ENVIROLOGIX

QuantiPlate[™] Kit for Microcystins High Sensitivity

Highlights:

- Quantitative laboratory detection of Microcystin toxin in potable water
- Detects from 0.1 to 1.2 ppb

Contents of Kit:

- 12 strips of 8 antibody-coated wells each, in plate frame
- 1 vial of Negative Control
- 1 vial of 0.1 ppb Microcystin LR Calibrator
- 1 vial of 0.3 ppb Microcystin LR Calibrator
- 1 vial of 0.6 ppb Microcystin LR Calibrator
- 1 vial of 1.2 ppb Microcystin LR Calibrator
- 1 bottle of Assay Diluent
- 1 bottle of Microcystin-enzyme Conjugate
- 1 packet of Wash Solution salts
- 1 bottle of Substrate
- 1 bottle of Stop Solution

Precision

	Recovery (%CV)	OD (%CV)
	Intra-Assay	n=11
0.2 ppb	4.6%	2.3%
0.6 ppb	3.0%	3.7%
	Inter-Assay	n=11
0.2 ppb	6.4%	4.3%
0.6 ppb	1.9%	8.9%

Cross-Reactivity

Compound	50% B ₀	80% B ₀
Microcystin LR	0.28	0.11
Microcystin LA	1.4	0.36
Microcystin RR	0.30	0.10
Microcystin YR	0.43	0.17
Nodularin	0.19	0.07

Catalog Number EP 022 HS

Intended Use

The EnviroLogix QuantiPlate Kit for Microcystins High Sensitivity is designed for the quantitative laboratory detection of Microcystin toxin in potable water samples, with an assay quantitation range from 0.1 to 1.2 parts per billion (ppb)

How the Test Works

This QuantiPlate Kit for Microcystins 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 wells.

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 this Kit is 0.071 ppb. The LOD was determined by interpolation at 87.8% B_0^* from a standard curve. 87.8% B_0 was determined to be 3 standard deviations from the mean of a population of negative potable 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). % $B_0 = (OD \text{ of Sample or Calibrator/OD of Negative Control}) x 100.$

Limit of Quantification

The Limit of Quantification (LOQ) of this Kit was validated at 0.15 ppb. The LOQ was determined by fortifying a population of negative potable water samples at 0.15 ppb. The mean recovery was 98.0% with a coefficient of variation (CV) [(standard deviation/mean) x 100] of 8.5%.

Precision

Microcystin-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).

Cross-Reactivity

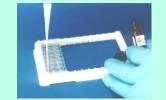
This Kit does not distinguish between the Microcystin toxin variants, but detects their presence to differing degrees. The accompanying table shows the value for 50% B_0 and the value for the 80% B_0 for four Microcystin toxin variants and nodularin toxin. Concentration is in ppb. Humic acid did not interfere in the assay up to a concentration of 100 ppm.



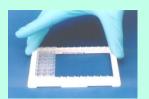
Remove unneeded strips



Select Calibrators and Control



Add controls/calibrators/sample



Mix plate



Incubate



Bottle Wash method

Materials Needed

- disposable tip adjustable air-displacement pipette which will measure 50 μ L and 100 μ L
- marking pen (indelible)
- tape or Parafilm®
- timer (30 minutes)
- distilled water for preparing Wash Solution
- glassware for storing Wash Solution
- wash bottle for washing strips with Wash Solution
- microtiter plate reader or strip reader
- microtiter plate washer (optional)
- twelve or 8-channel pipette that will measure 50 μ L and 100 μ L (optional)
- racked (glass) dilution tubes for loading samples into the plate with a 12channel pipette (optional)
- orbital plate shaker (optional)

Preparation of Solutions

Wash Buffer:

To make 1 L, add the contents of one packet of phosphate-buffered saline - Tween 20, pH 7.4 (**Wash Solution salts**) to 1 L of distilled water. Mix thoroughly to dissolve the salts. This can be stored at room temperature.

