ENVIROLOGIX™

# QualiPlate™ Kit for Cry2A

***Highlights:***

- *Qualitative results in less than 2 hours*
- *Volume packaged (V10)*

***Contents of Kit:***

- *10 96-well Cry2A antibody-coated solid plates*
- *Cry2A Positive Control*
- *Cry2A-Enzyme Conjugate*
- *Substrate*

***Note:*** *To handle bulk packaged Cry2A-Enzyme Conjugate and Substrate, pour 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.*

*Prepare Solutions*

*Catalog Number AP 005 CT NW V10*

## Intended Use

The QualiPlate Kit for Cry2A is designed for the non-quantitative laboratory detection of Cry2A endotoxins in corn and cotton leaf and seed tissue. Note: This is a very sensitive test for Cry2A — at the customer's discretion, it may be utilized in quantitative applications with user-supplied calibrators.

## How the Test Works

This 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 Cry2A toxin. Any residues present in the sample extract bind to the antibodies, and are then detected by addition of enzyme (horseradish peroxidase)-labeled Cry2A antibody.

After a simple wash step, the results of the assay are visualized with a color development step; color development is proportional to Cry2A concentration in the sample extract.

*Lighter color = Lower concentration*
*Darker color = Higher concentration*

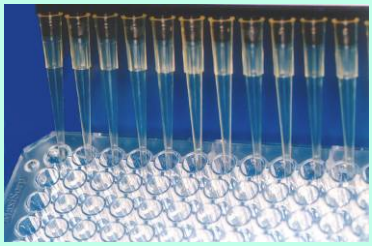
## Materials Not Provided

- Wash Buffer (preparation instructions below)
- Extraction Buffer (preparation instructions below)
- Stop Solution (preparation instructions below)
- disposable tip, adjustable air-displacement pipettes which will measure 100 microliters (µL), preferably of multi-channel style
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate reader or strip reader with 450 nm filter
- wash bottle, or microtiter plate or strip washer
- orbital plate shaker (optional)

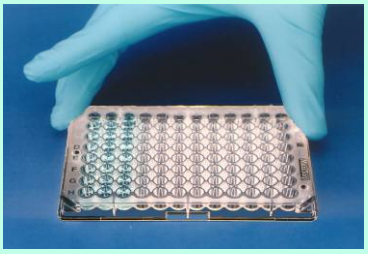
## Preparation of Solutions

- **Wash Buffer**: 0.01M Phosphate/0.15M Saline/ 0.05% Tween-20 Solution, pH 7.4. This can be purchased in 1L dry packets from Sigma Chemicals, Cat#P-3563, or prepared from salts and Tween-20 on site. Store at controlled ambient temperature for up to one week, then discard.
- **Extraction Buffer**: Prepare Extraction Buffer by adding 0.5 mL Tween-20 to 100 mL of Wash Buffer, for a final solution that is 0.01M Phosphate/ 0.15M Saline/0.55% Tween-20 Solution, pH 7.4.
- 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.

*Obtain Samples, Extract*



*Add Conjugate, Control, and sample extract*



*Mix plate*

*Incubate*

*Wash*

# Sample Preparation

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.

Note: It is recommended that the user prepare known negative and positive seed or leaf samples to be run in every assay as controls, in addition to the kit Positive Control.

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 performed. *Use extreme caution to prevent sample-to-sample cross-contamination with plant tissue or exudate.* Allow solids to settle before transferring extract to the assay plate. Dispensing particles into the assay plate can cause false positive results.

2. Single seed samples: Crush corn kernels or cotton seeds and extract each with **1 mL** of **Extraction Buffer**. Allow solids to settle before transferring extract to the assay plate. Dispensing particles into the assay plate can cause false positive results.

# 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 strips and reagents at room temperature - do not remove strips 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 may be needed to adhere to this time constraint.
- Use a disposable-tip air-displacement pipette and a clean pipette tip to add Blank, Positive Control, and sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combitip for these three reagents.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. It is recommended that at least two wells each of Blank (Extraction Buffer) and Cry2A Positive 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.

**Procedure**

1. Add **50 µL** of **Cry2A Enzyme-Conjugate** to each well of the plate. Immediately follow with **50 µL** of Extraction Buffer **Blank**, **50 µL** of **Cry2A Positive Control,** and **50 µL** of each **user-supplied control/sample extract** to their respective wells. Follow this same order of addition for all reagents.

**NOTE:** In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 5 and 7 when more than 4 strips are used.

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 the contents!

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

3. Cover the wells with tape or Parafilm to prevent evaporation and **incubate at ambient temperature for 45 minutes.** If an orbital plate shaker is available shake plate at 200 rpm. (Alternate timing steps may be used with this kit; please consult EnviroLogix Tech Support.)

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, for a total of 4 washes. Slap the plate on a paper towel to remove as much water as possible. Alternatively, perform these four washes with a microtiter plate or strip washer set for sequential 300 µL fill/aspirate cycles.

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 minutes** at ambient temperature. Use orbital shaker if available. (NOTE: Alternate timing steps may be used with this kit; please consult EnviroLogix Tech Support.)

**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.

   *General test criteria:*

   The mean OD of the BLANK wells should not exceed 0.15.

   The mean, blank-subtracted OD of the Positive Control wells should be at least 0.2.

   The coefficient of variance (%CV) between the duplicate Positive Control wells should not exceed 15%:

   $$\%CV = \frac{\text{std. deviation of OD's}}{\text{mean Pos.Ctl. OD}} \times 100$$

If the results of an assay fail to meet these criteria, consult EnviroLogix' Technical Service for suggestions on improving the test when you repeat the assay.

## Calculate the Positive Control Ratio

Divide the OD of each sample extract by the mean OD of the Positive Control wells. This number is the "Positive Control Ratio".

## Interpret the Qualitative Results

**Single Corn or Cotton Leaf and Seed samples:**
If the Positive Control Ratio calculated for a sample is less than 1.0, the sample does not contain Cry2A protein.

If the Positive Control Ratio of a sample is greater than or equal to 1.0, the sample does contain Cry2A protein.

Leaf and seed samples are by their nature either 100% positive or 100% negative. Any low level positive results from single seed or leaf samples must be due to either some form of sample cross-contamination (stray particles or dust from positive corn or cotton, leaf residue on leaf punch, etc.) or can be caused by transfer of particulate matter from leaf or seed extracts into the assay wells. If there is any question of the latter occurring, re-extraction and re-testing is recommended.

**Figure 1A. Example of a typical Qualitative 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" = Cry2A 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 well strips from one Kit with reagents or test well strips from a different Kit.
- **Do not expose Substrate to sunlight** during pipetting or while incubating in the test wells.
- The assay has been optimized for use with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- Cry2A endotoxins are proteins which can be degraded by heat and sunlight. Take samples from green, actively growing leaves. Samples that cannot be extracted immediately may be stored frozen for up to 1 week prior to analysis.
- 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***

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