

## Highlights:

- Less than 2 hours to run

## Contents of Kit:

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

## Precision

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

Catalog Number AP 007

## Intended Use

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

## How the Test Works

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

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

*Lighter color = Lower concentration*

*Darker color = Higher concentration*

## Performance

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

### Limit of Detection

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

### Limit of Quantification

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

### Precision

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

### Fortification and Recovery

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



Prepare Wash and  
Extraction Buffers

## Materials Not Provided

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

## Preparation of Solutions

1. **Wash Buffer:** Add the contents of the packet of **Buffer Salts** (phosphate buffered saline, pH 7.4 - Tween 20) to 1 liter of distilled or de-ionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.
2. **1X Extraction/Dilution Buffer:** To prepare 1X working Extraction/Dilution Buffer, add the entire contents of the bottle of 5X (35 mL) supplied in the kit to 140 mL of distilled or deionized water in a suitable container. Mix thoroughly to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.

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

## Sample Preparation

### Sample Extraction:

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

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

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

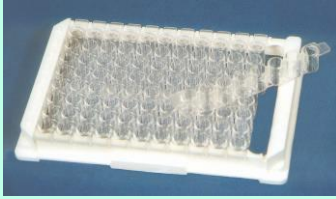
2. Add 0.5 mL of 1X Extraction/Dilution Buffer to the tube.



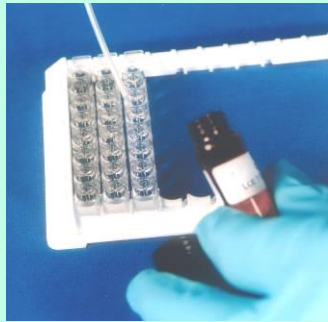
Obtain leaf tissue



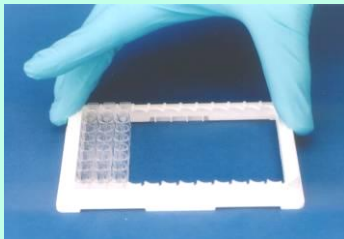
Grind tissue, add buffer,  
grind again



Remove unneeded strips



Add calibrators and sample extracts



Mix plate



Incubate



Bottle Wash method

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

#### Sample Dilution:

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

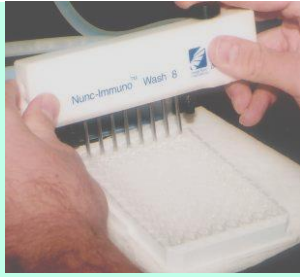
For a 1:51 dilution: add 1 mL 1X Extraction/Dilution Buffer to dilution tubes labeled for each sample. Add 20  $\mu$ L sample extract and mix.

## How to Run the Assay

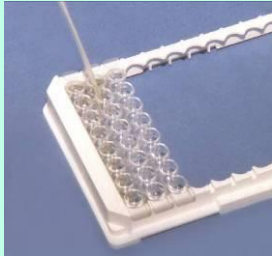
- Read all of these instructions before running the kit.
  - Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed strips and reagents at room temperature - do not remove strips from bag with desiccant until they have warmed up).
  - Organize all Calibrators and sample extracts, and pipettes so that step 1 can be performed in 15 minutes or less.
  - If more than four strips are to be run at one time, the 15 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
  - If four or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add each Calibrator and diluted sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combitip for these three reagents.
  - If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the foil bag provided, and refrigerate.
  - Use the well identification markings on the plate frame to guide you when adding the samples and reagents. In a qualitative (semi-quantitative) assay, the Negative Control (NC), three non-zero calibrators, and 88 diluted sample extracts (S) may be run on one plate. (See the Qualitative Assay Example Plate Layout - Figure 1A). For a quantitative assay the Negative Control (NC) and three Calibrators (C1-C3), along with 44 diluted sample extracts (S) may be run in duplicate wells on one plate. (See the Quantitative Assay Example Plate Layout - Figure 1B).
1. Add **50  $\mu$ L** of **Negative Control**, **50  $\mu$ L** of each **Calibrator**, and **50  $\mu$ L** of each diluted **sample extract** to their respective wells, as shown in the Example Plate Layouts (Figures 1A and 1B). Follow this same order of addition for all reagents.

