

Importance of Bt Cry1Ab/Cry1Ac Determination

Bt Cry1Ab/Cry1Ac, a protein derived from the bacteria *Bacillus thuringiensis*, is expressed in certain genetically modified (GM) corn and cotton plants, such as YieldGard® corn and Bollgard®, WideStrike® cotton. The Cry1Ab/Cry1Ac protein targets the larvae of multiple Lepidopteran (moth) species by binding to specific receptors in the larva's gut with cascading effects that ultimately leads to death (1).

Routes of exposure affecting regulatory decisions include direct contact, such as non-target organisms feeding on crop tissues, seeds, or plant residues containing Cry1Ab/Cry1Ac, and indirect contact, such as exposure to the toxin in pollen, soil contaminated by decomposing plant material containing Cry1Ab/Cry1Ac, and predators consuming target species (2).

Although initial testing showed low environmental impact and toxicity, controversy has arisen over the long-term impact of GM crops on the environment and whether or not GM foods are safe for consumption. Several animal studies have shown serious health risks, such as infertility, immune problems, accelerated aging, faulty insulin regulation, and changes in major organs including the gastrointestinal system, can be associated with the consumption GM products (3).

Bt proteins have been shown to leach into the soil and streams (4).

Efforts like the Non-GMO Project are raising awareness towards the growing number of problems associated with this technology and generating a larger public demand for manufacturers to label their products accordingly.

The Abraxis Bt Cry1Ab/1Ac ELISA allows for the analysis of 46 samples in duplicate determination. The test can be performed in 90 minutes.

Performance Data

Standard Curve:

NOTE: Quantitative standard curve

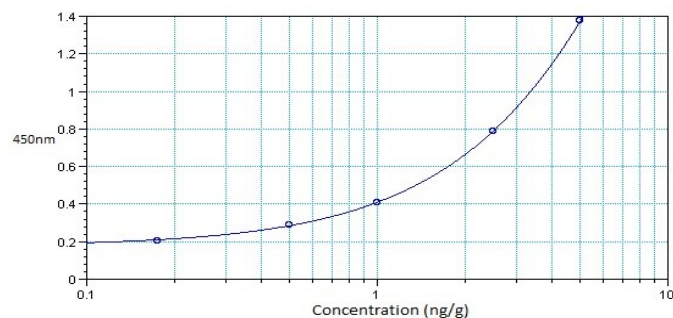
Is for illustration purposes only and

To demonstrate assay sensitivity.

The limit of detection is 0.175 ng/g.

The kit is offered with positive and

Negative controls only.



For demonstration purposes only. Not for use in sample interpretation.

This kit may be used with known percentages of Bt Cry1Ab/Cry1Ac-expressing corn samples, such as MON810 or Bt11 which are available from the European Commission Joint Research Centre, Institute for Reference Materials and Measurements. They can be used as standards or calibrators to test for measurement of ground corn samples. This kit can also be calibrated with pure Cry1Ab or Cry1Ac protein (PN 300003, 250020), available from Abraxis, LLC. This kit shows <1% cross reactivity with Bt Cry9Ac, Bt Cry2A, Bt Cry1Ab, Bt Cry1Ac, Bt Cry3B, and CP4 EPSPS.

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Bollgard® and Yieldgard® are registered trademarks of the Monsanto Company.

Pellet Pestles® is a registered trademark Kimble Chase Life Science and Research Products LLC.

Acrodiso® is a registered trademark of Pall Corporation.

GIPSA Sample Prep. References: USDA Grain Inspection Handbook, Book 1, Grain Sampling:

http://www.gipsa.usda.gov/fqis/handbook/qihbk1_inspec.aspx

Guidance document entitled Sampling for the Detection of Biotech Grains:

<http://www.gipsa.usda.gov/fqis/biotech/sample2.htm>

Practical Application of Sampling for the Detection of Biotech Grains:

<http://www.gipsa.usda.gov/fqis/biotech/sample1.htm>

Sample Planner Spreadsheet download:

www.gipsa.usda.gov/fqis/biotech/samplingplan1.xls

References: (1) Hellmich, R. L. & Hellmich, K. A. (2012) *Use and Impact of Bt Maize*. Nature Education Knowledge 3 (10):4

(2) "Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants". *Transgenic Research* (2010) 20 (1): 1– 22

(3) "GMO Dangers – Health Risks Brochure." Institute for Responsible Technology. www.responsibletechnology.org/gmo-dangers.

(4) "Occurrence of Maize Detritus and a Transgenic Insecticidal Protein (Cry1Ab) within the Stream network of an Agricultural Landscape." *PNAS* Oct 2010; Vol 107, No. 41:17645-17650. "Occurrence and Persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt Corn Cry1Ab gene from an Aquatic Environment." *Ecotoxicol Environ Saf.* 2007 Feb; 66(2):195-203.

