

*Event specific Soybean***Quantitative PCR method for detection of soybean event MON87701 (Charels et al., 2011)****1. GENERAL INFORMATION**

<b>Target genetic element</b>	5' integration border region (IBR) between the insert of soybean event MON87701 and the soybean host genome
<b>PCR Assay</b>	Simplex Real Time
<b>Detection Chemistry</b>	TaqMan®
<b>Compendium Reference</b>	QT-EVE-GM-010

**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 soybean event MON87701 seeds

**Tested GM Events****Event Name**

MON87701

**Unique Identifier**

MON-87701-2

**Crop Name***Glycine max L.***Collaborative Trial Description**

The participants received 20 blind samples representing five GM levels, namely 0.085%, 0.26%, 0.9%, 2.7%, and 8.1% of soybean event MON87701 DNA in non-GM soybean DNA. In addition the laboratories received five calibration samples, reaction reagents, primers and probes for the soybean lectin (*Le1*) reference gene and the MON87701 specific systems. 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**

<b>Test Level (%)</b>	0.085	0.26	0.9	2.7	8.1
<b>Mean Value (%)</b>	0.09	0.28	0.95	2.9	8.1
<b>RSDr (%)</b>	18%	21%	15%	14%	10%
<b>RSDR (%)</b>	20%	22%	15%	14%	23%
<b>Bias (%)</b>	8.6%	6.4%	5.2%	5.6%	0.1%

Unit of Measurement Test Level copy/copy

	<b>GMO Target</b>	<b>Taxon Target</b>
<b>Mean Slope</b>	-3.5	-3.4
<b>Mean PCR Efficiency %</b>	93	97
<b>Mean R2</b>	0.99	1.00

**Comment**

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

**3. REFERENCES**

Charels D, Mazzara M, Grazioli E, Van den Eede G. Event-specific Method for the Quantification of Soybean Line MON 87701 Using Real-time PCR - Validation Report and Protocol. EUR 25136 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2011. JRC68071 (ISBN 978-92-79-22477-5)

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**4. PRIMERS AND PROBES SEQUENCES**

<b>GM-target(s)</b>	5' integration border region (IBR) between the insert of soybean event MON87701 and the soybean host genome
<b>Primer Forward</b>	5'-TGGTGATATGAAGATACATGCTTAGCAT-3'
<b>Target element</b>	5'-host genome
<b>Primer Reverse</b>	5'-CGTTTCCCGCCTTCAGTTTAAA-3'
<b>Target element</b>	insert
<b>Amplicon length</b>	89 bp
<b>Probe</b>	5'-FAM-TCAGTGTTTGACACACACACTAAGCGTGCC-TAMRA-3'

<b>Taxon-target(s)</b>	lectin (Le1) gene
<b>Primer Forward</b>	5'-CCAGCTTCGCCGCTTCCTTC-3'
<b>Target element</b>	Le1
<b>Primer Reverse</b>	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
<b>Target element</b>	Le1
<b>Amplicon length</b>	74 bp
<b>Probe</b>	5'-FAM-CTTCACCTTCTATGCCCTGACAC-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	0,15 µmol/L
Primer Rev	0,60 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,25 µmol/L	Probe	0,050 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200 ng	Template DNA	maximum 200 ng
Final Volume	50 µL	Final Volume	50 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