ENVIROLOGIX™

QualiPlate™ Kit for Cry3Bb1 Corn

Catalog Number AP 015

Intended Use

The EnviroLogix QualiPlate Kit for Cry3Bb1 Corn is designed for the qualitative laboratory detection of Cry3Bb1 protein in single leaf, single seed, or bulk grain samples. This test will detect Cry3Bb1 protein found in 0.1% YieldGard Rootworm corn (1 seed in 1000) and requires 1 hour to run.

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 Cry3Bb1 protein. Any Cry3Bb1 protein present in the sample extract binds to the antibodies and is then detected by addition of enzyme (horseradish peroxidase)-labeled Cry3Bb1 antibody.

After a simple wash step, the results of the assay are visualized with a color development step. Color development increases with increasing Cry3Bb1 sample concentration from 0.1% to 25% YieldGard Rootworm corn, then levels and drops off.

Light color = Low concentration
Darker color = High concentration

How the Kit Performs

This QualiPlate Kit is a strictly qualitative (yes/no) assay. Samples are interpreted in comparison with Positive and Negative Control ground corn samples provided in each kit.

Precision

Cry3Bb1-fortified control solutions were repetitively analyzed in different assays on different days. The fortification levels used are roughly equivalent to 0.05% and 0.2% YieldGard Rootworm corn, respectively.

The data is expressed as % CV for both the absorbance (OD) and the Positive Control Ratio (OD of sample divided by the OD of the Positive Control ground corn).

Error Rate

Validation of this QualiPlate Kit 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 QualiPlate Kit manufacturing lots, generating 3 data points per corn sample.

1000-kernel grain samples

0 false positive results out of 360 non-YieldGard Rootworm data points, for a best estimate of false positive rate of 0%.

3 false negative results out of 342 0.1% YieldGard Rootworm data points, for a best estimate false negative rate of 0.88%.

Highlights:

- *Will detect 0.1% (1 seed in 1000) of YieldGard Rootworm corn*
- *Results in one hour*

Contents of Kit:

- *1 antibody-coated 96-well plate*
- *Positive Control ground corn*
- *Cry3Bb1 Enzyme Conjugate*
- *1 packet of Buffer Salts*
- *Substrate*
- *Stop Solution*

Precision

	OD (%CV)	**Pos. Ctl. Ratio** (%CV)
Inter-Assay		**n=30**
0.05%	15.5%	20.6%
0.20%	13.6%	22.6%

Single seed samples

3 false positive results out of 360 non-YieldGard Rootworm seed data points, for a best estimate false positive rate of 0.83%.

0 false negative results out of 338 YieldGard Rootworm seed data points, for a best estimate false negative rate of 0%.

Single leaf punch samples

0 false positive results out of 360 non-YieldGard Rootworm leaf data points, for a best estimate false positive rate of 0%.

5 false negative results out of 360 YieldGard Rootworm leaf data points, for a best estimate false negative rate of 1.39%.

NOTE: These are best estimates of expected false positive/false negative rates based upon the data available; actual results may vary.

Prepare Wash/Extraction buffer

Materials Not Provided

- distilled or deionized water for preparing Wash/Extraction Buffer
- glass bottles or flask plus graduated cylinder with 1 liter capacity for preparation and storage of Wash/Extraction Buffer
- Waring 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# ACC 002, 100/package) (optional)
- centrifuge capable of 5000 x g (optional)
- disposable tip, adjustable air-displacement pipettes which will measure 50 and 100 microliters (µL)
- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate reader with 450 nm filter
- wash bottle, or microtiter plate 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)
- orbital plate shaker (optional)

Preparation of Solutions

Wash/Extraction Buffer: Add the contents of the packet of Buffer Salts (phosphate buffered saline, pH 7.4 - Tween 20) to 1 liter of distilled or deionized water, and stir to dissolve. Store refrigerated when not in use; warm 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.

Positive Control ground corn extracts: Extracts of these controls must be run in every assay. To extract, add 5 mL of Wash/Extraction Buffer to each tube containing 2 grams of ground Control corn. 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, then clarify by allowing to settle 10 minutes or by centrifuging 5 minutes at 5000 x g.

