Microcystin-LR ELISA Kit

Catalog Number KA1496

96 assays

Version: 14

Intended for research use only

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Introduction

Intended Use

The Microcystin-LR ELISA Kit is intended for the detection of Microcystin - LR (MC-LR) in environmental samples - potable water and surface water samples.

Principle of the Assay

The Microcystin-LR ELISA Kit is based on the use of monoclonal antibody anti-microcystin that binds MC-LR or MC-enzyme conjugate (MC-LR-Px). MC present in the sample and assay calibrators are bound during the first incubation by the anti-MC-LR antibodies, which are immobilized to the wells. Second incubation is with MC-LR-Px. After a second incubation, the unbound MC-LR and MC-LR-Px is decanted and the wells are thoroughly washed. Finally, a clear solution of chromogenic substrate (TMB) is then added to the wells. In the presence of MC-LR-Px conjugate, the clear substrate is converted to a blue color. The reaction is stopped by adding of Stop solution and the blue substrate is converted to a yellow color. The high concentration of MC-LR will allow fewer MC-LR-Px conjugate molecules to be bound by the antibodies, resulting in a lighter yellow color. The low concentration of MC produces a dark yellow color.

Note: Color development is inversely proportional to MC concentration.

- ✓ Darker color = Lower Concentration
- ✓ Lighter color = Higher Concentration

The determination of the MC level in an unknown sample is interpreted relative to the assay calibrator levels using spectrophotometer.

General Information

Materials Supplied

List of component

Component	Amount
ELISA strips (colourless) coated with specific antibody.	1 microplate
Standard A, r.t.u. 0 µg/L	0.6 mL
Standard B, r.t.u. 0.1 µg/L	0.6 mL
Standard C, r.t.u. 0.5 µg/L	0.6 mL
Standard D, r.t.u. 1 µg/L	0.6 mL
Standard E, r.t.u. 2.5 µg/L	0.6 mL
MC-LR labeled with horseradish peroxidase, r.t.u (Px-conjugate)	2.5 mL
Wash buffer concentrate, 10x concentrated.	55 mL
Dilution buffer, r.t.u.	15 mL
Chromogenic substrate (TMB substrate), r.t.u.	13 mL
Stop solution, r.t.u.	13 mL

r.t.u: ready to use

Storage Instruction

- ✓ Store the kit reagents at +2 to +10°C, in a dry place and protected from the light. Avoid freezing. Expiration date is indicated at the ELISA kit label and at all reagent labels.
- ✓ Store unused strips in the sealable pouch and keep the desiccant inside. Transport in thermo bags until 72 hours. Any damages of packaging of kit reagents advise to the producer without delay.
- ✓ The ELISA kit should be used within three months after opening.

Materials Required but Not Supplied

- ✓ Distilled or deionised water for dilution of the Wash buffer concentrate.
- ✓ Appropriate equipment for pipetting, liquid dispensing and washing.
- ✓ Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

Precautions for Use

For research use only.

✓ Safety Precautions

- All ingredients of the kit are intended for laboratory use only.
- Standards and Px conjugate contain Microcystin, which is toxic, highly irritating.
- Safety accumulating bottle, used strips and used MC-LR standards and MC-LR-Px conjugate handle as with hazardous waste. They should be regarded as toxic and handled and disposed of according to the appropriate regulations.
- Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution. Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
- Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

✓ Handling Precautions

- Avoid contamination of samples and kit reagents.
- Avoid cross-contamination of reagents.
- Standards and conjugate contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3:1)).
- Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
- Follow the assay procedure indicated in the protocol.
- Variations in test results are usually due to:
    1. Insufficient mixing of reagents and samples.
    2. Inaccurate pipetting and inadequate incubation times.
    3. Poor washing technique or spilling the rim of well with sample.
    4. Use of identical pipette tip for different solutions.

Assay Protocol

Reagent Preparation

- ✓ Allow all kit components to reach room temperature and vortex the components in order to ensure homogeneity.
- ✓ Prepare Wash buffer by diluting the concentrate 10 times with an appropriate volume of distilled or deionised water (50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt presented in the concentrated Wash buffer, warm up the vial to 32 to 37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.
- ✓ Do not dilute standards, Px-conjugate, dilution buffer, TMB substrate and Stop solution, they are ready to use.
- ✓ Do not store diluted samples and diluted MC-LR-Px conjugate. Always prepare fresh.

Sample Preparation

Just before use thoroughly mix tested of the standards and samples (water samples). If needed, the samples may be diluted with distilled water 2, 5 or 10 times to increase the clarity. The grade of dilution is important to estimate the exact concentration of the sample.

