

QualiPlate™ Kit for Roundup Ready® Corn Event 603 and Cotton

Catalog Number AP 010

Highlights:

- Will detect 0.1% (1 seed in 1000) of Event 603 corn
- Test Roundup Ready corn or cotton seed lot purity in 1 hour

Contents of Kit:

- 1 antibody-coated 96-well plate
- Roundup Ready Enzyme Conjugate
- 1 packet of Buffer Salts
- Substrate
- Stop Solution

Intended Use

The EnviroLogix QualiPlate Kit for Roundup Ready Corn Event 603 and Cotton is designed for the qualitative laboratory detection of CP4 EPSPS enzyme (CP4) coded for by the Roundup Ready gene in Corn Event 603 grain, leaf, or seed, and cotton leaf and single seed. For the detection of Roundup Ready in soy bulk grain or soy flour, please refer to the Product Application Guide (Page 9). This test will detect the CP4 enzyme found in 0.1% Event 603 corn (one seed in 1000) and requires 1 hour to run.

NOTE: In corn, this kit can ONLY be used to detect RR Corn Event 603. It will NOT detect RR corn with the Event known as GA21.

How the Test Works

The EnviroLogix QualiPlate Kit for Roundup Ready Corn Event 603 and Cotton is a “sandwich” Enzyme-Linked ImmunoSorbent Assay (ELISA).

In the test, corn or cotton sample extracts are added to test wells coated with antibodies raised against CP4. Any CP4 present in the sample extract binds to the antibodies and is then detected by addition of enzyme (horseradish peroxidase)-labeled CP4 antibody.

After a simple wash step, the results of the assay are visualized with a color development step. Color development increases with increasing CP4 sample concentration.

Lighter color = Low concentration
Darker color = High concentration

How the Kit Performs

The EnviroLogix QualiPlate Kit for Roundup Ready Corn Event 603 and Cotton is a strictly qualitative (yes/no) assay. Samples are interpreted in comparison with Positive and Negative Controls. Instructions for interpreting results based upon these controls start on page 6.

Precision

CP4-fortified control solutions were repetitively analyzed in different assays on different days (inter-assay). The fortification levels used are roughly equivalent to 0.15% and 0.4% Event 603 corn, respectively. The data is expressed as % CV for both the optical density absorbance (OD) and the Positive Control Ratio (OD of sample divided by the OD of the Positive Control ground corn).

Error Rate

Event 603 Corn

Validation of this QualiPlate Kit for corn involved in-house and beta-site (non-EnviroLogix users) components. Five different in-house operators and five different beta-sites participated. Each corn sample extract was tested in three different Plate Kit manufacturing lots, generating 3 data points per corn sample.

	OD (%CV)	Pos. Ctl. Ratio (%CV)
Inter-Assay n=33		
0.15%	23.9%	21.1%
0.4%	22.1%	20.8%

1000-kernel seed/grain samples

- 2 false positive results out of 378 non-Corn Event 603 data points, for a best estimate false positive rate of 0.53%.
- 2 false negative results out of 360 0.1% Corn Event 603 data points, for a best estimate false negative rate of 0.55%.

Single seed samples

- 0 false positive results out of 387 non-Corn Event 603 seed data points, for a best estimate 0% false positive rate.
- 0 false negative results out of 366 Corn Event 603 seed data points, for a best estimate 0% false negative rate.

Single leaf punch samples

- 0 false positive results out of 378 non-Corn Event 603 leaf data points, for a best estimate 0% false positive rate.
- 0 false negative results out of 378 Corn Event 603 leaf data points, for a best estimate 0% false negative rate.

IMPORTANT NOTE: The presence of Roundup Ready Soybean in a corn sample WILL cause a positive result in this assay.

Roundup Ready Cotton

Validation of this QualiPlate Kit for cotton involved in-house and beta-site (non-EnviroLogix users) components. Four different in-house operators and five different beta-sites participated. Each cotton sample extract was tested in three different Plate Kit manufacturing lots, generating 3 data points per cotton sample.

Single seed samples

- 0 false positive results out of 591 non-Roundup Ready cotton seed data points, for a best estimate 0% false positive rate.
- 7 false negative results out of 555 Roundup Ready cotton seed data points, for a best estimate 1.3 % false negative rate.

Single leaf punch samples

- 13 false positive results out of 1593 non-Roundup Ready cotton leaf data points, for a best estimate 0.8 % false positive rate.
- 0 false negative results out of 567 Roundup Ready cotton leaf data points, for a best estimate 0% false negative rate.

