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## **Certified Reference Materials**

### **AOCS 0215-B**

Report of the certification process for

MON 87411

Maize Certified Reference Materials

First Batch

OECD Unique ID MON-87411-9

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ISO 17034:2016  
A2LA Certificate 3438.01

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## Abstract

This report describes the preparation and certification of the maize CRM AOCS 0215-B produced by AOCS Technical Services in 2019. The CRMs have been prepared according to ISO Guide 17034:2016 and are intended to serve as control material for third party testing of maize for transformation events. Devitalized seed of MON 87411 was provided by Monsanto Company, St. Louis, MO (hereinafter “Monsanto”). The MON 87411 seed was milled by grinding the bulk source according to maize processing protocols at Texas A&M University. The certified value of AOCS 0215-B was based on the purity of the bulk seed material and with 95% confidence, the true value is  $\geq 996$  g/kg. The powder was aliquoted and bottled in 27-mL glass headspace vials and sealed under a nitrogen gas environment at Illinois Crop Improvement Association. The presence of MON 87411 in the maize was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory). CRM samples should be stored in a dry, sealed container at ambient or cooler conditions in the dark.

## **Acknowledgements**

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## Glossary

|                   |   |
|-------------------|---|
| AOCS              | American Oil Chemists' Society  |
| Conventional Crop | Crop variety with no history of transgenic technology and is produced through traditional plant-breeding techniques that rely on selecting and mating parent plants possessing promising traits and repeatedly selecting for superior performance among their offspring   |
| DNA               | Deoxyribonucleic Acid is the linear, double-helix macromolecule that makes up the genetic material of most organisms  |
| Detection Limit   | Lowest level at which target DNA can be detected in a sample.   |
| EC                | European Commission   |
| Genome            | The full set of genes and associated DNA characteristic of an organism  |
| ISO               | International Organisation for Standardisation  |
| GMO               | Organism that has had genetic sequences modified using molecular-level techniques   |
| PCR               | Polymerase Chain Reaction: technique used to determine whether a sample of plant tissue contains a particular DNA sequence. PCR relies on primer sets that zero in on a particular target DNA sequence and a special DNA-copying enzyme (DNA polymerase) that makes enough copies of the target sequence for identification and measurement |
| Qualitative PCR   | PCR methods that determine the presence or absence of a specific target DNA sequence at a particular level of detection   |

|                    |  |
|--------------------|--|
| Quantitation Limit | Lowest level at which the amount of target DNA sequence in a sample can be reproducible.           |
| Quantitative PCR   | PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules |
| Trait : MON 87411  | Corn rootworm resistance and glyphosate tolerance  |

## **Introduction**

Plant genetic modification is an extension of traditional plant breeding. It allows plant breeders to develop crops with specific traits including insect, disease, and herbicide resistance; processing advantages; and nutritional enhancement. An important component for identifying these new traits is a Certified Reference Material created from leaf, seed, or grain containing the new trait as well as a CRM created from the conventionally bred matrix. The European Commission has mandated that from 18 April 2004, a method for detecting a new event derived from transgenic technology and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new crop derived from transgenic technology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above regulatory requirements for GMO determination, AOCS 0215-B was manufactured from maize according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

## **Material Processing**

MON 87411 maize seeds used to prepare AOCS 0215-B were hemizygous through successive breeding generations, and the donor for the MON 87411 maize event was the female parent. Monsanto devitalized 15 kg of MON 87411 maize seed, and delivered the bulk seed to AOCS. The MON 87411 seed was milled by Texas A&M University according to industry standard maize processing procedures. Illinois Crop Improvement Association was contracted for packaging the samples. The powder was aliquoted and bottled in 27-mL glass headspace vials and sealed under a nitrogen gas environment.