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01292016

Bt Cry1Ab/Cry1Ac ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of
Bt Cry1Ab/Cry1Ac in Seeds, Leaf Tissue, Water, and Soil Samples

Product No. 510001



1. General Description

The Bt Cry1Ab/1Ac ELISA is an immunoassay for the qualitative screening detection of Bt Cry1Ab/1Ac protein residues. The *Bacillus thuringiensis* (Bt) Cry1Ab/1Ac protein is an insecticidal crystalline protein expressed by the Cry1Ab/1Ac gene in certain strains of genetically modified plants. This test is suitable for the qualitative detection of Bt Cry1Ab/1Ac in MON810, Bt11, Bollgard®, Bollgard II®, or WideStrike® corn seed, cotton seed, leaf tissue, surface water, and soil samples (please refer to the appropriate sample preparation or extraction). If necessary, positive samples can be confirmed by PCR or other conventional methods.

2. Safety Instructions

The positive control solution in the test kit contain small amounts of Bt Cry1Ab/1Ac. In addition, the substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of substrate and stopping solutions with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Bt Cry1Ab/1Ac ELISA Kit should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a "sandwich" ELISA based on the recognition of Bt Cry1Ab/1Ac by specific antibodies. Controls, prepared samples, and extracts are added to microtiter wells coated with anti-Bt Cry1Ab/1Ac polyclonal antibodies. The Bt Cry1Ab/1Ac in the controls and samples or extracts will be bound to the antibody coated wells. After a 30-minute incubation proceeded by a washing step, the "sandwich" is completed by the addition of an enzyme-labeled anti-Bt Cry1Ab/1Ac polyclonal antibody. The enzyme-labeled conjugated antibody is washed from the wells after a 30-minute incubation. After a final washing step, the substrate solution is added to produce a color signal. The intensity of the blue color is directly proportional to the concentration of the Bt Cry1Ab/1Ac present in the sample. The color reaction is stopped after 20 minutes and the color is evaluated using an ELISA plate reader. The sample is considered positive when the absorbance value is above the absorbance value of the negative control reagent.

5. Limitations of the Bt Cry1Ab/1Ac ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Samples must be extracted and diluted as instructed in the sample preparation section (Section C) or appropriate technical bulletin before testing in the ELISA.

Mistakes in handling the test also can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis Bt Cry1Ab/1Ac ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), positive samples should be confirmed by an alternative method, such as PCR.

A. Reagents and Materials Provided

1. Microtiter plate coated with an anti-Bt Cry1Ab/1Ac polyclonal antibody, in a resealable aluminum pouch, 96 wells
2. Bt Cry1Ab/1Ac Positive Control Solution, 1 mL
3. Negative Control Solution, 1 mL
4. Anti-Bt Cry1Ab/1Ac polyclonal antibody-HRP conjugate, 4 vials (lyophilized)
5. Antibody conjugate diluent, 12 mL
6. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
7. Extraction Solution/Sample Diluent (5X) Concentrate, 2 x 25 mL, must be diluted before use, see Test Preparation (Section D)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Timer
5. 5 mL microcentrifuge vials
6. Scoopula
7. Analytical 3 place balance (optional)
8. Pipette bulbs
9. Vortex mixer
10. 4 mL glass vials with Teflon-lined caps
11. Centrifuge capable of spinning at 3,000 rpm (1500 x g) (optional)
12. 15 mL or 50 mL centrifuge vials (optional)
13. Low protein binding syringe filter (0.8/0.2 µm Pall Acrodisc® PN 4905 or equivalent) with syringe (optional)
14. Small plastic bags or wax paper
15. Pliers, hammer, seed crusher, or disposable PELLET PESTLES® with microcentrifuge tubes (Kimble® PN 749520-0000 or equivalent)

C. Sample Preparation (To collect a composite sample according to the USDA/GIPSA guidelines, follow the links found in the "Sample Prep. References" in the final page of this insert.)

Corn, Cotton, Soybean Seed

1. Place a single seed in a small plastic bag and crush with a pliers or hammer (or place in seed crusher if available). Transfer the crushed sample to a 5 mL centrifuge vial. Note: Take precautions to avoid sample cross-contamination. If a quantitative result is desired, the seed must be weighed.
2. Add 4.0 mL of the 1X Extraction Solution (see Section D) to the centrifuge vial. Cap the vial and vortex for 30 seconds.
3. Let the sample settle for at least one minute.
4. The extract is ready to be analyzed (Section F. Assay Procedure, step 1).
5. Note: If a lower limit of detection is needed, 1.0 mL of 1x Extraction Solution can be used.