Items Not Provided

- distilled or deionized water for preparing Wash/Extraction Buffers
- glass bottles or flask plus graduated cylinder with 1 liter capacity for preparation and storage of Wash/Extraction Buffer
- Tween® 20 (Sigma cat# P 1379, or equivalent), Sodium tetraborate (Borax, Sigma cat# S 9640, or equivalent, optional) for cotton sample extraction
- **Positive Control.** It is recommended that the user prepare a known positive control sample to run in each assay. A ground corn Positive Control may be purchased through EnviroLogix (CON-105, Part #10764).
- Waring laboratory blender (model 31BL91 or equivalent), glass jar adapter (Eberbach # E8495) and 32 oz. glass Mason jars for ground corn samples
- snap-cap tubes and pestles for extraction of leaf samples (EnviroLogix Cat No. ACC 002 / Part#11213, 100/package)
- centrifuge capable of 5000 x g (optional)



Prepare wash buffer and grain extraction solutions

USDA Websites

- www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
- www.gipsa.usda.gov/fgis/biotech/sample2.htm - Guidance document entitled Sampling for the Detection of Biotech Grains.
- www.gipsa.usda.gov/fgis/biotech/sample1.htm - Practical Application of Sampling for the Detection of Biotech Grains.
- www.gipsa.usda.gov/fgis/biotech/samplingplan1.xls - This website provides a simple to use Sample Planner (29K Excel Spreadsheet). The planner allows you to enter different assumptions in terms of sample size, number of samples, acceptable quality level and to determine the probability of accepting lots with given concentration levels. It also plots the probabilities in graph form for easy interpretation. Specific data can be saved for documentation and future analyses.

- disposable tip, adjustable air-displacement pipettes which will measure 50 and 100 microliters (μL)
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter ELISA plate reader
- wash bottle, or microtiter plate or strip washer
- multi-channel pipette that will measure 50 and 100 μL
- racked dilution tubes for loading samples into the plate with a multi-channel pipette, or the equivalent
- orbital plate shaker (optional)

Preparation of Solutions

Wash/Extraction Buffer:

Add the contents of the packet of **Buffer Salts** to 1 liter of distilled or deionized water and stir to dissolve. Store refrigerated when not in use; allow to come to room temperature prior to assay. If more Wash/Extraction buffer is needed, order item # P-3563 from Sigma Chemical Co. (St. Louis, MO), or prepare the equivalent. Use this buffer for the wash step of the assay, and to extract all corn samples.

Cotton Extraction Buffers:

Cotton leaf and seed samples may be extracted with either of the following buffers:

PBS-0.55% Tween: Add 0.5 mL Tween 20 to 100 mL Wash/Extraction Buffer. Store refrigerated when not in use; allow to come to room temperature prior to assay.

Borate-Tween: Prepare 0.1 M sodium tetraborate/0.5% Tween 20 (38.1 grams per liter of de-ionized water plus 5 mL Tween 20). Adjust pH to 7.5. Store refrigerated when not in use; allow to come to room temperature prior to assay.

Sample Preparation

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

Sampling Ground Corn Grain/Seed

This protocol requires that a small sample (20 to 50 grams) be analyzed. It is essential that this sample be well mixed and representative of the larger bulk. The test will detect 0.1% Event 603 corn (one positive kernel in a sample of 1000 kernels).

NOTE: Thorough mixing of the bulk grain sample and determination of an appropriate sampling plan are critical to the results of this testing, and are the responsibility of the user of this test kit. The USDA/GIPSA has prepared several guidance documents to address the issues involved in obtaining representative grain samples from static lots - such as trucks, barges, and railcars - and for taking samples from grain streams.

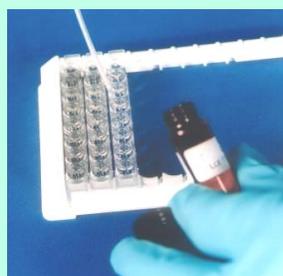
Sampling plans should be chosen that best meet the needs of both the buyer and seller in terms of acceptable risks. Increasing the number of kernels in the sample and taking multiple samples will increase the likelihood of obtaining representative samples, and maximize the probability of detecting any contamination in the grain lot. For further information on USDA/GIPSA guidelines for obtaining representative samples and assessing detection probabilities for biotech grain, see the websites listed to the left.



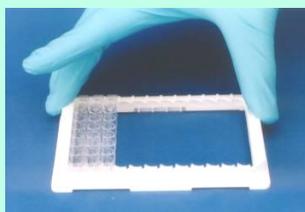
Allow all reagents to reach room temperature before beginning



Remove unneeded strips



Add controls and sample extracts



Mix plate



Incubate

Grind and Extract the Samples

Ground Corn Grain/Seed:

Once representative samples have been obtained from a truck or container, they can be reduced in size using a splitter and uniformly ground and mixed. **The finer the grind, the faster and more efficient the extraction.**

1. For 1000 kernel samples, grind in a 32 ounce "Mason" jar on a blender at high speed for 1 minute. Shake jar to mix, then repeat the grinding a second time. Thoroughly clean the grinding equipment between samples to prevent cross-contamination.
2. Weigh at least 20 grams of ground corn sample into a jar or cup.
3. Add 50 mL of Wash/Extraction Buffer to each 20 gram sample. For all other grain sample sizes, add Wash/Extraction Buffer at the rate of 2.5 mL per gram of grain. Cap and shake vigorously by hand or vortex for 20-30 seconds. Let stand at room temperature for 1 hour to extract. Mix again at the end of the hour.
4. For best results, clarify the extracts by centrifuging at 5000 x g for 5 minutes. Alternatively, allow them to settle out for at least 10 minutes. Insert a pipette tip below any floating lipid layer and above the pellet to remove the clarified sample. Dispensing particles into the test plate can cause false positive results.