If running the assay at a later date, or more than one assay per plate, freeze 0.5 mL aliquots of each clarified extract. Thaw just prior to use.

USDA Websites:
Guidance on bulk grain testing

www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx
USDA Grain Inspection Handbook, Book 1, Grain Sampling. This document provides a comprehensive overview of recommended sampling guidelines for static lots and grain streams. It reviews the various types of equipment and strategies that can be used to obtain a representative grain sample from different types of containers.

www.gipsa.usda.gov/fgis/biotech/sample2.htm
Guidance document entitled Sampling for the Detection of Biotech Grains, which provides important statistical sampling considerations when testing for the presence of biotech grains. It covers the basis for making probability determinations in accepting lots based upon different assumptions with respect to sample size, number of samples, sample preparation, etc.

www.gipsa.usda.gov/fgis/biotech/sample1.htm
Practical Application of Sampling for the Detection of Biotech Grains. This one-page application guide provides a table that gives sample sizes for selected lot concentrations and probability of rejecting the specified concentrations. It also provides a formula for making the calculation for other combinations.

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.

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, in addition to the Positive Control ground corn supplied with the kit.

Ground Bulk Grain Samples:

Testing of bulk grain for Cry3Bb1 protein is an indicator of the presence or absence of YieldGard Rootworm corn in a given sample. A negative test with this kit is not an indicator of the absence of other genetic modifications.

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

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 ground corn sample sizes, add Wash/Extraction Buffer at the rate of 2.5 mL per gram of corn. 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. **NOTE:** Dispensing particles into the test plate can cause false positive results.

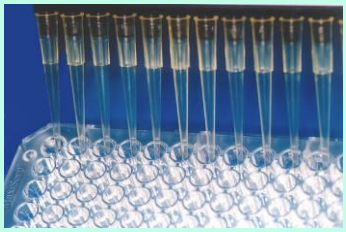
Single Seed Samples

1. Crush seeds: Seeds may be placed in a re-sealable 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.
2. Add 1 mL of Wash/Extraction Buffer to each crushed corn seed. Mix for at least 30 seconds, then allow particles to settle. Dispensing particles into the test plate can cause false positive results.

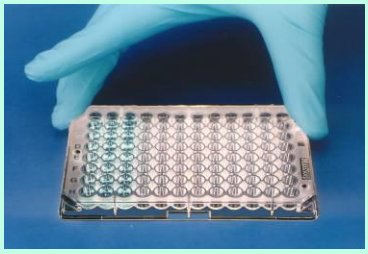
Single Leaf Punch Samples:

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).

Allow all reagents to reach room temperature before beginning



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



Mix plate

Bottle Wash method

2. Add 0.25 mL of Wash/Extraction Buffer per 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).
- 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 Wash/Extraction Buffer blank (BL), user-supplied known-negative control (NC), and the 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 1A).

1. Add **50 µL** of **Cry3Bb1 Enzyme Conjugate** to each well, followed immediately by **50 µL of Wash/Extraction Buffer Blank (BL)**, **50 µL** of **Negative** and **Positive Control ground corn** extracts **(PC and NC)** and **50 µL** of each **sample extract (S)** to their respective wells, as shown in Figure 1A.

   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 bench top 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 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** to each well.

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 15 minutes**. If an orbital plate shaker is available shake plate at 200 rpm.

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

8. 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.

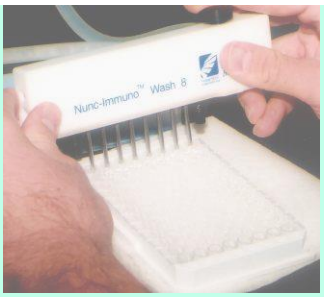
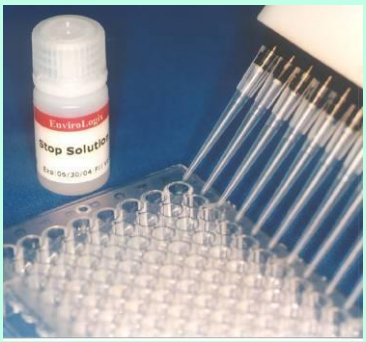


Plate Wash option



Complete protocol and add Stop Solution

Read plates in a Plate Reader 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 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 0.1 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.