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 samples, reagents and pipettes so that steps 1 and 2 can be performed in 10 minutes or less.
- If more than three strips are to be run at one time, the 10 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
- If three 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 to the wells. Assay Diluent, 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 plastic bag provided.
- Use the well identification markings on the plate frame to guide you when adding the samples and reagents. Two strips may be used to run the Negative Control (NC), three Calibrators (C1-C3) and four samples, in duplicate. More samples require more strips. For an example plate layout see Figure 1.
- 1. Rapidly add 50 μ L of Microcystin Assay Diluent to each well that will be used, preferably with a repeating or multi-channel pipetter.
- Immediately add 50 μL of Negative Control (NC), 50 μL of each Calibrator (C1-C3) and 50 μL of each sample (S1-S8) to their respective wells, as shown at left. (Follow this same order of addition for all reagents.) Do not add Microcystin-enzyme Conjugate in this step.

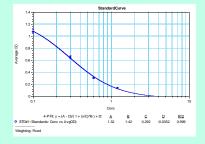
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Read plates in a Plate Reader within 30 minutes of the addition of Stop Solution

Illustrative standard curve



3. 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!

NOTE: In order to minimize setup time it is recommended that a multi-channel pipette be used in steps 1, 2, 5, 8 and 10 when more than 3 strips are used.

- 4. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 30 minutes. If an orbital shaker is available shake at 200 rpm.
- 5. Add **50 µL** of **Microcystin-enzyme Conjugate** to each well. Do not empty the well contents or wash the strips at this time.
- 6. Thoroughly mix the contents of the wells as in step 3. Cover the wells with tape or Parafilm and incubate at ambient temperature for 30 minutes. Use orbital shaker if available.
- 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 Solution**, then shake to empty. Repeat this wash step four times. Slap the plate on a paper towel to remove as much Wash Solution as possible. Alternatively, use a microtiter plate washer with **Wash Solution** for the wash step.
- 8. Add 100 μL of Substrate to each well.
- 9. Thoroughly mix the contents of the wells, as in step 3. Cover the wells with <u>new</u> tape or Parafilm and incubate for 30 minutes at ambient temperature. Use orbital shaker if available.

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

10. Add **100** μ L 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. If the plate reader does not auto-zero on air, zero the instrument against $200 \,\mu\text{L}$ water in a blank well. Measure and record the optical density (OD) of each well's contents. Alternatively, measure and record the OD in every well, then subtract the OD of the water blank from each of the readings.
- 3. A 4 parameter curve fit should be used to create the standard curve, use the software provided with your plate reader to interpolate sample concentrations off this 4 parameter curve. A semi-Log curve fit is not recommended.

If the OD of a sample is <u>lower</u> than that of the <u>highest</u> Calibrator, the sample must be reported as greater than 1.2ppb. If a concentration must be determined for these high level samples, dilute the sample 1:8 in distilled water. Run this dilution in a repeat of the immunoassay. If the result now falls within the range of the ODs of the Calibrators, you must then multiply the concentration measured in the diluted sample by a factor of 8.

Precautions and Notes

- Store all components at 4°-8°C (39°-46°F) when not in use.
- Do not expose 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 QuantiPlate Kit with reagents or test well strips from a different QuantiPlate Kit.
- Do not expose Substrate to sunlight during pipetting or while incubating in the test wells.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- Observe any applicable regulations when disposing of samples and kit reagents.
- Microcystin LR in aqueous solution will stick to plastics such as polypropylene. Collect and process samples in glass containers. Clear samples free of organic material can be stored refrigerated for up to two weeks before analysis.

Figure 1a. Example of a typical plate setup. (1 x 8 strips)

	1	2	3	4	5	6	7	8	9	10	11	12
А	NC	NC										
В	C1	C1										
С	C2	C2										
D	C3	C3										
Е	S 1	S 1										
F	S 2	S 2										
G	S 3	S 3										
Н	S4	S4										

Figure 2a. Illustrative quantitative calculations

Well contents	OD	Average OD	%CV	%B0	Microcystin Concentration (ppb)
Negative	1.358	1.050	0	100	
Control	1.359	1.358	0	100	NA
0.1ppb	1.115				
Calibrator	1.106	1.11	0.60	81.7	NA
0.3ppb	0.672				
Calibrator	0.696	0.684	2.5	50.4	NA
0.6 ppb	0.327				
Calibrator	0.324	0.325	0.7	23.9	NA
1.2 ppb	0.151				
Calibrator	0.159	0.155	3.6	11.4	NA
	0.746				
Sample	0.772	0.759	2.4	55.9	0.248

