

Oilseed rape quantitative PCR methods

Quantitative PCR method for detection of oilseed rape event GT73

1. GENERAL INFORMATION

| | |
|-------------------------------|---|
| Target genetic element | 3' integration border region (IBR) between the insert of oilseed rape event GT73 and the oilseed rape host genome |
| PCR Assay | Simplex Real Time |
| Detection Chemistry | TaqMan® |
| Compendium Reference | QT-EVE-BN-004 |

2. VALIDATION DATA

| | |
|---|--|
| Collaborative trial coordinator | JRC-IHCP |
| Test material applied in collaborative trial | Oilseed rape seeds |
| Materials used for calibration/controls | Genomic DNA samples extracted from non-GM and GM oilseed rape event GT73 seeds |
| Tested GM events | |
| Event Name | GT73 (RT73) |
| Unique Identifier | MON-00073-7 |
| Crop Name | <i>Brassica napus</i> L. |

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 4.0% and 8.0% of oilseed rape event GT73 DNA in non-GM oilseed rape DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the cruciferin (*CruA*) reference gene and for the GT73 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 | 0.085% | LOQ Absolute | not reported |

Values determined in the collaborative trial

| | | | | | |
|----------------------------|--------------|--------------|--------------|-------------|-------------|
| Test Level (%) | 0.10% | 0.40% | 0.90% | 4.0% | 8.0% |
| Mean Value (%) | 0.08% | 0.35% | 0.85% | 4.2% | 8.4% |
| RSD_r (%) | 23% | 17% | 17% | 14% | 14% |
| RSD_R (%) | 28% | 24% | 19% | 17% | 16% |
| Bias % | -25% | -13% | -6% | 5.8% | 4.5% |

| | GM0 Target | Taxon Target |
|------------------------------|-------------------|---------------------|
| Mean Slope | -3.4 | -3.4 |
| Mean PCR Efficiency % | 95 | 95 |
| Mean R² | 0.99 | 0.99 |

Comment

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

3. REFERENCES

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Event-Specific Method for the Quantification of Oilseed Rape Line RT73 Using Real-Time PCR - Validation Report and Protocol - Seeds Sampling and DNA Extraction of Oilseed Rape. EUR 22918 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37550 (ISBN 978-92-79-06935-2)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

| | |
|------------------------|---|
| Primer Forward | 5'-CCATATTGACCATCATACTCATTGCT-3' |
| Target element | Insert |
| Primer Reverse | 5'-GCTTATACGAAGGCAAGAAAAGGA-3' |
| Target element | 3'-host genome |
| Amplicon length | 108 bp |
| Probe | 5'-FAM-TTCCCGGACATGAAGATCATCCTCCTT-TAMRA-3' |
| Probe Name | RT73 probe |
| Target element | DNA sequence in the 3' IBR |

Taxon-target(s)

| | |
|------------------------|---|
| Primer Forward | 5'-GGCCAGGGTTTCCGTGAT-3' |
| Target element | <i>cruA</i> |
| Primer Reverse | 5'-CCGTCGTTGTAGAACCATTGG-3' |
| Target element | <i>cruA</i> |
| Amplicon length | 101 bp |
| Probe | 5'-VIC-AGTCCTTATGTGCTCCACTTTCTGGTGCA-TAMRA-3' |
| Probe Name | TM003 |
| Target element | cruciferin A (<i>cruA</i>) gene |

5. PCR REACTIONS SETUP

GM-target(s) Taxon-target(s)

| Reagent | Final Concentration | Reagent | Final Concentration |
|----------------------------------|---------------------|----------------------------------|---------------------|
| TaqMan® Universal PCR Master Mix | 1x | TaqMan® Universal PCR Master Mix | 1x |
| Primer Fw | 0,15 µmol/L | Primer Fw | 0,20 µmol/L |
| Primer Rev | 0,15 µmol/L | Primer Rev | 0,20 µmol/L |
| Probe | 0,05 µmol/L | Probe | 0,20 µmol/L |
| Nuclease-free water | # | Nuclease-free water | # |
| Template DNA | maximum 200 | Template DNA | maximum 200 |
| Final Volume | 50 µL | Final Volume | 25 µL |

6. AMPLIFICATION CONDITIONS

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

| Stage | Temperature | Time | No Cycles |
|-----------------------------------|-------------|------|-----------|
| 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 |