**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® and 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/Cry1Ac Strip Test Kit allows for the analysis of 50 samples. The test can be performed in less than 30 minutes.

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.



GIPSA Sample Prep.

References:

USDA Grain Inspection Handbook, Book 1, Grain Sampling:
http://www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx
Guidance document entitled Sampling for the Detection of Biotech Grains:
http://www.gipsa.usda.gov/fgis/biotech/sample2.htm
Practical Application of Sampling for the Detection of Biotech Grains:
http://www.gipsa.usda.gov/fgis/biotech/sample1.htm
Sample Planner Spreedsheet download:
www.gipsa.usda.gov/fgis/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.



For ordering or technical assistance contact:

Abraxis LLC
124 Railroad Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

01292016

# Bt Cry1Ab/Cry1Ac Strip Test

Immunochromatographic Strip Test for the Detection of
Bt Cry1Ab/Cry1Ac in Corn and Cotton Samples
Product No. 510300

## 1. General Description

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. The Bt Cry1Ab/1Ac ELISA is a rapid immunochromatographic test, designed solely for use in the qualitative screening to detect the presence of Bt Cry1Ab/1Ac in corn and cotton seeds and leaf 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

Discard samples according to local, state, and federal regulations.

## 3. Storage and Stability

The Bt Cry1Ab/Cry1Ac Strip Kit should be stored between 4-30°C. The test strips, test vials, and samples to be analyzed should be at room temperature before use.

## 4. Test Principle

The lateral flow test is based on a "sandwiched" format of the specific immuno-chemical reaction between a gold-labeled antibody, a protein, and an unlabeled-antibody. Bt Cry1Ab/Cry1Ac, if present in a sample solution, will bind with gold-labeled antibodies in the sample pad, forming an antibody-protein complex. The solution wicks up causing the fluid to pass over an area containing the immoblized antibodies specific to the protein on the nitrocellulose membrane forming a sandwich (test line). The excess unbound gold-labeled antibodies will continue to migrate up the strip and bind to different immobilized antibodies specific to the gold -labeled antibody, producing a second visible line (control line). If Bt Cry1Ab/Cry1Ac is present in a sample at a quantity above the limit of detection, it will saturate the antibody binding sites forming a line. If there is no Bt Cry1Ab/Cry1Ac present, it is considered a negative result. Any line delevops at the test line is considered a positive result. The control line is not influenced by the presence or absence of Bt Cry1Ab/Cry1Ac in the sample, and therefore should be present in all reactions.

## 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.

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/Cry1Ac Strip kit provides screening results. As with any analytical technique (GC, HPLC, etc.), positive samples should be confirmed by an alternative method, such as PCR.

## 6. Warnings and Precautions

-Use reasonable judgment when interpreting the test results.
-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
-For test strips packaged in a desiccant vial, the vial should be kept completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
-Avoid touching or bending the membrane on the dipstick.
-Store Test Kit between 4-30˚C. Do not freeze.
-Avoid cross-contamination of samples by using a new conical vial and disposable pipette for each sample.
-Use only the Test Strip reagents from one kit lot, as they have been adjusted in combination.
-Seeds are capable of absorbing liquid. Therefore less liquid would be available in the supernatant for strip testing. To avoid this, centrifugation can be used to separate the liquid with the seeds. If a centrifuge is not available, you can increase the amount of samples and buffer (while maintaining the same ratio).

