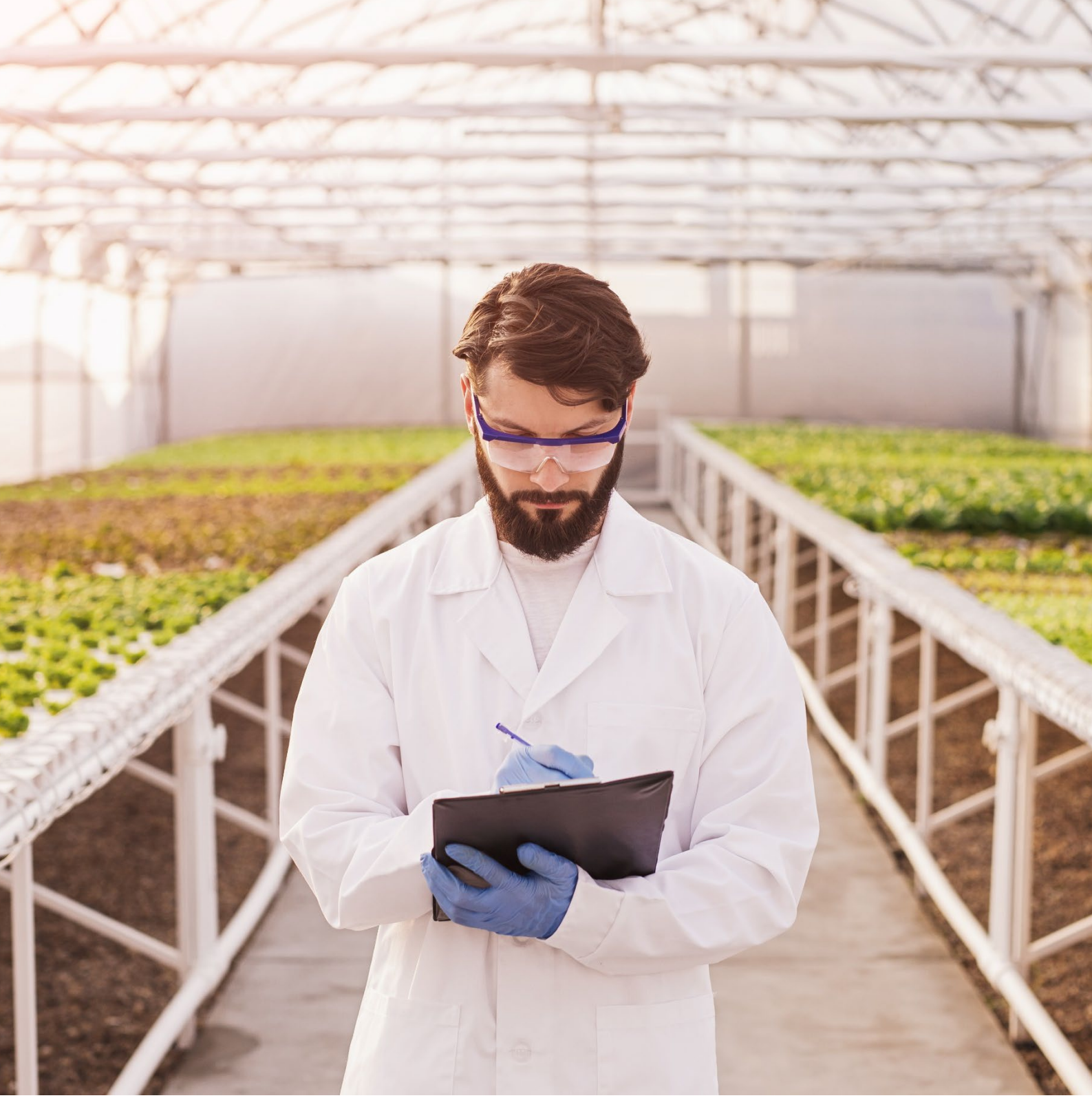

INSTRUCTIONS FOR USE

Imegen® GMO Screening Plus Kit

Ref. IMG-273

Manufactured by HEALTH IN CODE, S.L.
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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. 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® GMO Screening Plus 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.06	OCT 2024	Modifications in table 4: error in the No. of cycle's transcription and step removal (not necessary). Content revision in section 2.1
V.05	JUN 2023	Contents review
V.04	AUG 2022	Change of manufacturer's identification, going from Imegen to Health in Code, S.L. Update of the front page (logo ISO 9001 removed).
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, soy protein, etc.) are found in more than 60% of the food we eat.

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.

The promoter 35S obtained from cauliflower mosaic virus (CaMV) and the terminator NOS from Agrobacterium tumefaciens are the regulatory elements traditionally analysed during screening for transgenic material in foods. However, these regulatory elements do not cover such important transgenic events as MON89788 soy, H7-1 sugar beet or GT73 rape. Therefore, in order to ensure the widest detection spectrum possible, the TaqMan® GMO Screening kit includes the promoter 34S from Figwort Mosaic Virus (FMV) together with the P35S and TNOS regulatory regions.

