

Bt-Cry1Ab/Cry1Ac

• Intended Use

For the detection and quantitation of Bt Cry1Ab and Cry1Ac (MON810, Bt11, Bt176) endotoxin residues in corn tissues and cotton leaf tissues. For use with other sample matrices, contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Bt Cry1Ab/Cry1Ac Microtiter Plate Kit is a "sandwich" enzyme linked immunosorbent assay (ELISA). In the assay system, standards, controls, or sample extracts are added to wells coated with monoclonal antibodies raised against Cry1Ab/Cry1Ac endotoxin. Any endotoxin residues found in the standard or sample extracts bind to the antibodies on the wells. The "sandwich" is completed by the addition of polyclonal antibodies raised against the same endotoxin. An enzyme labeled conjugate is then added and the enzyme activity bound to the wells is measured using a substrate to develop a colored product. Since the formation of a "sandwich complex" occurs only in the presence of a Cry1Ac/Cry1Ab molecule, the enzyme activity of the bound sandwich complex is directly proportional to the amount of endotoxin in the sample.

A dose response curve of absorbance of the colored product formed vs. concentration is generated using results obtained from the standards. Concentration of Cry1Ab/Cry1Ac present in the control and sample extracts are determined directly from this curve.

Lighter color = Lower concentration
Darker color = Higher concentration

• Reagents

The Abraxis Bt Cry1Ab/Cry1Ac Kit contains the following items:

- 1. Cry1Ab/Cry1Ac Antibody coated wells**
Monoclonal antibody specific for Cry1Ab/Cry1Ac endotoxin adsorbed to plastic wells.
8 strips of 12 antibody coated wells and strip holder (1).
- 2. Cry1Ab/Cry1Ac Antiserum solution**
Polyclonal antibody (rabbit) specific for Cry1Ab/Cry1Ac endotoxin.
One vial containing 11 mL
- 3. Goat anti-rabbit Enzyme Conjugate (100x)**
Horseradish peroxidase (HRP) labeled goat anti-rabbit. Supplied as a liquid concentrate 100x with preservative and stabilizers.
One vial containing 0.25 mL
- 4. Conjugate Diluting Buffer**
Buffered solution with preservative and stabilizers used to dilute the conjugate
One bottle containing 12 mL
- 5. Bt Standards (Cry1Ab)**
Five concentrations (0, 0.25, 0.5, 1.0, 2.0, 4.0 ng/mL) of Bt calibrators in a buffered solution with preservative and stabilizers. Each vial contains 1.0 mL.
- 6. Control (Cry1Ab)**
A concentration (approximately 1.5 ng/mL) of Bt Cry1Ab in a buffered solution with preservative and stabilizers. One vial containing 1.0 mL.
- 7. Extraction Solution/Sample Diluent (5x)**
Buffered solution 5x concentrate with preservative and stabilizers without any detectable Bt endotoxin.
One bottle containing 30 mL.
- 8. Color Solution**
A solution of hydrogen peroxide and 3,3',5,5'-tetramethyl benzidine in an organic base.
One bottle containing 12 mL
- 9. Stopping Solution**
A solution of diluted acid.
One bottle containing 6 mL

10. Washing Buffer (5x) Concentrate
Preserved buffered 5x concentrate.
One bottle containing 110 mL

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box.

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

- Precision pipets capable of delivering 20, 100, 500, and 1000 μ L, and tips
 - Disposable Tissue Extractors, Abraxis PN 510010
 - Test tubes for dilution of sample extracts
 - Marking pen (indelible)
 - Tape or Parafilm®
 - Timer
 - Vortex mixer
 - Distilled or deionized water for diluting Wash buffer and the 10x Cry1Ab/Cry1Ac Extraction/Dilution Buffer
 - Storage bottles with 300 mL capacity for the storage of 1x Extraction/Dilution buffer and 1000 mL capacity for storage of 1x Wash buffer
 - Microplate or strip reader capable of reading absorbance at 450 nm
 - Wash bottle (Nalgene cat # 03-409-10E or equivalent) if performing manual plate washing
 - Test tube rack
- ## • Materials Recommended but Not Required
- Multi-channel pipette (100 μ L)
 - Reagent reservoirs for multi-channel dispensing
 - Automated plate washer

• Sample Information

This procedure is recommended for use with corn tissues and cotton leaf tissues. For testing of Cry1Ab and Cry1Ac in corn and cotton seeds, and in bulk corn grain, refer to application bulletin. Other samples may require modifications to the procedure and should be thoroughly validated.

