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Food Safety

User Guide

imegen™ P35S Maize quantification Kit

P35S versus maize quantification by real-time PCR

REF : IMG-275

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All products sold by the imegen are subjected to rigorous quality control. The ***imegen™ P35S 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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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, soy, corn and their derivatives (soy protein, corn starch, etc.) are the ingredients of more than the 60% of the food we meet.

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.

The ***imegen-GMO Maize Quantification Kit****, allows the number of copies of the P35S present in the most of the transgenic events to be determined with respect to total corn in a sample.*

The quantification of P35S with respect corn allows estimating the quantity of transgenic material present in a sample. If the sample contains corn events with several copies of P35S, or another transgenic vegetable species, which contain P35S, the results can be overestimated.

This kit uses Real-Time PCR technology and contains all the reagents required to quantify the P35S promoter 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 P35S promoter.

Content and storage of the kit

Sample analysis comprises two real-time PCR simultaneous processes:

- *One of them allows the total amount of corn DNA in the sample*
- *The other, allows the amount of P35S promoter DNA present in the sample*

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To perform the reaction to determine the total maize DNA amount in the sample, this kit includes a master mix with two primers and a TaqMan®-type probe labelled with the FAM™ fluorophore. This reaction specifically amplifies an endogenous maize gene known as MSS.

To perform the reaction to determine the P35S promoter DNA amount in the sample, this kit includes a master mix with two primers and a TaqMan®-type probe labelled with the FAM™ fluorophore. The reaction specifically amplifies P35S promoter.

The kit also includes a plasmid DNA standard containing a copy of each of the targets used during analysis. A comparison of the results obtained with the samples and this standard allows a relative quantification to be made and therefore the percentage of P35S maize with respect to the total maize present in the sample to be calculated.

The kit contents the necessary reagents to perform 50 reactions:

Reagents	Color	Amount	Storage
P35S Master Mix	Blue pad	375 µL	-20ºC
Maize Master Mix	Red pad	375 µL	-20ºC
General Master Mix	White pad	2 x 625 µL	4ºC
P35S standard	Blue cap	4 x 50 µL	-20ºC

Table 1**. Kit components and storage temperature of **imegen-P35S Maize quantification Kit

Equipment and material required but not supplied

*In the following table the equipment and material requirements for using **imegen-P35S Maize quantification 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

Limit of quantification: 20 copies of DNA
Detection limit of the PCR technique (Maize system): 3 copies of DNA
Detection limit of the PCR technique (P35S maize system): 3 copies of DNA

This Kit allows relative quantifications of up to 0.01% of P35S maize to be determined with respect to total maize in a sample. The relative limit of quantification varies depending on the sample analysed.

2. Real time PCR

PCR reactions preparation

Two absolute quantifications are performed during the course of the relative quantification of P35S promoter present in a sample. The first of these determines the total amount of maize present in the sample and the second determines the amount of P35S promoter. Preparation of the amplification reactions includes:

Preparation of the amplification reactions includes:

- *Standard dilutions*
- *Negative PCR and/or extraction controls*
- *Sample analysis in duplicate*

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 reactions is showed below:

1. *Thaw a vial of P35S standard and prepare four 1:10 serial dilutions of this standard. This process results in the quantitative standards with which the samples can be compared.*

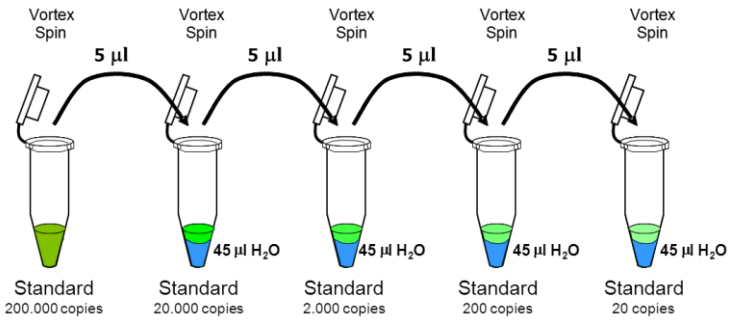

Figure 1: Four serial standard dilutions are made from P35S Standard to perform two standard curves

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2. *Thaw the Master Mixes, negative controls and DNA samples (if stored frozen).*
3. *Shake each of the reagents on the vortex whilst keeping them cold.*
4. *Add into a 1.5 mL tube, the following reagents:*

Reagents	Amount per reaction
Maize Master Mix	7.5 µL
General Master Mix	12.5 µL

***Table 3**. Reagents amount per reaction*

Add into another 1.5 mL tube, the following reagents:

Reagents	Amount per reaction
P35S Master Mix	7.5 µL
General Master Mix	12.5 µL

***Table 4**. Reagents amount per reaction*

5. *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).*
6. *Add 5 µl of each sample DNA (10-25 ng/µL) to the corresponding wells:*
    a. *Total maize reactions and,*
    b. *P35S amplification reactions*
7. *Add 5 µl of each standard dilution to the corresponding wells:*
    a. *Total maize reactions and,*
    b. *P35S amplification reactions*
8. *Add 5 µl of each control (negative control and DNA extraction control) to the corresponding wells:*
    a. *Total maize reactions and,*
    b. *P35S amplification reactions*
9. *Seal the plate with optical film and spin.*

10. *Load the plate into a thermal cycler and then perform a run using the conditions showed in the next section.*

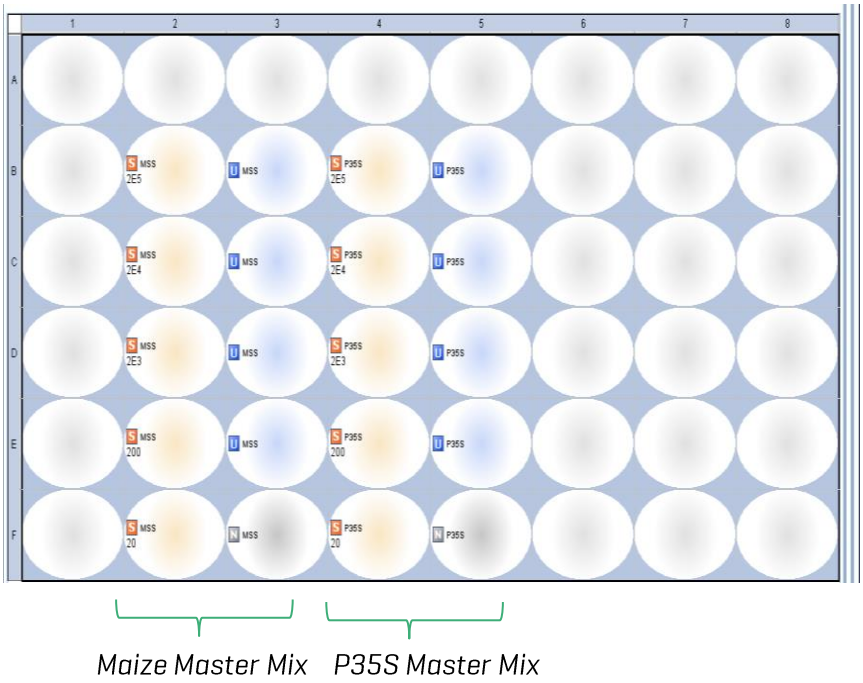

Figure 2: Proposed design analysis for 2 samples obtained from the same DNA extraction round.

** 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.

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Probes	Receptor	Quencher
MSS-P	FAM™	TAMRA
P35S-P	FAM™	MGB

***Table 4**. 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 sample results, it should be confirmed that the results obtained with the different controls are as expected:

- ***Negative controls:*** *No amplification should be detected in either the reaction corresponding to maize or that corresponding to P35S promoter. Amplification in a negative control indicates the presence of contamination and therefore that the assay should be repeated.*

- ***P35S standard:*** *Amplification should be detected for the five points corresponding to the maize standard and the five points corresponding to the P35S promoter standard. Furthermore, the curves obtained using the standard points should meet the following requirements:*