Single Corn or Cotton Seed Samples:

1. Crush seeds: Seeds may be placed in a resealable plastic bag and smashed with a hammer, then transferred to a tube; or, a seed crusher/48-well plate combination may be used (for example Hypure #HSC-100, PerkinElmer, Norton, OH, with Costar plate #3548, Corning Life Sciences, Acton, MA, or equivalent). Check to be sure that all seeds have been crushed. Take extreme care not to cross-contaminate between seed samples. If using the seed crusher, dip the crushing prongs in clean water, then shake off the excess prior to crushing. After crushing, slide a piece of paper between the plate and the crushing prongs as you remove them from the wells. These procedures help to prevent seed particles from jumping from one well to the next, reducing the risk of cross-contamination.
2. Add 1 mL of Wash/Extraction Buffer to each crushed corn seed; add 1 mL of PBS-0.55% Tween or Borate-Tween to each crushed cotton seed. Mix for at least 30 seconds, then allow particles to settle. Dispensing particles into the test plate can cause false positive results.

Single Corn or Cotton Leaf Punch Samples:

1. Take a single leaf punch of approximately 5 millimeters diameter, using a micro-tube cap or a paper punch. Mash the leaf tissue with a pestle matched to the micro-tube, or with a disposable pipette tip, or a Hypure cutter (HCT-200, PerkinElmer, Norton, OH) in a 96-well plate (Costar #3370, Corning Life Sciences, Acton, MA, or equivalent).
2. Add 0.25 mL of Wash/Extraction Buffer per corn leaf punch; add 0.25 mL of PBS-0.55% Tween or Borate-Tween to each cotton leaf punch. Mix for at least 30 seconds, then allow particles to settle. Take extreme care not to cross-contaminate between leaf samples. Dispensing particles into the test plate can cause false positive results.

How to Run the Assay

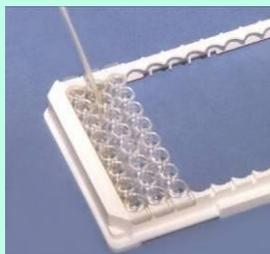
- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed plates and reagents at room temperature - do not remove plate from bag with desiccant until it has warmed up).



Bottle Wash method



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.*

- Organize all Control 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 Control(s) 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.
- 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. For this qualitative assay, duplicate wells of the Wash/Extraction Buffer Blank (BL), user-supplied Negative Control (NC) and Positive Control (PC), along with 90 sample extracts (S) in single wells may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1).

Procedure

1. Add **50 μ L** of **Roundup Ready Enzyme Conjugate** to each well, followed immediately by **50 μ L** of **Wash/Extraction Buffer Blank (BL)**, **50 μ L** of user-supplied **Negative and Positive Controls (PC and NC)** and **50 μ L** of each **sample extract (S)** to their respective wells, as shown in the Example Plate Layout (Figure 1).

NOTE: In order to minimize setup time 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 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 **45 minutes**. If an orbital plate shaker is available, shake plate at 200 rpm.
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/Extraction Buffer, then shake to empty. Repeat this wash step three times. Alternatively, perform these four washes (300 μ L/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 μ L** 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.
CAUTION: Stop Solution is 1.0N Hydrochloric acid. Handle carefully.
7. Add **100 μ L** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of the 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 Wash/Extraction Buffer Blank wells (this should automatically subtract the mean optical density (OD) of the Blank wells from each control and sample OD). If the reader cannot do this, it must be done manually.

General test criteria:

The mean OD of the BLANK wells should not exceed 0.2.

The mean, blank-subtracted OD of the Positive Control wells should be at least 0.2 and at least 3x greater than the mean, blank-subtracted OD of the Negative Control wells.

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 ground corn extract wells. This number is the "Positive Control Ratio".

Interpret the Qualitative Results

Ground corn samples

If the Positive Control Ratio calculated for a sample is less than 0.25, the ground corn contains less than 0.1% Event 603 corn.

If the Positive Control Ratio of a sample is greater than or equal to 0.25, the sample contains 0.1% or greater Event 603 corn.

NOTE: Ground corn samples containing more than 25% Event 603 corn may show decreasing OD's with increasing concentration. However, the OD's will be much greater than that of a 0.1% Event 603 sample. This test is to be used qualitatively only, with yes/no results at 0.1% Event 603 corn. For information on testing at different cutoff levels, please contact EnviroLogix' Technical Service.

Single Corn or Cotton Leaf and Seed samples:

If the Positive Control Ratio calculated for a sample is less than 1.0, the sample is not Event 603 corn or Roundup Ready cotton.

