Quantitative PCR method for detection of oilseed rape event T45

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of oilseed rape event T45 and the oilseed rape host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-BN-001

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 and GM event T45 oilseed rape

Tested GM events

Event Name	T45 (HCN28)
Unique Identifier	ACS-BN008-2
Crop Name	*Brassica napus* L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 1.8 % and 3.6% of oilseed rape event T45 DNA in non-GM oilseed rape DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the cruciferin (*CruA)* reference gene and for the T45 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤0.045%	≤ 0.04%	**LOD Absolute**	not reported
LOQ Relative	≤0.09%	0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	1.8%	3.6%
Mean Value (%)	0.09%	0.37%	0.88%	1.8%	3.6%
RSD_r (%)	16%	22%	17%	11%	17%
RSD_R (%)	26%	23%	20%	20%	25%
Bias %	-11%	-7.8%	-1.7%	-3.0%	-1.3%

	GMO Target
Mean Slope	not reported
Mean PCR Efficiency %	not reported
Mean R^2	not reported

Comment
The LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Bogni A, Mazzara M, Savini C, Van Den Eede G. Event-specific Method for the Quantification of Oilseed Rape Line T45 Using Real-time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Oilseed Rape. EUR 22357 EN. 2006. JRC34761 (ISBN 92-79-02987-8)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CAATGGACACATGAATTATGC-3'
Target element	5'-host genome
Primer Reverse	5'-GACTCTGTATGAACTGTTCGC-3'
Target element	insert
Amplicon length	123 bp
Probe	5'-FAM-TAGAGGACCTAACAGAACTCGCCGT-TAMRA-3'
Probe Name	TM026
Target element	DNA sequence in the 5'-IBR

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'
Probe Name	TM003
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	Template DNA	maximum 200
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