Assay Procedure

Manufacturer will not be held responsible for results if protocol is not followed exactly.

1. Allow the antibody coated strips to reach room temperature before opening in order to prevent water condensation within the wells. Withdraw an adequate number of antibody coated strips. Put the remaining strips back in the aluminium pouches and seal them if possible, keep the desiccant inside.
2. Wash and aspirate the wells three times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
3. Pipette 40 µL of Dilution buffer to each well that will be used. Then add 40 µL Standards stock solution of MC (Standard A, Standard B, Standard..) or tested samples (Sample 1, Sample 2, Sample..). If you want to exclude a possible laboratory error apply the standards and samples in doublets. See Plate Layout.
4. Incubate for 45 (+/- 5) minutes at room temperature.
5. Add 20 µL of MC-LR-Px conjugate to each well. Do not empty the well nor wash the strips at this time.
6. Incubate for 15 (+/- 5 sec.) minutes at room temperature, 240 rpm.
7. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells five times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells!

   If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last

remaining drops.

8. Dispense 100 µL of the TMB substrate into each well.
   Note: Pipette in a regular rhythm or use an appropriate dispensing instrument.
9. Incubate for 20 minutes (+/-5 seconds) at room temperature.
   The time measurement must be started at the beginning of TMB dispensing.
   Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
10. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.
11. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.

✓ Flow Chart

Step 1.	Prepare reagents and samples, dilute it
	↓
Step 2.	Wash 3 times (250 µL/ well), aspirate
	↓
	Dispense 40 µL/well of dilution buffer
	↓
	Add 40 µL/well of standards or samples
	↓
	Incubate 45 min at room temperature
	↓
Step 3.	Dispense 20 µL/ well of MC-LR-Px conjugate
	↓
	Incubate 15 min at room temperature, 240 rpm
	↓
	Wash 5 times (250 µL/well), aspirate
	↓
Step 4.	Dispense 100 µL/well TMB substrate
	↓
	Incubate 20 minutes at room temperature, dark
	↓
Step 5.	Dispense 100 µL/well of Stop solution
	↓
Step 6.	Read optical density for 450 nm (ref. 620- 690 nm) till 20 minutes

Data Analysis

Calculation of Results

Calculate the concentration for each sample:

1. Calculate the average values of OD of all standards and samples if you pipetted in parallels
2. Calculate the B/Bmax (towards Standard A=negative control) for each sample and standard
   (%) $B/B\max = (A_{sample} / A_{Neg.\ control}) \times 100$
3. Construct the standard curve by plotting the %B/Bmax (Y-axis) versus the MC standards concentration (X-axis). You can use suitable software, e.g. KIM, Origin, or Excel and choose the optimal trend line for the best concentration of calibration curve points for the standards.
4. Adding the %B/Bmax values of each sample into standard curve gives the MC-LR concentration in the samples.
5. If the sample has been diluted, the outcome has to be multiplied by the grade of dilution of the sample.
6. If %B/Bmax of the sample is lower than %B/Bmax of Standard E is necessary to dilute the sample for obtain the accurate concentration of MC in the sample.

Performance Characteristics

✓ Limits of assay

The kit uses monoclonal antibody, which has been produced in order to identify microcystines that have arginin in the position 4. This group includes routinely founded and determinated microcystin–LR. Detection limit of the kit is 0.1 µg/L.

✓ Validity of the test

The results of the test are valid if:

- The B/Bmax of MC-LR Standard E is not higher than 50%.
- The mean absorbance of MC-LR Standard A is not lower than 0.700.
- The mean B/Bmax of Standards can be lined up as follows Standard E < Standard D < Standard C < Standard B < Standard A.

✓ Intraassay variability

(n = number of parallels):

n	A	±σ	min – max	CV
8	1.243	0.070	1.12 – 1.322	5.5%
20	0.325	0.031	0.281 – 0.385	9.5%

✓ Interassay variability

(n = number of parallels):

n	B/Bmax	±σ	min – max	CV repro.
8	32.0	0.039	28.3-38.5	12.5 %
8	57.8	0.068	46.0-65.5	11.7 %

Resources

References

1. Anke Zeck, Anja Eikenberg, Michael G. Weller, Reihardt Niessner : Highly sensitive immunoassay based of monoclonal antibody specific for [4-arginine] microcystins, Analytica Clinica Acta 2001

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard A	Sample 4										
B	Standard B	Sample 5										
C	Standard C	Sample 6										
D	Standard D	Sample …										
E	Standard E											
F	Sample 1											
G	Sample 2											
H	Sample 3											