Certified Reference Materials

AOCS 0304-A3

Report of the certification process for

Non-modified Canola

Canola Certified Reference Materials

Third Batch

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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 Monsanto Company, St. Louis, MO. It was prepared by grinding the bulk seed at Monsanto. The certified value of AOCS 0304-A3 was based on the purity of the bulk seed material and with 95% confidence, the true value is < 0.9 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 at 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, Monsanto Company, 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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AOCS</td>
<td>American Oil Chemists' Society</td>
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<tr>
<td>Conventional Crop</td>
<td>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</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid is the linear, double-helix macromolecule that makes up the genetic material of most organisms</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>Lowest level at which target DNA can be detected in a sample.</td>
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<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>Genome</td>
<td>The full set of genes and associated DNA characteristic of an organism</td>
</tr>
<tr>
<td>GMO</td>
<td>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.</td>
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<tr>
<td>ISO</td>
<td>International Organisation for Standardisation</td>
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<tr>
<td>PCR</td>
<td>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</td>
</tr>
</tbody>
</table>
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. The CRMs are available from AOCS.

Material Processing

Monsanto 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 (Table 1).

**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.**

<table>
<thead>
<tr>
<th>AOCS 0304-A3 Sample</th>
<th>Trait Presence</th>
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<tbody>
<tr>
<td>Sample # 94</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 179</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 271</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 340</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 468</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 505</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 672</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 774</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 837</td>
<td>Negative</td>
</tr>
<tr>
<td>Sample # 923</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Certified Value and Measurement Uncertainty**

The genetic purity of the seed lot used to produce AOCS 0304-A3 was assessed by Monsanto. 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. Using a 95% confidence level, the true % impurity of 0304-A3 non-modified canola seed lot was < 0.09%. Consequently, with 95% confidence, the true value is < 0.9 g/kg. The Measurement Uncertainty was based on the upper bound of the true % purity and is the expanded uncertainty with a coverage factor of 1.65 and the confidence level of 95%. The expanded measurement uncertainty for AOCS 0304-A3 is 0 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**

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


