ENVIROLOGIX™

QualiTube™ Kit For Microcystin

Catalog Number ET 022

Intended Use

The EnviroLogix QualiTube Kit for Microcystin is designed for semi-quantitative field screening of Microcystin toxin in surface water samples. The kit is supplied with calibrators at 0.5 and 3 ppb. The assay range can easily be extended.

How the Test Works

The QualiTube Kit for Microcystin is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In the test, Microcystin toxin in the sample competes with enzyme (horseradish peroxidase)-labeled Microcystin for a limited number of antibody binding sites on the inside surface of the test tubes.

After a simple wash step, the outcome of the competition is visualized with a color development step. As with all competitive immunoassays, sample concentration is inversely proportional to color development.

Darker color = Lower concentration
Lighter color = Higher concentration

Limit of Detection

The Limit of Detection (LOD) of the EnviroLogix Microcystin Tube Kit is 0.3 ppb. The LOD was determined by interpolation at 81.5% B_0* from a standard curve. 81.5% B_0 was determined to be 2 standard deviations from the mean of a population of negative water samples.

*100% B_0 equals the maximum amount of Microcystin-enzyme conjugate that is bound by the antibody in the absence of any Microcystin in the sample (i.e. negative control). %Bo = (OD of Sample or Calibrator/OD of Negative Control) x 100.

Precision

Microcystin-fortified control solutions were repetitively analyzed within a single assay. The data is expressed as %CV for absorbance (OD) and %B_0.

False Positive/False Negative Rate

Six surface water samples were fortified with Microcystin to a concentration of one half and twice the 0.5 ppb low calibrator. All six samples (6/6) fortified with 0.25 ppb resulted in absorbances greater than the 0.5 ppb microcystin calibrator, for a 0% false positive rate. All six samples (6/6) fortified with 1.0 ppb produced absorbances between 0.5 and 3.0 ppb in microcystin concentration, for a 0% false negative rate.

Cross-Reactivity

The QualiTube Kit for Microcystin does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The table (left) shows the value for 50% B_0 and the value for the 81.5% B_0 limit of detection for four microcystin toxin variants and nodularin toxin. Concentration is in ppb.

Highlights:

- *Semi-quantitative field screening of Microcystin toxin in surface water*
- *Detects from 0.5 to 3 ppb*

Contents of Kit:

- *36 antibody-coated test tubes*
- *1 vial of 0.5 ppb Microcystin LR Calibrator*
- *1 vial of 3 ppb Microcystin LR Calibrator*
- *1 dropper bottle of Assay Diluent*
- *1 dropper bottle of Microcystin-enzyme Conjugate*
- *1 dropper bottle of Substrate*
- *1 bottle of Stop Solution*
- *36 sample pipettes*

Optional Accessory Item:

- *ACC 062 – 1 vial of 1.5 ppb Microcystin LR Calibrator*

Precision

Intra-Assay Precision (n=7)	
	%CV (OD)
Negative Control	8.4%
1.0 ppb Control	8.1%
Inter-Assay Precision (n=8)	
	%CV (B/B_0)
1.0 ppb Control	9.6%

Cross-Reactivity

Compound	**50% B_0**	**81.5% B_0 LOD**
Microcystin LR	0.94	0.30
Microcystin LA	0.78	0.43
Microcystin RR	1.53	0.65
Microcystin YR	2.53	0.69
Nodularin	1.44	0.53

Humic acid did not interfere in the assay up to a concentration of 100 ppm.

Materials Not Provided

- marking pen (indelible)
- timer (5, 20 and 10 minutes)
- cool tap or distilled water for rinsing tubes, in a wash bottle
- photometer for reading tubes (optional)
- test tube rack that can hold at least 6 tubes securely enough to flick out water after wash step (Contact EnviroLogix for information on obtaining an appropriate rack)
- disposable tip, adjustable air-displacement pipette which will measure 0.7 mL (optional)

How to Run the Assay

- Read all of the instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed tubes and reagents at room temperature - do not remove tubes from bag with desiccant until they have warmed up).
- Organize all samples and reagents so that steps 1 and 2 can be performed in 3 minutes or less.
- Do not run more than 6 tubes at a time.

1. Rapidly add **5 drops of Microcystin Assay Diluent** to each tube in the assay.

2. Using the sample pipette provided, immediately add two drops of **0.5 ppb Microcystin Calibrator** to the first tube. Add two drops of **3.0 ppb Microcystin Calibrator** to the second tube. Add **two drops** of sample to each of the subsequent tubes, up to a total of 4 samples. **Do not add Microcystin-enzyme Conjugate in this step**.

