

## GMOMETHODS: EU DATABASE OF REFERENCE METHODS

### Quantitative PCR method for detection of maize event T25 (Mazzara et al., 2013)

*Event specific*    *Maize*

#### 1. GENERAL INFORMATION

|                               |  |
|-------------------------------|--|
| <b>Target genetic element</b> | 3' integration border region (IBR) between the insert of maize event T25 and the maize host genome |
| <b>PCR Assay</b>              | Simplex Real Time  |
| <b>Detection Chemistry</b>    | TaqMan®  |
| <b>Compendium Reference</b>   | QT-EVE-ZM-011  |

#### 2. VALIDATION DATA

|   |  |
|---|--|
| <b>Collaborative trial coordinator</b>              | JRC-IHCP   |
| <b>Test material applied in collaborative trial</b> | DNA  |
| <b>Materials used for calibration/controls</b>      | Genomic DNA samples extracted from non-GM and GM maize event T25 |

#### Tested GM Events

| Event Name | Unique Identifier | Crop Name |
|------------|-------------------|-----------|
| T25        | ACS-ZM003-2       | Zea mays  |

#### Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.15%, 0.4%, 0.9%, 2.0% and 3.3 % of maize event T25 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the alcohol dehydrogenase 1 (*adh1*) reference gene and for the T25 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The  $\Delta C_t$  method was followed to calculate the GM content of the blind samples.

#### Method Performance

|                     |          |                     |              |
|---------------------|----------|---------------------|--------------|
| <b>LOD Relative</b> | ≤ 0.045% | <b>LOD Absolute</b> | not reported |
| <b>LOQ Relative</b> | ≤0.09%   | <b>LOQ Absolute</b> | not reported |

**Values determined in the collaborative trial**

|                                | Test Level (%) |       |       |      |     |
|--------------------------------|----------------|-------|-------|------|-----|
|                                | 0.15           | 0.4   | 0.9   | 2    | 3.3 |
| <b>Mean Value (%)</b>          | 0.11           | 0.38  | 0.82  | 1.8  | 3.5 |
| <b>RSDr (%)</b>                | 26%            | 22%   | 10%   | 22%  | 11% |
| <b>RSDR (%)</b>                | 26%            | 23%   | 21%   | 22%  | 18% |
| <b>Bias (%)</b>                | -27%           | -6.0% | -9.0% | -12% | 6%  |
| Unit of Measurement Test Level | % GMO          |       |       |      |     |

|                              | DCt   |
|------------------------------|-------|
| <b>Mean Slope</b>            | -3.44 |
| <b>Mean PCR Efficiency %</b> | 92    |
| <b>Mean R2</b>               | 0.97  |

**Comment**

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

**3. REFERENCES**

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Event-specific Method for the Quantification of Maize Line T25 Using Real-time PCR v. 1.01 - Validation Report and Validated Method. EUR 26151 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2013. JRC84152 (ISBN 978-92-79-33030-8)

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**4. PRIMERS AND PROBES SEQUENCES**

|                        |  |
|------------------------|--|
| <b>GM-target(s)</b>    | 3' integration border region (IBR) between the insert of maize event T25 and the maize host genome |
| <b>Primer Forward</b>  | 5'-ACAAGCGTGTCGTGCTCCAC-3'   |
| <b>Target element</b>  | insert   |
| <b>Primer Reverse</b>  | 5'-GACATGATACTCCTTCCACCG-3'  |
| <b>Target element</b>  | 3'-host genome   |
| <b>Amplicon length</b> | 102 bp   |
| <b>Probe</b>           | 5'-FAM-TCATTGAGTCGTTCCGCCATTGTCG-TAMRA-3'  |

|                        |   |
|------------------------|---|
| <b>Taxon-target(s)</b> | alcohol dehydrogenase1 (adh1) gene          |
| <b>Primer Forward</b>  | 5'-CGTCGTTTCCCATCTCTTCCTCCT-3'              |
| <b>Target element</b>  | adh1  |
| <b>Primer Reverse</b>  | 5'-CCACTCCGAGACCCTCAGTC-3'                  |
| <b>Target element</b>  | adh1  |
| <b>Amplicon length</b> | 135 bp                                      |
| <b>Probe</b>           | 5'-FAM-AATCAGGGCTCATTTTCTCGCTCCTCA-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                            | 0.40 µmol/L         | Primer Fw                            | 0.20 µmol/L         |
| Primer Rev                           | 0.40 µmol/L         | Primer Rev                           | 0.20 µmol/L         |
| Probe                                | 0.20 µmol/L         | Probe                                | 0.20 µmol/L         |
| Nuclease-free water                  | #                   | Nuclease-free water                  | #                   |
| Template DNA                         | maximum             | Template DNA                         | maximum             |
| Final Volume                         | 25 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       |