



**Mail Address:**

AOCS, P.O. Box 17190, Urbana, IL 61803-7190 USA

**Street Address:**

AOCS, 2710 S. Boulder Drive, Urbana, IL 618026996 USA

**Phone:** +1-217-359-2344; **Fax:** +1-217-351-8091;

**E-Mail:** [general@aocs.org](mailto:general@aocs.org); **Web:** [www.aocs.org](http://www.aocs.org)

# **Certified Reference Materials**

## **AOCS 0804-A2**

Report of the certification process for

Non-Modified Cotton

Certified Reference Materials

Second Batch

Denise Williams  
Technical Service Manager

Tiffanie West  
Technical Service Director



ISO 17034:2016  
A2LA Certificate 3438.01

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## **Abstract**

This report describes the preparation and certification of the cotton CRM AOCS 0804-A2 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 non-modified cotton powder was provided by Bayer CropScience, St. Louis, MO (hereinafter “Bayer CropScience”). It was prepared by grinding the bulk seed at Bayer CropScience. The certified value of AOCS 0804-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 1445, MON 88701, MON 531, MON 15985 and MON 88913 in AOCS 0804-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 dry in a 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 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
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 Organisation for Standardisation
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

## **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-A2 was manufactured from cottonseeds according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

## **Material Processing**

Bayer CropScience milled ~10 kg of non-modified cotton seed. All of the seed powder was passed through a 710  $\mu$ 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 1445, MON 88701, MON 531, MON 15985 and MON 88913 events in the non-modified cotton material was assessed on 10 random vials of AOCS 0804-A2. 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 1445, MON 88701, MON 531, MON 15985 and MON 88913 in the samples (Table 1).

**Table 1. Trait verification testing on AOCS 0804-A2 non-modified cotton performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for presence of MON 1445, MON 88701, MON 531, MON 15985 and MON 88913.**

<b>AOCS 0804-A2 Sample</b>	<b>Trait MON 1445, MON 88701, MON 531, MON 15985, MON 88913 Presence</b>
Sample # 71	Negative
Sample # 183	Negative
Sample # 219	Negative
Sample # 334	Negative
Sample # 477	Negative
Sample # 540	Negative
Sample # 655	Negative
Sample # 738	Negative
Sample # 862	Negative
Sample # 915	Negative

## Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0804-A2 was assessed by Bayer CropScience. A total of 3750 cottonseed seeds were subjected to individual seed testing for the presence of MON 1445, MON 88701, MON 531, MON 15985 and MON 88913 by qualitative event-specific PCR. 3750 of the 3750 seeds tested negative for the presence of MON 1445, MON 88701, MON 531, MON 15985 and MON 88913.

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 the MON 1445, MON 88701, MON 531, MON 15985 and MON 88913 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 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 ( $u_{lts,rel}$ ):

$$u_{CRM,rel} = \sqrt{u_{char,rel}^2 + u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2}$$
$$U_{CRM} = 1.65 \times u_{CRM,rel} \times 1000 \text{ g/kg}$$

The expanded measurement uncertainty for AOCS 0804-A2 is 0.4 g/kg.

## Homogeneity

The homogeneity of AOCS 0804-A2 is related to the purity of the seeds. 3750 out of 3750 cottonseed seeds tested negative for the MON 1445, MON 88701, MON 531, MON 15985 and MON 88913 cottonseed events by event-specific PCR. Based on the

sample impurity of 0%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of non-modified cotton was confirmed when 10 random vials of AOCS 0804-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 1445, MON 88701, MON 531, MON 15985 and MON 88913 events 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 °C (fridge), 25 °C (ambient), 60 °C (oven)) for 4 different durations (0, 1, 2, and 4 weeks). The study concluded that samples are stable at 4 °C (fridge) and 25 °C (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 °C (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperate studied is 25 °C 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 °C (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; <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

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