

Certified Reference Materials

AOCS 0304-A3

Report of the certification process for
Non-Modified
Canola
Third 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 canola CRM AOCS 0304-A3 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 canola for transformation events. Non-modified canola 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 0304-A3 was based on the purity of the bulk seed material and is 0 g/kg. The non-modified canola 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 GT73/RT73, MON 94100 and MON 88302 in the canola was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory). This CRM shall be stored dry in a sealed container in ambient or cooler conditions in the dark.

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Glossary

<i>AOCS</i>	American Oil Chemists' Society
<i>Conventional Crop</i>	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
<i>Cycle threshold (Ct)</i>	Number of PCR cycles required for the fluorescent signal to cross a threshold that exceeds background level
<i>DNA</i>	Deoxyribonucleic Acid
<i>Detection Limit</i>	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
<i>EC</i>	European Commission
<i>Genome</i>	The full set of genes and associated DNA characteristic of an organism
<i>GMO</i>	Organism that has had genetic sequences modified using molecular-level techniques
<i>ISO</i>	International Organization for Standardization
<i>PCR</i>	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
<i>Qualitative PCR</i>	PCR methods that determine the presence or absence of a specific target DNA sequence at a particular level of detection
<i>Quantitation Limit</i>	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
<i>Quantitative PCR</i>	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules

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 event derived from modern biotechnology and Certified Reference Material 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 0304-A3 was manufactured from canola according to ISO 17034:2016 and in accordance with EC No 1829/2003, EC No 641/2004 and EC No 619/2011. The CRM is available from AOCS.

Material Processing

Bayer CropScience milled ~10 kg of non-modified maize 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 GT73/RT73, MON 94100 and MON 88302 traits in the non-modified canola material was assessed on 10 random vials of AOCS 0304-A3. 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 Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to verify the absence of the traits in the samples (Table1).

Table 1. Trait verification testing on AOCS 0304-A3 non-modified canola performed by Eurofins- GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) for presence of GT73/RT73, MON 94100 and MON 88302.

AOCS 0304-A3 Sample	Trait Presence
Sample # 94	Negative
Sample # 179	Negative
Sample # 271	Negative
Sample # 340	Negative
Sample # 468	Negative
Sample # 505	Negative
Sample # 672	Negative
Sample # 774	Negative
Sample # 837	Negative
Sample # 923	Negative

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0304-A3 was assessed by Bayer CropScience. A total of 3500 maize seeds were subjected to individual seed testing for the presence of GT73/RT73, MON 94100 and MON 88302 by qualitative event-specific PCR. 3500 of the 3500 seeds tested negative for the presence of GT73/RT73, MON 94100 and MON 88302.

Purity estimation was calculated using SeedCalc8 (Remund *et al.*, 2008). The % impurity in the sample was 0%, when 3500 seeds were tested.

The measurement uncertainty is the expanded uncertainty using the value of the upper bound of impurity at 0.9 g/kg. The standard uncertainty can be obtained by dividing the expanded uncertainty by $2\sqrt{3}$ (rectangular distribution).

The standard measurement uncertainty for AOCS 0304-A3 is 0.2 g/kg.

Homogeneity

The homogeneity of AOCS 0304-A3 is related to the purity of the seeds. 3500 out of 3500 seeds tested negative for the GT73/RT73, MON 94100 and MON 88302 canola events by event-specific PCR. 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 canola was confirmed when 10 random vials of AOCS 0304-A3 were selected and were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to verify the absence of GT73/RT73, MON 94100 and MON 88302 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; <https://www.ilcrop.com/>

International Seed Testing Association, International Rules of Seed Testing: Seed Science and Technology Rules, 2012

ISO 17025:2005 and ISO 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories

ISO 17034:2016, General Requirements for the Competence of Reference Material Producers

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