

Certified Reference Materials

AOCS 0707-B16

Report of the certification process for

A2704-12

Soybean

Sixteenth Batch

Zoe Serelis
Technical Services Manager

Tiffanie West
Technical Services Director



ISO 17034:2016
A2LA Certificate 3438.01

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Abstract

This report describes the preparation and certification of the soybean CRM AOCS 0707-B16 produced by AOCS Technical Services in 2024. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of soybean for transformation events. The presence of A2704-12 in the soybean was verified using event-specific, qualitative PCR analysis by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory). AOCS 0707-B16 is available in 0.5 ml skirted screw-cap self-sealing tubes. The A2704-12 soybean DNA was provided by BASF Agricultural Solutions US LLC. The A2704-12 soybean leaf DNA extract was extracted from clean leaves that were individually tested and confirmed positive for A2704-12 soybean 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.

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 North America 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

<i>AOCS</i>	American Oil Chemists' Society
<i>Conventional Crop</i>	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
<i>DNA</i>	Deoxyribonucleic Acid
<i>Detection Limit</i>	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
<i>EC</i>	European Commission
<i>Genome</i>	The complete set of genes and DNA associated with an organism
<i>GMO</i>	Organism that has had genetic sequences modified using molecular-level techniques
<i>ISO</i>	International Organization for Standardization
<i>PCR</i>	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
<i>Qualitative PCR</i>	PCR methods that determine the presence or absence of a specific target DNA sequence at a particular level of detection
<i>Quantitation Limit</i>	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
<i>Quantitative PCR</i>	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
<i>Trait: A2704-12</i>	Phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium

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 (CRM) created from leaf, seed, or grain containing the new trait as well as a CRM created from the conventionally bred matrix. The European Commission (EC) 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 0707-B16 was manufactured from soybean according to ISO 17034:2016 and in accordance with EC No 1829/2003, EC No 641/2004 and EC No 619/2011. This CRM is available from AOCS.

Materials and Methods

BASF Agricultural Solutions US LLC prepared the bulk material by taking plants that had been individually tested using several quality standards and were grown from soybean seeds harvested from plants that had passed the same criteria. Only the leaf material confirmed as positive for the A2704-12 event and meeting all quality requirements were harvested, immediately frozen, and stored at -70 °C. Plants not meeting the quality standards were removed and destroyed.

Genomic DNA was extracted from A2704-12-positive leaf tissue obtained from one or more plants using a CTAB-based protocol (Doyle and Doyle, 1987). DNA integrity and concentration were evaluated by electrophoresis on a 1.0% agarose gel stained with ethidium bromide and compared against lambda molecular weight standards by digital imaging quantification. Concentration measurements were performed in triplicate across three independent gels. No evidence of physical degradation was observed, and the DNA migrated at positions greater than 40 kb. The leaf tissue used to produce the

AOCS 0707-B16 material was confirmed to contain the A2704-12 event and to be absent of A5547-127 (LOQ < 0.1%) and 3'nos sequences (LOQ < 0.05%) using PCR protocols at BASF Agricultural Solutions US LLC.

BASF Agricultural Solutions US LLC delivered 3.0 mg of A2704-12 soybean genomic DNA extract to AOCS. The A2704-12 soybean leaf DNA was packaged by SGS North America Inc., Brookings, SD in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of 10 µg.

For the verification of trait presence, homogeneity, and to rule out degradation during packaging, 10 random samples were collected and submitted for event-specific, qualitative PCR analysis for A2704-12 soybean: 5, 47, 78, 106, 132, 144, 223, 234, 265, and 273. Vial numbers were obtained by the Random Number Generator function of Microsoft Excel and the testing was conducted by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025-accredited laboratory). The results confirm the presence of the A2704-12 after the packaging (*Table 1*).

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, Chantilly, VA (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis. The results are presented in *Table 1*. The results show that no contamination occurred during the packaging of AOCS 0707-B16.

Table 1. Results on the verification and homogeneity of AOCS 0707-B16, A2704-12 soybean, event-specific qualitative PCR analysis conducted by FoodChain ID Testing

Sample	A2704-12 Presence
AOCS 0707-B16 #5	Positive
AOCS 0707-B16 #47	Positive
AOCS 0707-B16 #78	Positive
AOCS 0707-B16 #106	Positive
AOCS 0707-B16 #132	Positive
AOCS 0707-B16 #144	Positive
AOCS 0707-B16 #223	Positive
AOCS 0707-B16 #234	Positive
AOCS 0707-B16 #265	Positive
AOCS 0707-B16 #273	Positive

References

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

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*.

FoodChain ID Testing, LLC, 4150 Lafayette Center Drive, Suite 600, Chantilly, VA, 20151 Telephone: 1 703-222-8700 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; eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32003R1829&from=en

SGS- North America Inc., 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/>