If the Positive Control Ratio of a sample is greater than or equal to 1.0, the sample is Event 603 corn or Roundup Ready cotton.

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 Event 603 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 1. Example of a typical Qualitative assay setup.

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

Precautions and Notes

- Store all QualiPlate Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose QualiPlate 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 to sunlight during pipetting or while incubating in the test wells.
- The assay has been optimized to be used 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.
- Observe any applicable regulations when disposing of samples and kit reagents.
- Use caution to prevent sample-to-sample cross-contamination with samples, fluids, or disposables.



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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.

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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.

Parafilm is a registered trademark of American Can Corporation

Roundup Ready, 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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LICENSE

EnviroLogix has developed this kit using proprietary reagents as well as reagents licensed from the Monsanto Company.

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Safety Data Sheet

According to OSHA 29CFR 1910.1200

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

1.1 Product identifier	Wash Buffer Salts
Trade name:	50-0091, 10099
Part number:	
1.2 Relevant identified uses of the substance or mixture and uses advised against application of the substance or mixture	Laboratory chemicals
1.3 Details of the supplier of the safety data sheet	EnvirolLogix Inc., 500 Riverside Industrial Pkwy, Portland ME 04103, USA (207) 797-0300 (207) 797-0300 Technical Service
1.4 Emergency telephone number:	

SECTION 2. Hazards identification

2.1 Classification of the Substance or Mixture:	Not a hazardous substance or mixture
Classification according to OSHA 29CFR 1910.1200 (Hazard Communication):	
2.2 Label Elements:	None required according to 29CFR 1910.1200
Other indications:	None
2.3 Additional information:	No other information

SECTION 3. Composition/information on ingredients

3.2 Mixture:	Positively acid										
Synonym:	PBS										
Hazardous Components											
	<table border="1"> <thead> <tr> <th>Chemical name</th> <th>CAS No</th> <th>EC No</th> <th>Amount (%)</th> <th>Classification</th> </tr> </thead> <tbody> <tr> <td>Potassium Chloride</td> <td>7447-40-7</td> <td>231-211-8</td> <td>1-5 %</td> <td>Aquatic Acute 3; Aquatic Chronic 3, H412</td> </tr> </tbody> </table>	Chemical name	CAS No	EC No	Amount (%)	Classification	Potassium Chloride	7447-40-7	231-211-8	1-5 %	Aquatic Acute 3; Aquatic Chronic 3, H412
Chemical name	CAS No	EC No	Amount (%)	Classification							
Potassium Chloride	7447-40-7	231-211-8	1-5 %	Aquatic Acute 3; Aquatic Chronic 3, H412							

Based on the amount of hazardous ingredients in this product, it is not considered hazardous according to 29CFR 1910.1200

SECTION 4. First aid measures

4.1 Description of first aid measures:	
After inhalation:	Supply fresh air, consult doctor in case of breathing difficulties.
After skin contact:	Flush skin with plenty of water for at least 15 minutes. Remove contaminated clothing. Seek medical attention if irritation develops.
After eye contact:	Rinse opened eye for several minutes under running water. Seek medical attention if irritation develops.
After swallowing:	If swallowed, consult with medical staff or poison control center to determine if any immediate response or follow up actions are recommended. Never give anything by mouth to an unconscious person.
4.2 Most important symptoms and effects, both acute and delayed:	None
4.3 Indication of any immediate medical attention and special treatment needed:	No special treatment is required

SECTION 5. Firefighting measures

5.1 Extinguishing media:	Stable extinguishing agents: CO ₂ , extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
5.2 Special hazards arising from the substance or mixture:	Carbon oxides, Oxides of Phosphorus, Potassium, Sodium, Hydrogen Chloride gas
5.2 Advice for firefighters:	Wear protective equipment appropriate for fire conditions including respiratory protective gear

SECTION 6. Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures:	Use PPE, avoid dust formation, ensure adequate ventilation, avoid breathing dust
6.2 Environmental precautions:	Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge to the environment must be avoided.
6.3 Methods and material for containment and clean up:	Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable closed containers for disposal
6.4 Reference to other sections:	For safe handling refer to Section 7; For information on PPE refer to Section 8. For disposal, refer to Section 13.

SECTION 7. Handling and storage

7.1 Precautions for safe handling:	Practice good chemical hygiene when handling. Avoid contact with eyes, skin and clothing. Prevent formation of dust.
7.2 Conditions for safe storage, including any incompatibilities:	Keep containers closed, store in a dry, well ventilated space.
7.3 Specific end use(s):	Apart from the uses mentioned in section 1.2, no other end uses are stipulated.

