

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:

- 50 Cry1F and Cry34 antibody-coated solid plates
- Enzyme Conjugate
- Positive Control
- S1 Substrate (for Cry34 results)
- S2 Substrate (for Cry1F results)
- **Note:** To handle bulk packaged Enzyme Conjugate, S1 Substrate and S2 Substrate, pour off 6 mL of Conjugate and 11 mL of each Substrate per plate to be run each day. Use a multiple-channel pipette to dispense. Do not pour excess Substrates back into the reagent bottles.

Catalog Number AP 077 NW V50

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™ I, HERCULEX RW, HERCULEX XTRA, and SmartStax®.

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 solutions
- 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

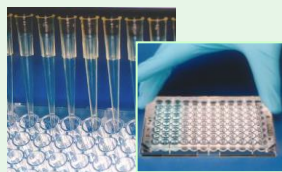
- Read all of these instructions before running the kit.
 - Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed plates and reagents at room temperature - do not remove plate from bag with desiccant until it has warmed up).
 - Organize all Control and sample 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 edge to guide you when adding the samples and reagents. It is recommended that at least two wells each of Blank (Extraction Buffer) and 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. See example of typical assay setup, Figure 1A, on page 4.
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 (PC)**, 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 S1 Substrate** to each well. **BE SURE TO USE S1 SUBSTRATE 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**. Mix plate manually, or set reader to shake for 2-5 seconds.
 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 S2 Substrate** to each well. **BE SURE TO USE S2 SUBSTRATE 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**. Mix plate manually, or set reader to shake for 2-5 seconds.
 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).



Prepare Wash and Extraction Buffers



Allow all reagents to reach room temperature before beginning



Add Enzyme-Conjugate, followed immediately by control and sample extracts, to the plate, then 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.

How to Interpret the Results

Compare the OD's of the sample extracts to those of the user-supplied Positive Control to determine presence or absence of Cry1F and Cry34 protein(s) in the sample extract. Samples with absorbances close to that of the Blank wells are presumed to be negative for that protein. Samples with absorbances significantly higher than those of the Blank wells are considered positive for that protein.

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.

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

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 S1 Substrate or S2 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.
- 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.

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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.

Parafilm is a registered trademark of American Can Corporation

HERCULEX is a trademark of Dow AgroSciences LLC

SmartStax is a registered trademark of Monsanto Technology LLC

Tween is a registered trademark of Uniqema, a business unit of ICI Americas Inc.

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Safety Data Sheet
According to 29CFR 1910.1200

SECTION 1. Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier
Trade name: N1 Substrate
Part number: 10034, 10011
Synonym: P975

1.2 Relevant identified uses of the substance or mixture and any advised special applications of the substance / or preparation
Laboratory chemicals

1.3 Details of all supplier of the safety data sheet
Manufacturer/supplier: EnviroLogix Inc, 590 Riverside Industrial Hwy, Fairfield NJ 07004, USA (207) 797-6300, 207-797-6306 (Technical Service)

1.4 Emergency telephone number

SECTION 2. Hazards identification

2.1 Classification of the substance or mixture
Classification according to 29CFR 1910.1200: Hazard Classes: Skin Irritation Cat. 2, Eye Contact Cat. 1, Carcinogenic Cat. 2, STOT 2H Cat. 2

2.2 Label elements
Labeling according to 29CFR 1910.1200:
Hazard pictograms:
Signal word: Danger
Hazard statements: H314 Causes skin irritation, H318 Causes serious eye damage, H351 Suspected of causing cancer, H373 May cause damage to organs (liver, kidney) through prolonged or repeated exposure
Precautionary statements: P201 Obtain special instructions before use, P202 Do not handle until all safety precautions have been read and understood, P203 Do not breathe vapor, fume, mist, P264 Wash hands, face, and feet thoroughly after handling, P273 Wipe up spills, protective clothing, face shield, protective gloves, P302 + P352 IF ON SKIN: Wash with plenty of water, P303 + P361 + P353 IF ON EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing, P308 + P313 IF EXPOSED or concerned: Get medical attention, P310 Immediately call a Poison Center or physician, P314 Get medical advice/attention (if you feel unwell), P321 Specific treatment (see first aid instructions on this label), P332 + P313 IF skin irritation occurs: Get medical advice/attention, P362 Take off contaminated clothing and wash before reuse, P501 Store locked up, P502 Dispose of contents/container through a licensed hazardous waste disposal contractor