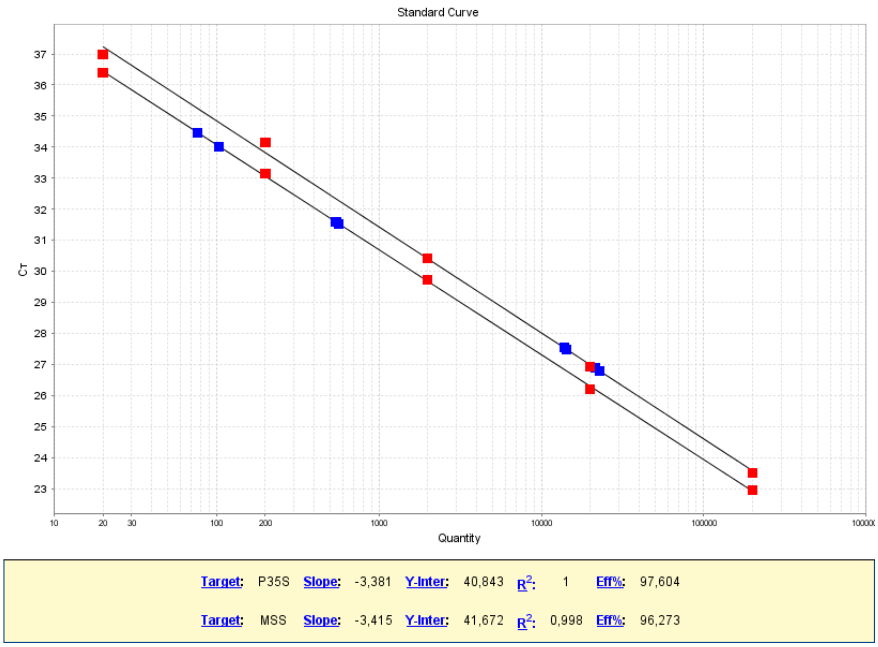

Figure 3: Standard curves for total maize and P35S targets. Red dots represent the dilutions of the standard.

Furthermore, the curves obtained using the standard points should meet the following requirements:

- *The efficiency of the curve should be between 80% and 110%.*
- *The slope of the curve should be between -3.1 and -3.9.*
- *The correlation coefficient (R^2) should be greater than 0.98.*

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 P35S promoter:

- ***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 P35S promoter.*

The following formula should be used to calculate the percentage of P35S promoter with respect to total maize present in the sample:

$$\%\ P35S = \frac{\text{No. of copies of P35S x 100}}{\text{No. of maize copies}}$$

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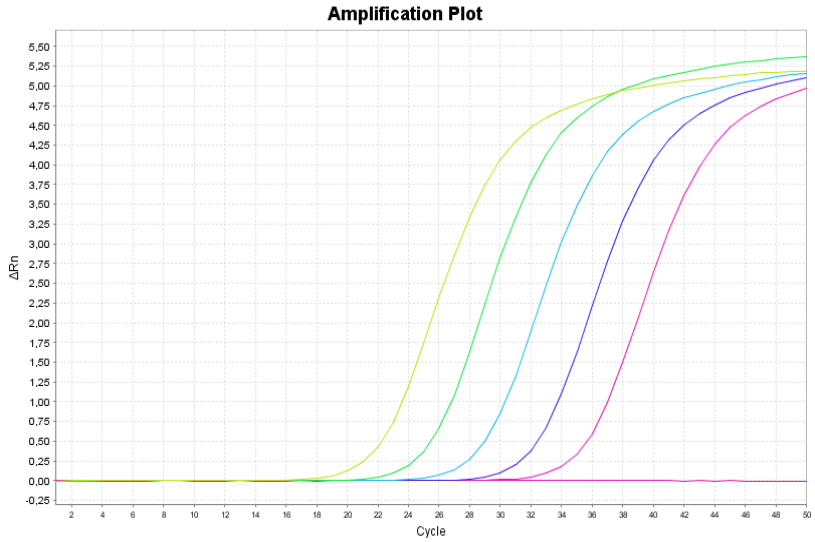

Figure 4: Amplification curves for each of the dilutions of the P35S Standard using P35S master mix

The following table shows the interpretation that should be done from the obtained result:

Maize	P35S	Interpretation
Quantifiable	Not detected	No P35S promoter detected in the sample.
Quantifiable	Not quantifiable	The amount of P35S promoter detected in the sample is lower than the limit of quantification.
Quantifiable	Quantifiable	The amount of P35S promoter with respect to total maize in the sample is X%.
Not quantifiable	Not detected	No P35S promoter detected in the sample, the amount of maize present in the sample is lower than the limit of quantification.
Not quantifiable	Not quantifiable	The amounts of maize or P35S promoter detected in the sample are lower than the limit of quantification.
Not detected	Not detected	No maize or P35S promoter detected in the sample.*

***Table 8**. Possible results and their interpretation*

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[*] *It is possible that the inability to detect maize DNA in a sample is due to the presence of inhibitors in the DNA used. To check for the absence of inhibitors in the sample, we recommend that you use an inhibition control consisting of amplification with Maize Master Mix in a well containing test sample DNA together with 1 µL of the inhibition control corresponding to the dilution containing 20,000 copies of the standard. Another well containing 5 µL of water and 1 µL of the same inhibition control should be amplified in parallel. If the amplification of both reactions is similar, it can be concluded that the sample is not inhibited.*

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