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

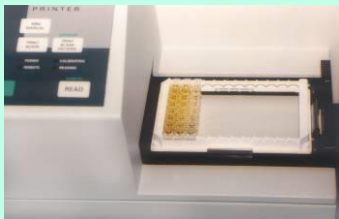
2. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!
3. Cover the wells with tape or Parafilm to prevent evaporation and **incubate** at ambient temperature for **15 minutes**.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



Strip Plate Wash option

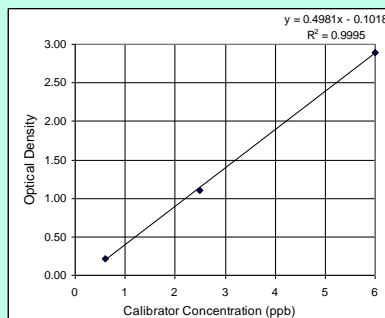


Complete protocol and add Stop Solution



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

Figure 3. Illustrative Cry1C standard curve



5. Thoroughly mix the contents of the wells as described in step 2. Be careful not to spill the contents!
6. Cover the wells with new tape or Parafilm to prevent evaporation and **incubate at ambient temperature for 1 hour.**
7. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink or other suitable container. Flood the wells completely with Wash Buffer, then shake to empty. Repeat this wash step three times. Slap the plate on a paper towel to remove as much Wash Buffer as possible. Alternatively, perform these four washes with a microtiter plate or strip washer.
8. Add **100 µL** of **Substrate** to each well.
9. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate for 30 minutes** at ambient temperature.

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

10. Add **100 µL** of **Stop Solution** to each well and mix thoroughly. This will turn the well contents yellow.

**NOTE:** Read the plate within 30 minutes of the addition of Stop Solution.

## How to Interpret the Results

### Spectrophotometric Measurement

1. Set the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)
2. Set the plate reader to blank on the Negative Control wells. If the reader cannot do this, measure and record the optical density (OD) of each well's contents, then subtract the average OD of the Negative Control wells from each of the readings.

General Test Criteria:

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

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

If the results of an assay fail to meet these criteria, consult EnviroLogix' Technical Service for suggestions on improving the test when you repeat the assay.

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

### How to Interpret the Semi-Quantitative Results

Compare the OD's of the diluted sample extracts to those of the Calibrators to obtain an estimate of the amount of Cry1C endotoxin in your sample extract.

### How to Calculate the Quantitative Cry1C Results

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

If the OD of a sample is lower than that of the Low Calibrator (0.6 ppb Cry1C), the sample must be reported as less than:  $(0.6 \text{ ppb} \times \text{dilution factor during extraction} \times \text{dilution of sample extract employed}) \div 1000 = x \text{ ppm Cry1C}$ .

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

$(6 \text{ ppb} \times \text{dilution factor during extraction} \times \text{dilution of sample extract employed}) \div 1000 = x \text{ ppm Cry1C}$ .

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



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

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

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

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



**Figure 2. Illustrative quantitative calculations**

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

\* Figures are after subtraction of Negative Control values.

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

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



## Precautions and Notes

- Store all Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Kit components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test well strips from one Kit with reagents or test well strips from a different Kit.
- **Do not expose Substrate to sunlight** during pipetting or while incubating in the test wells.
- The assay has been optimized for use with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- Cry1C endotoxins are proteins which can be degraded by heat and sunlight. Take samples from green, actively growing leaves. Samples that cannot be extracted immediately may be stored frozen for up to 1 week prior to analysis.
- Observe any applicable regulations when disposing of samples and kit reagents.



**For Technical Support  
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***www.envirologix.com***



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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**  
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 or 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 (207) 797-0300 Technical Service

**1.4 Emergency telephone number:**

**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**

**3.1 Mixture:** Potassium acid phosphate  
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: Breathe 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<sub>2</sub>, 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:**  
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 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: 7-8  
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**  
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 effects

*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.*

EFES Department  
EnviroLogix Inc.





Material Safety Data Sheet  
OSHA 29CFR 1910.1200

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

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

**SECTION 2. Hazards identification**

<b>2.1 Classification of the substance or mixture</b>	<b>Hazard Classes</b>
Classification according to OSHA 29 CFR 1910.1200	Metal Corrosive (Cat. 1) H290 Skin Irritation (Cat 2) H315 Serious Eye damage (Cat. 1) H318
<b>2.2 Label elements</b>	
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.
<b>2.3 Other Statements</b>	None

<b>6.3 Methods and material for containment and cleanup:</b>	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.
<b>6.4 References to other sections:</b>	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**