2.3 Other statements
None

SECTION 3. Composition/information on ingredients

3.1 Mixture

Chemical name	CAS No.	EC No.	Amount (%)
Dithioammine	131-42-2	209-568-0	7-13
Hydrogen Chloride	7647-01-0	231-597-7	0.3 - 1.0

SECTION 4. First aid measures

4.1 Description of first aid measures
In case of inhalation: Remove to fresh air. If not breathing give artificial respiration. In case of skin contact: Remove contaminated clothing and shoes immediately. Wash affected area with mild soap or detergent for at least 15 minutes or until no evidence of chemical remains. In case of eye contact: Immediately flush eyes with plenty of water for at least 15 minutes. Lifting eyelids occasionally, until no evidence of chemical remains. Call a physician. In case of ingestion: Rinse mouth, DO NOT induce vomiting unless directed to do so by medical personnel. Call a physician immediately.
May cause skin, eye, respiratory, and gastrointestinal damage/irritation. Repeated exposure may cause cancer and damage to target organs (liver, kidney)

4.2 Most important symptoms and effects, both acute and delayed:
No additional information available

4.3 Indication of any immediate medical attention and special treatment needed:
No additional information available

4.2 Most important symptoms and effects, both acute and delayed:
No additional information available

4.3 Indication of any immediate medical attention and special treatment needed:
No additional information available

SECTION 10. Stability and reactivity

10.1 Reactivity:
No dangerous reactions known under normal conditions of use
Stable under recommended storage conditions

10.2 Chemical stability:
No Data Available

10.3 Possibility of hazardous reactions:
None known

10.4 Conditions to avoid:
Light, elevated temperature, moisture

10.5 Incompatible materials:
Strong oxidizing agents, strong acids, and some metals

10.6 Hazardous decomposition products:
Hazardous fumes of nitrous oxides (NOx) and carbon oxides. Phosphorous oxides.

SECTION 11. Toxicological information

Information on toxicological effects
Acute effects (toxicity) test:

	Dithioammine	Hydrogen Chloride
LD50 Oral Rat	420 mg/kg	700 mg/kg
LD50 Inhaled Rat	7620 mg/kg	700 mg/kg
LC50 Inhalation rat (ppm)	3124 ppm/1h	

Skin corrosion/irritation: Causes skin irritation
Serious eye damage/irritation: Causes eye irritation
Sensitization skin, respiratory: None known
Additional toxicological information: CMR (carcinogenicity, mutagenicity and toxicity for reproduction) - Dithioammine IARC: 2B possible human carcinogen.

SECTION 12. Ecological information

12.1 Toxicity: No data available
12.2 Persistence and degradability: Not established
12.3 Bioaccumulative potential: Not established
12.4 Mobility in soil: No data available
12.5 Results of PBT and vPvB assessment: Not carried out
12.6 Other adverse effects: No data available

SECTION 13. Disposal considerations

13.1 Waste treatment method:
Send over to hazardous waste disposer.
Follow federal, state and local regulations for discharge for waste control regulations. US EPA guidelines for waste classification determination is listed in 40 CFR part 261.3.
Follow European Directive on waste, 2006/18/EC.

Unfilled package:
Treat as hazardous waste, disposal must be in accordance with official regulations.

SECTION 14. Transport information

14.1 UN Number (DOT, ADR, ADR, IMDG, IATA): N/A dangerous goods
14.2 UN Proper Shipping Name: Not dangerous goods
14.3 Transport hazard class(es): Not dangerous goods
14.4 Packing Group: Not dangerous goods
14.5 Environmental hazard: Avoid release into the environment.
14.6 Special precautions for user: None
14.7 Transport in bulk according to Annex 2 of MARPOL 73/78 and the IBC code: Not applicable.

SECTION 15. Regulatory information

15.1 US Federal Regulations:
TSCA: The ingredients of this product are listed on the TSCA inventory.
SARA Section 302 (Extremely Hazardous Substance): No chemicals in this material are subject to requirements of SARA Title III, Section 302.
SARA section 304 (Reportable Quantity): Dithioammine (111-42-2) 100 lb.; Hydrogen Chloride (7647-01-0) 5000 lb.

SECTION 5. Firefighting measures

5.1 Extinguishing media: CO2, extinguishing powder or water spray. Use extinguishing media appropriate to surrounding and circumstances.
5.2 Special hazards arising from the substance or mixture: None not flammable or explosive
5.3 Advice for firefighters: Wear protective gear suitable for the area including respiratory protection.

