Oilseed rape quantitative PCR methods

Quantitative PCR method for detection of oilseed rape event Ms1 (verified by the EURL-GMFF in the context of Commission Decision 2007/305/EC)

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of oilseed rape event Ms1 and the oilseed rape host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-BN-005

2. VALIDATION DATA

In-house study coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM maize event MS1 leaves
Tested GM events	
Event Name	MS1
Unique Identifier	ACS-BN004-7
Crop Name	*Brassica napus*

Experimental Design

The EURL-GMFF prepared samples representing five GM levels, namely 0.15%, 0.45%, 0.9%, 2.0% and 3.3% of oilseed rape event MS1 DNA in non-GM oilseed rape DNA. Eight runs were carried out. In each run, samples were analyzed in parallel with both the GM-specific system and the cruA reference system. Five GM levels per run were examined and two replicates for each GM level were analyzed. PCR analysis was performed in triplicate for all samples. The ΔCt method was followed to calculate the relative GM content of the unknown samples.

Method Performance

LOD Relative	≤ 0.045%	**LOD Absolute**	not reported
LOQ Relative	0.09%	**LOQ Absolute**	not reported

Values determined in the in-house study

Test Level (%)	0.15%	0.45%	0.90%	2.0%	3.3%
Mean Value (%)	0.14%	0.48%	0.91%	1.8%	3.0%
RSD_r (%)	10.6%	5.9%	5.1%	11.9%	15.5%
Bias (%)	-3.8%	6.8%	1.3%	-10.9%	-8.5%

	GMO Target
Mean Slope	-3.31
Mean PCR Efficiency %	101
Mean R^2	0.99

Comment
The LOD and LOQ values were provided by the method developer. The relative LOD and LOQ were not assessed in the in-house validation study.

3. REFERENCES

Savini, C. et al. In-house Validation of an Event-specific Method for the Quantification of Oilseed Rape MS1 Using Real-time PCR. Validation Report and Protocol. EUR XXXX EN. 2011. JRCXXXXX (ISBN XXX-XX-XX-XXXX-X)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-ACGCTGCGGACATCTACATT-3'
Target element	Insert
Primer Reverse	5'-CTAGATCGGAAGCTGAAGATGG-3'
Target element	3'-host genome
Amplicon length	187 bp
Probe	5'-FAM-CTCATTGCTGATCCACCTAGCCGACTT-TAMRA-3'
Target element	3' integration border region (IBR) between the insert of oilseed rape event Ms1 and the oilseed rape host genome

Taxon-target(s)

Primer Forward	5'-GGCCAGGGTTTCCGTGAT-3'
Target element	*cruA*
Primer Reverse	5'-CCGTCGTTGTAGAACCATTGG-3'
Target element	*cruA*
Amplicon length	101 bp
Probe	5'-VIC-AGTCCTTATGTGCTCCACTTTCTGGTGCA-TAMRA-3'
Target element	cruciferin A (*cruA*) gene

5. PCR REACTIONS SETUP

GM-target(s) Taxon-target(s)

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200 ng	Template DNA	maximum 200 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	No Cycles
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45