ENVIROLOGIX™

QualiPlate™ Kit for Cry1Ab/Cry1Ac

Catalog Number AP 003 CRBS

Highlights:

- *Screen single corn or cotton seed or leaf samples for the presence or absence of Cry1Ab or Cry1Ac*

Contents of Kit:

- *12 strips of 8 antibody-coated wells, in plate frame*
- *Cry1Ab/Cry1Ac Positive Control*
- *Cry1Ab/Cry1Ac Enzyme Conjugate*
- *Substrate*

Note: *To handle bulk packaged Cry1Ab/Cry1Ac Enzyme Conjugate and Substrate, pour off 5.5 milliliters of Conjugate and 11.5 mL Substrate per plate to be run each day. Use a multiple-channel pipette to dispense. Do not pour excess Substrate back into the reagent bottle.*

Intended Use

The EnviroLogix QualiPlate Kit for Cry1Ab/Cry1Ac is designed for the non-quantitative laboratory detection of:

- Cry1Ab protein in Bt11, MON810 or Bt176 corn leaf tissue, or in Bt11 or MON810 corn seed samples;
- Cry1Ac protein in Bollgard®, Bollgard II, or WideStrike™ cotton leaf or seed samples.

Note: This is a very sensitive test for Cry1Ab or Cry1Ac—at the customer's discretion, it may be utilized in quantitative applications with user-supplied calibrators.

How the Test Works

This EnviroLogix QualiPlate Kit is a "sandwich" Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, plant leaf or seed sample extracts are added to test wells coated with antibodies raised against Cry1Ab/Cry1Ac toxin. Any residues present in the sample extract bind to the antibodies, and are then detected by addition of enzyme (horseradish peroxidase)-labeled Cry1Ab/Cry1Ac antibody.

After a simple wash step, the results of the assay are visualized with a color development step; color development is proportional to Cry1Ab/Cry1Ac concentration in the sample extract.

Lighter color = Lower concentration
Darker color = Higher concentration

Materials Not Provided

- PBS/0.05% Tween-20 Wash Buffer (may be purchased in 1L dry packets from Sigma Chemicals, Cat#P-3563, or prepared from salts on site). Store at controlled ambient temperature for up to one week, then discard.
- Extraction Buffer – PBS/0.55% Tween-20. This may be prepared by adding 0.5 mL Tween-20 to 100 mL already prepared PBS/0.05% Tween-20 Wash Buffer. Store refrigerated when not in use; warm to room temperature prior to assay.
- 1 N Hydrochloric acid (HCl) Stop Solution. Prepare by adding 83 mL of concentrated HCl (36.5-38.0%) to 917 mL of distilled or deionized water; work in a fume hood and use proper protective gear. This reagent may be stored at room temperature for 2 years.
- disposable tip, adjustable air-displacement pipettes which will measure 50 and 100 microliters (µL), preferably of multi-channel style.
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate reader
- wash bottle, or microtiter plate or strip washer

Prepare wash buffer and extraction solutions

Punch leaf sample

Crush single seed

Remove unneeded strips

- orbital plate shaker (optional)
- Calibrators or Standards. This kit may be used in a quantitative fashion with user-supplied calibrators. For example, corn flour standards containing known percentages of Bt11- or MON810-expressing corn are available from the European Commission Joint Research Centre, Institute for Reference Materials and Measurements (Retieseweg, B-2440 Geel, Belgium), and can be used to calibrate this test for measurement of ground corn samples. In order for this to work, it is imperative that the samples be ground to the same consistency as the calibrators, and that both are extracted with the same extraction buffer, buffer-to-sample ratio, and extraction time. Alternatively, if the user can obtain pure Cry1Ab or Cry1Ac protein, the kit can be calibrated with these materials. In this instance, complete extraction of the protein from the sample is required to obtain the best estimate of the amount of Cry1 protein in the sample.

Sample Preparation

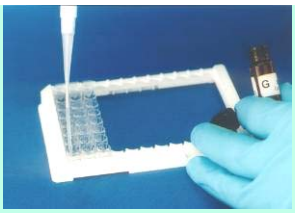
Sample Extraction:

Sample extraction protocols are to be designed and validated by the individual users of this kit. The following suggestions are guidelines, and define the manner in which the kit is performance tested by the manufacturer.

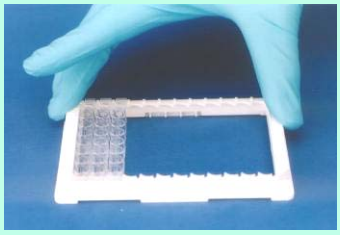
1. Green leaf samples: Extract green corn or cotton leaf samples that are 5-10 mm^2 in size with 250 µL of Extraction Buffer. The extraction efficiency will vary proportionately with the amount of tissue disruption and mixing performed. *Use extreme caution to prevent sample-to-sample cross-contamination with plant tissue or exudate.*
2. Single seed samples: Crush corn or cotton seeds and extract each with 0.75 to 1 mL of Extraction Buffer. Mix thoroughly, then allow solids to settle before transferring extract to the assay plate.

