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# Certified Reference Materials

# AOCS 0922-A

Report of the certification process for

Non-Modified Maize

Certified Reference Materials

First 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 0922-A produced by AOCS Technical Services in 2022. 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 Corteva Agriscience, Johnston, IA. It was prepared by grinding the bulk seed at Corteva Agriscience. The certified value of AOCS 0922-A was based on the purity of the bulk seed material and is 0 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 DAS-01131-3 in AOCS 0922-A was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan GmbH, Freiburg, Germany (an ISO 17025 accredited laboratory). CRM samples should be stored in a dry, sealed container at ambient or cooler conditions in the dark.

## Acknowledgements

The authors would like to express sincere appreciation and gratitude to several individuals and their companies for support and guidance throughout this project. Thanks go to Margit Ross, Corteva Agriscience, for offering AOCS the opportunity to manufacture and distribute these products; to Melissa Salazar at Illinois Crop Improvement Association for packaging the samples; and to Anna Holt and Franziska Peters, Eurofins-GeneScan GmbH for event-specific, qualitative PCR analysis including the provision of information on running the analyses and interpreting the results.

## Glossary

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| AOCS | American Oil Chemists' Society |
| Conventional/ 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 |

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|---|---|
| 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 0922-A was manufactured from maize seed according to ISO 17034:2016 and in accordance with EC No 1829/2003, EC No 641/2004 and EC No 619/2011. This CRM is available from AOCS.

## Material Processing

Corteva Agriscience milled ~4 kg of non-modified maize seed. All of the seed powder was passed through a 500 µM 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 DAS-01131-3 events in the non-modified maize material was assessed on 10 random vials of AOCS 0922-A. 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 GmbH, Freiburg, Germany (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 0922-A non-modified maize performed by Eurofins- GeneScan GmbH (an ISO 17025 accredited laboratory) for absence of DAS-01131-3.**

| AOCS 0922-A Sample | Trait Presence |
|---|---|
| Sample # 9 | Negative |
| Sample # 35 | Negative |
| Sample # 61 | Negative |
| Sample # 137 | Negative |
| Sample # 173 | Negative |
| Sample # 187 | Negative |
| Sample # 235 | Negative |
| Sample # 280 | Negative |
| Sample # 318 | Negative |
| Sample # 343 | Negative |

## Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0922-A was assessed by Corteva Agriscience. A total of 3200 maize seeds were subjected to seed pool testing for the presence of DAS-01131-3 by qualitative event-specific PCR. 3200 of the 3200 seeds tested negative for the presence of DAS-01131-3.
Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008). The % impurity in the sample was 0%, when 3200 seeds were tested.

The measurement uncertainty is the expanded uncertainty using the value of the upper bound of impurity of 0.9 g/kg. The standard uncertainty can be obtained by dividing the expanded uncertainty by $2\sqrt{3}$ (rectangular distribution).

The standard uncertainty for AOCS 0922-A is 0.3 g/kg.

## Homogeneity

The homogeneity of AOCS 0922-A is related to the purity of the seeds. 3200 out of 3200 seeds in seed pools tested negative for the DAS-01131-3 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 DAS-01131-3 trait was confirmed when 10 random vials of AOCS 0922-A were selected and were sent to Eurofins-GeneScan GmbH, Freiburg, Germany (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to verify the absence of DAS-01131-3 in the samples (See Trait Verification section and Table 1).

## Stability

Time, temperature and light are regarded as the most relevant influences on the stability of CRM (Linsinger, *et al*., 2001). The influence of light is mitigated by shipping and storing the vials in boxes, thus minimizing the possibility of degradation due to light. The influence of temperature is mitigated by storing the vials in a temperature-controlled room, and shipping vials at ambient temperature.

Stability of these CRMs has been listed as 1 year from the certification date. The materials have been ground and are stored at ambient temperature, under nitrogen gas in a sealed, 27-mL glass headspace vials. 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 GmbH, Engresserstraße 4, D-79108 Freiburg, Germany Telephone: +49 761 6400 4011 Online: https://www.eurofinsus.de/food-analysis

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

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