Samples containing gross particulate matter should be filtered using a low protein binding filter such as Pall Gelman Sciences cat # 4184 or equivalent. Alternatively the samples can be centrifuged at 5000 x g for 5 minutes.

Sample Extraction

- Take 2 leaf punch samples (approximately 10 mg each) by snapping the tube cap of the Sample extraction Device down on the leaf. Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with a twisting motion. Continue the process for about 30 seconds, or until the leaf tissue is well ground. *To prevent sample contamination, a new extraction device and pestle must be used with each plant tissue sample.*

NOTE: If a quantitation level of Cry1Ab/Cry1Ac endotoxin is needed (quantitative assay), the weight of each leaf punch sample must be determined and recorded.

- Add 0.5 mL of the 1x Sample Extraction/Dilution Buffer to the tube.
- Repeat the grinding step (described in step 1) to mix tissue with the Extraction/Dilution Buffer. Allow the solids to settle in each tube for a few minutes before proceeding.

Sample Dilution

If the Cry1Ab/Cry1Ac concentration of a sample exceeds 4 ng/mL and a quantitative result is desired, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Sample Extraction/Sample Diluent buffer. For example, in a separate test tube make a eleven-fold dilution by adding 100 μ L of the sample to 1000 μ L of Sample Extraction/ Sample Diluent buffer. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor e.g. 11.

• Reagent Preparation

- 1. Wash Buffer**
In a 100 mL container, dilute the wash buffer concentrate 1:5 by the addition of distilled or deionized water (i.e., 100 mL of wash buffer concentrate plus 400 mL of H₂O). This solution is used to wash the antibody coated wells. Store refrigerated when not in use.
- 2. 1x Cry1Ab/Cry1Ac Extraction/Dilution Buffer**
Add the entire contents of the 5x bottle supplied in the kit to 120 mL of distilled or deionized water in a suitable size container. Store refrigerated when not in use.
- 3. Anti-Rabbit HRP Conjugate**
Dilute the conjugate 1:100 with the conjugate diluent buffer just prior to use. (i.e., 0.10 mL conjugate + 9.90 mL of conjugate diluent buffer). Dilute only the amount needed per assay, diluted conjugate is not to be stored.

All reagents must be allowed to come to room temperature prior to use.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each well in an identical manner.

Add reagents directly to the bottom of the well while avoiding contact between the reagents and the pipet tip. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

If more than 3 strips are going to be run, the use of a multi-channel pipette is recommended.

If fewer than all eight strips are used, reseal the unused strips with the desiccant in the foil bag provided. Store refrigerated.

Use the well identification markings on the plate frame as a guide when adding samples and reagents. In a qualitative assay, the zero standard, four non-zero calibrators, a control and 84 sample extracts may be run in one plate. For a quantitative assay, the zero standard and four calibrators and a control along with 42 sample extracts may be run in duplicate wells on one plate.

Do not use any reagents beyond their stated shelf life. Each component used in any one assay should be of the same lot number and stored under identical conditions.

Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

This Abraxis Assay will detect Cry1Ab/ Cry1Ac and other related endotoxins to different degrees. Refer to specificity table for data on several of the Cry endotoxins.