SECTION 6. Accidental release measures

6.1 Personal protection, protective equipment and emergency procedures: In the case of spilled mixture wear safety gloves to prevent skin contact. In the case of a large spill, additional protection, including respiratory protection, is recommended.
6.2 Environmental precautions: Do not discharge mixture to sewer system or waterways.
6.3 Methods and material for containment and clean up: Small spills: wipe up or scrape up and discard in appropriate waste. Clean with water afterwards. Large spills: wipe up or scrape up material, wash area thoroughly with water. Dispose according to section 13.
6.4 Reference to other sections: For safe handling, refer to section 7; for information on PPE refer to section 8; for disposal information, refer to section 13.

SECTION 7. Handling and storage

7.1 Precautions for safe handling: Do not handle until all safety precautions have been read and understood. Practice good chemical hygiene when handling. Avoid contact with eyes, skin, and clothing.
7.2 Conditions for safe storage, including any incompatibilities: Keep container tightly closed in a dry and well-ventilated place. Store away from light and avoid elevated temperature.
7.3 Specific end uses: Apart from those mentioned in Section 1.2, no other specific end uses are stipulated.

SECTION 8. Exposure controls/personal protection

8.1 Control Parameters

Component	OSHA PEL	ACGIH TWA
Dithioammine	17 mg/m ³ as dust/fraction	1 mg/m ³ respirable fraction
Hydrogen chloride	5 mg/m ³ as gas (ceiling)	2 ppm

8.2 Exposure Controls
Engineering Controls: Utilize general industrial hygiene practice
Personal protective equipment: Safety gloves with side shields, goggles. Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU). Eye and face protection regulations are identified by OSHA (US) in 29CFR1910.133. Do not wear contact lenses when working with chemicals.
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with the product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practice. Wash and dry hands. The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.
Appropriate respiratory protection should be determined according to local conditions using risk analysis protocols. An approved disposable air purifying particulate respirator may be used as a backup air equipment condition. Always use equipment and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Environmental Controls: Contains acids, do not release to the environment.

SECTION 9. Physical and chemical properties

9.1 Information on Physical and Chemical Properties:
Appearance: Liquid
Color: None
Odor: Clear, light yellow
pH: 9.7 - 10.2
Melting point/Melting range: No Data Available
Boiling point/Boiling range: No Data Available
Flash point: No Data Available
Flammability (solid, gas): No Data Available
Vapor pressure: No Data Available
Vapor density: No Data Available
Relative density: No Data Available

US State Regulations:
California: Dithioammine is on California Proposition 65 Carcinogen list
Right to know list: Dithioammine (111-42-2); Hydrogen Chloride (7647-01-0) in Massachusetts, New Jersey, Pennsylvania Right to Know List.

SECTION 16. Other information

This information is true based on our present knowledge. However, EnviroLogix makes no representation of its accuracy or completeness. Permitting receiving this information may involve their independent judgment in determining the product's safety and suitability for its intended use. This document shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.
EHS Department
EnviroLogix Inc.
GHS: H314 Causes skin irritation, H318 Causes serious eye damage, H351 Suspected of causing cancer, H373 May cause damage to organs (liver, kidney) through prolonged or repeated exposure
P201 Obtain special instructions before use, P202 Do not handle until all safety precautions have been read and understood, P203 Do not breathe vapor, fume, mist, P264 Wash hands, face, and feet thoroughly after handling, P273 Wipe up spills, protective clothing, face shield, protective gloves, P302 + P352 IF ON SKIN: Wash with plenty of water, P303 + P361 + P353 IF ON EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing, P308 + P313 IF EXPOSED or concerned: Get medical attention, P310 Immediately call a Poison Center or physician, P314 Get medical advice/attention (if you feel unwell), P321 Specific treatment (see first aid instructions on this label), P332 + P313 IF skin irritation occurs: Get medical advice/attention, P362 Take off contaminated clothing and wash before reuse, P501 Store locked up, P502 Dispose of contents/container through a licensed hazardous waste disposal contractor
NFPA System:

NFPA health hazard: 2 - Intense or continued exposure could cause temporary incapacitation or possible chronic injury unless prompt medical attention is given.
NFPA fire hazard: 0 - Materials that will not burn.
NFPA reactivity: 0 - Normally stable, even under fire exposure conditions, and are not reactive with water.