

Bt Cry1F

• Intended Use

For the detection and quantitation of Bt Cry1F endotoxin residues in corn seeds and leaf tissues. For use with other sample matrices, contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis Bt Cry1F Microtiter Plate Kit is a “sandwich” enzyme linked immunosorbent assay (ELISA). In the assay system, standards and sample extracts are added to microtiter wells coated with monoclonal antibodies raised against Bt Cry1F endotoxin. Any endotoxin residues found in the standard or sample extracts bind to the antibody coated wells. The “sandwich” is completed by the addition of polyclonal antibodies raised against the same endotoxin. An enzyme-labeled conjugate is then added. The enzyme bound to the wells is measured using a substrate which develops into a colored product. Since the formation of a “sandwich complex” occurs only in the presence of a Cry1F molecule, the enzyme activity of the bound sandwich complex is directly proportional to the amount of endotoxin in the sample.

Lighter color = Lower concentration

Darker color = Higher concentration

A dose response curve of absorbance of the colored product formed vs. standard concentration is then generated. The concentrations of Cry1F present in the sample extracts are then determined directly from this curve.

• Reagents

The Abraxis Bt Cry1F Kit contains the following items:

1. Cry1F Antibody coated wells

Microtiter plate coated with monoclonal antibody specific for Cry1F endotoxin.

12 strips of 8 antibody coated wells
with strip holder (1)

2. Cry1F Antiserum Solution

Polyclonal antibody (rabbit) specific for Cry1F endotoxin.

One vial containing 11 mL

3. Goat anti-rabbit Enzyme Conjugate Solution

Horseradish peroxidase (HRP) labeled goat anti-rabbit in a buffered solution with preservative and stabilizers.

One bottle containing 11 mL

4. Bt Standards (Cry1F)

Seven concentrations (0, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0 ng/mL) of Bt calibrators in a buffered solution with preservative and stabilizers.

Each vial containing 1.0 mL

5. Extraction Solution/Sample Diluent (5X) Concentrate

5X concentrated buffered solution with preservative and stabilizers without any detectable Cry 1F.

One bottle containing 30 mL

6. Color Solution

A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.

One bottle containing 12 mL

7. Stopping Solution

A solution of diluted acid.

One bottle containing 12 mL

8. Wash Buffer (5X) Concentrate

5X concentrated buffered salts with detergent and preservatives.

One bottle containing 100 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.

• Additional Materials Required (Not Provided)

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

Precision pipettes* capable of delivering 20-200µL, and 200-1000 µL, with disposable tips.

Multi-channel or stepper pipette* capable of delivering 50-250µL with disposable tips.

Disposable Tissue Extractors, Abraxis PN 510010.

Disposable glass test tubes or glass vials with Teflon lined caps for dilution of sample extracts.

Balance,* Mettler model AE 100 or equivalent.

Small plastic bags or waxed paper.

Marking pen (indelible).

Tape or Parafilm®.

Timer.

Vortex Mixer,* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent.

Distilled or deionized water for diluting the 5X concentrated Extraction Solution/Sample Diluent and 5X concentrated Wash Buffer.

Bottles with 150 mL capacity for the storage of 1X Extraction Solution/Sample Diluent and 500 mL capacity for storage of 1X Wash Buffer.

Microplate or strip reader* capable of reading absorbance at 450 nm.

*Please contact Abraxis for supplier information.

• 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 pipette tip**. This will help to ensure consistent quantities of reagent in the test mixture.

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

The use of a multi-channel pipette is recommended.

The microtiter plate consists of 12 strips of 8 wells. The recommended run size is 4 strips or less, as drift may occur. Remove the unneeded strips and store in the re-sealable foil bag (with desiccant) provided.

Store refrigerated. Do not use reagents beyond their stated shelf life. Reagents used in any one assay should be of the same kit lot, as they have been adjusted in combination.

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

The Bt Cry1F Assay will detect Cry1F and other related endotoxins to different degrees. Refer to the Specificity section for data on various Bt Cry endotoxins.

• Quality Control

It is recommended that control solutions (negative and positive solutions) of Cry1F be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Reagent Preparation

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

1X Wash Buffer

In a 500 mL container, dilute the 5X concentrated Wash Buffer 1:5 with deionized or distilled water by adding the entire contents of the 5X concentrated Wash Buffer (100 mL) to 400 mL of deionized or distilled water. This solution is used to wash the antibody coated wells. Store refrigerated.

1X Cry1F Extraction Solution/Sample Diluent

In a 150 mL container, dilute the 5X concentrated Extraction Solution/Sample Diluent 1:5 with deionized or distilled water by adding the entire contents of the 5X concentrated Extraction Solution/Sample Diluent (30 mL) to 120 mL of deionized or distilled water. This solution is used for sample extraction and for sample dilution. Store refrigerated.

• Sample Information

Corn Seed Sample Extraction

1. Weigh a single seed. Record weight.
2. Place seed in a small plastic bag or waxed paper.
3. Crush seed with pliers or a hammer, then transfer to a labeled microcentrifuge tube or glass vial.

Note: Use caution when handling/extracting samples to avoid sample cross-contamination.