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



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SECTION 1. Identification of the substar 1.1 Product identifier Trade name: Part number:	According to OSILA 29CFR 1910 1200 reconstructure and 64 the company (and prinking Weaks Patter salut 50.002), 1009	5.1 Extinguishing media: Stituble extinguishing agents: 5.2 Special lazards arising from the substance o mixture: 5.2 Advice for firefighters:	Carbon exides, Oxides of Phosphoreus, Potassium, Sodium, Hydrogen Chloride g Wear protective equipment appropriate for fire conditions including respiratory
1.2 Relevant identified uses of the substance or m and uses advised against application of the su	éxture éstance		protective gear
/ the preparation : 1.3 Details of the supplier of the safety data sho	Lalovatory chemicals eet EnviroLogix Inc., 500 Riverside Industrial Plowy.	SECTION 6. Accidental release measures	
Manufacturer/Supplier:	Portland ME 04103, USA (207) 797-0300	6.1 Personal precautions, protective equipment and emergency procedures:	Use PPE, avoid dust formation, ensure adoptate ventilation, avoid breathing dust
1.4 Emergency telephone number:	(207) 797-0300 Technical Service	6.2 Environmental precautions:	Prevent further leakage or spillage if safe to do so. Do not let product enter drains.
SECTION 2. Hezards identification 2.1 Classification of the Substance or Mixture:		6.3 Methods and material for containment and clean up:	Discharge to the environment must be avoided. Pick up and arrange clisposal without oreating dust. Sweep up and shovel. Keep in
Classification according to OSHA 29CFR 19 (Hazard Communication):	10.1200 Not a hazardous substance or mixture		suitable closed containers for disposal
.2 Label Elements:	None required according to 29CFR 1910.1200	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.
Other indications	Nime	SECTION 7. Handling and storage	
		7.1 Precautions for safe handling: 7.2 Conditions for safe storage, including any	Practice good chemical hygiene when handling. Avoid contact with eyes, skin and elothing. Prevent formation of dust.
3 Additional Information:	No other information	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.
CTION 3. Composition/information on 2 Mixture: Powdered soli		SECTION 8. Exposure controls/personal pi	rolection
Synonyms PBS		8.1 Control parameters: Components with workplace control	
Hazardeus Components Chemica	(%)	Parameters	Contains no substances with occupational exposure limit values
Potassiu	m Chloride 7447-40-7 231-211-8 1-5 % Aquatic Acute 3; Aquatic Chronic 3; H412	8.2 Exposure controls 8.2.1 Appropriate angineering controls:	Ensure eyewash and safety shower are nearby; provide ventilation if necessary
ased on the amount of hazardous ingredients in	this product, it is not considered hazardous according to 29CFR 1910.1200	8.2.2 Personal Protective Equipment:	
KOTION (Research		Eyes	Safety glasses with side shields, gogglas. Use equipment for eye protection tested and approved under appropriate government standards such as MONH (US) er MN 166 (Eye and face protection regulations are described by OSITA (US) n29CTR[10].133
DEGITION 4. First still measures 1 Description of first aid measures:			not wear contact lenses when working with chemicals
After inhalation After skin contact	Supply fresh air; consult doctor in case of breathing difficulties. Flush skin with planty of water for at least 15 minutes. Remove contaminated	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
After eye contact	elefting, Seek medical attention if irritation develops. Rinse opened eye for several mimites under running water. Seek medical attention if irritation develops.		 product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. The selected protective gloves has
