

# Certified Reference Materials

## AOCS 0306-F9

Report of the certification process for

Ms8

Canola Certified Reference Materials

Ninth Batch

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## **Abstract**

This report describes the preparation and certification of the canola CRM AOCS 0306-F9 produced by AOCS Technical Services in 2022. The CRMs have been prepared according to ISO 17034:2106 and are intended to serve as control material for third party testing of canola for transformation events. The purity of the Ms8 canola was verified using event-specific, qualitative PCR analysis by FoodChain ID Test, LLC, Chantilly, VA (an ISO 17025 Accredited laboratory). AOCS 0306-F9 is available in 0.5 ml skirted screw-cap self-sealing tubes. The canola Ms8 DNA was provided by BASF Agricultural Solutions Seed US LLC. The canola leaf DNA extract was prepared by grinding the bulk sources according to standard canola processing protocols and were then packaged under a water environment. The leaf DNA extract sample shall be stored dry in a sealed container at +4 °C 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 Benoit Maes and Ray Shillito, BASF Agricultural Solutions Seed US LLC, for offering AOCS the opportunity to manufacture and distribute these products; to Heather Waxdahl, SGS Midwest Seed Services for packaging the samples; and to Dan Smith, Bernd Schoel and Joy Bolster, FoodChain ID Testing, LLC 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	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

Trait: Ms8 MS8 was produced by genetically engineering plants to be male sterile and tolerant to the herbicide glufosinate ammonium (as a selectable marker).

## 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 ranging from 0.90 to 5% of authorized biotech events before accepting a shipment.

To meet the above analytical requirements for GM determination, AOCS 0306-F9 was manufactured from canola according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

## Materials and Methods

BASF Agricultural Solutions Seed US LLC delivered 2 mg of Ms8 canola 0306-F9 to AOCS. Ten (10) working samples of DNA 10 µg each were prepared from the composite and sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for the presence of the intended event, Ms8. This testing was for purity as well as homogeneity purposes.

The source leaf material was taken from plants which had been tested individually using a number of quality standards and was grown from seeds harvested from plants that had themselves passed the same criteria. Plants not meeting the quality standards were removed and destroyed. Leaf material was harvested from the plants which met the

quality standards and frozen immediately, stored at -70 °C. The genomic DNA was extracted from leaves of one or more plants according to CTAB-based (Doyle JJ and Doyle JL, 1987) protocol. The integrity and concentration of the genomic DNA was determined by electrophoresis in a 1.0% agarose gel and ethidium bromide-staining and compared to lambda molecular weight standards by digital imaging quantification. The concentration measurement was done in triplicate, repeated in three different gels. No indications for physical degradation were apparent and the DNA migrated at positions higher than 40 Kb.

The leaf used to manufacture the Ms8 materials was shown to contain the Ms8 event as well as the absence of P35S, BXN, 2mEPSPS, cp4EPSPS , cp4EPSPS, NPTII, Barstar, and PAT sequences using PCR protocols at BASF Agricultural Solutions Seed US LLC. The Ms8 canola leaf DNA was packaged by SGS-Midwest Seed Services in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of 10 µg.

AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of purity, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 0306-F9: 139, 172, 191, 208, 238, 244, 263, 279, 297 and 301 were sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for Ms8 presence in the samples.

## **Stability**

Stability of these CRMs has been listed as 1 year from the certification date. The materials were sealed and stored under refrigerated conditions, therefore not exposed to air and are expected to be stable for longer than the estimated expiration date. The stability of the leaf DNA extract material will be reevaluated annually. If the samples are still representative of the certified value, the certificates will be extended.

## Results and Discussion

### Sample Homogeneity and Prepared Sample Verification

Once the bulk material was processed and packaged, ten (10) samples were identified by the Microsoft Excel Random Number Generator and sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis. These results are presented in Table 1. This data confirms the presence and homogeneity of the Ms8 gene after the packaging of AOCS 0306-F9.

<b>Sample</b>	<b>Ms8 Presence</b>
AOCS 0306-F9 139	Positive
AOCS 0306-F9 172	Positive
AOCS 0306-F9 191	Positive
AOCS 0306-F9 208	Positive
AOCS 0306-F9 238	Positive
AOCS 0306-F9 244	Positive
AOCS 0306-F9 263	Positive

AOCS 0306-F9 279	Positive
AOCS 0306-F9 297	Positive
AOCS 0306-F9 301	Positive

## References

Center for Environmental Risk Assessment GM Database

[http://www.cera-gmc.org/?action=gm\\_crop\\_database](http://www.cera-gmc.org/?action=gm_crop_database)

FoodChain ID Testing, LLC, 4150 Lafayette Center Drive, Suite 600, Chantilly, VA 20151

[www.foodchainid.com](http://www.foodchainid.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 174034:2016 (E) General Requirements for the Competence of Reference Material Producers

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>

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