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Certified Reference Materials

AOCS 0911-A

Report for the certification process for

Non-Modified

Soybean

First Batch

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ISO 17034:2016
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Abstract

This report describes the preparation and certification of the soybean certified reference material (CRM) AOCS 0911-A produced by AOCS Technical Services in 2011. The CRM has been prepared according to ISO Guides 30 through 35 and is intended to serve as control material for third-party testing of soybeans for biotechnology-derived events. The purity of the Non-modified soybeans was verified using BPS-CV127-9 event-specific, qualitative PCR analysis by Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory). The certified value of AOCS 0911-A was based on the purity of the bulk seed material and is 0 g/kg. AOCS 0911-A is available in 27-mL glass headspace vials. The soybeans ("non-modified") were provided by BASF Agricultural Solutions US LLC and were clean grain. AOCS devitalized the bulk soybeans at BASF Agricultural Solutions US LLC and then transferred the coarsely milled material to AOCS. The soybeans were further processed by grinding the bulk sources according to standard soybean processing protocols by Texas A&M University and were then packaged under a nitrogen gas environment at Illinois Crop Improvement Association. The powder sample shall be stored dry in a sealed container at ambient or lower temperature and in the dark.

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Glossary

AOCS	American Oil Chemists' Society
BPS-CV127-9	Tolerance to the herbicide imidazolinone herbicide, derived from a single transformation event
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 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 corresponding Non-modified crop. The European Commission (EC) has mandated that from 18 April 2004, a method for detecting a new event derived from modern biotechnology and Reference Material must be available before the EC will consider authorizing a new food or feed derived from modern biotechnology. Several nations outside of Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above analytical requirements for biotechnology-derived event determination, AOCS 0911-A 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

BASF Agricultural Solutions US LLC delivered 14 kg of non-modified soybeans, devitalized by AOCS on the BASF Agricultural Solutions US LLC premises, to AOCS. The materials were clean grain. Before the materials were shipped to Texas A&M University for processing to a uniform particle size, primary samples were taken from randomly selected areas and depths to form a 3 kg composite sample in accordance with the International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 100 kg. Ten (10) working samples of 100 g each were prepared from the composite sample and sent to Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory) for BPS-CV127-9 event-specific, qualitative PCR analysis. The analyses performed by Eurofins GeneScan were used to assess the purity and homogeneity of the seed lot.

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These non-modified soybeans were processed according to industry-standard soybean processing procedures, packaged in 27-mL glass headspace vials, and sealed under a nitrogen gas environment.

Trait Verification

The absence of the CV127 trait was assessed on ten (10) random vials of 0911-A. AOCS used the Microsoft Excel Random Number Generator function of Microsoft Excel to select samples for verification of the trait absence. Sample numbers that were randomly selected were sent to Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory) for BPS-CV127-9 event-specific, qualitative PCR analysis. These results are presented in Table 1. This data shows that no contamination occurred during the packaging of AOCS 0911-A.

Table 1. Results for the verification of AOCS 0911-A [non-modified soybean] material as tested by Eurofins GeneScan with BPS-CV127-9 event-specific, qualitative PCR analysis.

Sample	BPS-CV127-9 Presence
AOCS 0911-A 34	Negative
AOCS 0911-A 58	Negative
AOCS 0911-A 59	Negative
AOCS 0911-A 161	Negative
AOCS 0911-A 175	Negative
AOCS 0911-A 233	Negative
AOCS 0911-A 331	Negative
AOCS 0911-A 373	Negative
AOCS 0911-A 401	Negative
AOCS 0911-A 466	Negative

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The AOCS 0911-A CRM was prepared from non-modified soybeans. Sample heterogeneity was not considered because there was no blending of non-modified and modern biotechnology-derived soybeans into defined mixtures.

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0911-A was assessed by BASF Agricultural Solutions US LLC A total of 3000 soybean seeds were subjected to testing for the presence of CV127 by qualitative event-specific PCR. 3000 of the 3000 seeds tested negative for the presence of CV127.

Purity estimation was calculated using SeedCalc8 (Remund et al., 2008). The % impurity in the samples was 0% when 3000 seeds were tested.

The measurement uncertainty is the expanded uncertainty using the value of the upper bound of impurity at 1 g/kg. The standard uncertainty can be obtained by dividing the expanded uncertainty by $2\sqrt{3}$ (rectangular distribution).

The standard uncertainty for this CRM is 0.3 g/kg.

Homogeneity

The homogeneity of AOCS 0911-A is related to the purity of the seeds. 3000 out of 3000 seeds tested negative for the CV127 soybean trait by event-specific PCR. Based on the sample impurity of 0%, as determined using SeedCalc8, the batch was expected to be homogenous.

After the non-modified soybean seed was ground and bottled as described above, ten samples of AOCS 0911-A were randomly selected using the Microsoft Excel Random Number Generator function and were sent to Eurofins GeneScan, Metairie, LA (an ISO 17025-accredited laboratory) for BPS-CV127-9 event-specific, qualitative PCR analysis. The test results for the non-modified soybean bulk material are presented in Table 2 and further confirm the homogeneity of AOCS 0911-A.

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Table 2. Results of the homogeneity testing performed by Eurofins GeneScan on the 0911-A non-modified soybean material.

Sample	BPS-CV127-9 Presence
Homogeneity Sample 1	Negative
Homogeneity Sample 2	Negative
Homogeneity Sample 3	Negative
Homogeneity Sample 4	Negative
Homogeneity Sample 5	Negative
Homogeneity Sample 6	Negative
Homogeneity Sample 7	Negative
Homogeneity Sample 8	Negative
Homogeneity Sample 9	Negative
Homogeneity Sample 10	Negative

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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Illinois Crop Improvement Association, 3105 Research Road, Champaign, IL 61826; Telephone: +1 217 359 4053 Fax: +1 217 359 4075; www.ilcrop.com/

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