SECTION 8. Exposure controls/personal protection

8.1 Control parameters:	Components with workplace control Parameters: Contains no substances with occupational exposure limit values
8.2 Exposure controls:	Ensure eyewash and safety shower are nearby; provide ventilation if necessary
8.2.1 Appropriate engineering controls:	
8.2.2 Personal Protective Equipment:	Eyes Safety glasses 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 equipment are described by OSHA (US) in 29CFR1910.133. Do not wear contact lenses when working with chemicals
	Hands 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 this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. 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.
	Respiratory protection 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 to engineering controls. Always use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
	Body Use body protection relative to its type and amount of material being handled
8.2.3 Environmental controls:	Sweep or wipe up spills, do not allow into sewers or drains

SECTION 9. Physical and chemical properties

9.1 Information on basic physical and chemical properties:	
a) Appearance:	White powder.
b) Odor:	None
c) Odor Threshold:	No data available
d) pH:	7-4
e) Melting point/freezing point:	No data available
f) Boiling point/boiling range:	No data available
g) Flash point:	No data available
h) Evaporation rate:	No data available
i) Flammability (solid, gaseous):	No data available
j) Upper/lower flammability or explosive limits:	No data available
k) Vapor pressure:	No data available
l) Vapor density:	No data available
m) Relative density:	No data available
n) Solubility(ies):	Water soluble
o) Partition Coefficient: n-Octanol/water:	No data available
p) Auto-ignition temperature:	No data available
q) Decomposition temperature:	No data available
r) Viscosity:	No data available
s) Explosive properties:	No data available
t) Oxidizing properties:	No data available
9.2 Other information:	No further relevant information available.

SECTION 10. Stability and reactivity

10.1 Reactivity:	No data available.
10.2 Chemical stability:	Stable under normal recommended storage conditions.
10.3 Possibility of hazardous reactions:	No data available.
10.4 Conditions to avoid:	No data available.
10.5 Incompatible materials:	Strong oxidizing agents and strong acids.
10.6 Hazardous decomposition products:	No data available.

SECTION 11. Toxicological information

Acute toxicity:	No data available
Inhalation:	No data available
Dermal:	No data available
Skin corrosion/irritation:	No data available
Serious eye damage:	No data available
Respiratory or skin sensitization:	No data available
Mutagenicity and toxicity for reproduction:	No data available
Carcinogenicity:	No component of this product at levels greater than 0.1 % is identified as probable, possible, or confirmed human carcinogen by IARC, ACGIH, NTP, or OSHA.

SECTION 12. Ecological information

12.1 Toxicity:	No data available.
12.2 Persistence and degradability:	No data available.
12.3 Bio accumulative potential:	No data available.
12.4 Mobility in soil:	No data available.
12.5 Results of PBT and vPvB assessment:	Not available as a chemical safety assessment, not required/not conducted.
12.6 Other adverse effects:	No data available.

SECTION 13. Disposal considerations

Dispose of excess or unused product in accordance with Local, State and Federal regulations. Contact a licensed professional waste disposal service to dispose of this material.

SECTION 14. Transport information

14.1 UN-number (DOT, ADR, ADN, IMDG, IATA):	Not dangerous goods
14.2 UN proper shipping name (DOT, ADR, ADN, IMDG, IATA):	Not dangerous goods
14.3 Transport hazard classes (DOT, ADR, ADN, IMDG, IATA):	Not applicable
14.4 Packing group (DOT, ADR, IMDG, IATA):	Not applicable
14.5 Environmental hazards	Not applicable
14.6 Special precautions for user:	Not applicable
14.7 Transport in bulk according to Annex II of MARPOL 73/78	Not applicable

SECTION 15. Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture	
US Federal Regulations	
SAHA Section 302 (Extremely Hazardous Substances):	Not listed
Clean Air Act:	Not listed
Clean Water Act:	Not listed
OSHA:	Not listed
US State Regulations	
Massachusetts Right to Know:	Disodium Hydrogenorthophosphate CAS No 7558-79-4 Rev. Date: 2007-03-01
California Prop. 65 Components:	Contains no chemicals known to cause cancer, birth defects, or reproductive harm.
15.2 Chemical Safety Assessment	Not carried out

SECTION 16. Other information

Hazard Code
H412 Harmful to aquatic life with long lasting effects

This information is true based on our present knowledge. However, EnvirolLogix makes no representation of its accuracy or completeness. Persons receiving this information must exercise 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.

ENVIROLOGIX
EnvirolLogix Inc.



Material Safety Data Sheet
OSHA 29CFR 1910.1200

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

1.1 Product identifier	Stop Solution
Trade name:	L O N HCl
Synonyms:	10825, 10827, 10828, 11193, 11776 (XGDD007)
Part number:	Laboratory chemicals
1.2 Relevant identified uses of the substance or mixture and uses advised against application of the substance / the preparation :	
1.3 Details of the supplier of the safety data sheet	Envirol ogix Inc., 500 Riverside Industrial Pkwy, Portland ME, 04103, USA Phone: (207) 7974300
1.4 Emergency telephone number:	(207) 797-0300 Technical Service

SECTION 2. Hazards identification

2.1 Classification of the substance or mixture	Hazard Classes
Classification according to OSHA 29 CFR 1910.1200	Metal Corrosive (Cat. 1) H290 Skin Irritation (Cat 2) H315 Serious Eye damage (Cat. 1) H318
2.2 Label elements	
Labeling according to OSHA 29CFR 1910.1200	
Hazard pictograms :	
Signal word :	Warning
Hazard statements:	H290 May be corrosive to metals H315 Causes skin irritation H318 Causes serious eye damage
Precautionary statements:	P281 Use personal protective equipment as required P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
2.3 Other Statements	None

6.3 Methods and material for containment and cleanup:	Absorb in paper towel and discard in appropriate waste. Clean with water afterwards. Large spills may be neutralized with dilute solutions of sodium carbonate or calcium oxide.
6.4 References to other sections:	For safe handling refer to Section 7. For information on PPE refer to Section 8. For disposal refer to Section 13.

