AOCS
STREET ADDRESS
3356 Big Pine Trail, Ste. C&D,
Champaign, IL 61822 USA
MAILING ADDRESS
PO Box 7230,
Champaign, IL 61826 USA
+1 217-359-2344
general@aocs.org
aocs.org

Certified Reference Materials

AOCS 0804-C2

Report of the certification process for

MON 531

Cotton

Second Batch

OECD Unique ID MON-ØØ531-6

Zoe Serelis
Technical Services Manager

Tiffanie West
Technical Services Director

A2LA ACCREDITED
ISO 17034:2016
A2LA Certificate 3438.01

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Table of Contents

Abstract 4

Acknowledgements 5

Glossary 6

Introduction 7

Material Processing 7

Trait Verification to Certify Presence of MON 531 7

Certified Value and Measurement Uncertainty 8

Homogeneity 9

Stability 9

References 11

Abstract

This report describes the preparation and certification of the cotton CRM AOCS 0804-C2 produced by AOCS Technical Services in 2021. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of cottonseeds for transformation events. The cotton MON 531 powder was provided by Bayer CropScience, St. Louis, MO. It was prepared by grinding the bulk seed at Bayer CropScience. The certified value of AOCS 0804-C2 was based on the purity of the bulk seed material and with 95% confidence, the true value is ≥ 963 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 531 in AOCS 0804-C2 was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory). CRM samples should be stored dry in a 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
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
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
Trait: MON531	Bollgard® cotton

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 modern biotechnology and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new crop derived from modern biotechnology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold before accepting a shipment.

To meet the above analytical requirements for GM determination, AOCS 0804-C2 was manufactured from cottonseeds 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

The MON 531 cotton seed used to prepare AOCS 0804-C2 were homozygous resulting from several cycles of self-pollination. Bayer CropScience milled ~4 kg of MON 531 cotton seed. All the seed powder was passed through a 710 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 to Certify Presence of MON 531

The presence of the MON 531 trait was assessed on 10 random vials of AOCS 0804-C2. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait presence. 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 presence of MON 531 in the samples (Table 1).

Table 1. Trait verification testing on AOCS 0804-C2 MON 531 cotton performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory).

AOCS 0804-C2 Sample	Trait MON 531 Presence
Sample # 25	Positive
Sample # 89	Positive
Sample # 121	Positive
Sample # 170	Positive
Sample # 193	Positive
Sample # 225	Positive
Sample # 258	Positive
Sample # 281	Positive
Sample # 327	Positive
Sample # 374	Positive

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0804-C2 was assessed by Bayer CropScience. A total of 664 cottonseed seeds were subjected to individual seed testing for the presence of MON 531 by qualitative event-specific PCR. 656 of the 664 seeds tested positive for the presence of MON 531.

Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008) and corresponds to the lower bound of true % purity. The % purity in the sample was 98.8%, when 664 seeds were tested. Using a 95% confidence level, the true % purity of the MON 531 seed lot was at least 96.3%. Consequently, with 95% confidence, the true value is ≥ 963 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 2 and confidence level of 95%. It is obtained by combining the uncertainties from the purity assessment ($u_{char,rel}$), the homogeneity assessment ($u_{bb,rel}$), the transport stability assessment ($u_{sts,rel}$) and the long-term stability assessment ($u_{lts,rel}$):

$$u_{CRM,rel} = \sqrt{{u_{char,rel}}^2 + {u_{bb,rel}}^2 + {u_{sts,rel}}^2 + {u_{lts,rel}}^2}$$

$$U_{CRM} = 2 \times u_{CRM,rel} \times 1000\ g/kg$$

When using an asymmetric uncertainty, the reported measurement uncertainty is truncated on the right side such that the value does not exceed 1000 g/kg. Consequently, the expanded measurement uncertainty for AOCS 0804-C2 is +12 g/kg, -25 g/kg.

Homogeneity

The homogeneity of AOCS 0804-C2 is related to the purity of the seeds. 656 out of 664 cottonseed seeds tested positive for the MON 531 cottonseed event by event-specific PCR. Based on the sample purity of 98.8%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of the MON 531 trait was confirmed when 10 random vials of AOCS 0804-C2 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 531 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.

The effect of temperature and time are investigated.

A transport (short-term) stability study is conducted to assess the stability of maize CRM during transport. The temperature and time conditions in the study cover the typical conditions and the not so rare situations. The outcome of the study is considered transferable to other CRMs of similar property. Samples were subject to 3 different temperatures (4 ℃ (fridge), 25 ℃ (ambient), 60 ℃ (oven)) for 4 different durations (0, 1, 2, and 4 weeks). The study concluded that samples are stable at 4 ℃ (fridge) and 25 ℃ (ambient) for 4 weeks. The estimated uncertainty contribution from transport (short-term) stability is 1.0%.

A long-term stability study is conducted to assess the stability of maize CRM during storage. Samples are stored at 25 ℃ (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperate studied is 25 ℃ and the length of time to be studied is 10 years. The outcome of the study is considered transferable to other CRMs of similar property. In the initial 1-year stability study, samples were subject the storage condition for 4 different durations (0, 1, 3, 6 and 12 months). The study concluded that samples are stable at 25 ℃ (ambient) for 12 months. The estimated uncertainty contribution from long-term stability is 0.42%.

Stability of these CRMs has been listed as 1 year from the certification date. The materials were processed and are stored at ambient or lower temperature, under nitrogen, in 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 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; https://www.ilcrop.com/

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

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

ISO 17034:2016, General Requirements for the Competence of Reference Material Producers

Linsinger, T., Pauwels, J., van der Veen, A. *et al.* Homogeneity and stability of reference materials. *Accred Qual Assur* 6**,** 20–25 (2001). doi.org/10.1007/s007690000261

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1829&from=en

Remund K, Simpson R, Laffont J-L, Wright D, and Gregoire S. Seedcalc8. 2008 https://www.seedtest.org/en/services-header/tools/statistics-committee/statistical-tools-seed-testing.html