The regulatory elements present in GMOs are found naturally in the organisms from which they are obtained (CaMV, A. tumefaciens and FMV), which is why the use of regulatory regions arouses such controversy when it comes to interpreting a positive result. The Imegen® GMO Screening Plus Kit incorporates the simultaneous detection of a genomic region exclusive to these three organisms, thus allowing a positive result to be interpreted unambiguously as being due to either the presence of genetically modified material or the natural presence of these organisms.

01.2 Intended use

The Imegen® GMO Screening Plus Kit allows the presence of transgenic DNA to be analyzed by detecting three regulatory regions present in the genetically modified organisms approved by the EU.

This kit uses Real-Time PCR technology and contains all the reagents required to detect GMOs in DNA obtained from any food or feed. Furthermore, it also contains an

internal positive control (IPC) that allows the presence of inhibition during the PCR process to be identified.

Four real-time PCR reactions are performed during sample analysis. Each of these reactions amplifies two independent regions by way of a single multiplex PCR reaction using two channels on the thermal cycler (FAM and VIC).

This kit allows certified standards containing 0.1% of different events and transgenic plant species to be detected.

01.3 Content and storage conditions of the kit

Imegen® GMO Screening Plus Kit contents the necessary reagents to perform 48 reactions.

P35S/CaMV	Master Mix with specific oligonucleotides and a hydrolysis probe labelled with the FAM™ fluorophore to amplify the CaMV P35S regulatory element, and other labelled with VIC™ fluorophore to detect a specific CaMV amplicon.
TNOS/A. tumefaciens	Master Mix with specific oligonucleotides and a hydrolysis probe labelled with the FAM™ fluorophore to amplify the TNOS regulatory element from A. tumefaciens, and other labelled with VIC™ fluorophore to detect a genomic region exclusive to this bacterium.
P34S/FMV	Master Mix with specific oligonucleotides and a hydrolysis probe labelled with the FAM™ fluorophore to amplify the P34S regulatory element from FMV, and other labelled with VIC™ fluorophore to detect amplicons from a genomic region exclusive to this virus.
Plant/IPC	Master Mix with specific oligonucleotides and a hydrolysis probe labelled with the FAM™ fluorophore to amplify detects plant DNA, and other labelled with VIC™ fluorophore to detect an internal positive control that is used to rule out the presence of inhibitors in the sample.
Master Mix	Master Mix of PCR with nucleotides, $MgCl_2$, DNA polymerase and buffer needed to carry out RT- PCR.
Positive Control	DNA with the transgenic events of interest.

Table 1. IMG-273 Imegen® GMO Screening Plus Kit components and description.

Reagents	Color indicator	Quantity	Conservation
P35S, Master Mix*	Red disc	400 µl	-20 °C
TNOS Master Mix	Blue disc	400 µl	-20 °C
P34S-FMV Master Mix	Yellow disc	400 µl	-20 °C
Plant Master Mix	Green disc	400 µl	-20 °C
General Master Mix*	White disc	3 x 880 µl	-20 °C upon receipt. 2 - 8 °C after initial use. Store protected from light.
Positive control*	Orange stopper	250 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 (not DEPC-Treated)

02 Methods

02.1 Preparation of the amplification reactions

Imegen® GMO Screening Plus Kit is designed to perform four reactions for each sample to be analyzed (P35S, TNOS, P34S-FMV and plant). Thus, four separate PCR masters, one for each region analyzed, should be prepared.

We recommend using, the positive control included in this kit for each run.

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

01. Thaw the P35S, TNOS, P34S-FMV and Plant Master Mixes, the Positive Control and the DNA samples (if stored frozen).
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 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)			
	P35S	TNOS	P34S	Plant
P35S Master Mix	7.5 µL			
TNOS Master Mix		7.5 µL		
P34S Master Mix			7.5 µL	
Plant Master Mix				7.5 µL
General Master Mix	12.5 µL	12.5 µL	12.5 µL	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 µl of each DNA sample at 10-25 ng/µl, into the appropriate wells.

06. Add 5 µl of Positive Control and Negative Controls* into the appropriate wells.

07. 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-273 Imegen® GMO Screening Plus Kit probes and specifications.

Reagents	Receptor	Quencher
P35S	FAM™	MGB
CaMV	VIC™	MGB
TNOS	FAM™	MGB
A. tumefaciens	VIC™	MGB
P34S	FAM™	MGB
IFMV	VIC™	MGB
Plant	FAM™	MGB
IPC-P	VIC™	MGB

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

- **Reaction volume:** 25 µL.
- **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**	
No. of cycles	1 cycle	50 cycles	
		Denaturation	Primers binding/extenion
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, both in the FAM™ channel as VIC™.
- **Negative controls:** amplification should only be detected in the VIC™ channel for the reaction performed with Plant Master Mix. This channel detects an internal positive control (IPC) that confirms the absence of inhibition in the sample.

