Certified Reference Materials AOCS 0906-A and AOCS 0906-B

Report of the certification process for

Conventional and MON89788

Soybean Seed Certified Reference Materials

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Abstract

This report describes the preparation and certification of the soybean seed CRMs AOCS 0906-A and AOCS 0906-B produced by AOCS Technical Services in 2006. These CRMs have been prepared according to ISO Guides 30-35 and are intended to serve as control material for third party testing of soybean seed for transformation events. The purity of the conventional and MON89788 soybean was verified using DNA- and protein- based detection methods. AOCS 0906-A and AOCS 0906-B are available in 27 -mL glass headspace vials. The conventional soybean (Line A3244) and Roundup RReady2Yield™ (MON89788; GLP-0504-16045) soybean were clean seed quality provided by Monsanto Company, St. Louis, MO, USA. The soybean seed was prepared by grinding the bulk sources according to standard soybean processing protocols and was then packaged under a Nitrogen environment. The ground sample shall be stored dry in a sealed container at +4° C in the dark.

™ Roundup RReady2Yield is a trademark of Monsanto Technology LLC.

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Glossary

AOCS	American Oil Chemists' Society
Conventional Variety	Crop variety with no history of genetic engineering 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
CP4 EPSPS	Glyphosate tolerance derived by inserting a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) encoding gene from *Agrobacterium tumefaciens* strain CP4
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 exist in a sample and be reliably tested by PCR methods. It is typically expressed as a percentage: the ratio of the number of transgenically 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
Genome	The full set of genes and associated DNA characteristic of an organism
IRMM	Institute for Reference Materials and Measurement
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

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 conventionally bred matrix. The European Commission has mandated that from 18 April 2004, a method for detecting a new biotech event and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new genetically modified crop. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level ranging from 0.90 to 5% of authorized biotech events before accepting a shipment.

To meet the above analytical requirements for GM determination, AOCS 0906-A and AOCS 0906-B were manufactured from soybean seed according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

Monsanto Company (St. Louis, MO) delivered 25 kg conventional soybean seed (Line: A3244) and 25 kg of MON89788 soybean (GLP-0504-16045) to AOCS. The materials were clean seed quality. Before the materials were shipped to Texas A&M University, primary samples were taken from randomly selected areas and depths to form a 5 kg composite sample in accordance with the International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 500 kg. Ten working samples of 100 g each were prepared from the composite sample and sent to Eurofins GeneScan, Metairie, LA (ISO 17025 Accredited laboratory) for qualitative PCR analysis. The analysis performed by Eurofins GeneScan was used to assess the purity and homogeneity of the seed lot.

Five hundred seeds were randomly selected from the composite sample and analyzed with Strategic Diagnostics Inc Trait✓ RUR Leaf and Seed Test Kit to verify seed-lot purity.

The conventional seed (Line: A3244) was processed according to standard soybean processing procedure, packaged in 27 -mL headspace vials, and sealed under a Nitrogen environment. AOCS used the Random Number Generator function of Microsoft Excel 2003 to select samples for verification of purity, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 0906-A: 1, 9, 21, 125, 175, 271, 427, 542, 694, and 704 were sent to Eurofins GeneScan (New Orleans, LA) for event-specific qualitative PCR analysis to screen for MON89788 presence in the samples.

After the non-modified seed was completely ground and packaged, the MON89788 soybean was processed according to standard soybean processing procedure, packaged in 27 -mL headspace vials, and sealed under a Nitrogen environment. AOCS used the Random Number Generator function of Microsoft Excel 2003 to select samples for verification of purity, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 0906-B: 40, 61, 370, 406, 525, 645, 656, 734, 742, and 797 were sent to Eurofins GeneScan USA (New Orleans, LA) for event-specific qualitative PCR analysis to screen for MON89788 presence in the samples.

Stability of these CRMs has been listed as 1 year from the introduction date. The materials have been ground and are stored frozen under Nitrogen gas in a sealed, glass vial. These materials are expected to be stable for longer than the estimated expiration date. The stability of the ground material will be reevaluated at time of expiration. If the samples are still representative of the certified value, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The following tables are the purity data for the homogeneity samples. The non-modified Soybean (Line: A3244) are presented in Table 1. Results for the MON89788 soybean are presented in Tables 2 and 3.

