

Highlights:

- Range of 1 to 10 ppb Cry2Aa in sample extract
- Less than 2 hours to run

Contents of Kit:

- 12 strips of 8 antibody-coated wells each, in plate frame
- Cry2A Negative Control
- 1.0 ppb Cry2Aa Calibrator
- 5.0 ppb Cry2Aa Calibrator
- 10 ppb Cry2Aa Calibrator
- Cry2A-Enzyme Conjugate
- 5X Extraction/ Dilution Buffer for Bt Plate Kits
- 1 packet of Buffer Salts
- Substrate
- Stop Solution

Precision

| | Recovery (%CV) | OD (%CV) |
|------------------------|-------------------|-------------|
| Intra-Assay n=7 | | |
| 3 ppb | 4.4% | 4.1% |
| 7 ppb | 2.2% | 2.1% |
| Inter-Assay n=8 | | |
| 3 ppb | 6.4% | n/a |
| 7 ppb | 6.4% | n/a |

Catalog Number AP 005

Intended Use

The QuantiPlate Kit for Cry2A is designed for the semi-quantitative laboratory detection of Cry2Aa endotoxin in cotton leaf tissue samples.

How the Test Works

This kit is a “sandwich” Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, cotton leaf sample extracts are added to test wells coated with antibodies raised against Cry2Aa toxin. Any Cry2Aa protein present in the sample extract bind to the antibodies, and are then detected by addition of enzyme (horseradish peroxidase)-labeled Cry2 antibody.

After a simple wash step, the results of the assay are visualized with a color development step; color development is proportional to Cry2Aa concentration in the sample extract.

Lighter color = Lower concentration

Darker color = Higher concentration

Limit of Detection

The Limit of Detection (LOD) of this kit is 0.52 parts per billion (ppb) Cry2Aa in cotton leaf extract. The LOD was determined by interpolating an OD equal to three times the background OD from a Cry2Aa standard curve.

Limit of Quantification

The Limit of Quantification (LOQ) of the EnviroLogix Cry2A Plate Kit was validated at 0.5 parts per million (ppm) in cotton leaf. The LOQ was determined by fortifying a population of negative cotton leaf samples at 0.5 ppm Cry2Aa. The mean recovery was 78% with a coefficient of variation [CV, (standard deviation/mean) x 100] of 3.9%.

Precision

Cry2Aa-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

Eight cotton leaf samples were fortified with Cry2Aa to concentrations ranging from 0.7 ppm to 1.5 ppm. The average recovery was 93%.

Materials Not Provided

- Disposable Tissue Extractors, EnviroLogix Cat. # ACC 002
- distilled or deionized water for preparing Wash Buffer and diluting 5X Extraction/Dilution Buffer– see recipe below
- glass bottles or flasks with 175 mL capacity for storage of 1X Extraction/ Dilution Buffer and 1 liter capacity for Wash Buffer
- test tubes for dilution of sample extracts
- disposable tip, adjustable air-displacement pipettes which will measure 20, 100, 500 and 1000 microliters (µL)



Prepare Wash and Extraction Buffers



Obtain leaf tissue



Grind tissue, add buffer, grind again

- marking pen (indelible)
- tape or Parafilm®
- timer
- microtiter plate reader or strip reader
- wash bottle, or microtiter plate or strip washer
- multi-channel pipette that will measure 100 μ L (optional)
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)

Preparation of Solutions

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 deionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay.

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.

Sample Preparation

Sample Extraction:

1. Take 2 leaf punch samples (approximately 10 milligrams each) by snapping the tube cap of the Disposable Tissue 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.*

NOTE: If the assay is to be used to quantitate levels of Cry2A toxin in cotton 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.
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.

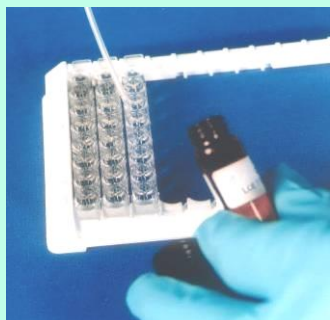
Sample Dilution:

Concentrations of Cry2A toxins will vary from plant to plant. Sample extracts must be diluted at least 1:11 prior to assay, but larger dilutions may be required in order to bring assay results within the range of calibration. Instructions follow for both 1:11 and 1:51 dilution schemes. If a more sensitive assay is required, contact EnviroLogix for technical assistance.

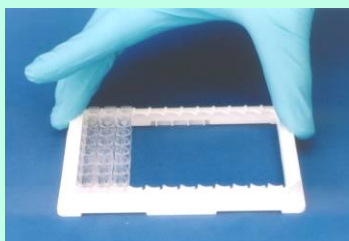
1. For a 1:11 dilution: add 0.5 mL 1X Extraction/Dilution Buffer to dilution tubes labeled for each sample. Add 50 μ L sample extract and mix.
2. For a 1:51 dilution: add 1 mL 1X Extraction/Dilution Buffer to dilution tubes labeled for each sample. Add 20 μ L sample extract and mix.



Remove unneeded strips



Add calibrators and sample extracts



Mix plate



Incubate



Bottle Wash method

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 diluted 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 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 as a guide when adding the samples and reagents. In a qualitative assay, the Negative Control (NC), the lowest calibrator and 46 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).