How to 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".

How to 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% YieldGard Rootworm corn.

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

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

Single Leaf and Seed samples

If the Positive Control Ratio calculated for a sample is less than 0.5, the sample is not YieldGard Rootworm corn.

If the Positive Control Ratio of a sample is greater than or equal to 0.5, the sample is YieldGard Rootworm corn.

These types of 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 (flying

particles or dust from YieldGard Rootworm corn, corn leaf residue on leaf punch, etc.) or can be caused by transfer of particulate matter from leaf or seed extracts into the assay wells. If there is any question of the latter occurring, re-extraction and re-testing is recommended.

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	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 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 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.
- Cry3Bb1 endotoxin is a protein that can be degraded by heat and sunlight. Samples that cannot be extracted immediately may be stored frozen for up to 1 week prior to analysis.
- 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:

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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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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.

LICENSE

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

ENVIROLOGIX™

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

SECTION 2. Hazards identification

2.1 Classification of the Substance or Mixture:
Classification according to OSHA 29CFR 1910.1200 (Hazard Communication): Not a hazardous substance or mixture
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: Powdered solid
Synonyms: PBS
Hazardous Components

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:
Suitable 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 Phosphorous, 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
8.2.1 Appropriate engineering controls: Ensure eyewash and safety shower are nearby; provide ventilation if necessary
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 regulations 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:	Not 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
Carcinogenity:	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 class(es) (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 MARPOL73/78 Not applicable.

SECTION 15. Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
US Federal Regulations

SARA 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 affects

This information is true based on our present knowledge. However, EnviroLogix 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

EHS Department
EnviroLogix Inc.

ENVIROLOGIX

Material Safety Data Sheet
OSHA 29CFR 1910.1200

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

1.1 Product identifier
Trade name: **Stop Solution**
Synonyms: **1.0 N HCl**
Part number: 10825, 10827, 10828, 11193, 11776 (XGD007)

1.2 Relevant identified uses of the substance or mixture and uses advised against application of the substance / the preparation : Laboratory chemicals

1.3 Details of the supplier of the safety data sheet
Manufacturer/Supplier: EnviroLogix Inc., 500 Riverside Industrial Pkwy. Portland ME 04103, USA Phone: (207) 797-0300

1.4 Emergency telephone number: (207) 797-0300 Technical Service

SECTION 2. Hazards identification

2.1 Classification of the substance or mixture
Classification according to OSHA 29 CFR 1910.1200 — **Hazard Classes**
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

SECTION 3. Composition/information on ingredients

3.2 Mixture
Aqueous solution 1N Hydrochloric Acid (1N HCl, 3 % HCl)

Chemical name	Amount (%)	CAS No / EC No	Classification According to OSHA 29CFR 1910.1200	
			Hazard Classification	Hazard Code
Hydrochloric acid	1-4 %	7647-01-0 / 231-595-7	May be Corrosive to Metals	H290
			Causes Skin Irritation	H315
			Causes Serious Eye Damage	H318

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.

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:

Hydrogen Chloride	European (Commission directive 96/94)	USA (OSHA)
	8Hr TWA = 5 ppm (7.5 mg/m3)	Ceiling Limit = 5 ppm (7.5 mg/m3)
	STEL = 10 ppm (15 mg/m3)	

8.2 Exposure Controls:
8.2.1Engineering 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.

8.2.2 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 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) Odor 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 decompositions products should not be produced.

SECTION 11. Toxicological information

Information on Toxicological Effects
Acute effects (toxicity tests):

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	LC 50=826 mg/L	96h	Leuciscus idus
Acute daphnia toxicity	No data		
Acute algae toxicity	No data		