Qualitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter

1. GENERAL INFORMATION

Target genetic element	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
PCR Assay	Single
Detection Chemistry	Agarose gelectrophoresis
Compendium Reference	QL-ELE-00-004

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	Biscuits (soybean)
Materials used for calibration/controls	In house produced processed food controls
Tested GM events	
Event Name	GTS 40-3-2
Unique Identifier	MON-04032-6
Crop Name	*Glycine max* L.

Collaborative Trial Description

All laboratories received a detailed method description for DNA extraction using either a CTAB method or a commercially available kit. PCR conditions had to be optimized for their local specific equipment. The method has been evaluated for the detection of genetically modified organisms in biscuits containing each 0%, 2%, and 10% of Roundup-Ready event GTS 40-3-2 soybeans. Each participant received control samples and unknown independent duplicates of GMO samples of which some contained 0% GMOs samples and others contained various percentages of the transgenic event. The participants were requested to analyse each sample once and to specify whether it was considered GMO positive or GMO negative using method for detecting the CaMV P-35S promoter.

Method Performance

LOD Relative	≤2%	**LOD Absolute**	50 HGE
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial

False positive (%)	6.7%
False negative (%)	1.5%

Test Level (%)	0%	2%	10%
Specificity %	93%	-	-
Sensitivity %	-	100%	97%

Comment
The samples were prepared by the collaborative trial coordinator following procedures that resemble as closely as possible the different processing conditions applied by the food industry. The absolute and relative LOD have not been determined for this method.

3. REFERENCES

M. Lipp, A. Bluth, F. Eyquem, L. Kruse, H. Schimmel, G. Van den Eede and E. Anklam (2001) Validation of a method based on polymerase chain reaction for the detection of genetically modified organisms in various processed foodstuffs Eur. Food Res. Technol. 212: 497-504

ISO/FDIS 21569:2005: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products--Qualitative nucleic acid based methods

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CCACGTCTTCAAAGCAAGTGG-3'
Target element	*CaMV P-35S*
Primer Reverse	5'-TCCTCTCCAAATGAAATGAACTTCC-3'
Target element	*CaMV P-35S*
Amplicon length	123 bp
Target element	CaMV 35S promoter

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
Water	#
AmpliTaq Gold® DNA Polymerase	0,8 IU
PCR Buffer 10x (with 15 mmol/L $MgCl_2$)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	640 µmol/L
Primer Fw	0,60 µmol/L
Primer Rev	0,60 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	25”	
Annealing	62°C	30”	
Extension	72°C	45”	
Denaturing, Annealing & Extension			50
Final Extension	72°C	420”	1