Street Address:
AOCS, 3356 Big Pine Trail Ste C/D
Champaign, IL 61822 USA
Phone: +1-217-359-2344
E-Mail: CRM@aocs.org; **Web:** www.aocs.org

Certified Reference Materials

AOCS 1022-A

Report of the certification process for

Non-Modified Rice

Certified Reference Materials

First Batch

Denise Williams
Technical Services Manager

Tiffanie West
Technical 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 http://www.aocs.org

Contents

Abstract

This report describes the preparation and certification of the rice CRM AOCS 1022-A 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 rice for transformation events. The non-modified rice powder was provided by Bayer CropScience, St. Louis, MO. It was prepared by grinding the bulk seed at Bayer CropScience. The certified value of AOCS 1022-A was based on the purity of the bulk seed material and is 0 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 absence of LLRice62 in AOCS 1022-A was verified using event-specific, qualitative PCR analysis by FoodChain ID, Chantilly, VA (an ISO 17025 accredited laboratory). CRM samples should be stored dry in a sealed container at ambient or cooler conditions 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 Jack Milligan and Richard Woo, Bayer CropScience, for offering AOCS the opportunity to manufacture and distribute these products; to Beverly Ryan, Charlie Drennan and the crew at Illinois Crop Improvement Association for packaging the samples; and to Dan Smith and Bernd Schoel, FoodChain ID for event-specific, qualitative PCR analysis including the provision of information on running the analyses and interpreting the results.

Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of transgenic technology and is produced through traditional 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 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
ISO	International Organization for Standardization
GMO	Organism that has had genetic sequences modified using molecular-level techniques
PCR	Polymerase Chain Reaction: technique used to determine whether a sample of plant tissue contains a specific DNA sequence. PCR relies on primer sets that zero in on a specific 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 specific level of detection
Quantitation Limit	Lowest level at which the amount of target DNA sequence in a sample can be reproducible.
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules

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 seed 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 seed and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above regulatory requirements for GMO determination, AOCS 1022-A was manufactured from rices 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.

Material Processing

Bayer CropScience milled ~5 kg of non-modified rice 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

The absence of LLRice2 rice event in the non-modified rice material was assessed on 10 random vials of AOCS 1022-A. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait presence. Sample numbers that were randomly selected were sent to FoodChain ID, Chantilly, VA (an ISO 17025

accredited laboratory) for event-specific qualitative PCR analysis to verify the absence or below limit of detection in the samples (Table 1).

Table 1. Trait verification testing on AOCS 1022-A non-modified rice performed by FoodChain ID, Chantilly, VA (an ISO 17025 accredited laboratory) for presence of LLRice62.

AOCS 1022-A Sample	Presence
Sample # 37	Negative
Sample # 74	Negative
Sample # 133	Negative
Sample # 180	Negative
Sample # 226	Negative
Sample # 251	Negative
Sample # 323	Negative
Sample # 388	Negative
Sample # 435	Negative
Sample # 472	Negative

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 1022-A was assessed by Bayer CropScience. A total of 3750 rice seeds were subjected to individual seed testing for the presence of LLRice62 by qualitative event-specific PCR. 3750 of the 3750 rice seeds tested negative for LLRice62 .
Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008). The % impurity in the sample was 0%, when 3750 seeds were tested.

The measurement uncertainty is the expanded uncertainty using the value of the upper bound of impurity at 0.8 g/kg. The standard uncertainty can be obtained by dividing the expanded uncertainty by $2\sqrt{3}$ (rectangular distribution).

The standard uncertainty for AOCS 1022-A is 0.8 g/kg.

Homogeneity

The homogeneity of AOCS 1022-A is related to the purity of the seeds. 3750 out of 3750 rice seeds tested negative for LLRice62 rice event by event-specific PCR. Based on the sample impurity of 0%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of non-modified rice was confirmed when 10 random vials of AOCS 1022-A were selected and were sent to FoodChain ID, Chantilly, VA (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to verify he absence of the LLRice62 event 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.

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 negative the presence of the intended trait(s), the certificates will be extended.

References

FoodChain ID Testing,LLC, 4150 Lafayette Center Drive, Suite 600, Chantilly, VA 20151 Telephone: 1 703-222-8700 www.foodchainid.com

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

ISO 17034:2016 (E) General requirements for the competence of reference material producers

ISO 17025:2005 and ISO 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories

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

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

Remund K., Simpson R., Laffont J-L., Wright D., and Gregoire S. Seedcalc8. 2008. https://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--3449--1102.html