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## **Certified Reference Materials**

### **AOCS 0607-A2**

Report of the certification process for

MIR604

Maize Certified Reference Materials

Second Batch

OECD Unique Identifier SYN-IR6Ø4-5

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**ISO 17034:2016**  
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## Table of Contents

<b>Abstract .....</b>	<b>4</b>
<b>Acknowledgements .....</b>	<b>5</b>
<b>Glossary .....</b>	<b>6</b>
<b>Introduction.....</b>	<b>7</b>
<b>Materials and Methods .....</b>	<b>7</b>
<b>Certified Value Assignment .....</b>	<b>8</b>
<b>Stability.....</b>	<b>8</b>
<b>Results and Discussion.....</b>	<b>10</b>
<b>Sample Homogeneity .....</b>	<b>10</b>
<b>Prepared Sample Verification .....</b>	<b>11</b>
<b>References .....</b>	<b>12</b>

## **Abstract**

This report describes the preparation and certification of the maize CRM AOCS 0607-A2 produced by AOCS Technical Services in 2011. The CRMs have been prepared according to ISO Guides 30, 31, 34 and 35 and are intended to serve as control material for third party testing of maize for transformation events. The presence of event MIR604 in the MIR604 maize was verified using event-specific, qualitative real-time PCR analysis at Eurofins GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). CRM AOCS 0607-A2 is available in 27-mL glass headspace vials. The MIR604 maize powder was provided by Syngenta Crop Protection, LLC and was prepared by grinding the bulk source according to maize processing protocols by Syngenta Crop Protection, LLC. The MIR604 maize powder was then aliquoted and packaged under a nitrogen gas environment at Illinois Crop Improvement Association. The powder samples shall be stored dry in a sealed container at ambient or cooler conditions in the dark.

## **Acknowledgements**

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## Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Conventional counterpart means 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
Trait: MIR604	Confers resistance to coleopteran insects

## 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 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 authorization 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 analytical requirements for GM determination, AOCS 0607-A2 was manufactured from maize seed 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

The MIR604 maize used in the preparation of AOCS 0607-A2 was derived from three different seed lots that were mixed at a ratio of 1:2:1 as described in Table 1.

Seed Material	Description	Amount of Seed
NP2222/NP2391 (MIR604)	GM seed, MIR604 male donor	5 Kg
NP2222 (MIR604) /NP2391	GM seed, MIR604 female donor	10 Kg
NP2222/NP2391	Non-GM seed	5 Kg

Syngenta Crop Protection, LLC delivered 20 kg of MIR604 maize powder to AOCS. Primary samples were taken from randomly selected areas and depths to form a 3 kg-composite sample in accordance with the International Seed Testing Association's (ISTA) Seed Science and Technology Rules for batches up to 500 kg, ten (10) working samples of 10 g each were prepared from the composite sample and sent to Eurofins GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR

analysis. The analyses performed by Eurofins GeneScan, New Orleans, LA were used to assess the trait presence and homogeneity of the lot.

The MIR604 maize was processed, packaged in 27-mL glass headspace vials and sealed under a nitrogen gas environment. AOCS used the random number generator function of Microsoft Excel to select samples for verification of trait presence, homogeneity, and to rule out degradation during packaging. Sample numbers AOCS 0607-A2: 11, 72, 108, 122, 181, 275, 299, 357, 386 and 483 were sent to Eurofins GeneScan, New Orleans, LA for event-specific, qualitative real-time PCR analysis to screen for MIR604 presence in the samples.

## **Certified Value Assignment**

The presence of event MIR604 in CRM 0607-A2 was determined using a qualitative, MIR604-specific real-time PCR method. This CRM, AOCS 0607-A2, is intended for use as quality control material in qualitative methods for the detection and identification of event MIR604. CRM 0607-A2 IS NOT INTENDED for the use as a calibrant for the quantification of event MIR604.

## **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 maize 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 °C (fridge), 25 °C (ambient), 60 °C (oven)) for 4 different durations (0, 1, 2, and 4 weeks). The study concluded that samples are stable at 4 °C (fridge) and 25 °C (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 maize CRM during storage. Samples are stored at 25 °C (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperate studied is 25 °C 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 °C (ambient) for 12 months. The estimated uncertainty contribution from long-term stability is 0.42%.

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.

# Results and Discussion

## Sample Homogeneity

The PCR data for the MIR604 maize homogeneity samples is presented in Table 1.

<b>Table 1. Results of the homogeneity testing performed by Eurofins GeneScan, New Orleans, LA on the MIR604 bulk material provided by Syngenta Crop Protection, LLC</b>	
<b>Sample</b>	<b>MIR604 Presence</b>
Homogeneity Sample 1	Positive
Homogeneity Sample 2	Positive
Homogeneity Sample 3	Positive
Homogeneity Sample 4	Positive
Homogeneity Sample 5	Positive
Homogeneity Sample 6	Positive
Homogeneity Sample 7	Positive
Homogeneity Sample 8	Positive
Homogeneity Sample 9	Positive
Homogeneity Sample 10	Positive

## Prepared Sample Verification

After the bulk material was packaged, ten (10) samples were identified by the Microsoft Excel Random Number Generator and sent to Eurofins GeneScan, New Orleans, LA for event-specific, qualitative PCR analysis. These results are presented in Table 2. This data confirms the presence of MIR604 after the packaging of AOCS 0607-A2. These results are consistent with the homogeneity data presented in Table 1.

<b>Table 2. Results for the verification of AOCS 0607-A2 MIR604 maize as tested by Eurofins GeneScan, New Orleans, LA with MIR604 event-specific, qualitative PCR analysis.</b>	
<b>Sample</b>	<b>MIR604 Presence</b>
AOCS 0607-A2 11	Positive
AOCS 0607-A2 72	Positive
AOCS 0607-A2 108	Positive
AOCS 0607-A2 122	Positive
AOCS 0607-A2 181	Positive
AOCS 0607-A2 275	Positive
AOCS 0607-A2 299	Positive
AOCS 0607-A2 357	Positive
AOCS 0607-A2 386	Positive
AOCS 0607-A2 483	Positive

## References

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

Eurofins GeneScan; 2219 Lakeshore Drive, Suite 400, New Orleans, LA 70122;  
Telephone: +1 504 297 4330 Toll Free: +1 866 535 2730 Fax: +1 504 297 4335  
<https://www.eurofinsus.com/food-testing/testing-services/gmo/>

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

ISO Guide 30:1992 (E/F), Reference Materials – Selected Terms and Definitions

ISO Guide 31:2000 (E), Reference Materials- Contents of Certificates, Labels and  
Accompanying Documentation

ISO Guide 34:2009 (E), Reference Materials – General Requirements for the  
Competence of Reference Material Producers

ISO Guide 35:2006 (E) Reference Materials – Guidance for Characterization and  
Assessment of Homogeneity and Stability

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:32003R1829&from=en>)