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User's Manual

# Microcystin-LR ELISA Kit

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## 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.

## Principles of Testing

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.

## Reagents And Materials Provided

1. ELISA strips (colourless) coated with specific antibody [STRIPS Ab]: 1 microplate
2. Standard A [STANDARD A]: 0.6 mL, 0 ug/L, ready to use, 1 vial
3. Standard B [STANDARD B]: 0.6 mL, 0.1 ug/L, ready to use, 1 vial
4. Standard C [STANDARD C]: 0.6 mL, 0.5 ug/L, ready to use, 1 vial
5. Standard D [STANDARD D]: 0.6 mL, 1 ug/L, ready to use, 1 vial
6. Standard E [STANDARD E]: 0.6 mL, 2.5 ug/L, ready to use, 1 vial
7. MC-LR labeled with horseradish peroxidase [CONJ]: 2.5 mL, ready to use, 1 vial
8. Wash buffer concentrate, 10x concentrated [WASH 10X]: 55 mL, 1 vial
9. Dilution buffer [DIL-MC]: 15 mL, ready to use, 1 vial
10. Chromogenic substrate (TMB substrate) [TMB-BF]: 13 mL, ready to use, 1 vial
11. Stop solution [STOP]: 13 mL, ready to use, 1 vial
12. Instruction manual

## Materials Required But Not Supplied

a. Distilled or deionised water for dilution of the Wash buffer concentrate.

b. Appropriate equipment for pipetting, liquid dispensing and washing.

c. Spectrophotometer/colorimeter (microplate reader - wavelength 450 nm).

## Storage

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.

Do not store diluted samples and diluted MC-LR-Px conjugate. Always prepare fresh.

## Reagent Preparation

a. Allow all kit components to reach room temperature and vortex the components in order to ensure homogeneity.

b. 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.

c. 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 week if stored at +2 to +10°C.

d. Do not dilute standards, Px-conjugate, dilution buffer, TMB substrate and Stop solution, they are ready to use.

## Assay Procedure

a. 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.

b. 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.

c. Pipette 40 µL of Dilution buffer (DIL-MC) to each well that will be used. Then add 40 µL Standards stock solution of MC (STA, STB, ST..) or tested samples (S1, S2, S..). If you want to exclude a possible laboratory error apply the standards and samples in doublets.

See Figure 1.

d. Incubate for 45 (+/- 5) minutes at room temperature.

e. Add 20 µL of MC-LR-Px (Conj.) conjugate to each well. Do not empty the well nor wash the strips at this time.

f. Incubate for 15 (+/- 5 sec.) minutes at room temperature, 240 rpm.

g. 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.

h. Dispense 100 µL of the TMB-BF substrate into each well.

Pipette in a regular rhythm or use an appropriate dispensing instrument.

i. 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.

j. 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.

k. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.

Figure 1: Samples and Standards pipetting scheme

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | STA | S4 | | | | | | | | | | |
| B | STB | S5 | | | | | | | | | | |
| C | STC | S6 | | | | | | | | | | |
| D | STD | S … | | | | | | | | | | |
| E | STE | | | | | | | | | | | |
| F | S1 | | | | | | | | | | | |
| G | S2 | | | | | | | | | | | |
| H | S3 | | | | | | | | | | | |

## Calculation

Calculate the concentration for each sample:

1. calculate the average values of OD if you pipetted in parallels

ST A / ST B/ ST C / ST D / ST E / samples

2. calculate the B/Bmax (towards ST A=negative control) for each sample and standard

B/Bmax = ( Asample/ANeg. control ) x 100

3. Construct the standard curve by plotting the B/Bmax (Y-axis) versus log of the MC standards concentration (X-axis).
4. Read the concentration of the unknowns from the standard curve.
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 ST E is necessary to dilute the sample for obtain the

accurate concentration of MC in the sample.

## Performance Characteristics

Validity of the test

The results of the test are valid if:

The B/Bmax of MC-LR standard ST E is not higher than 50%.

The mean absorbance of MC-LR standard ST A is not lower than 0.700.

The mean B/Bmax of Standards can be lined up as follows ST E < ST D < ST C < ST B < ST A

## Precision

**Intraassay variability**

(N = number of parallels):

| n | A | ±δ | 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 paralells):

| n | B/Bmax | ±δ | 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 % |

## Precautions

SAFETYPRECAUTIONS:

All ingredients of the kit are intended for laboratory use only.

Standards and Px conjugate contain Microcystin, which is toxic, highly irritating, see Material safety data sheet.

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.

HANDLINGPRECAUTIONS:

Avoid contamination of samples and kit reagents.

Avoid cross-contamination of reagents.

Chromogenic substrate (TMB substrate) contains preservative ProClin 300®. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces. Follow the assay procedure indicated in the Instruction

manual.

Variations in test results are usually due to:

* Insufficient mixing of reagents and samples.

* Inaccurate pipetting and inadequate incubation times.

* Poor washing technique or spilling the rim of well with sample.

* Use of identical pipette tip for different solutions.

## Limitations

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