## A. Reagents and Materials Provided

1. 2 containers of 25 test strips
2. 5x Extraction Solution/Sample Diluent
3. Instructions

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

1. Timer
2. 2 mL and 5 mL microcentrifuge vials
3. Deionized water
4. Scoopula
5. Pipette bulbs
6. Vortex mixer
7. Centrifuge capable of spinning at 3,000 rpm (1500 x g) - optional
8. 15 mL or 50 mL centrifuge vials - optional
9. Low protein binding syringe filter (0.8/0.2 µm Pall Acrodisc® PN 4905 or equivalent) with syringe - optional
10. Small plastic bags or wax paper
11. Pliers, hammer, seed crusher, or disposable PELLET PESTLES® with microcentrifuge tubes (Kimble® PN 749520-0000 or equivalent)

## C. Sample Preparation

**Corn and Cotton Seeds***

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 2 mL or 5 mL centrifuge vial. ***Note:*** *Take precautions to avoid sample cross-contamination.*
2. Add 1.0 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. Centrifuge if necessary.
4. The extract is ready to be analyzed (Section E. Test Procedure, step 1).

**Leaf Samples**

1. Take 2-3 leaf punch samples by snapping the cap of the tube closed on the leaf. ***Note:*** *Take precautions to avoid sample cross-contamination.*
2. Grind the tissue by twisting and rotating the pestle in the tube until pulverized.
3. Add 1 mL 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 E. Test Procedure, step 1).

***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.**

## D. Test Preparation

Please only use the reagents and strips from one package lot in one test, as they have been adjusted in combination. Allow the reagents, strips, and samples to reach room temperature before use.

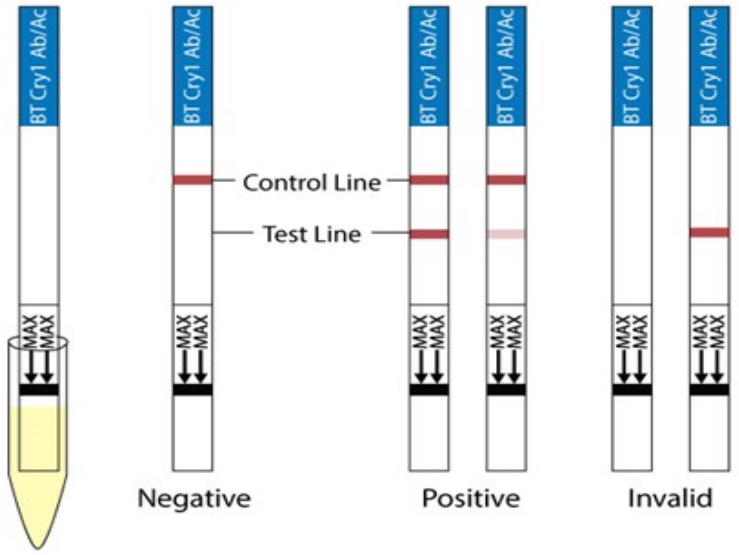
1. Dilute the Extraction Solution/Sample Diluent (5X) Concentrate at a ratio of 1:5 (i.e. 1 mL of solution added to 4 mL of deionized or distilled water and mix thoroughly) before extracting seed/leaf tissue samples or diluting samples/extracts.
2. Remove the number of strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).

## E. Test Procedure

1. Remove the testing device from the foil pouch by tearing at the notch. Hold the strip at the colored end. Do not touch the arrow end or the test window (the middle part of the strip).
2. Holding the strip vertically, immerse the end of the strip with the arrows into the sample liquid. Do not immerse past the MAX line.
3. Take the strip out when the sample has completely migrated to the test window (about 10 seconds). Lay the strip (MAX side facing up) flat on a clean, dry, non-absorbent surface.
4. Wait 15 minutes for the results. Results visible after 30 minutes are considered invalid.

## F. Evaluation

1. A positive result is the presence of two purple bands: one on the test (T) line and one on the control (C) line.
2. A negative result is the presence of one purple band on the control (C) line.
3. An invalid result: no band is present on the control (C) line. This may suggest the procedure was done incorrectly. Re-test with another strip.




## G. Sensitivity

A visible test line from an extracted sample constitutes at least 0.1% (one seed in 1000 conventional seeds) corn or cotton seeds. Bt Cry1Ab/Cry1Ac Strip Test has been validated to detect the presence MON810 and Bt11.