After swallowing	If swallowed, consult with medical staff or person control center to determine if any inumediate response or follow up actions are recommended. Never give anything by		to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.
2 Most important symptoms and effects, both	mouth to an unconscious person.	Respiratory protection	Appropriate respiratory protection should be determined according to local condition using risk analysis protocols. An approved disposable air purifying particulare respira
acute and delayed:	Nime		may be used as a backup to engineering controls. Always use respirators and compon tested and approved under appropriate government standards such as NIOSH (US) or
3 Indication of any immediate medical attent and special freatment needed:	No special treatment is required		CEN (EU).
		Body 8.2.3 Environmental controls:	Lise body protection relative to its type and amount of material being handled Sweep or wipe up spills, do not allow into sewers or drains
DPTION 6 - Block and a book a second			
SCTION 9. Physical and chemical prop 9.1 Information on basic physical and chemical properties;	ettie	SECTION 13. Disposal considerations Dispose of eases or unset product in accordance service to discove of timesterial.	with Local, State and Federal regulations. Contect a licensed professional water disposal
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	SECTION 1. Identification of the substan	ce/mixture and of	the company/undertaking			CTION 3. Composit	ion/Inform:	ation on ingr	edients	
	.1 Product identifier		2007		3.2	Mixture Aqueous solution	IN Hydrochi	wie Aeid (IN)	HC1, 3 % HC1)	
	Synonyms: Part nomber	1.0 N HC	C1	20070		Chemical name		CAS No	Classification According to O	SHA 29CFR 1910.1200
	1.2 Relevant identified uses of the substance or mixture and uses advised against application	m				Hydrachlaric seid		EC No	Howard Closel Reation	Harrard Code
	L3 Details of the supplier of the safety data shee	et	• • • • • • • • • • • • • • • • • • • •	10000000		injuction in the lost	100.00	7647-01-0		
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		00			4.1 E A	Description of first ald After inhalation :	l measures		In case of inhalation. Remove to fresh air.	If not breathing give artificial
	Mazard rictoments :				А	After skin contact :			In case of skin contact. Remove contamina	ted clothing and shoes immediately.
	Papara pictograms .	A 8	>						evidence of chemical remains.	
	Sumul word -	×			A	After eye contact :			minutes. Lifting evelids occasionally, unti-	I no evidence of chemical remains
	States Control of States	Warning			A	After swallowing :			In case of ingestion. DO NOT Induce von	iting unless directed to do so by
	Theorem and an and the states.	H315 C	auses skin irritation						medical personnel. Never give anything b a physician immediately.	y mouth to an inconscious person. (
	Precautionary statements:			tive automation required	4.2 M A	fost important sympt And delayed:	oms and effe	ets, both acut	e May cause skin irritation and eye damage	
		P302 + P	2352 IF ON SKIN: Wash	with plenty of soap and water.	4.3 1	ndication of any imm	ediate medic	al attention an	d	
		. 363 ° F.	minutes. Remove or	ontact lenses if present and easy to do.	s	pecial treatment need	led:		IX) NOT use sodium bicarbonate in an atte	empt to neutralize the acid.
	2.2 Culue Statement	None			1977	TION 5. Einefighti	no measure	5		
	2.5 Other Statements						and a state state		CO2, extinguishing powder or water spray. Figh	t larger fires with water spray or ale
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QuantiPlate Kit for Microcystins-High Sensitivity Page 8 of 8

