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validation report for
TaqMan® GMO screening kit

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Contents

2. Purpose

The current document is intended to describe the series of analyses performed to validate the TaqMan® GMO Screening kit, thus ensuring its quality.

3. References

Guideline UNE-EN ISO/IEC 17025:2005
Guideline UNE-EN ISO/IEC 21570:2005

4. Definitions

Validation: Confirmation by examination and the provision of objective evidence to demonstrate compliance with certain requirements for the expected specific use (source: ISO17025)

Precision: The degree of concordance between the results of independently obtained measurements under the conditions established.

Repeatability: Precision under conditions in which the results of a measurement are obtained using the same method, by the same operative, and using the same measurement instrument.

Reproducibility: Precision under conditions in which the results of a measurement are obtained using the same method and same measurand, but with different operatives and different measurement instruments.

Specificity: The degree to which a method can determine a particular analyte in a complex mixture without interference from other components in the mixture.

Limit of detection: The lowest value of an analyte in a sample that can be examined, that can be detected but not necessarily quantified exactly.

Sensitivity: The ability of an analytical method to detect small variations in the concentration of transgenic material in a specific matrix.

5. Introduction

The TaqMan® GMO Screening kit allows **all transgenic events authorised by the European union**, as well as the majority of such events described in worldwide databases, to be detected.

The promoter 35S obtained from the cauliflower mosaic virus (CaMV) and the terminator NOS from *Agrobacterium tumefaciens* are the regulatory elements that have traditionally been analysed when screening foodstuffs for transgenic material. However, these regulatory elements do not currently cover such important transgenic events as soy MON89788, sugarbeet H7-1 or oilseed rape GT73. In order to achieve a broader detection spectrum, the TaqMan® GMO Screening kit, includes the promoter 34S from *Figwort Mosaic Virus (FMV)*, together with the regulatory regions P35S and TNOS

The regulatory elements present in GMOs are found naturally in the organisms from which they are obtained (CaMV, *A. tumefaciens* y FMV). For this reason, the use of regulatory regions leads to some controversy when it comes to interpreting a positive result. The TaqMan® GMO Screening kit permits the simultaneous detection of a genomic region that is only found in these three organisms, thus meaning that a positive result can be assigned unequivocally to either the presence of genetically modified material or to the natural presence of these organisms.

Four real-time PCR steps are performed during the analysis of each sample. Each of these reactions amplifies two independent regions by way of a single multiplex PCR that uses two channels on the thermal cycler (FAM and VIC®). The amplification reactions involved are described below:

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

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

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

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

6. Validation Assay

6.1. 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 (Table 1), and graphical representation of one such assay (chart 1), are provided below. Varieties containing different percentages of transgenic material were used during this assay.

Table 1. Varieties used d during the specificity assay for the TaqMan® GMO Screening Kit

Standard transgenic varieties				
Maize Soy	**Soy**	**Oilseed Rape**	**Cotton**	**Sugarbeet**
GA21	RR	T45	MON1445	H7-1
MON810	A2704-12	GT63	MON531	
MON863				
BT176				
BT11				
NK603				
TC1507				
T25				

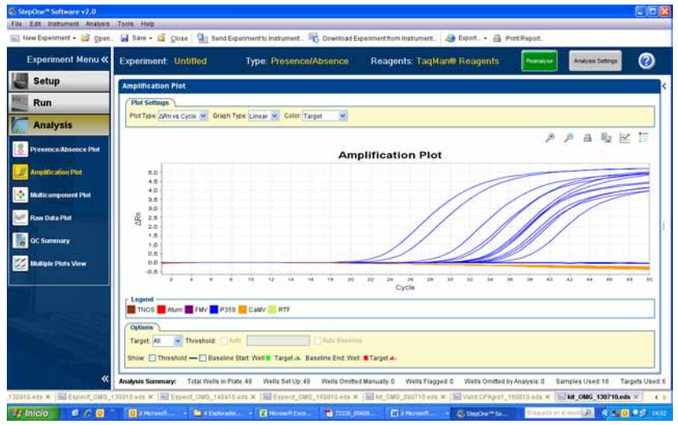

Chart 1: Specificity for P35S; target P35S blue and target CaMV orange.

