GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of maize event DAS-40278-9 (Savini et al., 2012)

Event specific ***Maize***

Last updated 15/7/2016

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event DAS-40278-9 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-ZM-004

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from non-GM and GM maize event DAS -40278-9

Tested GM Events

Event Name	Unique Identifier	Crop Name
DAS-40278-9	DAS-40278-9	*Zea mays*

Collaborative Trial Description

The participants received 20 blind test samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.0%, and 5.0% of maize event DAS-40278-9 DNA in non-GM maize DNA. In addition the laboratories received four calibration samples, reaction reagents, primers and probes for the maize high-mobility-group (*hmg*) reference gene and for the DAS-40278-9 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	≤0.04%	**LOD Absolute**	not reported
LOQ Relative	≤0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

	Test Level (%)				
	0.1	0.4	0.9	2	5
Mean Value (%)	0.12	0.38	0.91	2	5.2
RSDr (%)	15%	11%	11%	11%	9%
RSDR (%)	27%	19%	17%	14%	12%
Bias (%)	15%	-4.4%	1.3%	-0.5%	3.5%

Unit of Measurement Test Level % GMO copy N./genome copy N.

	GMO Target	Taxon Target
Mean Slope	-3.33	-3.45
Mean PCR Efficiency %	100	95
Mean R2	1.00	1.00

Comment

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

3. REFERENCES

Savini C, Bogni A, Foti N, Mazzara M, Kreysa J. Event-specific Method for the Quantification of Maize DAS-40278-9 by Real-time PCR - Validation Report and Validated Method - Sampling and DNA Extraction of Maize TC15O7. EUR 25585 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2012. (ISBN 978-92-79-27307-0)

DOI 10.2788/64013

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of maize event DAS-40278-9 and the maize host genome
Primer Forward	5'-CACGAACCATTGAGTTACAATC-3'
Target element	5'-host genome
Primer Reverse	5'-TGGTTCATTGTATTCTGGCTTTG-3'
Target element	insert
Amplicon length	98 bp
Probe	5'-FAM-CGTAGCTAACCTTCATTGTATTCCG-3'-TAMRA

Taxon-target(s)	high-mobility-group (*hmg*) gene
Primer Forward	5'-TTGGACTAGAAATCTCGTGCTGA-3'
Target element	*hmg*
Primer Reverse	5'-GCTACATAGGGAGCCTTGTCCT-3'
Target element	*hmg*
Amplicon length	79 bp
Probe	5'-FAM-CAATCCACACAAACGCACGCGTA-TAMRA-3'

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan Universal PCR Master Mix No UNG	1x
Primer Fw	0.35 µmol/L
Primer Rev	0.35 µmol/L
Probe	0.15 µmol/L
Nuclease-free water	#
Template DNA	5 µL
Final Volume	25 µL

Taxon-target(s)

Reagent	Final Concentration
TaqMan Universal PCR Master Mix No UNG	1x
Primer Fw	0.30 µmol/L
Primer Rev	0.30 µmol/L
Probe	0.18 µmol/L
Nuclease-free water	#
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

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

Stage	Temperature	Time	NoCycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45