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 knowledge. However, EnviroLogis makes no representation of its accuracy or completeness.
evenestation and	No Data Available	Persons receiving this information must exercise their independent judgment in determining the product's safety and mitability for its intended use. This document shall not constitute a guarantee for any specific product features and shall not establish a legisly valid contractual
com c Necescretoren d	Not available as a chemical safety assessment, not required/not conducted.	relationship EHS Department
		Enviral agix lae.
2.6 Other adverse effects:	No Data Available	Codes: H290 May be Cerrosive to Metals P281 Use Personnel Protective equipment as Required
ECTION 13. Disposal considerations		H315 Causes Skin Irritation P302 + P352 IF ON SKIN: Wash with planty of scop and water H318 Causes Serious Eye Damage P305 - P351 + P338 IF IN EYES: Rinse cautiously with water for several
	Contact a licensed professional waste disposal service to dispose of this material.	minutes. Remove contact lenses if present and easy to do so
	Disposal of surplus or waste solutions must be in accordance with applicable loca and national lows and regulations.	al, state,
ECTION 14. Transport information 4.1 UN-Number DOT, ADR, ADN, IMDG, IATA ;	UNI789	
 UN-Number DOT, ADR, ADN, IMDG, IATA : UN proper shipping name DOT, ADR, ADN, I 		
 Transport hazard class(cs) DOT, ADR, ADN, i 4.4 Packing group (DOT, ADR, IMDG, IATA): 	MDG, IATA): 8 III	
4.5 Environmental hazards	Not hazardous to the environment.	
4.6 Special precautions for user :	None	
4.7 Transport in bulk according to Annex II of M and the IBC code:	ARPOL73/78 No information available,	
CETION 15. Regulatory information		
5.1 Safety, health and environmental regulations/legislation specific for the		
substance or mixture US Federal Regulations		
	CAS# 7647-01-0 is not listed on the TSCA inventory. None listed.	
TSCA Haalth and Sufaty Parasition List		
Health and Safety Reporting List Chemical Test Rule	None under a Chemical Test Rule.	
Health and Safety Reporting List Chemical Test Rule CHRCLA SARA Section 302 (Extremely Hazardous	None under a Chemical Test Rule. CAS# 7647-01-0: 5000 lb final RQ; 2270 Kg final RQ. CAS# 7647-01-0: 500 lb TPQ.	
Health and Safety Reporting List Chemical Test Rule CERCLA	CAS# 7647-01-0: 5000 lb final RO; 2270 Kg final RO.	
Health and Safety Reporting List Chemical Test Rule CHRCLA SARA Section 302 (Extremely Hazardous Sabstances)	CAS# 7647-01-0: 5000 lb final RQ; 2270 Kg final RQ; CAS# 7647-01-0: 500 lb TPQ;	
Health and Sofey Reporting List Chemical Test Rule CERCI.A SARA Section 302 (Extremely Hazardous Substance) Clean Air Act Clean Water Act	CASF 7647-04-05 0000 lb fmal RC; 2270 Kg fmal RQ; CASF 7647-01-05 000 lb TPQ. CASF 7647-01-06 500 lb TPQ. CASF 7647-01-06 is listed as a hazardous air pollorant (HAP). CASF 7647-01-06 is located as hazardous Substance under the (WAR). CASF 7647-01-06 is considered highly hazardous by OSILA. CASF 7647-01-06 cam be located on the following rate right to know in	
Hadhi and Safey Reporting List Chemical Tork Rule (JRRCU A SARA Sertion 302 (Extremely Hazardons Salostance) Chem Vater Act OSHA	(ASF 7647-01-05 0001h fmd RC); 2270 Kg fimil RQ, CASF 7647-01-06 5001h TPQ (CASF 7647-01-06 is listed as a hazardous in pollutant (HAP). CASF 7647-01-06 is listed as a hazardous Substance under the CWA. CASF 7647-01-06 is is considered highly hazardous by OSILA.	inter Coly
Health and Safety Reporting List Chenical Text Note CHENIA SARA Section 920 (Estremely Hazardons Substances) Clean Mr Aut Clean Water Act Clean Water Act CSHA CSHA	(3A87 7617-01-2: 5000 1b Fmil (R); 2270 Kg fmil R); CA87 7617-01-3: 500 1b Tm2 (CA87 7617-01-3: b) index as harmfors introduced (IAV); CA87 7617-01-3: index as harmfore Subtractions (IAV); CA87 7617-01-3: index as harmfore Subtractions by OSILA; CA87 7617-01-3: index as harmfore CA87 and the Subtraction by OSILA; CA87 7617-01-3: index and the following state sight to know in XI, PA, MN, MA; C: ProperS on Segnificiant Bial: Acear and more the subtraction in this fit.	sale CA, product constant of the second
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Hashi and Safay Jegoring List Chrnival Totle (DRU), A SARA Settion 322 (Estremely Ilazardors Chara Marka Chara Marka Chara Marka OSIA US State Regulations European/International Regulations	(3A87 F017-01-8: 3000 HF frait (K2; 2270 Kg fmil R4). CA87 F017-01-8: 5000 HF frait (K2; 2270 Kg fmil R4). CA87 F017-01-8: histoites a harmofros inty Tolhami (HAP). CA87 F017-01-8: inconsidered highly hazardam by OSILA. CA87 F017-01-8: genetic formed in the following state right to know in VL, PA NN, MA. CA Pop 657 no Staginizioni Rial. Lavoit more of the chemicals in this p are hold. A registration number is not stabilish for this software is not strong in registration muther is not stabilish for this software is not stopping registration for the registration is not signal (energy of the relative state stabilish).	inte CA, product or or its