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## **Certified Reference Materials**

### **AOCS 0610-A7**

Report of the certification process for

FG72

Soybean Certified Reference Materials

Seventh 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 soybean CRM AOCS 0610-A7 produced by AOCS Technical Services in 2023. 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 FG72 in the soybean was verified using event-specific, qualitative PCR analysis by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025 accredited laboratory). AOCS 0610-A7 is available in 0.5 ml skirted screw-cap self-sealing tubes. The soybean FG72 genomic DNA was provided by Syngenta Crop Protection, LLC and was extracted from clean seeds. The seed genomic DNA extract sample shall be stored dry in a sealed container at +4 °C in the dark.

## **Acknowledgements**

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## 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: FG72	Tolerance to glyphosate and isoxaflutole herbicides

## 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 (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 0610-A7 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. The CRM is available from AOCS.

## Materials and Methods

Bulk homozygous FG72 soybean seed provided to Syngenta Crop Protection, LLC (“Syngenta”) by M.S. Technologies, LLC (“MS Tech”) was used in the preparation of Certified Reference Material. At the request of Syngenta, Eurofins GeneScan GmbH, Freiburg, Germany (an ISO 17025 accredited laboratory) extracted the FG72 soybean genomic DNA sample from ground seed. The DNA extraction method used combined cetyltrimethyl ammonium bromide (CTAB)-based lysis, phenol/chloroform/isoamyl alcohol and chloroform/isoamyl alcohol extraction of the lysate with isopropanol precipitation of the DNA. After resuspension of the precipitated DNA, it was further purified by anion exchange chromatography and frozen immediately and stored at  $-70^{\circ}\text{C}$ . The integrity and concentration of the genomic DNA was determined by electrophoresis in a 1.5%



agarose gel and ethidium bromide-staining and the molecular-weight size marker ‘Gene-Ruler 1 kb Plus DNA Ladder’ (Thermo Scientific) was used as DNA size standard. The major fraction of the FG72 soybean DNA was of high molecular weight (i.e. greater than 20 kb) indicating integrity of the extracted DNA. The concentration measurement was done by ‘Quant-iT Picogreen dsDNA kit’ (Invitrogen).

At the request of Syngenta, Eurofins GeneScan delivered 3.5 mg of the FG72 soybean DNA to AOCS. The DNA from the composite was packaged by SGS North America, Inc., Brookings, SD in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of approximately 10 µg.

## Trait Verification to Certify Presence of FG72

After the bulk material was packaged, ten 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 of the FG72 event after the packaging of AOCS 0610-A7.

<b>Table 1. Results for the verification of AOCS 0610-A7 FG72 soybean material as tested by FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025 Accredited laboratory) with FG72 event-specific, qualitative PCR analysis.</b>	
<b>Sample</b>	<b>FG72 Presence</b>
AOCS 0610-A7 32	Positive
AOCS 0610-A7 70	Positive

AOCS 0610-A7 103	Positive
AOCS 0610-A7 135	Positive
AOCS 0610-A7 159	Positive
AOCS 0610-A7 188	Positive
AOCS 0610-A7 214	Positive
AOCS 0610-A7 236	Positive
AOCS 0610-A7 258	Positive
AOCS 0610-A7 281	Positive

## Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0610-A7 was assessed by Syngenta. A total of 396 soybean seeds were subjected to individual seed testing for the presence of FG72 by qualitative event-specific PCR. 396 of the 396 seeds tested positive for the presence of FG72.

Purity estimation was calculated using SeedCalc8 (Remund *et al.*, 2008) and corresponded to the lower bound of true % purity. The % purity in the sample was 100%, when 396 seeds were tested. Using a 95% confidence level, the true % purity of the FG72 seed DNA lot was 99.25%. Consequently, with 95% confidence, the true value is > 992.5 ng/μg.

The Measurement Uncertainty was based on the lower bound of the true % purity. The measurement uncertainty is the expanded uncertainty with a coverage factor of 1.65 and confidence level of 95%. The expanded measurement uncertainty for AOCS 0610-A7 is 3.6 ng/μg.

## **Homogeneity**

The homogeneity of AOCS 0610-A7 is related to the purity of the seeds. 396 out of 396 seeds tested positive for the FG72 soybean event by event-specific PCR. Based on the sample purity of 100%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of the FG72 trait was confirmed when 10 random vials of AOCS 0610-A7 were selected and were sent to FoodChain ID Testing, LLC, Chantilly, VA (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to verify the presence of FG72 in the samples (See Trait Verification section and 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 seed genomic DNA extract material will be reevaluated annually. If the samples still test positive for the presence of the trait and have not degraded, the certificates will be extended.

## References

Biosafety Clearing House Living Modified Organism (LMO) Registry  
<http://bch.cbd.int/database/lmo-registry/>

Eurofins-GeneScan GmbH, Engresserstraße 4, D-79108 Freiburg, Germany Telephone: +49 761 6400 4011 Online: <https://www.eurofinsus.de/food-analysis>

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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