GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of maize event MON863 (Mazzara et al., 2005)

Event specific ***Maize***

Last updated 24/7/2015

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MON 863 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-ZM-009

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	maize flour
Materials used for calibration/controls	CRM IRMM-413 (JRC-IRMM)

Tested GM Events

Event Name	Unique Identifier	Crop Name
MON863	MON-00863-5	*Zea mays*

Collaborative Trial Description

The participants received 10 blind maize flour samples representing 5 GM levels, namely 0%, 0.1%, 1.0%, 5.0% and 10.0% of maize event MON863 in non-GM maize (w/w) prepared by the JRC-IRMM. In addition the laboratories received a sample for calibration (10% MON 863 maize flour), two negative target controls (Bt176 maize DNA and non-GM maize flour), reaction reagents, primers and probes for the alcohol dehydrogenase 1 (*adh1)* reference gene and the MON863 specific system. For each blind and calibration sample the laboratories performed an enhanced CTAB DNA extraction followed by spectrophotometric quantification, a real-time PCR monitor run (inhibition test) and a quantitative real-time PCR analysis. Samples were analysed in triplicate (calibrators) or in quadruplicate (blind) on the same plate with both the reference and the transgenic specific system. Two replicates for each GM level were analysed in two separate runs.

Method Performance

LOD Relative	≤0.05%	**LOD Absolute**	not reported
LOQ Relative	0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

	Test Level (%)			
	0.1	1	5	10
Mean Value (%)	0.13	1.2	5	9.4
RSDr (%)	35%	17%	10%	13%
RSDR (%)	35%	18%	18%	21%
Bias (%)	28%	20%	0%	-6%

Unit of Measurement Test Level % GMO (w/w)

	GMO Target	Taxon Target
Mean Slope	-3.9	-3.6
Mean PCR Efficiency %	84	88
Mean R2	0.97	0.97

Comment

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

3. REFERENCES

Mazzara M, Foti N, Price S, Paoletti C, Van Den Eede G. Event-Specific Method for the Quantitation of Maize Line MON 863 Using Real-Time PCR - Validation Report and Protocol. EUR 21830 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2005. JRC32105. (ISBN 92-79-00111-6)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of maize event MON 863 and the maize host genome
Primer Forward	5'-TGTTACGGCCTAAATGCTGAACT-3'
Target element	5'-host genome
Primer Reverse	5'-GTAGGATCGGAAAGCTTGGTAC-3'
Target element	insert
Amplicon lenght	84 bp
Probe	5'-FAM-TGAACACCCATCCGAACAAGTAGGGTCA-TAMRA-3'

Taxon-target(s)	alcohol dehydrogenase1 (*adh1*) gene
Primer Forward	5'-CCTTCTTGGCGGCTTATCTG-3'
Target element	*adh1*
Primer Reverse	5'-CCAGCCTCATGGCCAAAG-3'
Target element	*adh1*
Amplicon lenght	70 bp
Probe	5'-FAM-CTTAGGGGCAGACTCCCGTGTTCCCT-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	0.15 µmol/L	Primer Fw	0.15 µmol/L
Primer Rev	0.15 µmol/L	Primer Rev	0.15 µmol/L
Probe	0.050	Probe	0.05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	max 280 ng	Template DNA	max 280 ng
Final Volume	50 µL	Final Volume	50 µL

Comment

Based on scientific evidence, the adh1 (70 bp) endogenous reference gene assay targets a region in the maize genome that shows a sequence polymorphism, which may affect the efficiency of amplification (Broothaerts et al., 2008). Users of this event-specific quantification method should, therefore, replace the maize adh1 (70 bp) gene assay with the hmg gene assay (or any other suitable maize reference gene assay). Bridging experiments by the EURL-GMFF [EU-RL GMFF validation report (13 November 2013): Report on the verification of the performance of 1507, 59122, MON 810 and NK603 event-specific PCR-based methods applied to DNA extracted from stack maize 1507 x 59122 x MON 810 x NK603 (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx)] have demonstrated that this event-specific quantification method performs adequately in combination with hmg as reference gene target.

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