Certified Reference Materials

AOCS 0216-A

Report of the certification process for

MON 87403

Maize Certified Reference Materials

First Batch

OECD Unique ID MON-874Ø3-1

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Abstract

This report describes the preparation and certification of the maize CRM AOCS 0216-A produced by AOCS Technical Services in 2019. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of maize for transformation events. Coursely processed seed of MON 87403 was provided by Monsanto Company, St. Louis, MO (hereinafter “Monsanto”). The coursely ground MON 87403 seed powder was further milled by grinding the bulk source according to maize processing protocols at Texas A&M University. The certified value of AOCS 0216-A was based on the purity of the bulk seed material and with 95% confidence, the true value is ≥ 991.3 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 MON 87403 in the maize was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory). CRM samples should be stored in a dry, 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, Monsanto Company, for offering AOCS the opportunity to manufacture and distribute these products; to Richard Clough, Texas A&M University for processing the samples; to Sandra Harrison and Charlie Drennan at Illinois Crop Improvement Association for packaging the samples; and to Frank Spiegelhalter, Greg Ditta, E. Pearce Smith, and Daniel Thompson, Eurofins-GeneScan 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 Organisation for Standardisation

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 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 reproducible.

**Quantitative PCR**
PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules.

**Trait : MON 87403**
Through introduction of the ATHB17 gene, MON 87403 has increased ear biomass at an early reproductive phase (R1) compared to conventional control maize.
**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 0216-A was manufactured from maize according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

**Material Processing**

MON 87403 maize seeds used to prepare AOCS 0216-A were hemizygous through successive breeding generations, and the donor for the MON 87403 maize event was the female parent. Monsanto coursey processed ~15 kg of MON 87403 maize seed, and delivered the seed powder to AOCS. The coarsely ground MON 87403 seed powder was further milled by Texas A&M University according to industry standard maize processing procedures. Illinois Crop Improvement Association was contracted 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 MON 87403**

Prior to packaging, bulk seed powder 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, five (5) 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 (an ISO 17025 Accredited laboratory) were used to verify the presence of MON 87403 (Table 1).

Table 1. Trait verification testing on random composite samples of MON 87403 maize performed by Eurofins-GeneScan on bulk material provided by Monsanto

<table>
<thead>
<tr>
<th>Sample</th>
<th>MON 87403 Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite Sample 1</td>
<td>Positive</td>
</tr>
<tr>
<td>Composite Sample 2</td>
<td>Positive</td>
</tr>
<tr>
<td>Composite Sample 3</td>
<td>Positive</td>
</tr>
<tr>
<td>Composite Sample 4</td>
<td>Positive</td>
</tr>
<tr>
<td>Composite Sample 5</td>
<td>Positive</td>
</tr>
</tbody>
</table>

After the bulk material was packaged, the presence of the MON 87403 trait was assessed on 5 random vials of AOCS 0216-A. AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of trait presence and to rule out degradation during packaging. AOCS 0216-A sample numbers 520, 544, 658, 771, and 933 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for MON 87403 event-specific, qualitative PCR analysis (Table 2). This data confirms the presence of the MON 87403 in vials of AOCS 0216-A.

Table 2. Trait verification testing on AOCS 0216-A MON 87403 maize performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory).

<table>
<thead>
<tr>
<th>Sample</th>
<th>MON87403 Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOCS0216-A 520</td>
<td>Positive</td>
</tr>
<tr>
<td>AOCS 0216-A 544</td>
<td>Positive</td>
</tr>
<tr>
<td>AOCS 0216-A 658</td>
<td>Positive</td>
</tr>
<tr>
<td>AOCS0216-A 771</td>
<td>Positive</td>
</tr>
<tr>
<td>AOCS 0216-A 933</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0216-A was assessed by Monsanto. A total of 719 maize seeds were subjected to individual seed testing for the presence of MON 87403 by qualitative event-specific PCR. 717 of the 719 seeds tested positive for the presence of MON 87403.

Purity estimation was calculated using SeedCalc8 (Remund et al., 2008). The % purity in the sample was 99.72%, therefore, the assigned Certified Value was 997.2 g/kg. The Measurement Uncertainty was based on the lower bound of the true % purity. Using a 95% confidence level, the true % purity of the MON 87403 seed lot was 99.13%. Consequently, with 95% confidence, the true value is ≥ 991.3 g/kg.

Homogeneity

The homogeneity of AOCS 0216-A is related to the purity of the seeds. 717 out of 719 seeds tested positive for the MON 87403 maize event. Based on the sample purity of 99.72%, as determined using SeedCalc8, the batch was considered to be homogeneous.

Stability

Stability of these CRMs has been listed as 1 year from the introduction 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.
References

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; http://www.ilcrop.com/index.htm

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


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