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

**SECTION 8. Exposure controls/personal protection**

<b>8.1 Exposure limits:</b>	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/m<sup>3</sup>)</td> <td>Ceiling Limit = 5 ppm (7.5 mg/m<sup>3</sup>)</td> </tr> <tr> <td></td> <td>STEL = 10 ppm (15 mg/m<sup>3</sup>)</td> <td></td> </tr> </tbody> </table>	Hydrogen Chloride	European (Commission directive 96/94)	USA (OSHA)		8hr TWA = 5 ppm (7.5 mg/m <sup>3</sup> )	Ceiling Limit = 5 ppm (7.5 mg/m <sup>3</sup> )		STEL = 10 ppm (15 mg/m <sup>3</sup> )	
Hydrogen Chloride	European (Commission directive 96/94)	USA (OSHA)								
	8hr TWA = 5 ppm (7.5 mg/m <sup>3</sup> )	Ceiling Limit = 5 ppm (7.5 mg/m <sup>3</sup> )								
	STEL = 10 ppm (15 mg/m <sup>3</sup> )									
<b>8.2 Exposure Controls:</b>	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**

<b>3.2 Mixture</b>	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**

<b>4.1 Description of first aid measures</b>	
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.
<b>4.2 Most important symptoms and effects, both acute and delayed:</b>	May cause skin irritation and eye damage
<b>4.3 Indication of any immediate medical attention and special treatment needed:</b>	DO NOT use sodium bicarbonate in an attempt to neutralize the acid.

**SECTION 5. Firefighting measures**

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

**SECTION 6. Accidental release measures**

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

**SECTION 9. Physical and chemical properties**

<b>9.1 Information on basic physical and chemical properties:</b>	
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
<b>9.2 Other information:</b>	No further relevant information available.

**SECTION 10. Stability and reactivity**

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

**SECTION 11. Toxicological information**

<b>Information on Toxicological Effects</b>			
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**

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

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**

Waste treatment methods:	Contact a licensed professional waste disposal service to dispose of this material. Disposal of surplus or waste solutions must be in accordance with applicable local, state, and national laws and regulations.
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**SECTION 14. Transport information**

14.1 UN-Number DOT, ADR, ADN, IMDG, IATA :	UN1789
14.2 UN proper shipping name DOT, ADR, ADN, IMDG, IATA :	HYDROCHLORIC ACID SOLUTION
14.3 Transport hazard class(es) DOT, ADR, ADN, IMDG, IATA):	8
14.4 Packing group (DOT, ADR, IMDG, IATA):	III
14.5 Environmental hazards	Not hazardous to the environment.
14.6 Special precautions for user :	None
14.7 Transport in bulk according to Annex II of MARPOL 73/78 and the IBC code:	No information available.

**SECTION 15. Regulatory information**

<b>15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture</b>	
<b>US Federal Regulations</b>	
TSCA	CAS# 7647-01-0 is not listed on the TSCA inventory.
Health and Safety Reporting List	None listed.
Chemical Test Rule	None under a Chemical Test Rule.
CERCLA	CAS# 7647-01-0: 5000 lb final RQ, 2270 Kg final RQ.
SARA Section 302 (Extremely Hazardous Substances)	CAS# 7647-01-0: 500 lb TPQ.
Clean Air Act	CAS# 7647-01-0: is listed as a hazardous air pollutant (HAP).
Clean Water Act	CAS# 7647-01-0: is listed as a hazardous Substance under the CWA.
OSHA	CAS# 7647-01-0: is considered highly hazardous by OSHA.
<b>US State Regulations</b>	
	CAS# 7647-01-0: can be found on the following state right to know lists: CA, NJ, PA, MN, MA.
	CA Prop 65, no Significant Risk Level – none of the chemicals in this product are listed.
<b>European/International Regulations</b>	
REACH No	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
<b>Canada – DSL/NDSL</b>	
Canada – WHEMS	CAS# 7647-01-0: 1
Canadian Ingredient Disclosure List	WHEMS classification of E, D2A.
	CAS# 7647-01-0 is listed on the Canadian Ingredient Disclosure List.
15.2 Chemical Safety assessment	Not carried out

**SECTION 16. Other information**

*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.

<b>Codes:</b>			
H250	May be Corrosive to Metals	P281	Use Personal Protective equipment as Required
H315	Causes Skin Irritation	P302 + P352	IF ON SKIN: Wash with plenty of soap and water
H318	Causes Serious Eye Damage	P205+ P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do so Continue rinsing.