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Certified Reference Materials

AOCS 0723-A

Report of the certification process for

Non-Modified

Maize

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 0723-A produced by AOCS Technical Services in 2023. 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 Pioneer Hi-Bred International, Inc., Johnston, IA. It was prepared by grinding the bulk seed at Pioneer Hi-Bred International, Inc.. The certified value of AOCS 0723-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 DP-051291-2 in AOCS 0723-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.

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Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of modern biotechnology 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
Cycle threshold (Ct)	Number of PCR cycles required for the fluorescent signal to cross a threshold that exceeds background level
DNA	Deoxyribonucleic Acid
Detection Limit	Lowest level at which target DNA can be detected in a sample and be reliably tested by PCR methods. It is typically expressed as a percentage: the ratio of the number of modern biotechnology derived genomes to the number of crop genomes times 100 percent
EC	European Commission
Genome	The full set of genes and associated DNA characteristic of an organism
GMO	Organism that has had genetic sequences modified using molecular-level techniques
ISO	International Organization for Standardization
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 reliably quantified. 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

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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 0723-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

Pioneer Hi-Bred International, Inc. 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 DP-051291-2 events in the non-modified maize material was assessed on 10 random vials of AOCS 0723-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).

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Table 1. Trait verification testing on AOCS 0723-A non-modified maize performed by Eurofins- GeneScan, Freiburg, Germany (an ISO 17025-accredited laboratory) for absence of DP-051291-2.

AOCS 0723-A Sample	Trait Presence
Sample # 12	Negative
Sample # 56	Negative
Sample # 90	Negative
Sample # 119	Negative
Sample # 146	Negative
Sample # 161	Negative
Sample # 236	Negative
Sample # 283	Negative
Sample # 314	Negative
Sample # 383	Negative

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0723-A was assessed by Pioneer Hi-Bred International, Inc.. A total of 3200 seeds were subjected to seed testing for the presence of DP-051291-2 by qualitative event-specific PCR. 3200 of the 3200 seeds tested negative for the presence of DP-051291-2.

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 was based on the upper limit of impurity at 0.9 g/kg (rectangular distribution). The upper limit of impurity is used as the upper boundary of the standard uncertainty. The standard uncertainty is obtained by dividing it by $2*\sqrt{3}$.

The standard uncertainty for AOCS 0723-A is 0.3 g/kg.

Homogeneity

The homogeneity of AOCS 0723-A is related to the purity of the seeds. 3200 out of 3200 seeds were subjected to seed testing and all tested negative for the DP-051291-2 maize event by event-specific PCR. Based on the sample impurity of 0%, as determined using SeedCalc8, the batch was expected to be homogenous.

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In addition, the homogeneity of the DP-051291-2 trait was confirmed when 10 random vials of AOCS 0723-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 DP-051291-2 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 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 negative for the presence of the intended trait, the certificates will be extended.

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References

Eurofins-GeneScan GmbH, Engesserstraße 4, D-79108 Freiburg, Germany; Telephone: +49 761 6400 4022 Fax: +49 761 6400 4011 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; https://www.ilcrop.com/

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