The kit only produces amplification when the transgenic varieties contain the corresponding promoter for each variety.

6.2. PCR sensitivity and 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.

A total of 6 replicates for the dilutions 100, 10, 5 and 1 copies/reaction were performed to determine the PCR limit. The assay was subsequently repeated using 6 replicates of the 10 and 5 copies/reaction dilutions. The results obtained after real-time PCR can be found in Table 2 and show that the limit of detection for the PCR is 5 DNA copies per reaction for all the regions analysed with this kit.

Table 2. Results corresponding to the amplification performed to determine the PCR limit.

Results for PCR limit				
	100 copies/reaction	**10 copies/reaction**	**5 copies/reaction**	**1 copies/reaction**
P68S / CaMV	100% (6/6)	100% (12/12)	100% (12/12)	33% (4/6)
TNOS / A tumefaciens	100% (6/6)	100% (12/12)	>90% (11/12)	33% (4/6)
P34S/FMV	100% (6/6)	100% (12/12)	>90% (11/12)	17% (2/6)
Plant / IPC	100% (6/6)	100% (12/12)	>90% (11/12)	33% (4/6)

The following graphs are an example of the PCR limit results obtained for 10 copies/reaction in each determination using the kit:

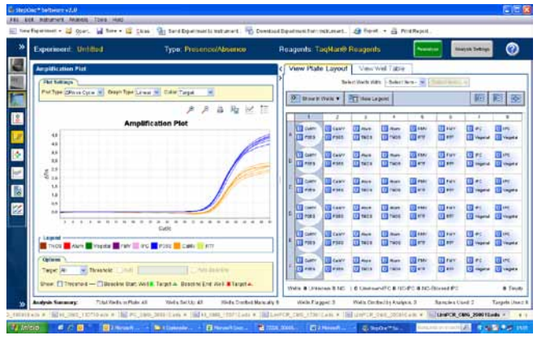

Chart 2: Target P35S blue and target CaMV orange

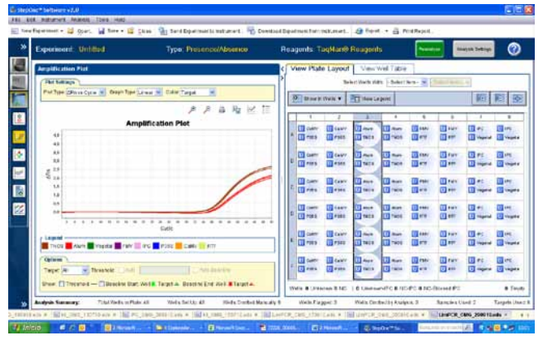

Chart 3: Target TNOS brown and target A.tumefaciens red

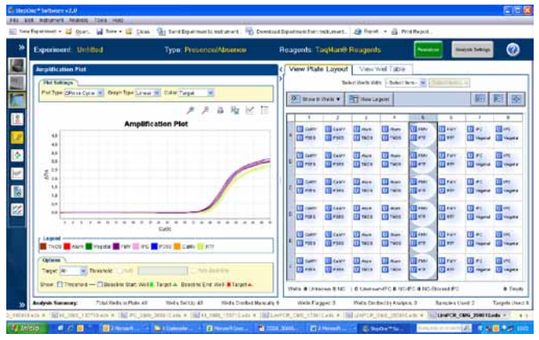

Chart 4: Target P34S purple and target FMV yellow

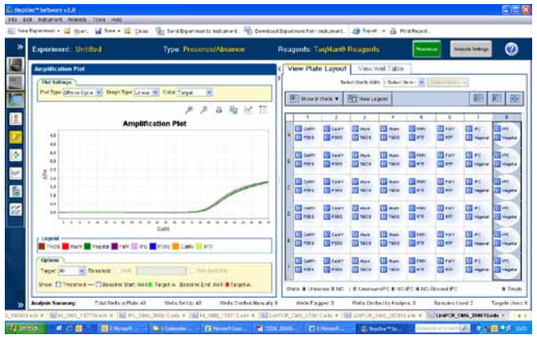

Chart 5: Target Plant green and target IPC purple

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

125 ng of total DNA obtained from any 0.1% certified GMO reference material has more than 50 copies of transgenic DNA. The absolute limit of detection of this kit are 5 copies of DNA, so, as shown in charts 1 to 5 0.01% of any transgenic material could be always created. In raw material it is possible to detect 0.01% of any transgenic material. In case of processed products, the detection limit varies depending on the composition and degradation of DNA produced during the food manufacturing.

