ENVIRCLOGIX

QualiTube[™] Kit For Microcystin

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 Conjugate1 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 Precis	sion (n=7)
	%CV (OD)
Negative Control	8.4%
1.0 ppb Control	8.1%
Inter-Assay Precis	sion (n=8)
	%CV (B/B ₀)
1.0 ppb Control	9.6%

Cross-Reactivity

Compound	50% Bo	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

Catalog Number ET 022

Intended Use

The EnviroLogix QualiTube Kit for Microcystin is designed for semiquantitative 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% B₀ 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 %B₀.

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.

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.
- 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	Less than
color of 0.5 ppb	0.5 ppb
Calibrator	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	More than
color of 3.0 ppb	3 ppb
Calibrator	Microcystins

TABLE 2 The following table illustrates results interpretation of water

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.)
- 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.

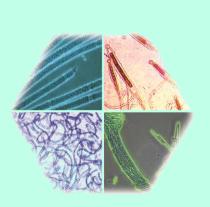
Figure 1. Illustrative results interpretation using tube photometer

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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	nce/mixture and of	the company/undertakin	a]			ion/informati	on on ingre	edients		
1.1 Product identifier Trade name:	Stop Sol			3.2	Mixture Aqueous solution	IN Hydrochlori	e Acid (INI	HCl, 3 % HCl)		
Synonyms: Part number	1.0 N HC		GD007)		Chemical name	Amount (%)	CAS No	Classification A	ccording to OSHA 2	9CFR 1910.1200
1.2 Relevant identified uses of the substance or mixture and uses advised against application	on				Hydrochloric acid		EC No	Hazard Classificatio	0	Hazard Code
of the substance / the preparation : 1.3 Details of the supplier of the safety data she	Laborator				Tryatoeneoire acra		647-01-0	May be Corrosive to M		H290
Manufacturer/Supplier:	Portland M	gix Inc., 500 Riverside Industr dE 04103, USA	ial Pkwy.			3	231-595-7	Causes Skin Irritatio		H315
1920 ISS 0 0		07) 797-0300						Causes Serious Eye Dar	2014	H318
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 19	Hazard (910.1200 Metal Ce	prosive (Cat. 1) H290								
	Skin Irrit Serious I	ation (Cat 2) H315 Eye damage (Cat. 1) H318		500	CTION 4. First aid 1	neasures				
2.2 Label elements Labeling according to OSHA 29CFR 1910.12	200			4.11	Description of first aid After inhalation :	measures		In case of inhalation. Remo	we to fresh air. If not	breathing give artificial
					After skin contact :			respiration. Get medical atto In case of skin contact. Rem	ention immediately. 10ve contaminated clc	thing and shoes immediately.
Hazard pictograms :	4.6	>						enidence of chemical remain	ns	or at least 10 minutes or until 1
Signal word :	\sim			3	After eye contact :			minutes. Lifting eyelids occ	casionally, until no ev	th plenty of water for at least i idence of chemical remains.
Signal word : Hazard statements:	Warning				After swallowing :			medical attention immediate In case of ingestion. DO NO	OT Induce vomiting u	nless directed to do so by
Hazard statements.	H315 Ca	ay be corrosive to metals auses skin irritation		6	the swallowing .			medical personnel. Never g a physician immediately.	give anything by mou	th to an unconscious person. (
The second se		uses serious eye damage		4.2 5	Most important sympt And delayed:	oms and effect	s, both acute	e May cause skin irritation an	d ana damana	
Precautionary statements:	P281 P302 + P	352 IF ON SKIN: Wa	ective equipment as required sh with plenty of soap and water.	100000	ndication of any imm	ediate medical	attention an			
	P305+ P.	351+P338 IF IN EYES: Rin minutes. Remove Continue rinsing.	se cautiously with water for several contact lenses if present and easy to do.		special treatment need			DO NOT use sodium bicarb	sonate in an attempt to	neutralize the acid.
2.3 Other Statements	None	- modig.	5 	15120	TION 5. Firefighti	ng measures				
and counter the					Extinguishing media:			CO2, extinguishing powder or wa	ater spray. Fight large	r fires with water spray or alco
				5.2 5	Special hazards arisin mixture:	g from the sub	stance or	resistant toam. Hydrogen Chloride gas		
					mixture: Advice for firefighters			Hydrogen Chloride gas Wear protective gear appropriate	for fire conditions	chaling respirators
				0.01	Auvice for intelliginers	50.		gear.	ior me conductions m	chung respiratory protocuve
					DS: Stop Solution (M	(GD007)		N 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		Page 2
SDR : Stop Solution (XGD007)		Revision : 13 April, 2015			CTION 9. Physical	and chemical	properties		3 April, 2015	rage 4
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2.2 Persistence and degradability :	No Data Available		SECTION 16. Other information		
2.3 Bio accumulative potential:	No Data Available		This information is true based on our present know		
2.4 Mobility in soil :	No Data Available		Persons receiving this information must exercise if use. This document shall not constitute a guarantee		
12.5 Results of PBT and vPvB assessment:	Not available as a chemical safety assessment, not required/not cond	iducted.	relationship EHS Department		
12.6 Other adverse effects:	No Data Available		EnviroLogix Inc.		
			Codes: H290 May be Corrosive to Metals		Use Personnel Protective equipment as Requ
