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Certified Reference Materials

AOCS 0124-B

Report of the certification process for

COR-23134-4

Soybean

First Batch

OECD Unique ID COR-23134-4

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ISO 17034:2016
A2LA Certificate 3438.01

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Table of Contents

Abstract 4

Acknowledgements 5

Glossary 6

Introduction 7

Material Processing 7

Trait Verification to Certify Presence of COR-23134-4 7

Certified Value and Measurement Uncertainty 8

Stability 9

Certificate Validity & Extension 10

References 11

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Abstract

This report describes the preparation and certification of the soybean CRM AOCS 0124- B 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 soybean COR-23134-4 powder was provided by Pioneer Hi-Bred International, Inc, Johnston, IA. It was prepared by grinding the bulk seed at Pioneer Hi-Bred International, Inc. The certified value of AOCS 0124-B was based on the purity of the plant leaves from the bulk seed material and the is 1000 g/kg. With 95% confidence, the true value is ≥ 976 g/kg. The powder was aliquoted and bottled in 27-mL glass headspace vials and sealed under a nitrogen gas environment at Illinois Crop Improvement Association. The presence of COR-23134-4 and the homogeneity in AOCS 0124-B was verified using event-specific, quantitative PCR analysis by Eurofins-GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory). CRM samples should be stored in a dry, sealed container at ambient or cooler conditions in the dark.

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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
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: COR-23134-4	Resistance to the insect lepidopteran

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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 0124-B 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.

Material Processing

COR-23134-4 soybean seeds used to prepare AOCS 0124-B were homozygous through successive breeding generations in soybean variety 93Y21. Pioneer Hi-Bred International, Inc milled ~4 kg of COR-23134-4 soybean seed. All of the seed powder was passed through a 500 µM mesh sieve. The seed powder was delivered to AOCS who contracted Illinois Crop Improvement Association for packaging the samples. The powder was aliquoted and bottled in 27-mL glass headspace vials and sealed under a nitrogen gas environment.

Trait Verification to Certify Presence of COR-23134-4

The presence of the COR-23134-4 trait was assessed on 10 vials of AOCS 0124-B. Sample numbers that were selected were sent to Eurofins-GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory) for event-specific, quantitative PCR analysis to verify the presence of COR-23134-4 in the samples (Table 1).

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Table 1. Trait verification testing on AOCS 0124-B COR-23134-4 soybean performed by Eurofins-GeneScan GmbH (an ISO 17025-accredited laboratory).

AOCS 0124-B Sample	Trait COR-23134-4 Presence
Sample # 5	Positive
Sample # 51	Positive
Sample # 128	Positive
Sample # 190	Positive
Sample # 203	Positive
Sample # 262	Positive
Sample # 296	Positive
Sample # 304	Positive
Sample # 337	Positive
Sample # 383	Positive

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0124-B was assessed by Pioneer Hi-Bred International, Inc. A total of 176 soybean seeds were germinated, sampled, and were subjected to individual plant testing for the presence of COR-23134-4 by qualitative event-specific PCR. 176 of the 176 seedling samples tested positive for the presence of COR-23134-4.

Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008). The % purity in the sample was 100%, when 176 seedling samples were tested and the true value of the COR-23134-4 seed source is ≥ 976 g/kg with 95% confidence.

The measurement uncertainty (U_{CRM}) is the expanded uncertainty with a coverage factor of 2 and a confidence level of 95%. It is obtained by combining the uncertainties from the purity assessment ($u_{char,rel}$), the homogeneity assessment ($u_{bb,rel}$), the transport stability assessment ($u_{sts,rel}$)and the long-term stability assessment ($u_{lts,rel}$):

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$$u_{CRM,rel} = \sqrt{u_{char,rel}{}^2 + u_{bb,rel}{}^2 + u_{sts,rel}{}^2 + u_{lts,rel}{}^2}$$

$$U_{CRM} = 2 \times u_{CRM,rel} \times 1000\ g/kg$$

The expanded measurement uncertainty for AOCS 0124-B is - 24 g/kg.

Homogeneity

The homogeneity of AOCS 0124-B is related to the purity of the seeds. 176 out of 176 soybean seedling samples were subjected to individual plant testing and all tested positive for the COR-23134-4 soybean event by event-specific PCR. Based on the sample purity of 100%, as determined using SeedCalc8, the batch was expected to be homogenous.

In addition, the homogeneity of the COR-23134-4 trait was confirmed when 10 random vials of AOCS 0124-B were selected and were sent to Eurofins-GeneScan GmbH, Freiburg, Germany (an ISO 17025-accredited laboratory) for event-specific, qualitative PCR analysis to verify the presence of COR-23134-4 in the samples (See Trait Verification section and Table 1).

Stability

Time, temperature and light are regarded as the most relevant influences on the stability of CRM (Linsinger, et al., 2001). The influence of light is mitigated by shipping and storing the vials in boxes, thus minimizing the possibility of degradation due to light. The influence of temperature is mitigated by storing the vials in a temperature-controlled room, and shipping vials at ambient temperature.

The effect of temperature and time are investigated.

A transport (short-term) stability study is conducted to assess the stability of soybean CRM during transport. The temperature and time conditions in the study cover the typical conditions and the not so rare situations. The outcome of the study is considered transferable to other CRMs of similar property. Samples were subject to 3 different temperatures (4 ℃ (fridge), 25 ℃ (ambient), 60 ℃ (oven)) for 4 different durations (0, 1,

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2, and 4 weeks). The study concluded that samples are stable at 4 ℃ (fridge) and 25 ℃ (ambient) for 4 weeks. The estimated uncertainty contribution from transport (short-term) stability is 1.0%.

A long-term stability study is conducted to assess the stability of soybean CRM during storage. Samples are stored at 25 ℃ (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperature studied is 25 ℃ and the length of time to be studied is 10 years. The outcome of the study is considered transferable to other CRMs of similar property. In the initial 1-year stability study, samples were subject the storage condition for 4 different durations (0, 1, 3, 6 and 12 months). The study concluded that samples are stable at 25 ℃ (ambient) for 12 months. The estimated uncertainty contribution from long-term stability is 0.42%.

Certificate Validity & Extension

Stability of these CRMs has been listed as 1 year from the certification date. The materials were processed and are stored at ambient temperature, under nitrogen gas, in 27-mL glass headspace vials. These materials are expected to be stable for longer than the estimated expiration date. The stability of the powder material will be reevaluated at time of expiration. If the samples still test positive for the presence of the intended trait, the certificates will be extended.

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References

Eurofins-GeneScan GmbH, Engesserstraße 4, D-79108 Freiburg, Germany; Telephone: +49 761 6400 4022 Fax: +49 761 6400 4011 https://www.eurofinsus.de/food-analysis/

Illinois Crop Improvement Association, 3105 Research Road, Champaign, IL 61826; Telephone: +1 217 359 4053 Fax: +1 217 359 4075; https://www.ilcrop.com/

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