6.3. Repeatability and reproducibility

The repeatability assay for the TaqMan GMO Screening kit was performed by amplifying 7 replicates of three different standards for each transgenic region (P35S, TNOS and P34S) by real-time PCR. The standards used were Mon810, Bt11 and H7-1, with a percentage of transgenic material of 0.1%. These assays were performed by varying parameters such as the operative who performed them and the batch of reagents used.

The results obtained in both assays can be found in Table 3 and show that the TaqMan GMO Screening kit has a high degree of repeatability and reproducibility.

Table 3. Results for the repeatability and reproducibility assays.

Event analysed	Standard used	Operative 1	Operative 2
P35S / CaMV	Mon 810 (0.1%100% (7/7)	100% ((7/7)	100% (7/7)
TNOS/A.tumefaciens	Bt 11 (0.1%)	100% (7/7)	100% (7/7)
P34S/FMV	H7-1 (0.1%)	100% (7/7)	100% (7/7)

The following charts show the repeatability results obtained for the GMO Screening Kit for the events analysed.

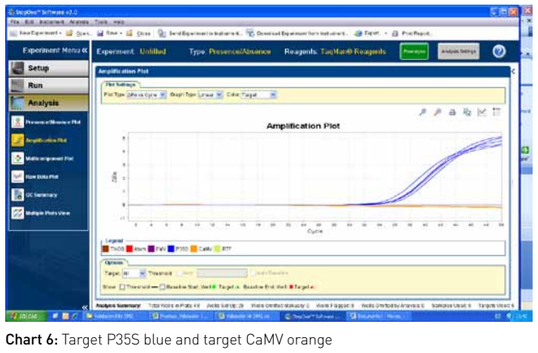

Chart 6: Target P35S blue and target CaMV orange

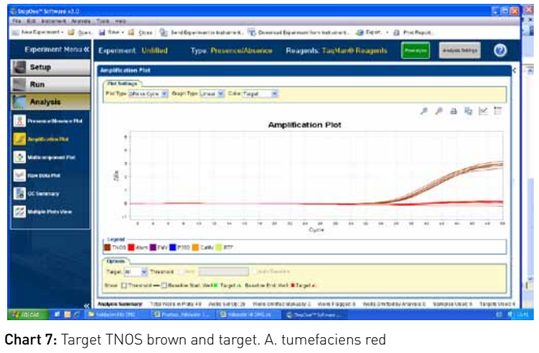

Chart 7: Target TNOS brown and target. A. tumefaciens red

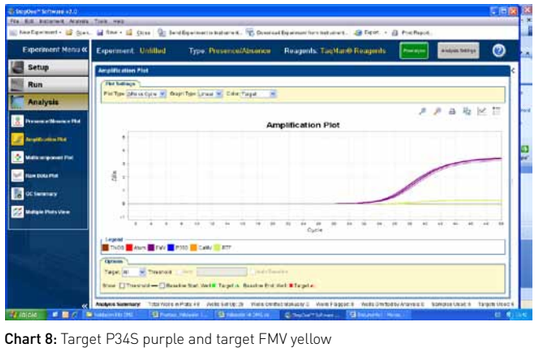

Chart 8: Target P34S purple and target FMV yellow

7. Conclusion

All the results obtained in this validation report allow us to assess the suitability of the TaqMan® GMO Screening kit to detect the presence or absence of genetically modified organism in DNA samples obtained from any food or feed. The innovative design of the kit allows to assign positive and negative results unequivocally.