Microcystin ELISA kit

Cat #: orb59527 (manual)

General description

The ELISASreening Kit is an immunochemical test for the quantitation of Microcystins (MCY) in algae or water samples. The test principle is based on a direct competitive enzyme-linked Immunosorbent assay (dcELISA). In the assay, MCY toxin in the sample competes with horseradish peroxidase-conjugated MCY for a limited amount of antibody which has been coated on the bottom of the test wells.

Materials and reagents supplied

1. One microtiter plate containing 96 test wells coated with polyclonal antibody specific to MCY
2. One vial of 【Negative control】, 1.5 mL
3. One vial each of 0.1, 0.5, 1.0, 2.0, and 5.0 ppb MCY-LR standard (calibrator), 1.5 mL
4. One vial of【HRP conjugate】, 6 mL
5. One vial of 【Substrate】, 12 mL
6. One vial of 【Stop solution】, 12 mL
7. One vial of 【10 x Wash buffer】, 30 mL

Additional materials not provided

1. 10 ml syringes
2. 0.45-micron, non-sterile filter units
3. Pipette and centrifuge tubes
4. Marking pen
5. Tape or Parafilm
6. Distilled water or tap
7. Microtiter plate reader or strips reader
8. Absorbent paper towels
9. Timer
10. Extraction buffer: 0.1 M ammonium bicarbonate
11. Calculator (Optional)
12. A multi-channel pipette (Optional)
13. Orbital shaker (Optional)
14. Solid phase extraction devices (Optional)
15. Microtiter plate washer (Optional)

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Sample preparation

Solid sample

1. Weigh out 2 g lyophilized algae sample and add 20 mL extraction buffer
2. Homogenize for 3 minutes with a tissue homogenizer
3. Centrifuge (3000 rpm for 3 minutes) to collect supernatant or pass through a filter to collect filtrate
4. Take one mL of clear supernatant solution (or filtrate) and add 9 mL (1:10 dilution) or 99 mL of distilled water (1:100 dilution)
5. Use this diluted solution in the ELISA

Water sample

1. Water samples should be filtered to remove particulate
2. If a lower sensitivity for microcystins is required (<0.01ppb), a solid-phase extraction device such as C18 reverse phase could concentrate the sample.

Assay Procedure

1. Format the microplate's wells for each standard or sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag and stored at 2-8°C.
2. Wash buffer preparation

   Dilute 10 x washing buffer with distilled water to make 1 x washing buffer by 1:9 ratio. Check if the 10X Wash buffer is at room temperature and the crystal at the bottom is dissolved thoroughly before use.
3. Add 50 µL of Negative control and standard solution (0.1, 0.5, 1.0, 2.0, and 5.0 ppb) or 50 µL of each sample into the assigned well.
4. Add 50 µL of MCY-HRP enzyme conjugate solution (HRP conjugate) to all wells. Swirl the plate gently to mix the content thoroughly. Now the volume of each well is 100 µL.
5. Incubate 30 minutes at room temperature (25-37°C) under dark.
6. Remove liquid from all wells.
7. Flood the wells with at least 300 –350 µL of 1 x washing buffer, and then decant the liquid from all wells.
8. Repeat the step 7 at least three times.
9. Invert and gently pat the plate on absorbent paper towels to remove remaining solution in wells .
10. Add 100 µL of substrate solution to each well and shake gently.
11. Incubate 30 minutes at room temperature (25-37°C) under dark. Blue color develops in the wells with Negative control.
12. Add 100 µL of stop solution to each well and mix gently (Now the volume of each well is 200 µL). Solution will turn from blue into yellow immediately.
13. Read color at OD 450 nm in an ELISA reader within 3-15 minutes after adding the stop solution.

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Example of typical plate setup.

	1	2	3	4	5	6	7	8	9	10	11	12
A			S1	S1	S9	S9						
B	NC	NC	S2	S2	S10	S10						
C	C1	C1	S3	S3								
D	C2	C2	S4	S4								
E	C3	C3	S5	S5								
F	C4	C4	S6	S6								
G	C5	C5	S7	S7								
H			S8	S8								

NC: Negative Control

C1= 0.1 ppb MCY-LR standard

C2= 0.5 ppb MCY-LR standard

C3= 1.0 ppb MCY-LR standard

C4= 2.0 ppb MCY-LR standard

C5= 5.0 ppb MCY-LR standard

S1, S2, S3, S4, S5 etc.=Samples

Calculating Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the $B/B_0\%$ as follows:

$$B/B_0\% = \frac{\text{average OD of Calibrator or sample}}{\text{average OD of Negative Control}} \times 100$$

The $\%B_0$ calculation is used to equalize different runs of assay. While the raw OD values of Negative Control, Calibrators, and samples may different from run to run, the $\%B_0$ relationship of calibrators and samples to Negative Control should remain fairly constant.

2. Graph the $B/B_0\%$ of each Calibrator against its Microcystin concentration on a semi-log scale
3. Determine the Microcystin concentration of each sample by finding its B/B_0 value and the corresponding concentration level on the graph.

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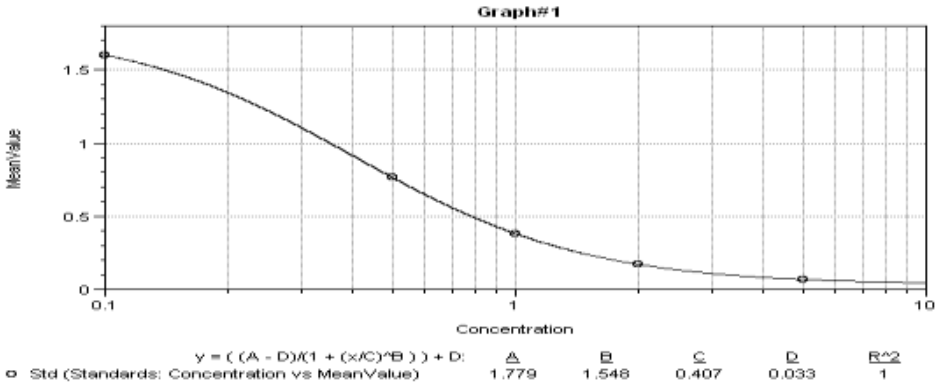
4. Interpolation of sample concentration is only valid if the B/B_0% of sample falls within the range of the B/B_0%'s set by calibrators.

Limit of detection

The detection limit for this assay based on MCY-LR is 0.01 ppb (ng/mL).

Performance data

1. MCY-LR standard curve



2. Cross -reactivity

variant	50 % B/B_0
MCY-LR	0.48
MCY-YR	0.67
MCY-RR	4.0
Nodularin	1.78

Precision

Well Contents	%CV(n=7)
Negative Control	4.3
0.1 ppb Calibrator	3.7
0.5 ppb Calibrator	4.8
1.0 ppb Calibrator	4.6
2.0 ppb Calibrator	4.9
5.0 ppb Calibrator	8.4

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