ENVIROLOGIX™

QuantiPlate™ Kit for Cry1C

Catalog Number AP 007 V10

Highlights:

- *Less than 2 hours to run*

Contents of Kit:

- *Ten (10) QuantiPlates, 12 strips of 8 antibody-coated wells each, in plate frame*
- *Cry1C Negative Control*
- *0.6 ppb Cry1C Calibrator*
- *2.5 ppb Cry1C Calibrator*
- *6 ppb Cry1C Calibrator*
- *Cry1C-Enzyme Conjugate*
- *5X Extraction/Dilution Buffer*
- *Buffer Salts*
- *Substrate*
- *Stop Solution*

Precision

	Recovery (%CV)	OD (%CV)
Intra-Assay		**n=18**
1.5 ppb	3.1%	3.3%
4.0 ppb	2.5%	2.8%
Inter-Assay		**n=18**
1.5 ppb	4.9%	3.9%
4.0 ppb	5.3%	4.1%

Intended Use

The QuantiPlate Kit for Cry1C is designed for the quantitative laboratory detection of Cry1C residues in plant leaf tissue samples.

How the Test Works

This kit is a "sandwich" Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, plant leaf sample extracts are added to test wells coated with antibodies raised against Cry1C toxin. Any residues present in the sample extract bind to the antibodies, and are then detected by addition of enzyme (horseradish peroxidase)-labeled Cry1C antibody.

After a simple wash step, the results of the assay are visualized with a color development step; color development is proportional to Cry1C concentration in the sample extract.

Lighter color = Lower concentration
Darker color = Higher concentration

Performance

Performance parameters below were all measured with a *Bacillus thuringiensis*-produced protein (present in the kit calibrators). Results will vary with Cry1C protein from different sources.

Limit of Detection

The lowest recommended calibrator to use with this kit is 0.6 parts per billion (nanograms/mL, ppb) Cry1C. The Limit of Detection (LOD) of this kit is 0.2 ppb Cry1C. The LOD was determined by interpolating an OD equal to three standard deviations above the mean of a population of negative leaf samples, from a Cry1C standard curve.

Limit of Quantification

The Limit of Quantification (LOQ) of the EnviroLogix Cry1C Plate Kit was validated at 0.75 ppb in various leaf matrices. The LOQ was determined by fortifying a population of Cry1C negative leaf samples at 0.75 ppb Cry1C. The mean recovery was 110% with a coefficient of variation [CV, (standard deviation/mean) x 100] of 8.6%.

Precision

Cry1C-fortified control solutions were repetitively analyzed both within a single assay, and in different assays on different days. The data is expressed as % CV for both the recovered concentration and for absorbance (OD).

Fortification and Recovery

Twelve Cry1C negative leaf samples were fortified with Cry1C to concentrations ranging from 0.75 ppb to 4.0 ppb. The average recovery was 110%, with CV's of 5.8 to 8.6%.

Prepare Wash and Extraction Buffers

Materials Not Provided

- Disposable Tissue Extractors, EnviroLogix Cat. # ACC 002
- disposable tip, adjustable air-displacement pipettes which will measure 50 and 100 microliters (µL)
- marking pen (indelible)
- tape or Parafilm®
- timer (15 minutes, 1 hour, and 30 minutes)
- distilled or de-ionized water for preparing Wash Buffer and diluting 5X Cry1C Extraction/Dilution Buffer
- glass bottles or flasks with 175 mL capacity for storage of 1X Extraction/ Dilution Buffer and 1 liter capacity for Wash Buffer
- microtiter plate reader or strip reader
- wash bottle, or microtiter plate or strip washer
- multi-channel pipette that will measure 50 and 100 µL (optional)
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)

Preparation of Solutions

1. **Wash Buffer:** Add the contents of one packet of **Buffer Salts** (phosphate buffered saline, pH 7.4 - Tween 20) to 1 liter of distilled or de-ionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.

2. **1X Extraction/Dilution Buffer:** To prepare 1X working Extraction/ Dilution Buffer, add the entire contents of the bottle of 5X (350 mL) supplied in the kit to 1.4 L of distilled or deionized water in a suitable container. Mix thoroughly to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.

NOTE: The Extraction-Dilution Buffer supplied in this kit is identical to that supplied in the EnviroLogix Cry1Ab/Cry1Ac Plate Kit (Cat# AP 003) and the Cry2A Plate Kit (Cat# AP 005). Therefore, extracted leaf samples can be diluted and tested in all three of these plate kits.