## **Trait Verification to Certify Presence of MON 87411**

Prior to packaging, bulk seed powder samples were taken from randomly selected areas and depths to form a 3 kg composite sample in accordance with the International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 500 kg, five (5) working samples of 10 g each were prepared from the composite sample and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for

event-specific, qualitative PCR analysis. The analyses performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) were used to verify the presence of MON 87411 (Table 1).

**Table 1. Trait verification testing on random composite samples of MON 87411 maize performed by Eurofins-GeneScan on bulk material provided by Monsanto**

| Sample             | MON 87411 Presence |
|--------------------|--------------------|
| Composite Sample 1 | Positive           |
| Composite Sample 2 | Positive           |
| Composite Sample 3 | Positive           |
| Composite Sample 4 | Positive           |
| Composite Sample 5 | Positive           |

After the bulk material was packaged, the presence of the MON 87411 trait was assessed on five (5) random vials of AOCS 0215-B. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait presence and to rule out degradation during packaging. AOCS 0215-B sample numbers, 614, 287, 240, 552, and 60 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for MON 87411 event-specific, qualitative PCR analysis (Table 2). This data confirms the presence of the MON 87411 in vials of AOCS 0215-B.

**Table 2. Trait verification testing on AOCS 0215-B MON 87411 maize performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory).**

| Sample          | MON 87411 Presence |
|-----------------|--------------------|
| AOCS 0215-B 614 | Positive           |
| AOCS 0215-B 287 | Positive           |
| AOCS 0215-B 240 | Positive           |
| AOCS 0215-B 552 | Positive           |
| AOCS 0215-B 60  | Positive           |

## Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0215-B was assessed by Monsanto. A total of 720 maize seeds were subjected to individual seed testing for the presence of MON 87411 by qualitative event-specific PCR. 720 of the 720 seeds tested positive for the presence of MON 87411.

Purity estimation was calculated using SeedCalc8 (Remund *et al.*, 2008) and the Certified Value corresponds to the lower bound true % purity. The % purity in the sample was 100% when 720 seeds were tested. Using a 95% confidence level, the true % purity of the MON 87411 seed lot was 99.6%. Consequently, with 95% confidence, the true value is  $\geq 996$  g/kg.

The Measurement Uncertainty was based on the lower bound of the true % purity and is the expanded uncertainty with a coverage factor of 1.65 and confidence level of 95%. The expanded measurement uncertainty for AOCS 0215-B is 2 g/kg.

## Homogeneity

The homogeneity of AOCS 0215-B is related to the purity of the seeds. 720 out of 720 seeds tested positive for the MON 87411 maize event. Based on the sample purity of 100%, as determined using SeedCalc8, the batch was considered to be homogeneous.

In addition, the homogeneity of the MON 87411 trait was confirmed when 5 random vials of AOCS 0215-B were selected and were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to verify the presence of MON 87411 in the samples (See Trait Verification section and Table 2).

## Stability

Stability of these CRMs has been listed as 1 year from the introduction date. The materials were processed and are stored at ambient temperature, under nitrogen gas, in 27 -mL glass headspace vials. These materials are expected to be stable for longer than the estimated expiration date. The stability of the powder material will be reevaluated at time of expiration. If the samples still test positive for the presence of the intended trait, the certificates will be extended.

## References

Eurofins-GeneScan; 2219 Lakeshore Drive, Suite 400, New Orleans, LA 70122; Telephone: +1 504 297 4330 Toll Free: +1 866 535 2730 Fax: +1 504 297 4335 <https://www.eurofinsus.com/food-testing/testing-services/gmo/>

Illinois Crop Improvement Association, 3105 Research Road, Champaign, IL 61826; Telephone: +1 217 359 4053 Fax: +1 217 359 4075; <http://www.ilcrop.com/index.htm>

ISO Guide 17034:2016 (E) General requirements for the competence of reference material producers

ISO 17025:2005 and ISO 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories

International Seed Testing Association, International Rules of Seed Testing: Seed Science and Technology Rules, 2012

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