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Certified Reference Materials

AOCS 0208-A8

Report of the certification process for

T45

Canola

Eighth Batch

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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 0208-A8 produced by AOCS Technical Services in 2022. 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 presence of T45 in the canola was verified using event-specific, qualitative PCR analysis by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory). AOCS 0208-A8 is available in 0.5 ml skirted screw-cap self-sealing tubes. The canola T45 DNA was extracted from clean leaves by BASF Agricultural Solutions US LLC. The leaf DNA extract sample shall be stored dry in a sealed container at +4 ˚C in the dark.

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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 Ray Shillito and Benoit Maes, BASF Agricultural Solutions US LLC, for offering AOCS the opportunity to manufacture and distribute these products; to Heather Waxdahl, SGS-Midwest, 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.

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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: T45	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium

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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 0208-A8 was manufactured from canola according to ISO 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.

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BASF Agricultural Solutions US LLC delivered 2.0 mg of T45 canola leaf DNA extract to AOCS. The 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, T45. This testing was for presence confirmation as well as homogeneity purposes.

The leaf used to manufacture the T45 materials was shown to contain the T45 event; as well as the homozygosity of the trait; and absence of L62 GM event, EPSPS sequences, BXN sequences, GS-40/90-1 GM event, GS-40/90-2 GM event, PSsuAra-bar sequences, T177 GM event, and Topaz 19/2 GM event using PCR protocols at BASF Agricultural Solutions US LLC. The T45 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 0208-A8: 20, 30, 108, 131, 193, 200, 230, 254, 302, and 306 were sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to screen for T45 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 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 and Prepared Sample Verification

After the bulk material was 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.

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These results are presented in Table 1. This data confirms the presence of the T45 gene after the packaging of AOCS 0208-A8.

Table 1. Results for the verification and homogeneity of AOCS 0208-A8 T45 canola material as tested by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory) with T45 event-specific, qualitative PCR analysis.

Sample	T45 Presence
AOCS 0208-A8 20	Positive
AOCS 0208-A8 30	Positive
AOCS 0208-A8 108	Positive
AOCS 0208-A8 131	Positive
AOCS 0208-A8 193	Positive
AOCS 0208-A8 200	Positive
AOCS 0208-A8 230	Positive
AOCS 0208-A8 254	Positive
AOCS 0208-A8 302	Positive
AOCS 0208-A8 306	Positive

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References

Center for Environmental Risk Assessment GM 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

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 17034:2016, 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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