How to Run the Assay

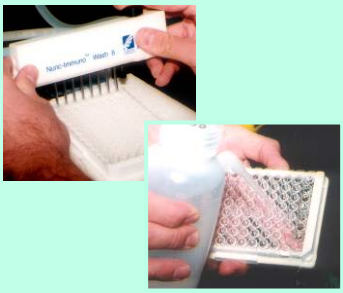
- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed plates and reagents at room temperature - do not remove plates from bag with desiccant until they have warmed up).
- Organize all reagents, sample extracts, and pipettes so that step 1 can be performed in 15 minutes or less. The use of a multichannel pipette is strongly recommended.
- If more than four strips are to be run at one time, the loading time will most likely exceed 15 minutes, and the use of a multi-channel pipette is recommended.
- If four or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add each Calibrator and diluted sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette for these three reagents.
- If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the foil pouch provided, and refrigerate.
- Use the well identification markings on the plate edge to guide you when adding the samples and reagents. It is recommended that at least two wells each of Blank (Extraction Buffer) and Cry1Ab Positive



Add Conjugate, Control, and sample extract

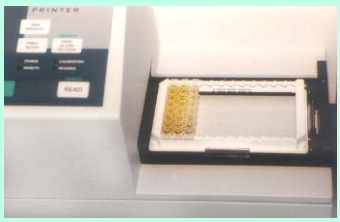


Mix plate



Strip Plate or Bottle Wash method

Complete protocol, then add Stop Solution



Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution

Control be run on each plate. Additional quality control samples may be added at the discretion of the user. Sample extracts may be run in either single or duplicate wells. See example of typical assay setup, Figure 1A, on page 4.

1. Add **50 µL** of **Cry1Ab/Cry1Ac Enzyme Conjugate** to each well of the plate. Immediately follow with **50 µL** of Extraction Buffer **Blank**, **50 µL** of **Cry1Ab Positive Control,** and **50 µL** of each **sample extract** to their respective wells. Follow this same order of addition for all reagents.

   NOTE: It is strongly recommended that a multi-channel pipette be used in steps 1, 5, and 7.

2. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the bench top for a full 20-30 seconds. Be careful not to spill the contents!

3. Cover the wells with tape or Parafilm to prevent evaporation and **incubate at ambient temperature for 1 to 2 hours.** If an orbital plate shaker is available shake plate at 200 rpm.

   NOTE: Users shall determine appropriate incubation times to give the best results with the tissue disruption/extraction methods in use.

4. After incubation, carefully remove 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 three times. Alternatively, perform these four washes (300 µL/well) with a microtiter plate or strip washer. Slap the plate on a paper towel to remove as much water as possible.

5. Add **100 µL** of **Substrate** to each well.

6. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate for 15 to 30 minutes at ambient temperature**. Use orbital shaker if available.

   NOTE: Users shall determine appropriate incubation times to give the best results with the tissue disruption/extraction methods in use.

Caution: Stop Solution is 1.0N Hydrochloric acid. Handle carefully.

7. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

NOTE: Read the plate within 30 minutes of the addition of Stop Solution.

How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)
2. Set the plate reader to blank on the Extraction Buffer **Blank** wells. If the reader cannot do this, measure and record the optical density (OD) of each well's contents, then subtract the average OD of the **Blank** wells from each of the readings.

Interpreting the Results

Compare the OD's of the sample extracts to those of the Positive Control to determine presence or absence of Cry1Ab/Cry1Ac endotoxin in your sample extract. Samples with absorbances close to that of the Blank wells (and less than that of the Positive Control wells) are presumed to be free of Bt endotoxin. Samples with absorbances significantly higher than those of the Blank wells are positive for Bt endotoxin content.

Figure 1A. Example of a typical assay setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	BL
H	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	PC

"BL" = Blank wells (Extraction Buffer)
"PC" = Cry1Ab Positive Control Wells
"S.." = sample extracts

Precautions and Notes

- Store all Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Kit components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test plates from one Kit with reagents or test plates from a different Kit.
- **Do not expose Substrate to sunlight** during pipetting or while incubating in the test wells.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- Cry1Ab and Cry1Ac proteins can be degraded by heat and sunlight. Take samples from green, actively growing leaves. Leaf samples that cannot be extracted immediately may be stored frozen for up to 1 week prior to analysis. Seeds may be stored for at least 6 months under cool, dry conditions.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- Observe any applicable regulations when disposing of samples and kit reagents.

For Technical Support Contact Us At:

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103-1486
USA

Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com

LIMITED WARRANTY

EnviroLogix Inc. ("EnviroLogix") warrants the products sold hereunder ("the Products") against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. If the Products do not conform to this Limited Warranty and the customer notifies EnviroLogix in writing of such defects during the warranty period, including an offer by the customer to return the Products to EnviroLogix for evaluation, EnviroLogix will repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

ENVIROLOGIX MAKES NO OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EnviroLogix products appearing in EnviroLogix published catalogues and product literature are EnviroLogix' sole representations concerning the Products and warranty. No other statements or representations, written or oral, by EnviroLogix' employees, agents or representatives, except written statements signed by a duly authorized officer of EnviroLogix Inc., are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

EnviroLogix does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by EnviroLogix, or against damages resulting from such non-EnviroLogix made products or components. EnviroLogix passes on to customer the warranty it received (if any) from the maker thereof of such non-EnviroLogix made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by EnviroLogix.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of EnviroLogix shall be to repair or replace the defective Products in the manner and for the period provided above. EnviroLogix shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall EnviroLogix be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of EnviroLogix with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Parafilm is a registered trademark of American Can Corporation
Bollgard is a registered trademark of the Monsanto Company
WideStrike is a trademark of Dow AgroSciences
EnviroLogix, the EnviroLogix logo, and QualiPlate are trademarks of EnviroLogix Inc.

© EnviroLogix 2007