Table 2 includes the data generated from the Strategic Diagnostics Inc Trait✓ RUR Leaf and Seed Test Kit. Five hundred seeds were test and (500 out of 500) were positive for the Monsanto Roundup RReady2Yield trait that produces the CP4 EPSPS protein. The 10 (100 g) samples sent to GeneScan USA for MON89788 screening by qualitative PCR analysis are presented in Table 3. These results, coupled with the results from seeds tested by the Strategic Diagnostics' test strips, conclude that the only quantifiable biotech event present in AOCS 0906-B soybean is MON89788.

Table 1. Results from Eurofins GeneScan for the homogeneity of conventional soybean seed (Line: A3244).

Sample	MON89788 Presence (LOQ = 0.05; Std. Dev = 0.05)
MC-A-1	Negative
MC-A-2	Negative
MC-A-3	0.2 %
MC-A-4	0.1 %
MC-A-5	Negative
MC-A-6	Negative
MC-A-7	0.2 %
MC-A-8	Negative
MC-A-9	Negative
MC-A-10	Negative

Table 2. Results from administering Strategic Diagnostics Inc Trait✓ RUR Leaf and Seed Test Kit for the presence of CP4 EPSPS in 500 MON89788 soybean seeds.

Seeds Tested	Results
Conventional	0
MON89788*	500
Unreacted Strips	0
* All the seeds in this line exhibit the trait (500/500) with 95% confidence.	

Table 3. Results from Eurofins GeneScan for the homogeneity of MON89788 soybean (GLP-0504-16045) seeds.

Sample	MON89788 Presence
MC-B-11	Positive
MC-B-12	Positive
MC-B-13	Positive
MC-B-14	Positive
MC-B-15	Positive
MC-B-16	Positive
MC-B-17	Positive
MC-B-18	Positive
MC-B-19	Positive
MC-B-20	Positive

Prepared Sample Verification

Once the seeds were packaged, 10 samples of each variety were identified by the Microsoft Excel Random Number Generator and sent to Eurofins GeneScan (New Orleans, LA) for event-specific qualitative PCR analysis. Table 4 verifies that no contamination was introduced during the packaging phase of AOCS 0906-A. The 10 MON89788 soybean samples are presented in Table 5. These data show no contamination occurred during the packaging of AOCS 0906-B. These results are in agreement with the homogeneity data presented in Tables 1, 2, and 3.

Table 4. Results for the verification of AOCS 0906-A [conventional soybean seed (Line: A3244)] as tested by Eurofins GeneScan.

Sample AOCS 0906-A	MON89788 Presence (LOQ = 0.05)
1 of 1000	< 0.05
9 of 1000	< 0.05
21 of 1000	< 0.05
125 of 1000	< 0.05
175 of 1000	< 0.05
271 of 1000	< 0.05
427 of 1000	< 0.05
542 of 1000	< 0.05
694 of 1000	< 0.05
704 of 1000	< 0.05

Table 5. Results for the verification of AOCS 0906-B [MON89788 soybean (GLP-0504-16045)] seeds as tested by Eurofins GeneScan.

Sample AOCS 0906-B	MON89788 Presence
40 of 1000	Positive
61 of 1000	Positive
370 of 1000	Positive
406 of 1000	Positive
525 of 1000	Positive
645 of 1000	Positive
656 of 1000	Positive
734 of 1000	Positive
742 of 1000	Positive
797 of 1000	Positive

References

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ISO Guide 30:1992 (E/F), Terms and definitions used in connection with reference materials

ISO Guide 31:2000 (E), Reference Materials- Contents of certificates and labels

ISO Guide 32:1997 (E) Calibration in analytical chemistry and use of certified reference materials

ISO Guide 33:2000 (E) Uses of certified reference materials

ISO Guide 34:2000 (E) General requirements for the competence of reference material producers

ISO Guide 35:1989 (E) Certification of reference materials-General and statistical principles

International Seed Testing Association, International Rules of Seed Testing: Seed Science and Technology Rules, Volume 21, Supplement, Rules, 1993

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