Certified Reference Materials

AOCS 0906-A2

Report of the certification process for

Non-Modified Soybean

Certified Reference Materials

Second Batch

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Contents

Abstract ........................................................................................................................................... 4
Acknowledgements ......................................................................................................................... 5
Glossary ........................................................................................................................................ 6
Introduction .................................................................................................................................. 8
Material Processing ......................................................................................................................... 8
Trait Verification ............................................................................................................................. 8
Certified Value and Measurement Uncertainty ............................................................................. 10
Stability ....................................................................................................................................... 11
References ..................................................................................................................................... 11
Abstract

This report describes the preparation and certification of the soybean CRM AOCS 0906- A2 produced by AOCS Technical Services in 2021. 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 non-modified soybean powder was provided by Monsanto Company, St. Louis, MO (hereinafter “Monsanto”). It was prepared by grinding the bulk seed at Monsanto. The certified value of AOCS 0906-A2 was based on the purity of the bulk seed material and with 95% confidence, the true value is < 0.8 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 MON 88701, MON 87708, MON 87751 and MON 87769 and below limit of quantification (LOQ) of MON 89788 and MON 87705 in AOCS 0906-A2 was verified using event-specific, qualitative and quantitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (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, Monsanto Company, for offering AOCS the opportunity to manufacture and distribute these products; to Sandra Harrison, Charlie Drennan and the crew 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

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>AOCS</td>
<td>American Oil Chemists’ Society</td>
</tr>
<tr>
<td>Conventional Crop</td>
<td>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</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid is the linear, double-helix macromolecule that makes up the genetic material of most organisms</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>Lowest level at which target DNA can be detected in a sample.</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>Genome</td>
<td>The full set of genes and associated DNA characteristic of an organism</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>GMO</td>
<td>Organism that has had genetic sequences modified using molecular-level techniques</td>
</tr>
<tr>
<td>PCR</td>
<td>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</td>
</tr>
</tbody>
</table>
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 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 0906-A2 was manufactured from soybeans according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

**Material Processing**

Monsanto milled ~10 kg of non-modified soybean seed. All of the seed powder was passed through a 710 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 MON 88701, MON 87708, MON 87751 and MON 87769 and below limit of quantification (LOQ) of MON 89788 and MON soybean events in the non-modified soybean material was assessed on 10 random vials of AOCS 0906-A2. 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 Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative and quantitative PCR analysis to verify the absence or below limit of quantification (LOQ = .05%) of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 in the samples (Table 1).

Table 1. Trait verification testing on AOCS 0906-A2 non-modified soybean performed by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for presence of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769.

<table>
<thead>
<tr>
<th>AOCS 0906-A2 Sample</th>
<th>Trait MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample # 47</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 101</td>
<td>Negative (below LOQ for MON 87705)</td>
</tr>
<tr>
<td>Sample # 213</td>
<td>Negative (below LOQ for MON 87705)</td>
</tr>
<tr>
<td>Sample # 375</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 442</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 539</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 665</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 723</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 804</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
<tr>
<td>Sample # 936</td>
<td>Negative (below LOQ for MON 89788 and MON 87705)</td>
</tr>
</tbody>
</table>
Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0906-A2 was assessed by Monsanto. A total of 3750 soybeans were subjected to individual seed testing for the presence of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 by qualitative event-specific PCR. 3750 of the 3750 soybeans tested negative for the presence of MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769.

Purity estimation was calculated using SeedCalc8 (Remund et al., 2008). The % impurity in the sample was 0%, when 3750 seeds were tested. Using a 95% confidence level, the true % impurity of the MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 seed lot was at least 0.08%. Consequently, with 95% confidence, the true value is < 0.8 g/kg.

The Measurement Uncertainty was based on the upper bound of the true % purity and is the expanded uncertainty with a coverage factor of 1.65 and confidence level of 95%. The expanded measurement uncertainty for AOCS 0906-A2 is 0 g/kg.

Homogeneity

The homogeneity of AOCS 0906-A2 is related to the purity of the seeds. 3750 out of 3750 soybeans tested negative for the MON 89788, MON 88701, MON 87705, MON 87708, MON 87751 and MON 87769 soybean events 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 soybean was confirmed when 10 random vials of AOCS 0906-A2 were selected and were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 accredited laboratory) for event-specific, qualitative and quantitative PCR analysis to verify he absence of MON 88701, MON 87708, MON 87751 and MON 87769 and below limit of quantification (LOQ= .05%) of MON 89788 and MON 87705 events 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 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

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