Procedure

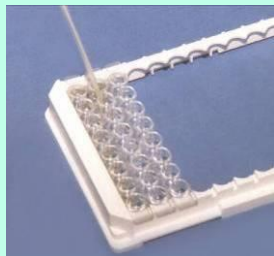
1. Add **100 μ L** of **Negative Control**, **100 μ L** of each **Calibrator**, and **100 μ L** of each **diluted sample extract** to their respective wells, as shown in the Example Plate Layouts (Figures 1A and 1B). Follow this same order of addition for all reagents.

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.

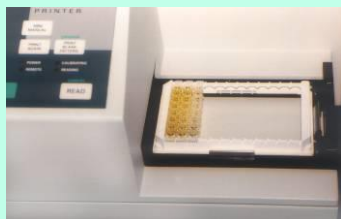
2. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents!
3. Cover the wells with tape or Parafilm to prevent evaporation and **incubate at ambient temperature for 15 minutes**.
4. Add **100 μ L** of **Cry2A-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.
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 water as possible. Alternatively, perform these four washes with a microtiter plate or strip washer (set to 300 μ L fill volume).
8. Add **100 μ L** of **Substrate** to each well.



Strip Plate Wash option

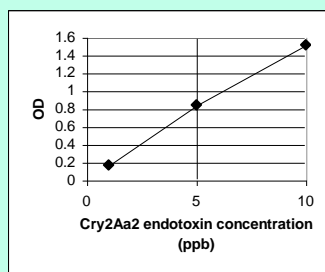


Complete protocol and add Stop Solution



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

Figure 3.
Illustrative standard curve



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%.

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

3. For a quantitative Cry2A 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 Cry2A 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 Cry2A endotoxin in your sample extract, expressed in terms of Cry2Aa reactivity.

How to Calculate the Quantitative Cry2A Results

NOTE: Although Cry2Ab calibrators are not provided with this kit, it is possible to use this kit to quantitate Cry2Ab in cotton samples. To do this, substitute the Cry2Ab calibrator values shown in the table below for the corresponding Cry2Aa calibrator concentration. Use these Cry2Ab concentrations to prepare the standard curve. Interpret results from this standard curve as ppb Cry2Ab.

1. After reading wells, average the OD of each set of calibrators and samples.
2. Graph the mean OD of each Calibrator against its Cry2Aa concentration on a linear scale (see Figure 3).
3. Determine the Cry2Aa 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 ($500 \mu\text{L} \div x \text{ mg leaf tissue}$) multiplied by the 1:11 or 1:51 dilution of sample extract employed, and divide by 1000. Report results as micrograms Cry2Aa 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.

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.
- Cry2A 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.

If the OD of a sample is lower than that of the Low Calibrator (1.0 ppb Cry2Aa), the sample must be reported as less than: $(1.0 \text{ ppb} \times \text{dilution factor during extraction} \times \text{dilution of sample extract employed}) \div 1000 = x \text{ ppm Cry2Aa}$.

If the OD of a sample is higher than that of the High Calibrator (10 ppb Cry2Aa), the sample must be reported as greater than: $(10 \text{ ppb} \times \text{dilution factor during extraction} \times \text{dilution of sample extract employed}) \div 1000 = x \text{ ppm Cry2Aa}$.

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.

| Kit Cry2Aa Calibrators | Equivalent Cry2Ab Calibrators |
|------------------------|-------------------------------|
| Negative Control (NC) | Negative Control (NC) |
| 1.0 ppb Cry2Aa (C1) | 2 ppb Cry2Ab (C1) |
| 5.0 ppb Cry2Aa (C2) | 10 ppb Cry2Ab (C2) |
| 10 ppb Cry2Aa (C3) | 20 ppb Cry2Ab (C3) |

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

| | | | | | | | | | | | | |
|---|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | C0 | C0 | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| B | C1 | C1 | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |
| C | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 | S41 | S41 |
| D | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 | S42 | S42 |
| E | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 | S43 | S43 |
| F | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 | S44 | S44 |
| G | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 | S45 | S45 |
| H | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 | S46 | S46 |

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

| | | | | | | | | | | | | |
|---|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | C0 | C0 | 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 | Cry2A Conc. (ppb) |
|--------------------|----------------|-----------------|------|-------------------|
| Negative Control | 0.053 - 0.053 | 0.053 ± 0.0 | 0 | NA |
| 1.0 ppb Calibrator | 0.166* - 0.172 | 0.169 ± 0.004 | 2.5 | NA |
| 5.0 ppb Calibrator | 0.847* - 0.840 | 0.844 ± 0.005 | 0.6 | NA |
| 10 ppb Calibrator | 1.510* - 1.520 | 1.515 ± 0.007 | 0.5 | NA |
| Sample | 0.523* - 0.528 | 0.526 ± 0.003 | 0.7 | 3.2 ppb** |

* Figures are after subtraction of Negative Control values.

**Concentration from curve = 3.2 ppb Cry2A, multiplied by 1:11 dilution of sample extract = 35.2 ppb, multiplied by 1:25 dilution during extraction, and divided by 1000 = 0.880 ppm Cry2A in cotton leaf.

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



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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: 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: CO₂, 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

| | |
|---|--|
| 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): | 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 | | |

| | |
|--|--|
| 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. |
|--------------------------|---|

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

| | |
|--|---|
| 15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture | |
| US Federal Regulations | |
| 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. |
| US State Regulations | |
| | 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. |
| European/International Regulations | |
| 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. |
| Canada – DSL/NDSL | |
| Canada – WHMIS | CAS# 7647-01-0: 1 |
| Canadian Ingredient Disclosure List | WHMIS 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.

| | | | |
|---------------|----------------------------|-----------------|---|
| Codes: | | | |
| 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. |