• Quality Control

A control solution at approximately 1.5 ng/ml of Cry1Ab is provided with this Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Add 100 uL of standard zero, 100 uL of each Calibrator or Control, and 100 uL of each diluted sample extract to their respective wells. Follow the same order of addition for all reagents. Cover plate to prevent contamination and evaporation.
2. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill contents.
3. Incubate at ambient temperature for 30 minutes.
4. After incubation, carefully removed the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with Wash Buffer then shake to empty. Repeat this wash step two times. Tap the strips on to a stack of paper towels to remove residual wash buffer. Alternative, perform these three washes with a microtiter plate or strip washer.
5. Add 100 uL of Cry1Ab/Cry1Ac antibody solution.
6. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill contents.
7. Incubate at ambient temperature for 30 minutes.
8. Repeat step 4.
9. Add 100 uL of enzyme conjugate.
10. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill contents.
11. Incubate at ambient temperature for 30 minutes.
12. Repeat step 4.
13. Add 100 uL of color solution.
14. Incubate at ambient temperature for 20 minutes.
15. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill contents
16. Add 50 uL of stopping solution. This will turn the well contents to yellow.
17. Read results at 450 nm within 15 minutes after adding the Stopping Solution. Set the plate reader to blank on the zero standard wells.

• Results

Data Interpretation

Average the absorbance readings for the duplicate calibrators, control, and samples.

Semi-Quantitative Results

Compare the OD's of the diluted sample extracts to those of the calibrators to obtain an estimate of the amount of Cry1Ab/ Cry1Ac endotoxin in the sample extract.

Quantitative Results

For a quantitative Cry1Ab/Cry1Ac assay, a linear curve fit for the standard curve should be used if the plate reader used has data reduction capabilities. Otherwise, calculate the results as described in the manual calculations.

Manual Calculations

1. Average the absorbance value for each of the calibrators, control, and samples.
2. Construct a standard curve by plotting the mean OD of each calibrator on the vertical linear (Y) axis against its corresponding

Cry1Ab concentration on the horizontal linear (X) axis on the graph paper provided.

3. Determine the endotoxin concentration for controls and samples by finding its OD value and its corresponding concentration level on the graph. Multiply the results by dilution factor incurred during extraction (500 uL ÷ x mg leaf tissue)/1000 to report as micrograms (ug) of endotoxin per gram of tissue.

• Performance Data

Precision

Cry1Ab fortified samples were analyzed both within a single assay, and in different assays. The following results were obtained using buffer, leaf and kernel extracts:

Buffer

Control	1	2	3
Mean (ppb)	1.04	2.12	3.94
% CV (within assay)	6.3	5.6	3.4
% CV (between assay)	3.9	4.0	1.9

Leaf Extract

Control	1	2	3
Mean (ppb)	1.30	2.08	3.96
% CV (within assay)	5.4	7.9	7.7
% CV (between assay)	3.2	5.1	4.7

Corn Kernel Extract

Control	1	2	3
Mean (ppb)	1.24	2.16	4.04
% CV (within assay)	6.7	3.5	8.1
% CV (between assay)	4.6	2.6	7.9

Limit of Detection

The Abraxis Bt Cry1Ab/Cry1Ac Assay limit of detection is 0.125 ng/ml (ppb) Cry1Ab in corn leaf extract. The Limit of Detection (LOD) was determined by calculating 3 standard deviations (OD units) from a negative corn leaf sample population and by interpolating from a Cry1Ab standard curve.

Recovery

Corn leaf extract samples were fortified with various levels of Cry1Ab endotoxin and then assayed using the Abraxis Cry1Ab/Cry1Ac Assay. The following results were obtained:

Amount of Cry1Ab Added (ppb)	Recovery		
	Mean (ppb)	S.D. (ppb)	%
0.750	0.718	0.026	96
1.50	1.49	0.092	99
3.0	2.95	0.090	98
Average			98

Specificity

The Abraxis Cry1Ab/Cry1Ac detects the presence of various Bt endotoxin to differing degrees. The following table shows the concentration of various Bt endotoxin equivalent to the given concentration of Cry1Ab.

Bt Endotoxin (ng/ml)				
Cry1Ab	0.50	1.0	2.0	4.0
Cry1Ac	0.28	0.49	0.95	2.0
Cry1F	18	24	57	460
Cry9C	72	317	1429	> 2700
Cry2A	> 2500	> 2500	> 2500	> 2500

• Ordering information

Abraxis Cry1Ab/Cry1Ac Kit, 96T	PN 510001
Sample Extraction/Dilution Buffer	PN 510002
Disposable Extraction Device	PN 510010

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

(215) 357-3911 * Fax(215) 357-5232
WEB: www.abraxiskits.com
Email: info@abraxiskits.com

• General Limited Warranty

Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.

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