QualiPlate™ Combo Kit for Cry1F & Cry34

Highlights:

- *Use a single test well to screen corn seeds or leaf samples for presence/ absence of Cry1F and Cry34*
- *Results for two analytes in 2 hours*

Contents of Kit:

- *Cry1F and Cry34 antibody-coated solid plate*
- *Enzyme Conjugate*
- *Positive Control*
- *Substrate 1 (for Cry34 results)*
- *Substrate 2 (for Cry1F results)*

Catalog Number AP 077

Intended Use

The EnviroLogix QualiPlate Combo Kit for Cry1F & Cry34 is designed for the qualitative laboratory detection of the presence or absence of these proteins in corn single leaf or single seed samples, with both analytes measured in the same well of the assay plate. This assay can be used to detect the presence of these proteins in corn products including HERCULEX™ XTRA. Herculex I (expresses Cry1F protein) and HERCULEX RW (expresses Cry34Ab1 protein) will also be detected by the appropriate portion of this test kit.

How the Test Works

This QualiPlate Kit is a "sandwich" Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, **corn** sample extracts are added to test wells coated with antibodies raised against Cry1F and Cry34 proteins. Any Cry1F or Cry34 protein present in the sample extract binds to the antibodies and is then detected by addition of alkaline-phosphatase-labeled Cry34 antibody, or horseradish peroxidase-labeled Cry1F antibody.

After a simple wash step, the results of the Cry34 assay are visualized via the addition of a pNPP Substrate. Once the yellow color develops and is read, the wash step is repeated, and a TMB substrate is added. The Cry1F results are visualized via the development of the resulting blue color.

Light color = Low concentration
Darker color = High concentration

Sample Preparation

Single Seed Samples

1. Crush seeds: Seeds may be placed in a plastic bag or tube and crushed with a rubber mallet or pliers, then transferred to a tube for extraction; alternately, a drill-press based machine or bead-beating device may be used.

   NOTE: Cry34 and Cry1F proteins are expressed at very high concentrations in corn seed. There is serious potential for cross-contamination between samples during seed crushing. Use the utmost care to avoid this.

2. Add **1 mL** of Extraction Buffer to each crushed corn seed. Mix for at least 30 seconds, let stand **up to 10 minutes**, then mix again. Longer extraction times are not desirable.

Single Leaf Punch Samples:

1. Take two leaf punches of approximately 5 mm diameter or a single punch of 10 mm diameter, using a paper punch or a micro-tube cap. Mash the leaf tissue with a pestle matched to the micro-tube, or beat with beads in a reciprocating shaker to the point of liquefaction of the leaf. The extraction efficiency of whatever method used will vary proportionately with the amount of tissue disruption performed.
2. Add **0.5 mL** of Extraction Buffer per sample and macerate again. Assay immediately. Use extreme care not to cross-contaminate between leaf samples.

Note: It is recommended that known positive and negative seed or leaf samples be run in every assay as controls.

Items Not Provided:

- *PBS/0.05% Tween-20* ***Wash Buffer, pH 7.4*** *(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.*
- **PBS/0.55% Tween-20 Extraction Buffer** *This may be prepared by adding 0.5% (5 mL per liter) Tween-20 to already prepared PBS/0.05% Tween-20 Wash Buffer. Prepare only enough for a few days' usage. Store refrigerated when not in use; warm to room temperature prior to assay.*
- *distilled or deionized water for preparing above solution*
- *EnviroLogix Tissue Extraction Kit (ACC 002) or other suitable equipment for taking and extracting leaf punch samples*
- *equipment for pulverizing seeds or leaves*
- *disposable tip, adjustable air-displacement multi-channel pipettes which will measure 50 and 100 microliters (µL)*
- *marking pen (indelible)*
- *tape or Parafilm®*
- *timer*
- *wash bottle, or microtiter plate washer*
- *microtiter plate reader with 405 and 650 nm filters*

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 plate from bag with desiccant until it has warmed up).
- Organize all Control and sample extracts and pipettes so that Step 1 can be performed in 15 minutes or less, using a multi-channel pipette.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. For this qualitative assay, duplicate wells of the Extraction Buffer blank (BL) and Positive Control Solution, along with 92 sample extracts (S) (including user-supplied controls) in single wells may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1A).

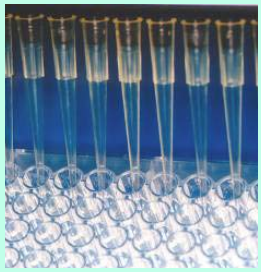
1. Add **50 µL** of **Cry1F& Cry34 Enzyme Conjugate** to each well, followed immediately by **50 µL of Extraction Buffer Blank (BL), 50 µL Positive Control,** and **50 µL** of each **sample/control extract (S)** to their respective wells, as shown in Figure 1A. **Caution**: Dispensing particles into the test plate can cause false positive results.

