



Food Ingredient Testing Bt Maize Kit User's Guide

A guide for using the **GMO** Bt Maize Test Kit to detect thresholds of **Genetically Modified Organisms (GMOs)** in food ingredients.

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IMPORTANT NOTICE:

All kit components must be stored at 2-8°C.

Allow kit components to warm up to room temperature before use in the ELISA procedure.

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INTRODUCTION

The **GMO** Bt Maize Test Kit detects the Cry1Ab protein produced by a gene derived from *Bacillus thuringiensis* (*Bt*). This gene has been incorporated into insect-resistant corn including YieldGard[®] brands from Monsanto (MON810) and Novartis (Bt11). The kit has been developed to identify this protein in Maize Processed Food Fractions such as, flours, meals, grits and glutens. Each kit contains Reference Standards includes a negative control and three (3) positive references containing the labeled percentage of a YieldGard[®] (MON810) corn flour. The Corn Flour Reference Standards can be used with other processed fractions such as ground corn, corn meal, and corn glutens (requires 5X dilution of gluten extracts).

Each run must meet the accept/reject criteria in the procedure to be valid. The run will consist of the following: one extraction each of the GMO Positive References, Negative Control and each sample. All extracts will be run in duplicate along with an Assay Blank. If a run does not meet the “run” acceptance criteria, the entire run must be repeated. Samples that do not pass the acceptance criteria in any run must be re-run a second time. Any additional runs must also include the GMO negative and positive Reference Standards. Kit components provide enough reagents for one entire plate run. Data may be reported on the included reporting sheets or through a suitable data management software package

Reagents and Materials Provided

1. Buffer Concentrate (10X): Qty: 200 mL.
2. Coated Strip Wells: Qty: 8 strips & Strip Holder: Qty: 1.
3. Plate sealers: Qty: 3.
4. Bt Maize Conjugate 1: Qty: 15 mL.
5. Bt Maize Conjugate 2: Qty: 15 mL.
6. Color Solution: Qty: 15 mL.
7. Stop Solution: Qty: 15 mL.
8. **GMO** Bt Maize Reference Standards at 0%, 0.15%, 0.5% and 2.0%. Standard levels are based on YieldGard[®] (Mon810) corn flour.
Quantity – 3 grams per standard level.

Materials Required But Not Provided

1. 15 mL polypropylene conical centrifuge tubes. Quantity-50.
2. Transfer pipettes. Quantity- 50.
3. Weigh boats or Equivalent. Quantity-50 without cleaning.
4. Spatulas. Quantity-50 without cleaning.

5. Plastic Tape for manual plate washing.
6. Wash bottle (Fisher Scientific 18oz -500 mL Nalgene Wash Bottle #03-409-10E or Equivalent) if doing manual plate washing.

Equipment Required But Not Provided

1. Precision pipettes capable of delivering 100-1000 μ L.
2. Pipettes capable of delivering 4.0 mL.
3. 40 US mesh screen
4. Vortex mixer.
5. Balance capable of 0.01 gram measurement.
6. Centrifuge capable of 5,000 to 10,000 rpm.
7. Microtiter plate reader capable of reading absorbance at 450 nm (preferably with subtraction of 650nm absorbance capability).

Materials Recommended But Not Required

1. Multi-channel pipette (100 μ L).
2. Reagent reservoirs for multi-channel dispensing.
3. Automated plate washer.
4. Test tube rack, 15 mL centrifuge tubes

TEST PREPARATION

Note: Allow all reagents to warm to room temperature before using.

Preparation of Bt Maize Coated Strips for automated or manual plate washing:

If using an automated plate washer: Remove the Bt Maize Coated Strips and Strip Holder from the foil bag. If less than a full plate of samples needs to be run, replace the required number of Bt Maize Coated Strips with Uncoated Strips (not included with the kit). Place unused Bt Maize Coated Strips back into the foil bag and seal the ziploc end. As illustrated in Figure 1, six (6) wells of one strip are required for the Reference Standards and Assay Blanks. For additional runs, a new set of Reference Standards and Assay Blanks must be run. Always seal the foil bag, immediately, after removing the Bt Maize Coated Strips.

