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Certified Reference Materials

AOCS 0906-A2

Report of the certification process for

Non-Modified Soybean

Certified Reference Materials

Second 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 the soybean CRM AOCS 0906- 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 soybean for transformation events. The non-modified soybean 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 0906-A2 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 MON 88701, MON 87708, MON 87751 and MON 87769 and below limit of quantification (LOQ) of MON 89788 and MON 87705 in AOCS 0906-A2 was verified using event-specific, qualitative and quantitative 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 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
ISO	International Organization for Standardization
GMO	Organism that has had genetic sequences modified using molecular-level techniques
PCR	Polymerase Chain Reaction: technique used to determine whether a sample of plant tissue contains a specific DNA sequence. PCR relies on primer sets that zero in on a specific 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 specific 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

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 0906-A2 was manufactured from soybeans 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

Bayer CropScience milled ~10 kg of non-modified soybean seed. All of 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

The absence of MON 88701, MON 87708, MON 87751 and MON 87769 and below limit of quantification (LOQ) of MON 89788 and MON soybean events in the non-modified soybean material was assessed on 10 random vials of AOCS 0906-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 and quantitative PCR analysis to verify the absence or below limit of quantification (LOQ = .05%) of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 in the samples (Table 1).

Table 1. Trait verification testing on AOCS 0906-A2 non-modified soybean performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for presence of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769.

AOCS 0906-A2 Sample	Trait MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 presence
Sample # 47	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 101	Negative (below LOQ for MON 87705)
Sample # 213	Negative (below LOQ for MON 87705)
Sample # 375	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 442	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 539	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 665	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 723	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 804	Negative (below LOQ for MON 89788 and MON 87705)
Sample # 936	Negative (below LOQ for MON 89788 and MON 87705)

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0906-A2 was assessed by Bayer CropScience. A total of 3750 soybeans were subjected to individual seed testing for the presence of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 by qualitative event-specific PCR. 3750 of the 3750 soybeans tested negative for the presence of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769.

Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008). The % impurity in the sample was 0% when 3750 seeds were tested.

The measurement uncertainty is the expanded uncertainty using the value of the upper bound of impurity at 0.8 g/kg. The standard uncertainty can be obtained by dividing the expanded uncertainty by $2\sqrt{3}$ (rectangular distribution).

The standard uncertainty for AOCS 0906-A2 is 0.2 g/kg.

Homogeneity

The homogeneity of AOCS 0906-A2 is related to the purity of the seeds. 3750 out of 3750 soybeans tested negative for the MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 soybean 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 soybean was confirmed when 10 random vials of AOCS 0906-A2 were selected and were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative and quantitative PCR analysis to verify he absence of MON 88701, MON 87708, MON 87751 and MON 87769 and below limit of quantification (LOQ= .05%) of MON 89788 and MON 87705 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.

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.

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

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