   NOTE: In order to minimize setup time it is strongly recommended that a multi-channel pipette be used in steps 1, 5, and 10.

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 contents!
3. Cover the wells with tape or Parafilm to prevent evaporation and **incubate at ambient temperature for 1 hour**.
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 more times. Alternatively, perform these four washes (300 µL/well) with a microtiter plate or strip washer. Slap the inverted plate on a paper towel to remove as much liquid as possible.
5. Add **100 µL** of **Substrate # 1** to each well. BE SURE TO USE SUBSTRATE #1 AT THIS STEP!
6. Thoroughly mix the contents of the wells as described in step 2. Be careful not to spill the contents!
7. Cover the wells with new tape or Parafilm to prevent evaporation and **incubate** at **ambient temperature for 30 minutes**.
8. **Read and record the yellow Cry34 results using a microtiter plate reader at a wavelength of 405 nanometers.** Set the plate reader to blank on the Extraction Buffer Blank wells (this should automatically subtract the mean optical density (OD) of the Blank wells from each control and sample OD).
9. Wash the plate four times as described in step 4.
10. Add **100 µL** of **Substrate #2** to each well. BE SURE TO USE SUBSTRATE #2 AT THIS STEP!
11. Thoroughly mix the contents of the wells as described in step 2. Be careful not to spill the contents!
12. Cover the wells with new tape or Parafilm to prevent evaporation and **incubate** at **ambient temperature for 30 minutes**.

Prepare Wash and Extraction buffer

Allow all reagents to reach room temperature before beginning



Add Enzyme-Conjugate, followed immediately by Control and sample extracts, to the plate

13. **Read and record the blue Cry1F results with a microtiter plate reader at a wavelength of 650 nanometers.** Set the plate reader to blank on the Extraction Buffer Blank wells (this should automatically subtract the mean OD of the Blank wells from each control and sample OD).

How to Interpret the Results

Spectrophotometric Measurement

General test criteria:

The mean OD of the BLANK wells in the Cry34 portion of the test should not exceed 0.35. The mean OD of the BLANK wells in the Cry1F portion of the test should not exceed 0.15.

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

The coefficient of variance (%CV) between the duplicate Positive Control Solution 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

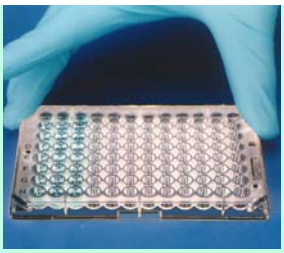
Cry34 Results: If the Positive Control Ratio calculated for a single seed or leaf punch sample in the Cry34 portion of the test is less than 0.75, the sample does not contain Cry34.

If the Positive Control Ratio of a sample is greater than or equal to 0.75, the sample is Cry34-expressing corn.

Cry1F Results: If the Positive Control Ratio calculated for a single seed or leaf punch sample in the Cry1F portion of the test is less than 1.0, the sample does not contain Cry1F.

If the Positive Control Ratio of a sample is greater than or equal to 1.0, the sample is Cry1F-expressing corn.

Single leaf and seed samples are by their nature either 100% positive or 100% negative, resulting in a clear delineation of color between negative and positive samples. Low level positive results may be due to insufficient extraction, or can be caused by some form of sample cross-contamination (flying particles or dust from cotton seed, cotton leaf residue on leaf punch, etc.) or by transfer of particulate matter from leaf or seed extracts into the assay wells. Cry34 seed PCR's are not necessarily proportional to seed concentration. The very high concentration of Cry34Ab1 protein present in HERCULEX XTRA seeds can cause a phenomenon known as hook effect in the assay. Light crushing of seeds and extraction for 10 minutes or less will minimize this problem. If needed, EnviroLogix can recommend an alternative assay protocol that will eliminate the phenomenon. Please contact EnviroLogix Technical Support for more information. Re-extraction and re-testing of questionable samples is recommended.



Mix

Wash Plate

Read plates in a Plate Reader at the appropriate wavelength:

- *405 nanometers for the Cry34 test result,*
- *650 nanometers for the Cry1F test results.*

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	S90	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

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 plates from one QualiPlate Kit with reagents or plates from a different QualiPlate Kit.
- **Do not expose Substrate 1 or Substrate 2 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.
- Do not use a stopping solution of any kind during this assay.
- 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 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.

This test kit has been validated and approved by Dow AgroSciences for detection of the Cry1F & Cry34Ab1 proteins expressed in corn products including HERCULEX XTRA.