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# Certified Reference Materials

# AOCS 1011-A2

Report for the certification process for

MON 88302

Canola Certified Reference Materials

Second Lot

OECD Unique ID MON-883Ø2-9

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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 1011-A2 produced by AOCS Technical Services in 2020. 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. The canola MON 88302 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 1011 A2 was based on the purity of the bulk seed material and with 95% confidence, the true value is ≥ 991 g/kg. The powder was aliquoted and bottled in 27-mL glass headspace vials and sealed under a nitrogen gas environment at Illinois Crop Improvement Association. The presence of MON 88302 in AOCS 1011-A2 was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory). CRM samples should 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, 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 | Crop variety with no history of transgenic technology and is produced through traditional plant-breeding techniques that rely on selecting and mating parent plants possessing promising traits and repeatedly selecting for superior performance among their offspring |
| 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 |
| ISO | International Organization for Standardization |
| GMO | Organism that has had genetic sequences modified using molecular-level techniques |
| 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 reproducible. |
| Quantitative PCR | PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules |
| Trait: MON 88302 | MON 88302 decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide |

## Introduction

Plant genetic modification 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 transgenic technology and a CRM must be available before the EC will consider authorizing acceptance of a new crop derived from transgenic technology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above regulatory requirements for GMO determination, AOCS 1011-A2 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

The MON 88302 canola used to prepare AOCS 1011-A2 were homozygous resulting from several cycles of self-pollination. Bayer CropScience milled ~4 kg of MON 88302 canola seed. All 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 to Certify Presence of MON 88302

The presence of the MON 88302 trait was assessed on 10 random vials of AOCS 1011-A2. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait presence. 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 presence and homogeneity of MON 88302 presence in the samples (Table 1).

**Table 1. Trait verification testing on AOCS 1011-A2 MON 88302 canola performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory).**

| AOCS 1011-A2 Sample | Trait MON 88302 Presence |
|---|---|
| Sample # 0058 | Positive |
| Sample # 0100 | Positive |
| Sample # 0135 | Positive |
| Sample # 0168 | Positive |
| Sample # 0199 | Positive |
| Sample # 0234 | Positive |
| Sample # 0287 | Positive |
| Sample # 0314 | Positive |
| Sample # 0350 | Positive |
| Sample # 0398 | Positive |

## Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 1011-A2 was assessed by Bayer CropScience. A total of 720 canola seeds were subjected to individual seed testing for the presence of MON 88302 by qualitative event-specific PCR. 718 of the 720 seeds tested positive for the presence of MON 88302.

Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008) and the Certified Value corresponded to the lower bound of true % purity. The % purity in the sample was 99.7% when 720 seeds were tested. Using a 95% confidence level, the true % purity of the MON 88302 seed lot was at least 99.1%. Consequently, with 95% confidence, the true value is ≥991 g/kg.

The Measurement Uncertainty was based on the lower bound of the true % purity and is the expanded uncertainty with a coverage factor of 1.65 and confidence level of 95%. It is obtained by combining the uncertainties from the purity assessment ($u_{char,rel}$), the homogeneity assessment ($u_{bb,rel}$), the transport stability assessment ($u_{sts,rel}$) and the long-term stability assessment ($u_{lts,rel}$):

$$u_{CRM,rel} = \sqrt{{u_{char,rel}}^2 + {u_{bb,rel}}^2 + {u_{sts,rel}}^2 + {u_{lts,rel}}^2}$$

$$U_{CRM} = 1.65 \times u_{CRM,rel} \times 1000\ g/kg$$

The expanded measurement uncertainty for AOCS 1011-A2 is 23 g/kg.

## Homogeneity

The homogeneity of AOCS 1011-A2 is related to the purity of the seeds. 718 out of 720 seeds tested positive for the MON 88302 canola event by event-specific PCR. Based on the sample purity of 99.7%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of the MON 88302 trait was confirmed when 10 random vials of AOCS 1011-A2 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 presence of 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.

The effect of temperature and time are investigated.

A transport (short-term) stability study is conducted to assess the stability of maize CRM during transport. The temperature and time conditions in the study cover the typical conditions and the not so rare situations. The outcome of the study is considered transferable to other CRMs of similar property. Samples were subject to 3 different temperatures (4 ℃ (fridge), 25 ℃ (ambient), 60 ℃ (oven)) for 4 different durations (0, 1, 2, and 4 weeks). The study concluded that samples are stable at 4 ℃ (fridge) and 25 ℃ (ambient) for 4 weeks. The estimated uncertainty contribution from transport (short-term) stability is 1.0%.

A long-term stability study is conducted to assess the stability of maize CRM during storage. Samples are stored at 25 ℃ (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperate studied is 25 ℃ and the length of time to be studied is 10 years. The outcome of the study is considered transferable to other CRMs of similar property. In the initial 1-year stability study, samples were subject the storage condition for 4 different durations (0, 1, 3, 6 and 12 months). The study concluded that samples are stable at 25 ℃ (ambient) for 12 months. The estimated uncertainty contribution from long-term stability is 0.42%.

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 positive 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

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