Obtain leaf tissue

Grind tissue, add buffer, grind again

Sample Preparation

Sample Extraction:

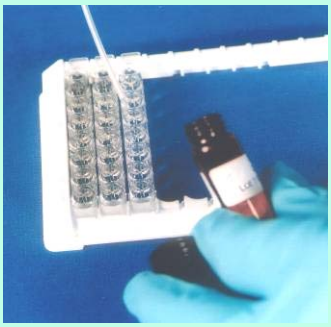
1. Take 2 leaf punch samples (approximately 10 milligrams each) by snapping the tube cap of the Disposable Sample Extractor down on the leaf. Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Continue this process for 20-30 seconds, or until the leaf tissue is well ground. *Use a new extraction device for each sample. Use extreme caution to prevent sample-to-sample cross-contamination with plant tissue or exudate.*

   Alternately, use a bead-beater device or homogenizer for more complete extraction.

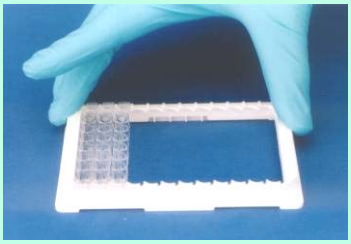
NOTE: If the assay is to be used to quantitate levels of Cry1C toxin in leaf tissue, the weight of each leaf punch sample must be determined and recorded.

2. Add 0.5 mL of 1X Extraction/Dilution Buffer to the tube.

Remove unneeded strips



Add calibrators and sample extracts



Mix plate

Incubate

Bottle Wash method

3. Repeat the grinding step to mix tissue with Extraction/Dilution Buffer. Repeat this protocol for each sample to be tested, using a new tube and pestle for each. Allow the solids to settle in each tube for a few minutes.

Sample Dilution:
Concentrations of Cry1C toxins will vary from plant to plant. Sample extracts may be tested without further dilution, with the results indicating whether or not the plant tested contains Cry1C protein. Dilution of positive extracts will be necessary in order to bring assay results within the range of calibration. For example:

For a 1:51 dilution: add 1 mL 1X Extraction/Dilution Buffer to dilution tubes labeled for each sample. Add 20 µL sample extract and mix.

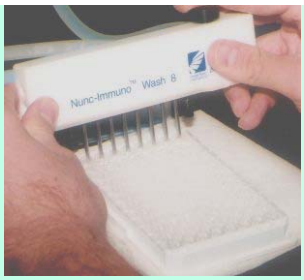
How to Run the Assay

- 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 Calibrators and sample extracts, and pipettes so that step 1 can be performed in 15 minutes or less.
- If more than four strips are to be run at one time, the 15 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
- 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 with a disposable tip on the end of the Combitip for these three reagents.
- If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the foil bag provided, and refrigerate.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. In a qualitative (semi-quantitative) assay, the Negative Control (NC), three non-zero calibrators, and 88 diluted sample extracts (S) may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1A). For a quantitative assay the Negative Control (NC) and three Calibrators (C1-C3), along with 44 diluted sample extracts (S) may be run in duplicate wells on one plate. (See the Quantitative Assay Example Plate Layout - Figure 1B).

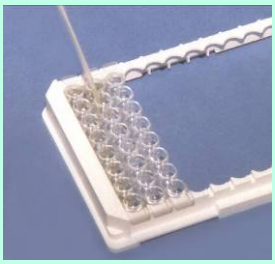
1. Add **50 µL** of **Negative Control, 50 µL** of each **Calibrator,** and **50 µL** of each diluted **sample extract** to their respective wells, as shown in the Example Plate Layouts (Figures 1A and 1B). Follow this same order of addition for all reagents.

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 4, 8 and 10 when more than 4 strips are used.

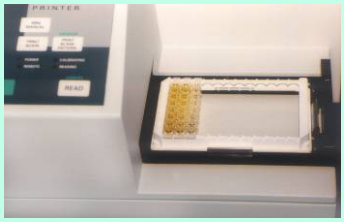
2. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!
3. Cover the wells with tape or Parafilm to prevent evaporation and **incubate** at ambient temperature for **15 minutes.**
4. Add **50 µL** of **Cry1C-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.