If using manual washing: Remove the Bt Maize Coated Strips and Strip Holder from the foil bag. Arrange the required number of Bt Maize Coated Strips into the strip holder. Place the unused Bt Maize Coated Strips back into the foil bag

and seal the ziploc end. Tape the edges of all (12) Strips to the Strip Holder to prevent strips from accidentally falling out of the strip holder during Wash Step. As illustrated in Figure 1, ten (10) wells of one strip are required for Reference Standards and Assay Blanks. For additional runs, a new Negative Control, Positive References and Assay Blanks must be run. Always seal the foil bag, immediately, after removing the Bt Maize Coated Strips.

Preparation of Bt Maize Buffer

1. Allow the 10X Buffer Concentrate to come to room temperature.
2. Dilute the 10X Buffer Concentrate in De-ionized water to prepare the Working Bt Maize Buffer. *Example: 50 mL 10X Buffer Concentrate into 450 mL deionized water.*
3. Add to the required volume to the Automatic Plate Washer or Wash Bottle (Fisher Scientific 18oz -500 mL Nalgene Wash Bottle #03-409-10E, or equivalent).

SAMPLE PREPARATION

NOTE: All samples, including processed food fractions, should be a particle size that passes through a 40 U.S. Mesh Screen before analysis.

1. Ground Maize

- (a) Determine a representative weight of maize (typically 500-1000 g) and grind in into a powder in an appropriate grinder. All the maize should be ground and the resulting powder must be a homogeneous mixture. Contact SDI Technical Support for recommended grinding protocols.
- (b) Sieve the resulting ground maize mixture through a 40 U.S. mesh screen. Hand pressure may need to be applied to pass through the 40 U.S. mesh screen.
- (c) Collect the resulting 40 U.S. mesh screened sample for use in the Extraction Procedure described in step 2. At least 1.0 grams are required per sample.
- (d) The ground and sieved maize unknown samples should be compared to the Maize Flour standards for analysis.

2. Extraction Procedure

- (a) Weigh out 1.0 g (± 0.05 g) of the Negative Control, Positive References and unknown samples using a weigh boat or equivalent and a spatula. *Note: Clean spatula and weigh boat between samples.*
- (b) Transfer into a labeled 15 mL polypropylene centrifuge tube.

- (c) Pipette 4.0 mL of Bt Maize Buffer into tube containing 1.0 g sample and **vortex for at least one (1) minute.**
- (d) Allow tubes to stand for 5 minutes then centrifuge at approximately 5000 rpm for 5 minutes.

3. Dilution of Maize Gluten Sample Extracts

Samples and Reference Controls do not require dilution prior to analysis except for maize gluten samples. Maize Gluten extracts should be diluted as described below and compared to undiluted extracts of the Reference Standards. Note: Figure 3 illustrates the dilution scheme required.

- (a) Pipette 400 μ L of the Bt Maize Buffer into the 12x75-mm test tube. Label each tube with the appropriate sample ID
- (b) Pipette 100 μ L of each sample extract, from step (d) of the “Extraction Procedure”, into the appropriately labeled and vortex to mix.
- (c) Use the diluted extract from step (b) as the sample extract in the Assay Procedure described below.

NOTE: Run Bt Maize extracts immediately after extraction. Bt Maize extracts and diluted extracts CANNOT be used for subsequent runs.

ASSAY PROCEDURE

ELISA Test Procedure

1. Sample Addition:

Add 100 μ L of extracts (from step (2d) of the “Extraction Procedure” above.) or diluted maize gluten extract to the appropriate wells in duplicate (Refer to Figure 1). Cover plate with supplied plate sealers to prevent contamination and evaporation. For Assay Buffer Blanks, add 100 μ L Bt Maize Buffer to each well.

2. Sample Incubation:

Incubate assay strips at room temperature for 1 hour.

3. Wash Cycle:

Wash 4X with Bt Maize Buffer (300 μ L /well). If an automated plate washer is not available, manual washing can be performed as follows:

- (a) Invert the Strip holder and discard the contents into a sink or suitable waste container. Tap the inverted Strip holder onto a stack of paper towels to remove residual sample.

- (b) Add Wash Buffer to each well of the Bt Maize Coated Strips with the Wash Buffer bottle (Fisher Scientific 18oz -500 mL Nalgene Wash Bottle #03-409-10E , or Equivalent). Fill each well with an overflow volume of Wash Buffer.
- (c) Invert the Strip holder and discard the contents into a sink or suitable waste container. Tap the inverted Strip holder onto a stack of paper towels to remove residual wash buffer.
- (d) Repeat steps (b) and (c) above three more times.

