

GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Qualitative PCR method for detection of tE9 terminator (Debode et al., 2016)

Element specific

Last updated 22/12/2016

1. GENERAL INFORMATION

Target genetic element	Terminator of the pea ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) E9 gene
PCR Assay	Simplex Real Time
Detection	TaqMan®
Compendium Reference	QL-ELE-00-024

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM commercial oilseed rape seeds and oilseed rape GT73 CRM (AOCS 0304-B)

Tested GM Events

Event Name	Unique Identifier	Crop Name
GT73 (RT73)	MON-00073-7	<i>Brassica napus</i>

Collaborative Trial Description

The participants received 40 blinded DNA samples representing four GM levels containing GT73 DNA at 0, 5, 10 and 20 target copies of tE9 terminator in a background of salmon sperm DNA (20ng/uL). The negative sample contained non-GM rapeseed DNA at 2500 copies of the *CruA* reference gene. In addition the laboratories received nuclease-free water, a positive control (target at 100 copies), reaction reagents and primers and probes for the tE9 terminator system. The laboratories tested each GM level in duplicate (20 measurements per level per lab) in one run.

Method Performance

LOD95%	5 copies	Confidence Interval	not reported
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Values determined in the collaborative trial

False positive rate (%)	1.92%
False negative rate (%)	0.64%

	Test Level			
	0	5	10	20
Average POD (%)	1.92	99.62	99.23	99.23
CI upper	n.r.	n.r.	n.r.	n.r.
CI lower	n.r.	n.r.	n.r.	n.r.

Unit of Measurement Test Level Target copy N.

3. REFERENCES

Debode F, Huber I, Macarthur R, Rischitor PE, Mazzara M, Herau V, Sebah D, Dobnik D, Broeders S, Roosens NH, Busch U, Berben G, Morisset D, Zel J. Inter-laboratory studies for the validation of two singleplex (tE9 and pea lectin) and one duplex (pat/bar) real-time PCR methods for GMO detection. Food Control 2016: Food Control 73: 452-461

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4. PRIMERS AND PROBES SEQUENCES

GM-target(s) Terminator of the pea ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) E9 gene

Primer Forward 5'-TTTTTATTCGGTTTTTCGCTATCG-3'

Target element T-E9

Primer Reverse 5'-TGAGAATGAACAAAAGGACCATATCA-3'

Target element T-E9

Amplicon length 87 bp

Probe 5'-FAM-TCATTA ACTCTTCTCCATCCATTTCCATTTACAGT-TAMRA-3'

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan Universal PCR Master Mix (2x)	1x
Primer Fw	0.34 $\mu\text{mol/L}$
Primer Rev	0.34 $\mu\text{mol/L}$
Probe	0.30 $\mu\text{mol/L}$
Distilled sterile water	#
Template DNA	5 μL
Final Volume	25 μL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	NoCycles
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Annealing	95°C	15"	
Extension	60°C	60"	
Annealing & Extension			50