SECTION 7. Handling and storage

7.1 Precautions for safe handling:	Practice good chemical hygiene when handling. Avoid contact with eyes, skin, and clothing.
7.2 Conditions for safe storage, including any incompatibilities:	Store in tightly closed, non-metal container, in a corrosive compatible area. Prevent direct sunlight and heat. Store in well aired storage rooms.
7.3 Specific end use(s):	Apart from the uses mentioned in section 1.2., no other specific uses are stipulated.

SECTION 8. Exposure controls/personal protection

8.1 Exposure limits:	Components with limit values that require monitoring at the workplace:									
	<table border="1"> <thead> <tr> <th>Hydrogen Chloride</th> <th>European (Commission directive 96/94)</th> <th>USA (OSHA)</th> </tr> </thead> <tbody> <tr> <td></td> <td>8hr TWA = 5 ppm (7.5 mg/m³)</td> <td>Ceiling Limit = 5 ppm (7.5 mg/m³)</td> </tr> <tr> <td></td> <td>STEL = 10 ppm (15 mg/m³)</td> <td></td> </tr> </tbody> </table>	Hydrogen Chloride	European (Commission directive 96/94)	USA (OSHA)		8hr TWA = 5 ppm (7.5 mg/m ³)	Ceiling Limit = 5 ppm (7.5 mg/m ³)		STEL = 10 ppm (15 mg/m ³)	
Hydrogen Chloride	European (Commission directive 96/94)	USA (OSHA)								
	8hr TWA = 5 ppm (7.5 mg/m ³)	Ceiling Limit = 5 ppm (7.5 mg/m ³)								
	STEL = 10 ppm (15 mg/m ³)									
8.2 Exposure Controls:	Facilities using this mixture should be equipped with an eyewash and safety shower. Use general or local exhaust ventilation to keep airborne concentrations below permissible exposure limits.									
8.2.1 Engineering controls										
8.2.2 General protective and hygienic measures:	The usual precautionary measures should be adhered to when handling chemicals.									
Eye Protection:	Safety glasses 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 described by OSHA (US) in 29CFR1910.133. Do not wear contact lenses when working with chemicals.									
Hand Protection:	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 this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. 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.									
Breathing Equipment:	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 to engineering controls. Always use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).									
8.2.3 Environmental exposure controls:	Contain spills, do not allow into environment									

SECTION 3. Composition/information on ingredients

3.2 Mixture	Aqueous solution 1N Hydrochloric Acid (1N HCl, 3% HCl)			
Chemical name	Amount (%)	CAS No		Classification According to OSHA 29CFR 1910.1200
		EC No		
Hydrochloric acid	1-4 %	7647-01-0		Hazard Classification May be Corrosive to Metals H290 Causes Skin Irritation H315 Causes Serious Eye Damage H318
		231-595-7		

SECTION 4. First aid measures

4.1 Description of first aid measures	
After inhalation :	In case of inhalation: Remove to fresh air. If not breathing give artificial respiration. Get medical attention immediately.
After skin contact :	In case of skin contact: Remove contaminated clothing and shoes immediately. Wash affected area with mild soap or detergent for at least 10 minutes or until no evidence of chemical remains.
After eye contact :	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. Get medical attention immediately.
After swallowing :	In case of ingestion, DO NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Call a physician immediately.
4.2 Most important symptoms and effects, both acute and delayed:	May cause skin irritation and eye damage
4.3 Indication of any immediate medical attention and special treatment needed:	DO NOT use sodium bicarbonate in an attempt to neutralize the acid.

SECTION 5. Firefighting measures

5.1 Extinguishing media:	CO ₂ , extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
5.2 Special hazards arising from the substance or mixture:	Hydrogen Chloride gas
5.3 Advice for firefighters:	Wear protective gear appropriate for fire conditions including respiratory protective gear.

SECTION 6. Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures:	In the case of spilled mixture wear gloves to prevent skin contact. In the case of a large spill, additional protection is recommended.
6.2 Environmental precautions:	Do not discharge mixture to sewer system or waterways.

