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Certified Reference Materials

AOCS 0304-A2

Report of the certification process for

Non-modified Canola

Canola Certified Reference Materials

Second Batch

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A2LA Certificate 3438.01

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Table of Contents

Abstract

This report describes the preparation and certification of the canola CRM AOCS 0304-A2 produced by AOCS Technical Services in 2016. The CRMs have been prepared according to ISO Guides 30, 31, 34 and 35 and are intended to serve as control material for third party testing of canola for transformation events. The absence of GT73/RT73 in the canola was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory). AOCS 0304-A2 is available in 27-mL glass headspace vials. Non-modified canola powder was provided by Monsanto Company, St. Louis, MO and was prepared by grinding the bulk source according to canola processing protocols by Texas A&M University. The non-modified canola powder was then packaged under a nitrogen gas environment at Illinois Crop Improvement Association. 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	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-A2 was manufactured from canola according to ISO Guides 30, 31, 34 and 35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

Monsanto Company, St. Louis, MO delivered non-modified canola to AOCS. The materials were clean seed quality. Before the materials were shipped for uniform processing, primary samples were taken from randomly selected areas and depths to form a 3 kg composite sample in accordance with the International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 500 kg, five (5) working samples of 10 g each were prepared from the composite sample and sent to Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis. The analyses performed by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) were used to assess the trait absence and homogeneity of the lot.

Fifty 100 gram packages of non-modifed canola seed were processed according to industry standard canola processing procedures, packaged in 27-mL glass headspace vials and sealed under a nitrogen gas environment. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait absence, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 0304-A2: 249, 301, 144, 273, and 373 were sent to Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for GT73/RT73 absence in the samples.

Stability

Stability of these CRMs has been listed as 1 year from the introduction 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.

Results and Discussion

Sample Homogeneity

The PCR data for the non-modifed homogeneity samples is presented in Table 1.

Table 1. Results of the homogeneity testing performed by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) on the non-modified bulk material 0304-A2 provided by Monsanto Company, St. Louis, MO

Sample	GT73/RT73 Presence
Homogeneity Sample 1	Negative
Homogeneity Sample 2	Negative
Homogeneity Sample 3	Negative
Homogeneity Sample 4	Negative
Homogeneity Sample 5	Negative

Prepared Sample Verification

Once the bulk material was packaged, five (5) samples were identified by the Microsoft Excel Random Number Generator and sent to Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis. These results are presented in Table 2. This data shows the absence of the GT73/RT73 gene after the packaging of AOCS 0304-A2. These results are in consistent with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0304-A2 non-modified canola 0304-A2 material as tested by Eurofins-GeneScan, Metairie, LA (an ISO 17025 Accredited laboratory) with event-specific, qualitative PCR analysis.	
Sample	**GT73/RT73 Presence**
AOCS 0304-A2 249	Negative
AOCS 0304-A2 301	Negative
AOCS 0304-A2 144	Negative
AOCS 0304-A2 273	Negative
AOCS 0304-A2 373	Negative

References

Center for Environmental Risk Assessment GM Database
http://www.cera-gmc.org/?action=gm_crop_database

Eurofins GeneScan; 2315 N Causeway Blvd, Metairie, LA 70001; Telephone: +1 504 846 2398; http://www.gmotesting.com

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

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