NOTE: Do not let wells dry out, as it may affect assay performance.

4. ***Addition of Bt Maize Conjugate 1:***
Add 100 μ L of the Bt Maize Conjugate 1 to each well of Bt Maize Coated Strips. Cover plate with supplied plate sealers to prevent evaporation.
5. ***Bt Maize Conjugate 1 Incubation:***
Incubate assay strips at room temperature for 1 hour.
6. ***Wash Cycle:***
Wash 4X with Wash Buffer as described in step 3 above.
7. ***Addition of Bt Maize Conjugate 2***
Add 100 μ L of the Bt Maize Conjugate 2 to each well of Bt Maize Coated Strips. Cover plate with supplied plate sealers to prevent evaporation.
8. ***Bt Maize Conjugate 2 Incubation:***
Incubate assay strips at room temperature for 45 minutes.
9. ***Wash Cycle:***
Wash 4X with Wash Buffer as described in step 3 above.
10. ***Color Development:***
 - (a) Add 100 μ L of Color Solution to each well of the Bt Maize Coated Strips and incubate for 20 minutes at room temperature.
 - (b) After the 20 minute incubation, stop color development, by adding 100 μ L of Stop Solution to each well of Bt Maize Coated Strips in the same sequence that the Color Solution was added.
 - (c) Read the absorbance of the developed color at 450 nm using a microtiter plate reader.

RAW DATA LOGS

If an automated plate reader print out is not available, record the raw data on the attached Data Logs, Figure 2.

ACCEPT/REJECT CRITERIA

The following Accept/Reject Criteria is **recommended**:

A run is considered acceptable only if the following criteria are met:

1. A run is considered acceptable only if the following criteria are met:
 - (a) The mean of duplicate determinations of the Assay Buffer Blank OD at 450 nm are ≤ 0.20 .
 - (b) The mean of duplicate determinations of the 0% Negative Standard OD at 450 nm is ≤ 0.30
 - (c) The mean of duplicate determinations of the 2.0% Reference Standard OD at 450 nm is ≥ 0.8
 - (d) The %CV of duplicates for the 0.15%, 0.5% and 2.0% Reference Standards must be $\leq 15\%$.
 - (e) The correlation coefficient (r^2) generated from the linear regression analysis of the Reference Standards must be greater than 0.96
2. Unknown sample results are considered acceptable if the %CV for the duplicates is $\leq 15\%$. This criteria applies **ONLY** when the mean sample OD is ≥ 0.30

DATA INTERPRETATION

Test Results

Average the absorbance readings for the duplicate reference standards, controls and unknowns.

Plot a standard curve of the mean absorbance (OD) vs. %GMO of the Negative and Positive Reference Standards on a linear graph. Perform a linear regression analysis of the standards, including the 0% standard to obtain a best-fit line. An EXCEL spreadsheet can be provided for the linear regression analysis by contacting the SDI Technical Service Department.

Interpolate the unknown sample concentrations from the reference standard curve using the regression estimate. Ensure the accept/reject results are within accepted quality control criteria prior to reporting sample results.

Corn Varieties

The **GMQ** Bt Maize Reference Standards are based on the most common Bt maize brand grown; Monsanto YieldGard[®] MON810 Bt maize event. Samples containing other events (or mixed events), such as Novartis YieldGard[®] Bt11 event or the Novartis KnockOut[®] and Mycogen NatureGard[®] from (Event 176) will require different interpretation. Contact SDI Technical Service Department for assistance with this interpretation.

PRECAUTIONS

- Store all test components refrigerated (2-8°C). Storage at ambient temperature on the day of use is acceptable.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Allow reagents to reach ambient temperature (18-27°C or 64-81°F) before beginning the test. This typically requires at least 1 hour to warm from recommended storage conditions.
- Do not use components after their expiration date.
- Do not expose Color Solution to direct sunlight.
- Read wells within 15 minutes after addition of Stop Solution.
- Do not mix reagents from different test kit lots.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure. This may give inaccurate results.

DISPOSAL OF RESIDUAL COMPONENTS

For residual reagents, samples and references flush down sink with copious amounts of water. All remaining plastic and paper components can be disposed of as standard solid waste.

TECHNICAL SUPPORT

For questions on the procedure or general technical support please contact:

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