control	-	-	Expected result
	+	+	[3]Contamination with Soya DNA or with the positive control

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

(2) Fail to amplify the DAN sample: An error to amplify the reference gene in the DNA sample might suggest the quality or the quality of the DNA sample is compromised. In this situation, a second analysis would be recommended before an interpretation of the results is made.

(3) PCR contaminations with Soya DNA or with Positive Control: 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 Quantification 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™ fluorophore.

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 Quantification 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.

Appendix A

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.

Table 7. Different species used during the specificity of IMG-256 Imegen® MON89788 Quantification Kit

Transgenic varieties	Result
MON89788[1]	Detected
RR[1]	Detected
MON87769[1]	Not detected
MON87705[1]	Not detected
A2704-12[1]	Not detected
A5547-127[1]	Not detected
DP305423[1]	Not detected
CV127[1]	Not detected
MON87708[1]	Not detected
DAS68416[1]	Not detected
GA21[2]	Not detected
MON87460[1]	Not detected
NK603[2]	Not detected
MON88017[1]	Not detected
DAS40278[2]	Not detected
Bt11[2]	Not detected
MON863[2]	Not detected
DAS01507[2]	Not detected
DAS21023[3]	Not detected
LLCotton25[3]	Not detected
MON1445[3]	Not detected
MON15985[3]	Not detected
T45[4]	Not detected

Ms8[4]	Not detected
GT73[4]	Not detected
MS1[4]	Not detected

Non-transgenic samples	**Result**
Maize *(Zea mays)*	Not detected
Carrot *(Daucus carota)*	Not detected
Lentil *(Ens culinaris)*	Not detected
Sesame *(Sesamum indicum)*	Not detected
Oats *(Avena sativa)*	Not detected
Wheat *(Triticum spp)*	Not detected
Cotton *(Gossypium hirstium)*	Not detected
Rapeseed *(Brassica napus)*	Not detected
Cow *(Bos taurus)*	Not detected
Sheep *(Ovis aries)*	Not detected
Buffalo *(Bubalus bubalis)*	Not detected
Atlantic cod *(Gadus morhua)*	Not detected
Common dab *(Limanda limanda)*	Not detected
Porc *(Sus scrofa)*	Not detected

AA.2 Detection and quantitation limit

The limit of relative quantification (LOQrel) of the Imegen® MON89788 Quantification Kit has been established in 0.1% (w/w), whereas the limit of absolute quantification (LOQabs), corresponding with the lowest value included in the standard curve, is established to be 20 total copies for each of the quantifiable systems (Soya lectin and MON89788). Detection limit of the PCR technique is 5 copies each of MON89788 and soya DNA.

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 polluters.
	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.

Appendix C

Documentation and support

AC.1 Food safety support

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

imegenagro.es

Health in Code certificates of analysis and other product documentation:

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

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