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Certified Reference Materials

AOCS 0806-A

AOCS 0806-B

AOCS 0806-C

AOCS 0806-D

Report of the certification process for

Conventional and EH92-527-1

Potato

First Batch

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A2LA ACCREDITED
ISO 17034:2016
A2LA Certificate 3438.01

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Abstract

This report describes the preparation and certification of the Potato CRM AOCS 0806-A, -B, -C, and -D produced by AOCS Technical Services in 2006. This CRM has been prepared according to ISO Guides 30-35 and is intended to serve as control material for third party testing of Potato for transformation event EH92-527-1. The purity of the genetically modified Potato was verified using DNA-based detection methods. AOCS 0806-A and -C are packaged in 10 g aliquots and are available in 27 -mL glass headspace vials. AOCS 0806-B and -D are packaged in 1 g aliquots and are available in 10 mL glass headspace vials. One lot, approximately 25 kg, of Solanum tubersum cultivar Prevalent-b was provided by Plant Science Sweden, a BASF Agricultural Solutions US LLC . The potato was prepared by lyophilizing and then grinding the bulk material according to standard potato processing protocols and was then packaged under a Nitrogen environment. One lot, approximately 25 kg, of genetically modified Solanum tubersum cultivar EH92-527-1 was provided by Plant Science Sweden, a BASF Agricultural Solutions US LLC . The potato was prepared by lyophilizing and then grinding the bulk material according to standard potato processing protocols and was then packaged under a Nitrogen environment. The ground samples shall 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 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, propagation material, 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 biotechnology-derived 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 biotechnology-derived events before accepting a shipment.

To meet the above analytical requirements for GM determination, AOCS 0806-A, -B, -C, and -D were manufactured from Potato tubers according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

AOCS received appropriate quality bulk material, 25 kg of Prevalent-b Potato, from Plant Science Sweden and made arrangements for Michigan State University to process the materials. Before the materials were shipped to Michigan State University, primary samples were taken from randomly selected areas and depths to form a 5 kg composite sample (approximately 32 potatoes). The composite sample was sent for separate processing and was then divided into ten working samples which were sent to Eurofins GeneScan, New Orleans, LA (ISO 17025-accredited laboratory) for qualitative PCR analysis. The analyses performed by Eurofins GeneScan were used to assess the purity and homogeneity of the lot.

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The conventional potato (Prevalent-b) was packaged both in 27 and 10 -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 0806-A: 18, 43, 61, 114, 154, 170, 173, 183, 189, 199, and AOCS 0806-B: 38, 40, 79, 81, 90, 113, 126, 171, 174, 192 were sent to Eurofins GeneScan (New Orleans, LA) for qualitative PCR analysis to screen for EH92-527-1 presence in the samples.

The genetically modified potato (EH92-527-1) was packaged both in 27 and 10 -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 0806-C: 1, 9, 19, 26, 28, 31, 33, 36, 53, 60, and AOCS 0806-D: 13, 29, 43, 51, 52, 70, 71, 115, 134, 140 were sent to Eurofins GeneScan (New Orleans, LA) for qualitative PCR analysis to screen for EH92-527-1 presence in the samples.

Stability

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.

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Results and Discussion

Sample Homogeneity

The following tables are the purity data for the homogeneity samples. The Prevalent-b potato data are presented in Table 1 and the EH92-527-1 potato data are presented in Table 2. The conventional potato, Prevalent-b, were all negative for presence of EH92-527-1 by event-specific Qualitative-PCR analysis and the genetically-modified potato, EH92-527-1, were all positive for the presence of EH92-527-1 by event-specific Qualitative-PCR analysis. These CRMs were prepared solely as identity preserved samples of genetically modified Potato. Sample heterogeneity was not considered because there was no blending of conventional and genetically modified potato into defined mixtures.

Table 1. Results from Eurofins GeneScan for the homogeneity of Prevalent-b potatoes.

Sample	EH92-527 Presence
BASF-1	Negative
BASF-2	Negative
BASF-3	Negative
BASF-4	Negative
BASF-5	Negative
BASF-6	Negative
BASF-7	Negative
BASF-8	Negative
BASF-9	Negative
BASF-10	Negative

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Table 2. Results from Eurofins GeneScan for the homogeneity of EH92-527-1 potatoes.	
Sample	**EH92-527-1 Presence**
BASF-11	Positive
BASF-12	Positive
BASF-13	Positive
BASF-14	Positive
BASF-15	Positive
BASF-16	Positive
BASF-17	Positive
BASF-18	Positive
BASF-19	Positive
BASF-20	Positive

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Prepared Sample Verification

Once the ground Potato was packaged, 10 samples of each variety were identified by the Microsoft Excel 2003 Random Number Generator and sent to Eurofins GeneScan (New Orleans, LA) for qualitative PCR analysis. Tables 3 through 6 verify that no contamination was introduced during the packaging phase of AOCS 0806-A, -B, -C, or -D. These results are in agreement with the homogeneity data presented in Tables 1 and 2.

Table 3. Results from Eurofins GeneScan for the 10-g package verification of Prevalent-b potatoes.

Sample	EH92-527-1 Presence
AOCS 0806-A 18	Negative
AOCS 0806-A 43	Negative
AOCS 0806-A 61	Negative
AOCS 0806-A 114	Negative
AOCS 0806-A 154	Negative
AOCS 0806-A 170	Negative
AOCS 0806-A 173	Negative
AOCS 0806-A 183	Negative
AOCS 0806-A 189	Negative
AOCS 0806-A 199	Negative

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Table 4. Results from Eurofins GeneScan for the 1-g package verification of Prevalent-b potatoes.	
Sample	**EH92-527-1 Presence**
AOCS 0806-B 38	Negative
AOCS 0806-B 40	Negative
AOCS 0806-B 79	Negative
AOCS 0806-B 81	Negative
AOCS 0806-B 90	Negative
AOCS 0806-B 113	Negative
AOCS 0806-B 126	Negative
AOCS 0806-B 171	Negative
AOCS 0806-B 174	Negative
AOCS 0806-B 192	Negative

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Table 5. Results from Eurofins GeneScan for the 10-g package verification of EH92-527-1 potatoes.	
Sample	**EH92-527-1 Presence**
AOCS 0806-C 1	Positive
AOCS 0806-C 9	Positive
AOCS 0806-C 19	Positive
AOCS 0806-C 26	Positive
AOCS 0806-C 28	Positive
AOCS 0806-C 31	Positive
AOCS 0806-C 33	Positive
AOCS 0806-C 36	Positive
AOCS 0806-C 53	Positive
AOCS 0806-C 60	Positive

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Table 6. Results from Eurofins GeneScan for the 1-g package verification of EH92-527-1 potatoes.	
Sample	**EH92-527-1 Presence**
AOCS 0806-D 13	Positive
AOCS 0806-D 29	Positive
AOCS 0806-D 43	Positive
AOCS 0806-D 51	Positive
AOCS 0806-D 52	Positive
AOCS 0806-D 70	Positive
AOCS 0806-D 71	Positive
AOCS 0806-D 115	Positive
AOCS 0806-D 134	Positive
AOCS 0806-D 140	Positive

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Eurofins GeneScan; 2315 N Causeway Blvd, Suite 200, Metairie, LA 70001; Telephone: +1 504 297 4330; Toll Free: +1 866 535 2730; Fax: +1 504 297 4335. http://www.eurofinsus.com/locations/genescan.html, accessed February 23, 2012

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