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## **Certified Reference Materials**

### **AOCS 0406-A2**

Report of the certification process for

Non-Modified Maize

Certified Reference Materials

Second Batch

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ISO 17034:2016

**A2LA Certificate 3438.01**

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## **Abstract**

This report describes the preparation and certification of maize CRM AOCS 0406-A2 produced by AOCS Technical Services in 2021. This CRM has been prepared according to ISO 17034:2016 and is intended to serve as control material for third party testing of maize or grain for transformation events. The non-modified maize powder was provided by Monsanto Company, St. Louis, MO (hereinafter “Monsanto”). It was prepared by grinding the bulk seed at Monsanto. The certified value of AOCS 0406-A2 was based on the purity of the bulk seed material and with 95% confidence, the true value is < 0.8 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 absence of MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 in AOCS 0406-A2 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 or Non-modified crop	Crop variety with no history of genetic engineering and is produced through 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 exist in a sample and be reliably tested by PCR methods. It is typically expressed as a percentage: the ratio of the number of transgenically derived genomes to the number of crop genomes times 100 percent
EC	European Commission
GMO	Organism that has had genetic sequences modified using molecular-level techniques
Genome	The full set of genes and associated DNA characteristics of an organism
ISO	International Organisation for Standardisation
ISTA	International Seed Testing Association

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. It is typically expressed as the ration of the number of transgenic genomes to the number of crop genomes times 100 percent.
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules

## **Introduction**

Plant biotechnology is an extension of traditional plant breeding. It allows plant breeders to develop crops with specific beneficial 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 non-modified bred matrix. The European Commission has mandated that from 18 April 2004, a method for detecting a new biotech event and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new genetically modified crop. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above analytical requirements for GM determination, AOCS 0406-A2 was manufactured from maize seed according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRMs is available from AOCS.

## **Material Processing**

Monsanto milled ~10 kg of non-modified maize seed. All of the seed powder was passed through a 500  $\mu$ m mesh sieve. The seed powder was delivered to AOCS who contracted Illinois Crop Improvement Association 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**

The absence of the MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 events in the non-modified maize material was assessed on 10 random vials of AOCS 0406-A2. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait absence. Sample numbers that were randomly selected were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to verify the absence of the traits in the samples (Table1).

**Table 1. Trait verification testing on AOCS 0406-A2 non-modified maize performed by Eurofins- GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for absence of MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419.**

<b>AOCS 0406-A2 Sample</b>	<b>Trait Presence</b>
Sample # 21	Negative
Sample # 105	Negative
Sample # 158	Negative
Sample # 276	Negative
Sample # 333	Negative
Sample # 475	Negative
Sample # 560	Negative
Sample # 617	Negative
Sample # 722	Negative
Sample # 854	Negative

### **Certified Value and Measurement Uncertainty**

The genetic purity of the seed lot used to produce AOCS 0406-A2 was assessed by Monsanto. A total of 3750 maize seeds were subjected to individual seed testing for the presence of MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 by qualitative event-specific PCR. 3750 of the 3750 seeds tested negative for the presence of MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419.

Purity estimation was calculated using SeedCalc8 (Remund *et al.*, 2008). The % impurity in the sample was 0%, when 3750 seeds were tested. Using a 95% confidence level, the true % impurity of MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 seed lot was at least 0.08%. Consequently, with 95% confidence, the true value is < 0.8 g/kg.

The Measurement Uncertainty was based on the upper bound of the true % purity and

is the expanded uncertainty with a coverage factor of 1.65 and a confidence level of 95%. The expanded measurement uncertainty for AOCS 0406-A2 is 0 g/kg.

## **Homogeneity**

The homogeneity of AOCS 0406-A2 is related to the purity of the seeds. 3750 out of 3750 seeds tested negative for the MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 maize event by event-specific PCR. Based on the sample impurity of 0%, as determined using SeedCalc8, the batch was expected to be homogenous.

In addition, the homogeneity of the MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 trait was confirmed when 10 random vials of AOCS 0406-A2 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 absence of MON 87411, MON 87403, MON 88017, MON 89034, MON 87460, MON 87427 and MON 87419 in the samples (See Trait Verification section and Table 1).

## **Stability**

Stability of these CRMs has been listed as 1 year from the certification date. The materials have been ground and is stored frozen under Nitrogen gas in a sealed, glass vial. These materials are expected to be stable for longer than the estimated expiration date. The stability of the ground material will be reevaluated at time of expiration. If the samples are still representative of the certified value, 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 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

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed; <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32003R1829&from=en>

Remund K., Simpson R., Laffont J-L., Wright D., and Gregoire S. Seedcalc8. 2008. <https://www.seedtest.org/en/statistical-tools-for-seed-testing- content---1--3449--1102.html>