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Certified Reference Materials

AOCS 1206-A2

Report of the certification process for

Non-Modified

Sugar Beet

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 sugar beet Certified Reference Material AOCS 1206-A2 produced by AOCS Technical Services in 2021. The CRM has been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of sugar beet for transformation events. Non-modified sugar beet powder was provided by Bayer CropScience LP and KWS SAAT SE & Co. KGaA. It was prepared by grinding the bulk seed at IBRL at University of Illinois. The certified value of AOCS 1206-A2 was based on the purity of the bulk seed material and with 95% confidence, the true value is < 3.0 g/kg for the H7-1 event only. The non-modified sugar beet powder was then packaged in 27-mL glass headspace vials and sealed under a nitrogen gas environment at Illinois Crop Improvement Association. The absence of H7-1 in the sugar beet was verified using event-specific, qualitative PCR analysis by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory). The absence of KWS20-1 in the sugar beet finished vials was verified using event-specific, quantitative PCR analysis by Eurofins GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory). With 95% confidence, the true value for the KWS20-1 event is < 0.25 g/kg. This CRM 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 (CRM) created from leaf, seed, or grain containing the new trait as well as a CRM created from the conventional counterpart. The European Commission has mandated that from 18 April 2004, a method for detecting a new event derived from modern biotechnology and CRM must be available before the EC will consider authorizing acceptance of a new crop derived from modern biotechnology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above analytical requirements for GMO determination, AOCS 1206-A2 was manufactured from sugar beet 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

IBRL at University of Illinois milled ~6 kg of non-modified sugar beet 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 the H7-1 trait in the non-modified sugar beet material was assessed on 10 random vials of AOCS 1206-A2. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait absence. Sample numbers that were randomly selected were sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to verify the absence of the H7-1 trait in the samples (*Table1*). Presence of amplifiable sugar beet DNA in the samples was verified by the amplification of glutamine synthetase (GS), a sugar beet endogenous reference gene.

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The absence of the KWS20-1 trait in the non-modified sugar beet material was assessed on 10 random vials of AOCS 1206-A2. Sample numbers that were randomly selected were sent to Eurofins GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory) for event-specific, quantitative PCR analysis to verify the absence of the traits in the samples (*Table 2*). Presence of amplifiable sugar beet DNA in the samples was verified by the amplification of glutamine synthetase (GS), a sugar beet endogenous reference gene.

Table 1. Trait verification testing on AOCS 1206-A2 non-modified sugar beet performed by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory) for presence of H7-1.

AOCS 1206-A2 Sample	Trait Presence
Sample # 25	Negative
Sample # 89	Negative
Sample # 107	Negative
Sample # 158	Negative
Sample # 230	Negative
Sample # 256	Negative
Sample # 283	Negative
Sample # 313	Negative
Sample # 378	Negative
Sample # 402	Negative

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Table 2. Trait verification testing on AOCS 1206-A2 non-modified sugar beet performed by Eurofins GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory) for presence of KWS20-1.

AOCS 1206-A2 Sample	Trait Presence
Sample # 11	<0.025%
Sample # 18	<0.025%
Sample # 138	<0.025%
Sample # 169	<0.025%
Sample # 252	<0.025%
Sample # 270	<0.025%
Sample # 344	<0.025%
Sample # 401	<0.025%
Sample # 407	<0.025%
Sample # 408	<0.025%

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 1206-A2 was assessed by AOCS. A total of 1000 sugar beet seeds were ground and tested in one pool, with an LOD of 0.05%, by real-time quantitative PCR for H7-1.

Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008). The % impurity for event H7-1 in the sample was 0% when a pool of 1000 grounded seeds were tested.

The measurement uncertainty for event H7-1 is the expanded uncertainty using the value of the upper bound of impurity at 3 g/kg. The standard uncertainty can be obtained by dividing the expanded uncertainty by $2\sqrt{3}$ (rectangular distribution).

The standard uncertainty for AOCS 1206-A2 event H7-1 is 0.9 g/kg.

The certified value of the KWS20-1 event was based on quantitative PCR testing of 10 sample vials of AOCS 1206-A2. With 95% confidence, the certified value of KWS20-1 is < 0.25 g/kg. The upper bound of impurity (0.25 g/kg) represents the upper boundary of the expanded uncertainty, with a coverage factor of 2 at a 95% confidence level.

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Homogeneity

The homogeneity of AOCS 1206-A2 is related to the purity of the seeds. A total of 1000 sugar beet seeds were ground and tested in one pool, with an LOD of 0.05%, by quantitative PCR for H7-1. Based on the sample impurity of 0%, as determined using SeedCalc8, the batch was expected to be homogenous.

In addition, the homogeneity of the non-modified sugar beet was confirmed when 10 random vials of AOCS 1206-A2 were selected and were sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory) for event-specific qualitative PCR analysis to verify the absence of H7-1 in the samples (see the *Trait Verification* section and *Table 1*) and were sent to Eurofins GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory) for event-specific quantitative PCR analysis to verify the absence of KWS20-1 in the samples (see the *Trait Verification* section and *Table 2*).

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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Eurofins-GeneScan GmbH, Engresserstraße 4, D-79108 Freiburg, Germany Telephone: +49 761 6400 4011 Online: https://www.eurofinsus.de/food-analysis

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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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