SECTION 9. Physical and chemical properties

9.1 Information on basic physical and chemical properties:	
a) Appearance:	Clear liquid, colorless to slight yellow.
b) Odor:	Pungent (slight)
c) Color Threshold:	No Data Available
d) pH:	pH 1
e) Melting point/freezing point:	No Data Available
f) Boiling point/Boiling range:	No Data Available
g) Flash point:	Not applicable
h) Evaporation rate:	0.36 (Water) compared with n-Butyl Acetate = 1
i) Flammability (solid, gaseous):	No Data Available
j) Upper/lower flammability or explosive limits:	No Data Available
k) Vapor pressure:	No Data Available
l) Vapor density:	No Data Available
m) Relative density:	No Data Available
n) Solubility(ies):	Fully miscible, water.
o) Partition Coefficient: n-Octanol/water:	No Data Available
p) Auto-ignition temperature:	No Data Available
q) Decomposition temperature:	No Data Available
r) Viscosity:	No Data Available but should be similar to that of water
s) Explosive properties:	No Data Available
t) Oxidizing properties:	No Data Available
9.2 Other information:	No further relevant information available.

SECTION 10. Stability and reactivity

10.1 Reactivity:	No data available
10.2 Chemical Stability:	Stable under normal temperatures and pressures.
10.3 Possibility of hazardous reactions:	Under normal conditions of storage and use, hazardous reactions will not occur.
10.4 Conditions to avoid:	No specific data
10.5 Incompatible materials:	Metals, Alkali metals, bases, Amines.
10.6 Hazardous decomposition products:	Under normal conditions of storage and use, hazardous decomposition products should not be produced.

SECTION 11. Toxicological information

Information on Toxicological Effects													
Acute effects (toxicity tests):	<table border="1"> <thead> <tr> <th>7647-01-0 HCl</th> <th>Effect Dose</th> <th>Species</th> </tr> </thead> <tbody> <tr> <td>Acute oral toxicity</td> <td>LD50=900mg/kg</td> <td>rabbit</td> </tr> <tr> <td>Acute dermal toxicity</td> <td>No data</td> <td></td> </tr> <tr> <td>Acute inhalative toxicity</td> <td>LC50 = 3124 mg/L</td> <td>rat</td> </tr> </tbody> </table>	7647-01-0 HCl	Effect Dose	Species	Acute oral toxicity	LD50=900mg/kg	rabbit	Acute dermal toxicity	No data		Acute inhalative toxicity	LC50 = 3124 mg/L	rat
7647-01-0 HCl	Effect Dose	Species											
Acute oral toxicity	LD50=900mg/kg	rabbit											
Acute dermal toxicity	No data												
Acute inhalative toxicity	LC50 = 3124 mg/L	rat											
Sensitization:	No sensitizing effects known												
CMR (carcinogenicity, mutagenicity and toxicity for reproduction) effects:	No CMR effects.												
Additional toxicological information:	No Additional Information												

SECTION 12. Ecological information

12.1 Toxicity:			
Aquatic toxicity (1N HCl)	Effect dose	Exposure time	Species
Acute fish toxicity	LC50=826 mg/L	96h	Lepomis idus
Acute daphnia toxicity	No data		
Acute algae toxicity	No data		

Intended Use

This Application Guide provides instruction for the use of the AP 010 QualiPlate Kit for Roundup Ready Corn and Cotton for qualitative or quantitative laboratory detection of CP4-EPSPS enzyme (CP4) coded for by the Roundup Ready gene in soybean **grain** or **flour**. This kit has not been validated for—and should not be used with—soy **meal** or any other soy product. This test will detect the CP4 enzyme in 0.1% Roundup Ready (RR1) or 0.2% Roundup Ready 2 Yield (RR2) soy grain or flour, and requires one hour to run. Follow instructions in the product insert for running the assay. This guide covers sample preparation, calibration, and data interpretation for the soybean/soy flour matrices.

Materials Required:

- EnviroLogix QualiPlate Kit for Roundup Ready Corn and Cotton (AP 010, or AP 010 NWV10)
- RR1 soy powder standard(s), sourced from the IRMM-JRC (European Commission Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg, B-2440 Geel, Belgium. www.irmm.jrc.be)
- RR2 soy powder standard (available as 100%), sourced from AOCS (American Association of Oil Chemists, Headquarters 2710 S. Boulder, Urbana, IL 61802-6996 USA; <https://secure.aocs.org/crm/index.cfm>)
- centrifuge capable of 5000 x g
- grinder or mill capable of reducing samples to a 40-mesh particle size
- test or centrifuge tubes for extraction of grain and dilution of sample extracts

Standard Extracts:

Recommended concentrations of RR Standards are Negative (0), 0.1, 1.0 and 2.0% RR1 soy powder, or Negative, 0.2, 2.0, and 4% RR2Y soy powder. These standards must be powders that will pass through a 40-mesh sieve. Mix positive and negative powders by weight to prepare the desired concentrations.

Standards must be extracted prior to performing the test. Standard extracts may then be aliquoted and frozen for use in later testing. Procedure:

1. Add 50 mL of water (distilled or deionized) to each 1 gram of soy powder Standard. Shake or vortex vigorously for 30 seconds, let stand for 1 hour, then shake again.
2. Centrifuge the extracts at 5000 x g for 5 minutes.
3. Pour the clarified extracts into a clean tube, and transfer 0.25 mL aliquots to suitable plastic, labeled, capped tubes for freezer storage (-20°C). These frozen extracts are stable for at least 6 months in a non-defrosting freezer.