Plant/IPC	It should be checked that the IPC (VIC) is positive for all samples and that the Ct in which it is detected is similar to that for the positive control. A negative result for the IPC indicates the presence of inhibitors in the sample. It should be noted that the IPC result may be negative in samples where a large amount of plant DNA (FAM) is detected as the PCR reagents are depleted before IPC amplification commences.

P35S/CaMV	P35S (FAM) amplification together with a lack of CaMV (VIC) amplification indicates the presence of transgenic material in the sample. The detection of both P35S and CaMV amplification indicates that the sample contains CaMV. This implies that the presence of transgenic material cannot be confirmed as it is impossible to distinguish between P35S arising from the CaMV present naturally in the sample or from genetically modified material.
TNOS/A. tumefaciens	TNOS (FAM) amplification together with a lack of A. tumefaciens (VIC) amplification indicates the presence of transgenic material in the sample. The detection of both TNOS and A. tumefaciens amplification indicates that the sample contains the bacterium Agrobacterium tumefaciens. This implies that the presence of transgenic material cannot be confirmed as it is impossible to distinguish between TNOS arising from the bacterium present naturally in the sample or from genetically modified material.
P34S/FMV	P34S (FAM) amplification together with a lack of FMV (VIC) amplification indicates the presence of transgenic material in the sample. The detection of both P34S and FMV amplification indicates that the sample contains FMV. This implies that the presence of transgenic material cannot be confirmed as it is impossible to distinguish between P34S arising from the FMV present naturally in the sample or from genetically modified material.

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. Results interpretation.

Plant Master Mix		**P35S Master Mix**		**TNOS Master Mix**		**P34S-FMV Master Mix**		**Interpretation**
Plant	**IPC**	**P35S**	**CaMV**	**TNOS**	**A.Tum**	**P34S**	**FMV**	
+	+	-	-	-	-	-	-	No transgenic material containing P35S, TNOS and P34S detected
+	+	+	-	-	-	-	-	Transgenic material containing P35S detected
+	+	-	-	+	-	-	-	Transgenic material containing TNOS detected

+	+	-	-	-	-	+	-	Transgenic material containing P34S detected
+	+	+	+	-	-	-	-	CaMV present in sample
+	+	-	-	+	+	-	-	A. tumefaciens present in sample
+	+	-	-	-	-	+	+	FMV present in sample
-	-	-	-	-	-	-	-	PCR inhibitors present in sample*
-	+	-	-	-	-	-	-	No plant DNA in sample
+	-	-	-	-	-	-	-	Sample contains large amount of plant 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 6. Possible results and their interpretation.

Plant Master Mix		P35S Master Mix		TNOS Master Mix		P34S-FMV Master Mix		Interpretation
Plant	**IPC**	**P35S**	**CaMV**	**TNOS**	**A.Tum**	**P34S**	**FMV**	
POSITIVE CONTROL								
+	+	+	+	+	+	+	+	Expected result
-	-	-	-	-	-	-	-	[1] Amplification error
NEGATIVE CONTROL								
-	+	-	-	-	-	-	-	Expected result
+	+	-	-	-	-	-	-	[2] Extract contaminated with plant material
+	+	+/-	-	+/-	-	+/-	-	[3] Contamination with transgenic material
NEGATIVE EXTRACTION CONTROL								
-	+	-	-	-	-	-	-	Expected result
+	+	-	-	-	-	-	-	[4] Extract contaminated with plant DNA
+	+	+/-	-	+/-	-	+/-	-	[5] Contamination with transgenic material DNA
+	+	+	+	+	+	+	+	[6] Contamination, possibly with positive control

(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 celery 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: 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® GMO Screening Plus 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)
- QuantStudio™ 5 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® GMO Screening Plus 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

As well as the theoretical specificity analyses performed when designing the oligonucleotides and probes used in the kit, specificity assays have been performed using transgenic varieties that contain P35S and/or TNOS and/or P34S have also been performed. The acceptance criterion used for the specificity parameter is that the PCR system developed should only produce amplification for the organisms for which the amplicon is expected to be detected.

A table listing the varieties used during this assay is provided below:

Table 7. Vegetable species used during the specificity assay for the Imegen® GMO Screening Plus Kit.

Reference Material				
Maize	**Soya**	**Rape**	**Cotton**	**Beetroot**
GA21	GTS-40-3-2	T45	MON1445	H7-1
MON810	A2704-12	GT73	MON531	
MON863	MON89788			
BT176				
BT11				
NK603				
TC1507				
T25				

AA.2 Detection limit

The PCR limit for the kit was calculated using the positive control provided with it. This control is a plasmid containing the targets for the different amplicons amplified by the kit.

The limit of detection for Imegen® GMO Screening Plus Kit is 5 DNA copies per reaction for all the regions analyzed with this kit. The kit enables the detection of 0.1% of GMO plant species in a background of non-GMO material, as demonstrated by the use of certified GMO reference standards.

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.
