Street Address:
3356 Big Pine Trail Ste C/D
Champaign, IL 61822 USA
Phone: +1-217-359-2344
E-Mail: CRM@aocs.org; **Web:** www.aocs.org

Certified Reference Materials

AOCS 0304-A3

Report of the certification process for

Non-modified Canola

Canola Certified Reference Materials

Third Batch

Denise Williams
Technical Services Manager

Tiffanie West
Technical Services Director

ISO 17034:2016
A2LA Certificate 3438.01

Legal Notice

Neither AOCS nor any person acting on behalf of AOCS is responsible for the use which might be made of the following information.

AOCS Mission Statement:

AOCS advances the science and technology of oils, fats, proteins, surfactants and related materials, enriching the lives of people everywhere.

More information regarding AOCS is available at http://www.aocs.org

Table of Contents

Abstract 4
Acknowledgements 5
Glossary 6
Introduction 8
Material Processing 8
Trait Verification 9
Certified Value and Measurement Uncertainty 9
Homogeneity 10
Stability 10
References 11

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.

Acknowledgements

The authors would like to express sincere appreciation and gratitude to several individuals and their companies for support and guidance throughout this project. Thanks go to Jack Milligan, Bayer CropScience, for offering AOCS the opportunity to manufacture and distribute these products; to Sandra Harrison and Charlie Drennan at Illinois Crop Improvement Association for packaging the samples; and to Frank Spiegelhalter, Greg Ditta, E. Pearce Smith, and Daniel Thompson, Eurofins-GeneScan for event-specific, qualitative PCR analysis including the provision of information on running the analyses and interpreting the results.

Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Conventional counterpart means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food
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 be detected in a sample.
EC	European Commission
Genome	The full set of genes and associated DNA characteristic of an organism
GMO	Genetically modified/engineered organism: an organism in which the genetic material has been changed through modern biotechnology in a way that does not occur naturally by multiplication and/or natural recombination.
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 bind to a particular target DNA sequence and a special DNA-copying enzyme

	(DNA polymerase) that exponentially amplifies 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 quantitated
Quantitative PCR	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; http://www.ilcrop.com/index.htm

ISO 17034:2016 (E) General requirements for the competence of reference material producers

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

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

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed; https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32003R1829&from=en

Remund K., Simpson R., Laffont J-L., Wright D., and Gregoire S. Seedcalc8. 2008. https://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--3449--1102.html