American Oil and Chemists' Society
Certified Reference Materials
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CERTIFICATE OF ANALYSIS

AOCS 0224-B | MZIR260 | Maize | Powder

OECD Unique Identifier SYN-ØØ26Ø-3

Certified Presence	Certified Value (g/kg)	Measurement Uncertainty	Test Method
MZIR260 DNA present	970 g/kg	+30 g/kg, -56 g/kg	Event-specific, real-time PCR

Description: This is the first batch of MZIR260 maize CRM prepared by AOCS for Syngenta Seeds, LLC. It was produced in June 2024. The certified value is based on a sample purity of 96.98% (386 out of 398 seeds tested positive for MZIR260 maize). With 95% confidence, the true value is ≥ 914 g/kg. The measurement uncertainty is the expanded uncertainty with a coverage factor of 2 and confidence level of 95%. This material is for limited purposes only: see "Intended Use" and "Terms and Conditions."

This certificate is valid through: July 2027

This validity may be extended when further evidence of stability becomes available. Customers will be notified by AOCS if a stability issue arises at AOCS Headquarters.

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AOCS Mission: AOCS advances the science and technology of oils, fats, proteins, surfactants and related materials, enriching the lives of people everywhere.

Characterization

Product Description AOCS 0224-B has been prepared by AOCS from genetically modified hemizygous MZIR260 maize seed. AOCS 0224-B is available in 27-mL glass headspace vials containing approximately 10 g of material. Users are informed that this reference material has been produced from seed of MZIR260 maize delivered by Syngenta Seeds, LLC. The maize hybrid used in the preparation of AOCS 0224-B resulted from the cross of female non-transgenic line IJ7010 and male line AX5707(MZIR260).

Homogeneity and Trait Verification The homogeneity of AOCS 0224-B is related to the purity of the seeds. 386 out of 398 MZIR260 maize seeds tested positive for the MZIR260 event by event-specific PCR. Based on the sample purity of 96.98% as determined using SeedCalc8, the batch was expected to be homogenous.

To further confirm homogeneity, ten vials of AOCS 0224-B were evaluated to determine the within-unit and between-unit homogeneity of the property value. The property value is defined as the ratio between copies of the event-specific target (MZIR260) and copies of a taxon-specific reference target (*adh1*). Variability of this ratio within CRM vials and between CRM vials is reported as the within-unit relative standard deviation (RSD_w), and the between-unit relative standard deviation (RSD_b), respectively. The results summarized below confirm that AOCS 0224-B is homogenous. The presence of Event MZIR260 in MZIR260 maize was verified in all ten vials.

CRM	RSD_w [%]	RSD_b [%]	$u^*_{bu\ rel}$ [%]
AOCS 0224-B	4.6	n.c.[1]	2.2

[1]n.c: RSD_b cannot be calculated as $MS_{between} < MS_{within}$. In this situation, maximum hidden inhomogeneity ($u^*_{bu\ rel}$) is provided as an alternative

Stability AOCS Certified Reference Materials (CRMs) are assessed for transport (short-term) and long-term stability and tested for stability on an annual or biennial basis, depending on the type of homogeneity testing that is required, and certificates may be extended based on the outcome of this testing. Customers may request extended certificates, but they are informed that results are based on samples that are obtained from AOCS' inventory. AOCS cannot guarantee the integrity of samples outside of AOCS control.

Analytical Method Used for Certification

The Certified value is based on the purity of the MZIR260 maize material used in production of this CRM. Eurofins-GeneScan, Freiburg, Germany (an ISO 17025-accredited laboratory) performed MZIR260-specific quantitative real-time PCR to determine the copy number ratio between the MZIR260-specific target and the taxon-specific reference target for the assessment of homogeneity. Purity, homogeneity and stability results were used to determine the expanded measurement uncertainty of the certified value.

Warnings and Precautions

This product is for laboratory use only and is not for consumption. The user of this CRM should follow safety requirements and rules issued by voluntary organizations and government agencies expert in the field of laboratory safety.

Intended Use

This CRM, AOCS 0224-B, is intended for use as quality control material or calibrant in methods for the detection, identification, and/or quantification of biotechnology-derived events.

