Street Address:
3356 Big Pine Trail, Ste C&D
Champaign, IL 61822 USA
Phone: +1-217-359-2344;
E-Mail: CRM@aocs.org; **Web:** www.aocs.org

Certified Reference Materials

AOCS 0113-A2

Report of the certification process for

MON 88701 Cotton

Certified Reference Materials

Second Batch

OECD Unique ID MON-887Ø1-3

Denise Williams
Technical Services Manager

Tiffanie West
Technical Services Director

ACCREDITED CERT #3438.01 ISO 17034:2016
A2LA Certificate 3438.01

Legal Notice

Neither AOCS nor any person acting on behalf of AOCS is responsible for the use which might be made of the following information.

AOCS Mission Statement

AOCS advances the science and technology of oils, fats, proteins, surfactants and related materials, enriching the lives of people everywhere.

More information regarding AOCS is available at http://www.aocs.org

Table of Contents

Abstract

This report describes the preparation and certification of the cotton CRM AOCS 0113-A2 produced by AOCS Technical Services in 2020. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of cotton for transformation events. The cotton MON 88701 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 0113-A2 was based on the purity of the bulk seed material and with 95% confidence, the true value is ≥ 977 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 88701 in AOCS 0113-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

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 Jack Milligan, Bayer CropScience, for offering AOCS the opportunity to manufacture and distribute these products; to Sandra Harrison and Charlie Drennan at Illinois Crop Improvement Association for packaging the samples; and to Frank Spiegelhalter, Greg Ditta, E. Pearce Smith, and Daniel Thompson, Eurofins-GeneScan for event-specific, qualitative PCR analysis including the provision of information on running the analyses and interpreting the results.

Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of transgenic technology and is produced through traditional 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 be detected in a sample.
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 reproducible.
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
Trait: MON 88701	Glufosinate herbicide and Dicamba herbicide tolerance

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

To meet the above regulatory requirements for GMO determination, AOCS 0113-A2 was manufactured from cotton 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.

Materials Processing

The MON 88701 cotton seed used to prepare AOCS 0113-A2 were homozygous resulting from several cycles of self-pollination. Bayer CropScience milled ~5 kg of MON 88701 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 88701

The presence of the MON 88701 trait was assessed on 10 random vials of AOCS 0113-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 88701 in the samples (Table 1).

Table 1. Trait verification testing on AOCS 0113-A2 MON 88701 maize performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory).

AOCS 0113-A2 Sample	Trait MON 88701 Presence
Sample # 12	Positive
Sample # 60	Positive
Sample # 106	Positive
Sample # 158	Positive
Sample # 216	Positive
Sample # 264	Positive
Sample # 301	Positive
Sample # 345	Positive
Sample # 397	Positive
Sample # 481	Positive

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0113-A2 was assessed by Bayer CropScience. A total of 350 cotton seeds were subjected to individual seed testing for the presence of MON 88701 by qualitative event-specific PCR. 350 of the 350 seeds tested positive for the presence of MON 88701.

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 100%, when 350 seeds were tested. Using a 95% confidence level, the true % purity of the MON 88701 seed lot was at least 97.7%. Consequently, with 95% confidence, the true value is ≥ 977 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 ($u_{lts,rel}$):

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

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

Consequently, the expanded measurement uncertainty for AOCS 0113-A2 is -23 g/kg.

Homogeneity

The homogeneity of AOCS 0113-A2 is related to the purity of the seeds. 350 out of 350 cotton seeds tested positive for the MON 88701 cotton seed event by event-specific PCR. Based on the sample purity of 100%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of the MON 88701 trait was confirmed when 10 random vials of AOCS 0113-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 presence of MON 88701 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

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