Strip Plate Wash option

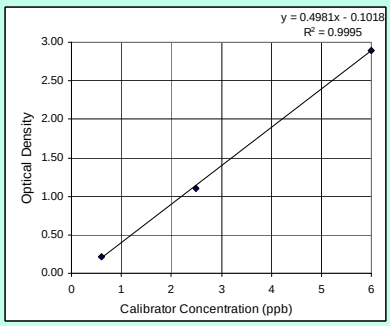


Complete protocol and add Stop Solution



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

Figure 3. Illustrative Cry1C standard curve



5. Thoroughly mix the contents of the wells as described in step 2. Be careful not to spill the contents!
6. Cover the wells with new tape or Parafilm to prevent evaporation and **incubate at ambient temperature for 1 hour.**
7. 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. Slap the plate on a paper towel to remove as much Wash Buffer as possible. Alternatively, perform these four washes with a microtiter plate or strip washer.
8. Add **100 µL** of **Substrate** to each well.
9. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate for 30 minutes** at ambient temperature.

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

10. 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 Negative Control 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 Negative Control wells from each of the readings.

   General Test Criteria:
   - The mean OD of the BLANK wells should not exceed 0.2.
   - The coefficient of variance (%CV) between the duplicate Calibrator and sample wells should not exceed 15%.

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

   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.

3. For a quantitative Cry1C assay, a **linear or quadratic** curve fit for the standard curve should be used if the microtiter plate reader you are using has data reduction capabilities. If not, calculate the results manually as described in the "How to Calculate the Quantitative Cry1C Results" section.

How to Interpret the 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 Cry1C endotoxin in your sample extract.

How to Calculate the Quantitative Cry1C Results

1. After reading the wells, average the OD of each set of calibrators and samples.
2. Graph the mean OD of each Calibrator against its Cry1C concentration on a linear scale (see Figure 3).
3. Determine the Cry1C concentration of each sample by finding its OD value and the corresponding concentration level on the graph. Multiply the result by the dilution factor incurred during extraction (500 µL ÷ *x* mg leaf tissue) and multiply by any dilution of sample extract employed, and divide by 1000. Report results as micrograms Cry1C toxin per gram of tissue (ppm).
4. Interpolation of sample concentration is only possible if the OD of the sample falls within the range of OD's of the Calibrators.

   If the OD of a sample is lower than that of the Low Calibrator (0.6 ppb Cry1C), the sample must be reported as less than: (0.6 ppb x dilution factor during extraction x dilution of sample extract employed) ÷ 1000 = *x* ppm Cry1C.

   If the OD of a sample is higher than that of the High Calibrator (6 ppb Cry1C), the sample must be reported as greater than:

   (6 ppb x dilution factor during extraction x dilution of sample extract employed) ÷ 1000 = *x* ppm Cry1C.

   If a concentration must be determined for these high level samples, dilute the sample extract 10-fold more than executed in the original assay in 1X Extraction/Dilution Buffer. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the OD's of the Calibrators, youmust then be sure to use this new dilution factor of sample extract in the calculations described above.

Figure 1A. Example of a typical Qualitative assay setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81
B	C1	C1	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82
C	C2	C2	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83
D	C3	C3	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
E	S1	S2	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
F	S3	S4	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
G	S5	S6	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
H	S7	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88

Figure 1B. Example of a typical Quantitative assay setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	C1	C1	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	C2	C2	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	C3	C3	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 2. Illustrative quantitative calculations

Well contents	OD	Average OD ± sd	%CV	Cry1C Concentration
Negative Control	0.035 0.037	0.036 ± 0.001	3.4	NA
0.6 ppb Calibrator	0.234* 0.243	0.239 ± 0.007	2.7	NA
2.5 ppb Calibrator	1.155* 1.151	1.153 ± 0.003	0.3	NA
6 ppb Calibrator	2.782* 2.731	2.756 ± 0.036	1.3	NA
Sample	0.760* 0.757	0.759 ± 0.002	0.3	1.7 ppb**

* Figures are after subtraction of Negative Control values.

**Sample is a positive extract which had been diluted 1:51 prior to assay:
Concentration from curve = 1.7 ppb Cry1C, multiplied by 1:51 dilution of sample extract = 86.7 ppb, multiplied by 1:25 dilution during extraction (20 mg leaf sample extracted with 0.5 mL), and divided by 1000 = 2.17 ppm Cry1C in leaf.

Actual values may vary; this data is for demonstration purposes only.

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.
- Cry1C 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.

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