Instructions for Use

Upon receipt the product should be stored in a sealed container in the dark at ambient or cooler conditions. The product may have settled during shipment, therefore, thoroughly mix the CRM before use to ensure homogeneity.

If the user of this CRM intends to use it multiple times, proper protocols must be followed to ensure that the sample retains its integrity. Use a clean laboratory spatula to remove the intended sample amount. After the sample has been removed, flush the headspace of the vial with nitrogen gas, then replace the rubber stopper. Place a new 20 mm tear-off aluminum unlined seal on top of the rubber stopper and crimp it to the vial using a crimping tool. Store the CRM in the dark at ambient or cooler temperatures and repeat this process for all subsequent uses.

Sample size:

The recommended **sample intake** suitable for DNA extraction and real time PCR is 1 g.

Protocols have been followed to ensure that this CRM has the presence of the MZIR260 trait. Please follow all instructions on this certificate to prevent contamination and be sure to store the CRM under the proper conditions.

Note: The AOCS 0224-B certification report is available online and a paper copy can be supplied upon request.

Terms and Conditions (i–ix)

i. The CRM AOCS 0224-B shall be used solely 1) in assays for (a) detecting the presence of MZIR260, or (b) quantification of MZIR260; or 2) for determining whether an assay cross-reacts with CRM AOCS 0224-B. CRM AOCS 0224-B shall be used for no other purpose. Specifically, the CRM may not be used to develop a detection method for MZIR260 nor trait(s) present therein. No other rights are conveyed by the sale of the CRM 0224-B to any purchaser, including any rights to any pending or granted Syngenta Seeds, LLC patents or other Syngenta Seeds, LLC intellectual property that may protect the CRM or MZIR260 or trait(s) present therein or a detection method for MZIR260.

ii. Neither the CRM AOCS 0224-B nor any extract or portion thereof shall be resold or redistributed by any purchaser, unless the resale or redistribution is required by national law in force in the purchaser's country.

iii. Neither the CRM AOCS 0224-B, nor any extract or portion thereof, shall be used for human or animal consumption or human or animal trials.

iv. Neither the CRM AOCS 0224-B nor MZIR260 DNA, nor any part of either of these, shall be used for transformation or breeding.

v. No characterization or derivation of MZIR260, of the trait or traits present in CRM AOCS 0224-B, or of the CRM AOCS 0224-B shall be performed, except as allowed for in section (i).

vi. All assay activities undertaken using the CRM AOCS 0224-B shall be conducted in strict compliance with all applicable laws governing such activities and shall comply with conditions of all permits and authorizations which may be required for such activities; and such activities shall be strictly limited to assays in contained facilities, for example, laboratories.

vii. Prior to disposal of any used or excess CRM AOCS 0224-B or residues thereof, such material or residue must be treated in a manner that degrades MZIR260 in the CRM material, such as by autoclaving.

viii. CRM AOCS 0224-B shall not be exported nor re-exported in violation of any applicable laws or without securing any necessary export or import clearances or permits.

ix. THE CRM 0224-B IS PROVIDED FOR THE PURPOSE OF IDENTIFYING, DETECTING AND QUANTIFYING MZIR260 AND FOR NO OTHER PURPOSE. AOCS HAS TESTED CRM 0224-B WITHIN THE PAST 24 MONTHS AND STATES THAT IT HAS DETERMINED IT TO BE OF SUFFICIENT QUALITY AND FIT FOR THE PURPOSES STATED HEREIN. NO SYNGENTA SEEDS, LLC WARRANTY IS PROVIDED WHETHER EXPRESS OR IMPLIED, IN RELATION TO THE CRM AOCS 0224-B AND SYNGENTA SEEDS, LLC MATERIALS. SYNGENTA SEEDS, LLC MAKES NO REPRESENTATION OR WARRANTY THAT THE USE OF THE CRM AOCS 0224-B, WHETHER BEFORE, OR AFTER THE EFFECTIVE DATE OF THE APPLICABLE CERTIFICATE, WILL NOT INFRINGE ANY PATENT OR OTHER PROPERTY RIGHTS.