ECTION 13. Disposal considerations			H315 Causes Skin Irritation H318 Causes Serious Eye Damage		IF ON SKIN: Wash with plenty of soap and wate IF IN EYES: Rinse cautiously with water for se
Waste treatment methods:	Contact a licensed professional waste disposal service to dispose of Disposal of surplus or waste solutions must be in accordance with ap and national laws and regulations.			STORED (*** ACLESCONSCON)	minutes. Remove contact lenses if present and or Continue rinsing.
ECTION 14. Transport information 14.1 UN-Number DOT, ADR, ADN, IMDG, IA	ATA : UN1789				
14.2 UN proper shipping name DOT, ADR, AI	DN, IMDG, IATA : HYDROCHLORIC ACID SOLUTION				
14.3 Transport hazard class(es) DOT, ADR, A 14.4 Packing group (DOT, ADR, IMDG, IATA	ADN, IMDG, IATA): 8 A): III				
14.5 Environmental hazards	Not hazardous to the environment.				
14.6 Special precautions for user :	None				
14.6 Special precautions for user : 14.7 Transport in bulk according to Annex II	None of MARPOL73/78				
14.6 Special precutions for user : 14.7 Transport in balk according to Annex II and the 18C code: ECTION 15. Regulatory information 15.5 More, balk and environmental regulations/legidation specific for the substance or mixture US Foderal Regulations TESA Chemical Test Bala CERCLA	None of MARPOL7378 No information available. CASH 76/1-78/ 6/8 not lated on the TSCA inventory. None initial None under a Chemical Tot Rule. CASH 76/1-6/1-6/0 SOB the Init RQ, 2270 Kg final RQ.	ler the CWA.			
14.6 Special precultions for user : 14.7 Transport in balk according to Annex II and the IBC code: 15.7 State, health and environmental regulations/legislation specific for the subdance or mixture 18. Forder, Regulations The Alth and State Regulations Chemical Test Kule Chemical State Regulations Vis State Regulations	None Inf MARPOL7297 No information available: CASE 76(2,9)-0 is not listed on the TSCA inventory. None issued CASE 76(2,9)-0 is not listed on the TSCA inventory. None issued CASE 76(2,9)-0 is not listed on the TSCA inventory. CASE 76(2,9)-0 is instel as a bacrobox strephilter AU CASE 76(2,9)-0 is instel as a bacrobox strephilter AU CASE 76(2,9)-0 is instel as a bacrobox strephilter AU	er the CWA. HA. ght to know lists: CA,			
14.6 Special precultions for user : 14.7 Ergmport In bulk according to Amer II and the IBC code: 14.7 Transport In bulk according to Amer II 15.8 Safety, bulk and environmental regulations/legislation acciliance acciliance 15.8 Safety Real and environmental 15.8 Safety Real and environmental 15.8 Safety Real and environmental 15.8 Safety Reapenting List Clearing To Kala Safety Reporting List Clearing To Kala Safety Reporting List Clearing To Kala Safety Reporting List Clearing To Kala Clearing To Kala Clearing Act Clearing Act Clearing Act Clearing Clearing Clearing Clearing Safety Real Safety Safety Real Safety Safety Real Safety Safety Real Safety Safety Real Safety Safety Real Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Safety Sa	None In MARPOLI297 No information available: CASE 7647-01-0 is not listed on the TSCA inventory. None issued. CASE 7647-01-0 5000 final 802, 2270 kg final 802. CASE 7647-01-0 5000 final 802, 2270 kg final 802. CASE 7647-01-01 5000 final 802, 2270 kg final 802. CASE 7647-01-01 is instead as a banchoos size pollutar(0). CASE 7647-01-01 is instead as a banchoos size pollutar(0). CASE 7647-01-01 is considered highly banchos by OSB CASE 7647-01-01, and final on on the following data; mill CASE 7647-01-01, and final on on the following data; mill CASE 7647-01-01, and final on on the following data; mill CASE 7647-01-01, and final on on the following data; mill CASE 7647-01-01, and final one of the following data; mill CASE 7647-01-01, and final one of the following data; mill CASE 7647-01-01, and final one of the following data; mill CASE 7647-01, and final one of the following data; mill CASE 7647-01, and final one of the following data; mill CASE 7647-01, and final one of the following data; mill CASE 7647-01, and final one of the following data; million data;	for the CWA. HA. Applit to know lists: CA, micals in this product as the substance or its does not require a			
14.6 Special precultions for user : 14.7 Erganport In bulk according to Amer II and the IBC code: 15.7 Interpret in bulk according to Amer II and the IBC code: 15.8 Article, bulk and environmental regulations/ligibation acciliance acci	None a MARPOLYDY CASH 76(1-6)1-0 is not histed on the TSCA inventory. None listed None under a Chernical Tset Rade. CASH 76(1-6)1-0 is instellated as heardness listed and the CASH 76(1-6)1-0 is instellated as heardness listed as locations (SIGN CASH 76(1-6)1-0 is instellated as heardness listed as locations (SIGN CASH 76(1-6)1-0 is instellated as heardness listed as locations (SIGN CASH 76(1-6)1-0 is instellated light) handhood listed is completed light) for address (SIGN CASH 76(1-6)1-0 is instellated light) handhood listed is listed as heardness listed listed CASH 76(1-6)1-0 is instellated light) handhood listed light) for address (SIGN CASH 76(1-6)1-0 is completed light) handhood listed listed. As 76(1-6)1-0 is completed light) handhood listed listed listed. As 76(1-6)1-0 is completed light) handhood listed	or the CWA. HA. glit to know lists: CA, micals in this product as the substance or its loss not require a situation deadline.			