Sample Extraction and Preparation:

- This protocol calls for a small sample (20 to 50 grams) to be analyzed. It is essential that this sample be well mixed and representative of the larger bulk. The test will detect 0.1% RR1 soy in soy flour (or 1 RR bean in a sample of 999 non-transgenic beans) or 0.2% RR2Y soy in soy flour (or 1 RR2Y bean in a sample of 499 non-transgenic beans).

- It is the responsibility of the user to ensure proper sampling and thorough mixing prior to analysis. Once representative samples have been obtained from the truck or container, they can be reduced in size using a splitter and uniformly ground and mixed.
- The finer the grind, the faster and more efficient the extraction. The commercial standards are a soy powder. In order for soybean samples to be measured against these standards, the ground/milled samples must be passed through a 40-mesh sieve. The fine sieved material is then extracted and tested. Failure to follow this procedure will result in falsely low reports of sample concentration.
- For 1000 bean samples grind in a 32 ounce “Mason” jar for 1 minute, on a blender at high speed. Shake jar to mix, then repeat the grinding a second time. Alternatively, pass through an appropriate mill.
- Thaw any frozen standard extracts (prepared according to the instructions on page 1).

NOTE: Thoroughly clean the grinding and sieving equipment between each sample to avoid cross-contamination.

1. Pour the entire ground sample onto a 40-mesh sieve. Sieve until a 20 to 50 gram sample has passed through. Weigh at least 20 grams of sieved ground soy sample into a jar or cup.
2. Add 100 mL of water to each 20 gram sample. For all other sample sizes, add water at the rate of 5 mL per gram of grain. Cap and shake vigorously by hand or vortex for 20-30 seconds. Let stand at room temperature for one hour to extract. Mix again at the end of the hour.
3. Clarify the extracts by centrifuging at 5000 x g for 5 minutes. Insert a pipette tip below any floating lipid layer and above the precipitate to remove the clarified sample.
4. Dilute the **sample** extract 1:50 in Wash Buffer: mix 20 µL clarified extract in 980 µL Wash Buffer. Sample extracts must be analyzed on the day they were extracted.
5. Dilute each thawed **standard** extract 1:5 in Wash Buffer: mix 100 µL extract plus 400 µL Wash Buffer.
NOTE: Thawed standard extracts should be used within 48 hours, and refrigerated when not in use.

Standards and samples are now ready to be added to the assay plate. Follow the instructions as described in the section on page 5 entitled “How to Run the Assay.” For a quantitative assay, use duplicate wells for each standard and sample.

How to Interpret the Results

Spectrophotometric Measurement

1. Set the wavelength of the microtiter plate reader to 450 nanometers (nm). If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.
1. Set the plate reader to blank on the RR Negative soy powder Standard wells (this should automatically subtract the mean optical density (OD) of the RR Negative soy powder Standard wells from each other Standard and sample OD). If the reader cannot do this, it must be done manually.
2. General test criteria:

The mean OD of the RR Negative soy powder Standard wells should not exceed 0.2. The coefficient of variance (%CV) of the duplicate Standard and sample wells should not exceed 15%:

$$\%CV = \frac{\text{std. deviation of ODs} \times 100}{\text{Mean OD}}$$

3. For a quantitative assay, a quadratic (or polynomial) 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 Results” section.

NOTE: Soy samples containing more than 10% Roundup Ready soy may show decreasing ODs with increasing concentration. Do not attempt to extrapolate sample concentrations beyond the range of the standard curve generated in this kit.

How to Interpret the Qualitative Results

Compare the ODs of the sample extracts to those of the Standards to obtain an estimate of the % RR sample. Samples with ODs greater than that of the lowest standard are considered positive. Those with OD's lower than that of the lowest standard contain less than 0.1% RR1 Soy or less than 0.2% RR2 Soy.

How to Calculate the Quantitative Results

1. After reading the wells, average the OD of each set of Standards and samples, and subtract the average OD of the RR Negative soy powder Standard wells from all (if your reader has not automatically done so).
2. Graph the mean OD of each Standard against its % RR content with a quadratic curve fit.
3. Determine the % RR content of each sample by finding its OD value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only possible if the OD of the sample falls within the range of OD's of the Standards.

If the OD of a sample is lower than that of the lowest Standard, the sample must be reported as less than 0.1% RR1 soy or 0.2% RR2 soy.

If the OD of a sample is higher than that of the highest Standard, the sample must be reported as greater than 2% RR1 Soy or 4% RR2 soy.

If a concentration must be determined for these high level samples, dilute the sample extract 1:10 more than executed in the original assay, in Wash Buffer. Run this dilution in a repeat of the assay. If the result now falls within the range of the OD's of the Standards, multiply the results from the standard curve by 10.