GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of maize event MON87460 (Savini et al., 2012)

Event specific ***Maize***

Last updated 19/5/2016

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MON87460 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-ZM-005

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 maize event MON87460 seeds

Tested GM Events

Event Name	Unique Identifier	Crop Name
MON87460	MON-87460-4	*Zea mays*

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 3.0%, and 8.0% of maize event MON87460 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, reaction reagents, primers and probes for the maize high mobility group (*hmg*) reference gene and for the MON87460 specific system. Four replicates for each GM level were analyzed in two runs with both the reference and the transgenic specific system.

Method Performance

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

Values determined in the collaborative trial

	Test Level (%)				
	0.09	0.4	0.9	3	8
Mean Value (%)	0.09	0.42	0.87	3.2	8.2
RSDr (%)	19%	11%	14%	8.1%	6.3%
RSDR (%)	28%	19%	27%	18%	17%
Bias (%)	-1.4%	5.1%	-3.4%	7.0%	2.2%

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

	GMO Target	Taxon Target
Mean Slope	-3.3	-3.3
Mean PCR Efficiency %	101	100
Mean R2	0.99	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, Mazzara M, Pinski G, Van den Eede G. Event-specific Method for the Quantification of Maize MON87460 Using Real-time PCR - Validation Report and Validated Method. EUR 25486 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2012. (ISBN 978-92-79-26179-4)

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4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of maize event MON87460 and the maize host genome
Primer Forward	5'-CACGTTGAAGGAAAATGGATTG-3'
Target element	5'-host genome
Primer Reverse	5'-TCGCGATCCTCCTCAAAGAC-3'
Target element	insert
Amplicon length	82 bp
Probe	5'-FAM-AGGGAGTATGTAGATAAATTTTCAAAGCGTTAGACGGC-TAMRA-3'

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)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan Universal PCR Master Mix (2x)	1x	TaqMan Universal PCR Master Mix (2x)	1x
Primer Fw	0.60 µmol/L	Primer Fw	0.30 µmol/L
Primer Rev	0.60 µmol/L	Primer Rev	0.30 µmol/L
Probe	0.25 µmol/L	Probe	0.16 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	max200 ng	Template DNA	max200 ng
Final Volume	50 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

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

Stage	**Temperature**	**Time**	**NoCycles**
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