STREET ADDRESS
3356 Big Pine Trail, Ste. C&D,
Champaign, IL 61822 USA

MAILING ADDRESS
PO Box 7230,
Champaign, IL 61826 USA

+1 217-359-2344
general@aocs.org
aocs.org

Certified Reference Materials

AOCS 0711-C3

Report of the certification process for

Rf2

Canola

Third Batch

Zoe Serelis
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 www.aocs.org

©AOCS, 2026

Table of Contents

Abstract 4
Acknowledgements 5
Glossary 6
Introduction 7
Materials and Methods 7
Stability 8
Results and Discussion 8
Sample Homogeneity 8
Prepared Sample Verification 9
References 10

©AOCS, 2026

Abstract

This report describes the preparation and certification of the canola CRM AOCS 0711-C3 produced by AOCS Technical Services in 2018. The CRMs have been prepared according to ISO Guide 17034:2016 and are intended to serve as control material for third party testing of canola for transformation events. The presence of Rf2 in the canola was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory). AOCS 0711-C3 is available in 0.5 ml skirted screw-cap self-sealing tubes. The canola Rf2 DNA was provided by BASF Agricultural Solutions US LLC and was extracted from clean leaves. The leaf DNA extract sample shall be stored dry in a sealed container at +4 ˚C in the dark.

©AOCS, 2026

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 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 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.

©AOCS, 2026

Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of modern biotechnology and is produced through 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
Detection Limit	Lowest level at which target DNA can be detected in a sample and be reliably tested by PCR methods. It is typically expressed as a percentage: the ratio of the number of modern biotechnology derived genomes to the number of crop genomes times 100 percent
EC	European Commission
Genome	The complete set of genes and DNA associated with an organism
GMO	Organism that has had genetic sequences modified using molecular-level techniques
ISO	International Organization for Standardization
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 reliably quantified. It is typically expressed as the ration of the number of transgenic genomes to the number of crop genomes times 100 percent
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
Trait: Rf2	Glufosinate ammonium herbicide tolerance and male sterility

©AOCS, 2026

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 Certified Reference Material 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 0711-C3 was manufactured from canola according to ISO Guide 17034:2016 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

Materials and Methods

BASF Agricultural Solutions US LLC prepared the bulk material by taking source leaf material from plants which had been tested individually using several 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 and 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 ethidium bromide-stained 1% agarose gel electrophoresis 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.

©AOCS, 2026

BASF Agricultural Solutions US LLC delivered 2 mg of Rf2 canola to AOCS. Five (5) working samples of DNA, 10 μg each, were prepared from the composite and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to screen for the presence of the intended event, Rf2. This testing was for presence confirmation as well as homogeneity purposes.

The leaf used to manufacture the Rf2 materials was shown to contain the Rf2 event using PCR protocols at BASF Agricultural Solutions US LLC. The Rf2 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 gene presence, homogeneity, and to rule out degradation during packaging. Sample numbers AOCS 0711-C3: 55, 139, 163, 171, and 187 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to screen for Rf2 presence in the samples.

Stability

Stability of these CRMs has been listed as 1 year from the introduction date. The materials were sealed and stored in the dark at 4 °C, 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 still test positive for the presence of the trait, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The PCR data for the Rf2 homogeneity samples is presented in Table 1.

©AOCS, 2026

Table 1. Results of the homogeneity testing performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) on the Rf2 bulk material 0711-C3 provided by BASF Agriculural Solutions US LLC	
Sample	**Rf2 Presence**
Homogeneity Sample 1	Positive
Homogeneity Sample 2	Positive
Homogeneity Sample 3	Positive
Homogeneity Sample 4	Positive
Homogeneity Sample 5	Positive

Prepared Sample Verification

After the bulk material was packaged, five (5) samples were identified by the Microsoft Excel Random Number Generator and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis. These results are presented in Table 2. This data confirms the presence of the Rf2 gene after the packaging of AOCS 0711-C3. These results are consistent with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0711-C3 Rf2 canola 0711-C3 material as tested by Eurofins-GeneScan, New Orleans, LA (an ISO 17025-accredited laboratory) with Rf2 event-specific, qualitative PCR analysis.	
Sample	**Rf2 Presence**
AOCS 0711-C3 55	Positive
AOCS 0711-C3 139	Positive
AOCS 0711-C3 163	Positive
AOCS 0711-C3 171	Positive
AOCS 0711-C3 187	Positive

©AOCS, 2026

References

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

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/gmotesting/

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

ISO Guide 30:2015 (E/F), Reference Materials – Selected Terms and Definitions

ISO Guide 31:2015 (E), Reference Materials – Contents of Certificates, Labels, and Accompanying Documentation

ISO Guide 17034:2016 (E) General Requirements for the Competence of Reference Material Producers

ISO Guide 35:2006 (E) Certification of Reference Materials – General and Statistical Principles

SGS Midwest Seed Services, 236 32nd Avenue, Brookings, South Dakota 57006 Telephone: + 1 605 692 7611 Toll Free: +1 877 692 7611 Fax: +1 605 692 7617
http://www.mwseed.com/

©AOCS, 2026