3. Thoroughly mix the contents of the tubes by moving the tube holder in a rapid circular motion on flat surface for a full 20-30 seconds.

4. Incubate tubes at ambient temperature for 5 minutes.

5. Add **5 drops** of **Microcystin-enzyme Conjugate** to each tube. Do not empty the tube contents or wash the tubes at this time. Thoroughly mix the contents of the tubes as in step 3.

6. Incubate tubes at ambient temperature for 20 minutes.

7. After incubation, vigorously shake the contents of the tubes into a sink or other suitable container. Flood the tubes completely with cool tap water, then shake to empty. Repeat this wash step three times. Invert the tubes on a paper towel and tap to remove as much water as possible.

8. Add **10 drops** of **Substrate** to each tube. Thoroughly mix the contents of the tubes, as in step 3. Incubate substrate in tubes for 10 minutes at ambient temperature.

NOTE: If blue color does not develop in the 0.5 ppb Calibrator tube, the assay is invalid and should be repeated.

TABLE 1
The following table illustrates results interpretation of water samples read visually:

Samples with blue color …	Contain …
Darker than the blue color of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystins
Between the blue color of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystins
Lighter than the blue color of 3.0 ppb Calibrator	More than 3 ppb Microcystins

TABLE 2
The following table illustrates results interpretation of water samples using a tube photometer:

Samples with OD values …	Contain …
Greater than OD of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystins
Between OD of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystins
Less than OD of 3.0 ppb Calibrator	More than 3 ppb Microcystins

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

9. This assay is designed to be read visually with un-stopped tubes (blue solution). If tubes are to be read using a tube photometer, pipette 0.7 mL of Stop Solution into each tube and mix thoroughly. This will turn the tube contents yellow.

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

10. **Interpret the results of un-stopped tubes immediately following the 10 minute substrate incubation.**

How to Interpret the Results

Reading Tubes Visually

1. Compare the intensity of the blue color of each sample tube to the intensity of the blue color in the 0.5 and 3.0 ppb calibrator tubes.

2. Score each sample tube as having less than, more than or equal color to the two calibrator tubes.

3. Use Table 1 (left) to determine the level of microcystin in the samples.

Spectrophotometric Measurement

1. Set the wavelength of your photometer to 450 nanometers (nm). (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)

2. If the photometer does not auto-zero on air, zero the instrument against 1 mL water in a blank tube. Measure and record the optical density (OD) of each tube's contents. Alternatively, measure and record the OD in every tube, then subtract the OD of the water blank from each of the readings.

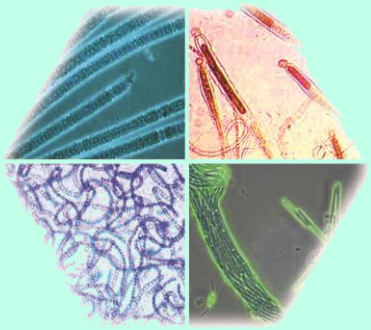
3. Use Table 2 (left) to determine the level of Microcystin in the sample.

4. For information on a field portable differential photometer contact EnviroLogix Technical Support. Contact information is at the end of these instructions.

Figure 1. Illustrative results interpretation using tube photometer

Well Contents	OD	Microcystin Concentration (ppb)
0.5 ppb Calibrator	0.984	NA
3.0 ppb Calibrator	0.306	NA
Sample	1.332	< 0.5 ppb
Sample	0.604	> 0.5 ppb, < 3.0 ppb

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



Precautions and Notes

- **While dropping solutions into tubes from dropper bottles, hold the top of each tube between your thumb and index finger. This will prevent the drops from adhering to the sides of the tube, allowing the drops to fall to the bottom of the tube.**
- **Hold pipette bulbs and dropper bottles vertically over the tube opening while dropping.**
- Store all Tube Kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose Tube 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 tubes from one Tube Kit with reagents or test tubes from a different Tube Kit.
- Do not expose **Substrate** to **sunlight** during pipetting or while incubating in the test tubes.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- As with all tests, it is recommended that results be confirmed by an alternate method if necessary.
- Microcystin LR in aqueous solution will stick to plastics such as polypropylene. Collect and process samples in glass containers.
- 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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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: 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: 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	LC50=826 mg/L	96h	Leuciscus 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 MARPOL73/78 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	CAS# 7647-01-0: 1
Canada – WHMIS	WHMIS classification of E, D2A.
Canadian Ingredient Disclosure List	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:

H290	May be Corrosive to Metals	P281	Use Personnel 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	P305+ P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do so Continue rinsing.