4. Add 1.0 mL of 1X Extraction Solution to each tube. Cap and shake for 30 seconds. Allow to soak for 2 hours.
5. To clarify the extract, centrifuge at 3000 g for 5 minutes.
6. Filter the clarified extract through a low protein binding syringe filter (0.8/0.2µm Pall Acrodisc® PN4905 or equivalent).
7. Pipette supernatant (top layer) into a clean vial. Analyze as sample (Assay Procedure, step 1).

The results obtained will need to be multiplied by the dilution factor incurred during sample extraction, calculated as follows:

$$\frac{(1000 \mu\text{L} \div x \text{ mg seed})}{1000} = \text{dilution factor}$$

Multiply the ELISA result by the dilution factor calculated above. This will yield results in micrograms of endotoxin per gram of seed (µg/g).

Sample Dilution

If the Cry1F concentration of a sample exceeds 8 ng/mL, the sample must be diluted and re-analyzed. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of 1X Sample Extraction/Sample Diluent. For example, in a separate test tube, make a ten-fold dilution by adding 100 µL of the sample to 900 µL of 1X Sample Extraction/Sample Diluent and mix thoroughly. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtained by the dilution factor incurred during the extraction and then by the additional dilution factor (e.g. 10).

• Assay Procedure

Read Sample Information, Reagent Preparation, and Procedural Notes and Precautions before proceeding.

Std. 0 - Std. 6: Standards
S1 - Sx : Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 0	Std. 4	S2									
B	Std. 0	Std. 4	S2									
C	Std. 1	Std. 5	S6									
D	Std. 1	Std. 5	S6									
E	Std. 2	Std. 6										
F	Std. 2	Std. 6										
G	Std. 3	S1										
H	Std. 3	S1										

1. Add 100µL of the appropriate standard or sample extract to the wells of the test strips according to the working scheme shown above. Analysis in duplicates or triplicates is recommended.
2. 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 at ambient temperature for 30 minutes.
3. After the incubation, carefully removed the covering and vigorously shake the contents of the wells into a sink or other suitable waste container. Wash the strips with the diluted Wash Buffer (see Reagent Preparation section) by adding a volume of at least 250µL of Wash Buffer to each well. Vigorously shake the contents of the wells into the waste container. Any remaining buffer should be removed by patting the plate on a stack of dry paper towels. Repeat this wash step two times, for a total of 3 rinses.
4. Add 100µL of Antibody Solution to each well successively using a multi-channel pipette or 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. Incubate at ambient temperature for 30 minutes.
5. Repeat step 3.
6. Add 100µL of Conjugate Solution to each well successively using a multi-channel pipette or 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. Incubate at ambient temperature for 30 minutes.
7. Repeat step 3.
8. Add 100µL of Color Solution to each well successively using a multi-channel pipette or 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. Incubate at ambient temperature for 20 minutes.
9. Add 100µL of stopping solution to each well using a multi-channel pipette or stepping pipette.
10. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Semi-Quantitative Results

To obtain an estimated amount of Cry1F endotoxin in the sample extracts, compare the mean absorbance value (OD) of each of the diluted sample extracts to the mean absorbance value of the standards. This will provide semi-quantitative results only.

Quantitative Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter is preferred). For a manual evaluation, calculate the mean absorbance value (OD) for each of the standards. Construct a standard curve by plotting the mean absorbance (OD) of each standard on the vertical (y) axis versus the corresponding Cry1F concentration of the horizontal (x) axis on graph paper. The OD for each sample will then yield levels of Cry1F in ppb by interpolation using the constructed standard curve.

The ELISA results obtained will then need to be multiplied by the dilution factor incurred during sample extraction, calculated as follows:

$$\frac{(1000 \mu\text{L} \div x \text{ mg seed})}{1000} = \text{dilution factor}$$

Multiplying the ELISA result by the dilution factor calculated above will then yield results in micrograms of endotoxin per gram of seed (µg/g).

• Performance Data

Precision

Cry1F fortified samples were analyzed and the following results were obtained:

Control	1	2	3
Mean (ppb)	0.52	0.82	3.38
% CV (within assay)	13.3	12.1	14.7
% CV (between assay)	10.0	10.0	12.6

Limit of Detection

The Abraxis Bt Cry1F Assay limit of detection is 0.1 ng/mL (ppb) Cry1F in corn seed extract. The Limit of Detection (LOD) was determined by calculating 3 standard deviations (OD units) from a negative corn seed sample population and by interpolation from the Cry1F standard curve.

Recovery

Corn seed extract samples were fortified with various levels of Cry1F endotoxin and then assayed using the Abraxis Bt Cry1F Assay. The following results were obtained:

Amount of Cry1F Added (ppb)	Recovery	
	Mean (ppb)	%
1.5	1.355	90.3
2.5	2.414	96.6
5.0	4.275	85.5
Average		90.8

Specificity

The Abraxis Bt Cry1F Assay detects the presence of various Bt endotoxins to different degrees. The following Bt endotoxins did not show activity in the Cry1F ELISA at concentrations < 1,000 ppb: Cry1Ab, Cry1Ac, Cry2A, Cry3B, and Cry9C.

• Ordering information

Abraxis Bt Cry1F Kit, 96T	PN 510006
Extraction Solution/Sample Diluent	PN 510002
Disposable Tissue Extraction Devices	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

Email: info@abraxiskits.com

WEB: www.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.**