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## **Certified Reference Materials**

### **AOCS 0113-A**

Report of the certification process for

MON88701

Cotton Certified Reference Materials

First Batch

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ISO Guide 34  
A2LA Certificate 3438.01

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## **Abstract**

This report describes the preparation and certification of the cotton CRM AOCS 0113-A produced by AOCS Technical Services in 2013. The CRMs have been prepared according to ISO Guides 30-35 and are intended to serve as control material for third party testing of cotton for transformation events. The presence of MON88701 in the cotton was verified using event-specific, qualitative PCR analysis by Eurofins-BioDiagnostics, River Falls, WI. AOCS 0113-A is available in 20 -mL glass headspace vials. The cotton MON88701 powder was provided by Monsanto Company, St. Louis, MO. The cotton MON88701 powder was prepared by grinding the bulk source according to cotton processing protocols by Texas A&M University and were then packed under a nitrogen gas environment at Illinois Crop Improvement Association. The powder sample shall 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 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 Anna Doornink, Eurofins BioDiagnostics 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: MON88701	Glufosinate herbicide and Dicamba herbicide tolerance

## **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 0113-A was manufactured from cotton according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRM is available from AOCS.

## **Materials and Methods**

Monsanto Company, St. Louis, MO delivered 20kg of MON88701 cotton to AOCS. Before the materials were shipped for uniform processing, 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 100 g each were prepared from the composite sample and sent to Eurofins-BioDiagnostics, River Falls, WI for event-specific, qualitative PCR analysis. The analyses performed by Eurofins-BioDiagnostics, River Falls, WI were used to assess the trait presence and homogeneity of the lot.

The MON88701 cotton was processed according to industry standard cotton processing procedures, packaged in 20 -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, and to rule out degradation during packaging. Sample numbers AOCS 0113-A: 50, 215, 256, 295, 364, 374, 529, 588, 599, and 625 were sent to Eurofins-BioDiagnostics, River Falls, WI for event-specific, qualitative PCR analysis to screen for MON88701 presence in the samples.

## **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 20 - 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 MON88701 homogeneity samples is presented in Table 1.

<b>Table 1. Results of the homogeneity testing performed by Eurofins-BioDiagnostics, River Falls, WI on the MON88701 bulk material 0113-A provided by Monsanto Company, St. Louis, MO</b>	
<b>Sample</b>	<b>MON88701 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-BioDiagnostics, River Falls, WI for event-specific, qualitative PCR analysis. These results are presented in Table 2. This data confirms the presence of the MON88701 gene after the packaging of AOCS 0113-A. These results are consistent with the homogeneity data presented in Table 1.

<b>Table 2. Results for the verification of AOCS 0113-A MON88701 cotton 0113-A material as tested by Eurofins-BioDiagnostics, River Falls, WI with MON88701 event-specific, qualitative PCR analysis.</b>	
<b>Sample</b>	<b>MON88701 Presence</b>
AOCS 0113-A 50	Positive
AOCS 0113-A 215	Positive
AOCS 0113-A 256	Positive
AOCS0113-A 295	Positive
AOCS 0113-A 364	Positive
AOCS0113-A 374	Positive
AOCS 0113-A 529	Positive
AOCS 0113-A 588	Positive
AOCS0113-A 599	Positive
AOCS 0113-A 625	Positive

The AOCS 0113-A CRM was prepared solely from an identity preserved cotton pro

duced by transgenic technology. Sample heterogeneity was not considered because there was no blending of conventional and derived cotton into defined mixtures.

## References

Center for Environmental Risk Assessment GM Database

[http://www.cera-gmc.org/?action=gm\\_crop\\_database](http://www.cera-gmc.org/?action=gm_crop_database)

Eurofins-BioDiagnostics, River Falls, WI

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 30:1992 (E/F), Terms and definitions used in connection with reference materials

ISO Guide 31:2000 (E), Reference Materials- Contents of certificates and labels

ISO Guide 34:2009 (E) General requirements for the competence of reference material producers

ISO Guide 35:2006 (E) Certification of reference materials-General and statistical principles

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

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