Leaf Samples

1. Take 2-3 leaf punch samples by snapping the cap of the tube closed on the leaf. Determine the sample weight in mg. Note: Take precautions to avoid sample cross-contamination. If a quantitative result is desired, the sample must be weighed.
2. Grind the tissue by twisting and rotating the pestle in the tube until pulverized.
3. Add 500µL of 1X Extraction Solution/Sample Diluent to the tube.
4. Grind the tissue in the extraction solution by twisting and rotating the pestle in the tube (about 30 seconds).
5. Re-seal the microcentrifuge tube, vortex for 30 seconds, and let settle for at least 1 minute.
6. The extract is ready to be analyzed (Section F. Assay Procedure, step 1).

For soil samples, please refer to the appropriate technical bulletins.

Water Samples (water samples should be collected in glass jars with Teflon-lined caps and preserved immediately.)

1. Add 1 mL of 5X Extraction Solution/Sample Diluent for every 4 mL of water sample, immediately after sample collection. Mix well.
2. After samples are diluted, those samples containing gross particulate matter should be settled, centrifuged, or filtered using a syringe and low protein binding syringe filter (ex. 0.8/0.2 µm Pall Acrodisc® PN 4905 or equivalent).
3. Analyze as sample (Section F. Assay Procedure, step 1).

The Bt Cry1Ab/Cry1Ac concentration in the water sample is determined by multiplying the ELISA result by a factor of 1.25. Highly contaminated samples, those outside of the calibration range of the assay, must be diluted further with 1X Extraction Solution/Sample Diluent and re-analyzed.

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the controls and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, substrate, and stop solutions. Please only use the reagents and controls from one package lot in one test, as they have been adjusted in combination.

1. Dilute the Extraction Solution/Sample Diluent (5X) Concentrate at a ratio of 1:5 (i.e. 1 mL of solution added to 4 mL of deionized or distilled water and mix thoroughly) before extracting seed/leaf tissue samples or diluting samples/extracts. Do not dilute if using to preserve water samples, see Sample Preparation (section C, Water Samples).
2. Adjust the microtiter plate and the reagents to room temperature before use.
3. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
4. The controls, substrate, and stop solutions are ready to use and do not require any further dilutions.
5. The HRP conjugate provided is lyophilized (4 vials). Once reconstituted, the conjugate solution will only remain viable for 1 week refrigerated and 4 weeks if stored at -20 °C. If additional samples are to be analyzed greater than the listed shelf, a new vial of conjugate must be prepared. To reconstitute, add 3 mL of Antibody Conjugate Diluent to each vial of conjugate required and vortex. If more than 1/4 of plate needs to be run, then combine the reconstituted HRP conjugate vials, vortex slowly and add to plate as described in the Assay procedure (Section F).
6. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
7. The stop solution must be handled with care as it contains diluted H2SO4.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards that have been determined in a test performed previously.

Positive Control (Pos)

Negative Control (Neg)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos	etc										
B	Pos	etc										
C	Neg											
D	Neg											
E	Samp1											
F	Samp1											
G	Samp2											
H	Samp2											

F. Assay Procedure

1. Add 100 µL of the **positive control, negative control, and samples or sample extracts** (Section C) into the wells of the test strips according to the working scheme given. Analysis in duplicate is recommended. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
2. Incubate the strips for 30 minutes at room temperature.
3. Remove the covering and decant the contents of the wells into a sink. Wash the strips **three times** using the **diluted washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
4. Add 100 µL of **Enzyme-conjugated anti-Bt Cry1Ab/Cry1Ac Antibody Solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
5. Incubate the strips for 30 minutes at room temperature.
6. Remove the covering and decant the contents of the wells into a sink. Wash the strips **three times** using the **diluted washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
7. Add 100 µL of **substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
8. Add 50 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

The interpretation of the qualitative results can be derived by simple comparison of the sample absorbances to the absorbances of the controls. Samples with absorbances that are above 0.200 are considered positive for Cry1Ab/Cry1Ac. Samples with absorbances below 0.200 are negative for Cry1Ab/Cry1Ac.

The mean absorbance of the negative control should not exceed the OD of 0.200. A valid test is when the positive control absorbance is above 0.200 and the negative control absorbance is below 0.200.

The coefficient of variance (%CV) between replicate positive control wells and negative control wells should not exceed 10%.

$$\text{To calculate the \%CV: } \%CV = \frac{\text{Standard Deviation OD}}{\text{Mean Pos. or Neg. OD}} \times 100$$