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Food Safety

User Guide

imegen™ GMO Screening Plus Kit

GMO Screening by real time PCR

REF : IMG-273

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Rev. 2

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All products sold by the imegen are subjected to rigorous quality control. 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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1. Product information

Kit description

Genetically modified organisms (GMOs) are widely distributed, with soy 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 ***imegen-GMO Screening Plus kit*** *allows the presence of transgenic DNA to be analysed 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.

The ***imegen-GMO Screening Plus kit*** *allows the most of the transgenic events approved by the EU to be detected, as well as the vast majority of such events described in other databases throughout the world.*

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.

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). These amplification reactions are described below:

P35S/CaMV: *This reaction uses two TaqMan® probes, one labelled with the FAM fluorophore, which detects amplicons of the CaMV P35S regulatory element, and the other labelled with VIC to detect a specific CaMV amplicon.*

TNOS/A. tumefaciens: *This reaction includes two TaqMan® probes. One of these is labelled with the FAM fluorophore and detects amplicons of the TNOS regulatory element from A. tumefaciens, whereas the other is labelled with VIC and detects amplicons from a genomic region exclusive to this bacterium.*

P34S/FMV: *This reaction includes two TaqMan® probes. One of these is labelled with the FAM fluorophore and detects amplicons of the P34S regulatory element from FMV, whereas the other is labelled with VIC and detects amplicons from a genomic region exclusive to this virus.*

Plant/IPC: *This reaction includes two TaqMan® probes. One of these is labelled with the FAM fluorophore and detects plant DNA, whereas the other is labelled with VIC and detects an internal positive control that is used to rule out the presence of inhibitors in the sample.*

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

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 ***Food Extraction Kit*** *(Part No: IMG-262).*

Content and storage of the kit

The kit contains needed reagents to perform the real time PCR reactions:

- *Amplification primers for the four PCR multiplex systems.*
- *The system contains two probes. One of them labelled with FAM™ for the specific construction DNA detection and other labelled with VIC® for the biological contamination or IPC DNA detection.*
- *General Master Mix which includes the enzyme of the PCR*
- *Positive control*

The kit contents the necessary reagents to perform 48 reactions:

Reagents	Color	Amount	Storage
P35S Master Mix	Red disc	400 µL	-20ºC
TNOS Master Mix	Blue disc	400 µL	-20ºC
P34S-FMV Master Mix	Yellow disc	400 µL	-20ºC
Plant Master Mix	Green disc	400 µL	-20ºC
General Master Mix	White disc	3 x 880 µL	4ºC
Positive Control	Orange stopper	250 µL	-20ºC

Table 1. *Kit components and storage temperature of* ***imegen-GMO Screening Plus Kit***

Equipment and material required but not supplied

In the following table the equipment and material requirements for using ***imegen-GMO Screening Plus Kit*** *are shown:*

Equipment	
1	Real-time PCR Thermal Cycler with channels for detection of FAM™ (520 nm) and VIC® (550 nm)
2	Micropipettes (10 µl, 20 µl and 200 µl)
3	Table top centrifuge with adaptors for 96 well PCR plates and/or 0.2 ml tubes
4	Vortex

Materials	
1	Optical 96-well reaction plates or 0.2 ml optical tubes
2	Optical adhesive film for 96 well plates or optical adhesive covers for 0.2 ml tubes
3	Disposable micropipette filter tips
4	1.5 ml sterile tubes
5	Powder-free latex gloves

Detection and quantification limits

The detection limit is the minimal amount of DNA that can be detected.

It has been shown that the PCR detection limit is 5 DNA copies per reaction for all the regions analysed by this kit.

2. Real time PCR

PCR reactions preparation

*The **imegen-GMO Screening Plus kit** is designed to perform four reactions for each sample to be analysed (P35S, TNOS, P34S-FMV and plant). Thus, four separate PCR masters, one for each region analysed, should be prepared.*

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

To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples and controls to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.

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

1. *Thaw the P35S, TNOS, P34S-FMV and Plant Master Mixes, the Positive Control and the DNA samples (if stored frozen).*
2. *Vortex each reagent and keep cold.*
3. *Add into a 1.5 mL tube, the following reagents:*

Reagents	Amount per reaction
P35S, TNOS, P34S-FMV or Plant Master Mix	7.5 µL
General Master Mix	12.5 µL

***Table 2.** Reagents amount per reaction*

4. *Vortex and spin the 1.5 mL tubes and dispense 20 µl per well or tube of 0.2 ml.*
5. *Add 5 µl of each DNA sample at 10-25 ng/µl, into the appropriate wells. We recommend making each sample analysis in duplicate.*

6. *Add 5 µl of Positive Control and Negative Controls* into the appropriate wells.*
7. *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.*

PCR amplification program

This kit is compatible with the Real-time PCR platforms 7500 FAST, StepOne Real-Time PCR System (Thermo Scientific), and any other Real-time PCR platforms equipped with FAM and VIC channels.

Probes	Receptor	Quencher
P35S	FAM™	MGB
CAMv	VIC®	MGB
TNOS	FAM™	MGB
Agrobaterium	VIC®	MGB
P34S	FAM™	MGB
FMV	VIC®	MGB
Plant	FAM™	MGB
IPC-P	VIC®	MGB

***Table 3**. Probes information*

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 or a StepOne Real-Time PCR system (Thermo Scientific) select Quantitation- Standard curve as a type of experiment and include ROX™ as a reference.*
- *Ramp rate: standard*
- *Optimal program:*

Fields	Step 1 Enzyme activation	Step 2 PCR	
Cycle Number	1 initial cycle	50 cycles	
		Denaturation	Primers binding/Extention
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute*

***Table 5.** Optimal PCR program*

**Fluorescence detection*

Note: This program has been validated on a StepOne Real-Time PCR System from Applied Biosystems. If you use another brand or model of thermal cycler, you may need the amplification program to be adjusted. Please contact our service department for advice.

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3. Results analysis

Before analysing 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:

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

***Table 5**. Possible results and their interpretation*

* *If inhibitors are detected in the sample, we recommend that you check whether an excess of DNA was added to the reaction (maximum recommended amount: 250 ng). If the amount of DNA is correct, we recommend that you repeat the DNA extraction using a new protocol (if necessary, you should contact our technical department for advice).*

The following table lists the results that can be obtained upon analysis of various assay controls and how they should be interpreted:

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

***Table 6**. Possible results and their interpretation*

Recommendations:

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

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[2-3] ***Contamination in the 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.*

[4-6] ***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.*