**Product Description**

Microcystin-LR ELISA Kit

**Intended Use**

Microcystin-LR ELISA Kit is intended for the detection of Microcystin - LR (MCLR) in environmental samples.

**Test Principle**

Microcystin-LR ELISA Kit is based on the use of monoclonal antibody antimicrocystin that bind 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.

**Kit Components**

| | | |
|---|---|---|
| 8-well break-away strips coated with the antibody within a plastic frame | | 1 microplate (96 wells) |
| 0,6 mL | MC-LR standard A (STA – concentration of MC-LR = 0 µg/L), r.t.u. * | 1 vial |
| 0,6 mL | MC-LR standard B (STB – concentration of MC-LR = 0.1 µg/L), r.t.u. * | 1 vial |
| 0,6 mL | MC-LR standard C (STC – concentration of MC-LR = 0.5 µg/L), r.t.u. * | 1 vial |
| 0,6 mL | MC-LR standard D (STD – concentration of MC-LR = 1 µg/L), r.t.u. * | 1 vial |
| 0,6 mL | MC-LR standard E (STE – concentration of MC-LR = 2.5 µg/L), r.t.u. * | 1 vial |
| 2,5 mL | MC-LR-Px enzyme conjugate r.t.u. * | 1 vial |
| 125 mL | Wash buffer concentrate, 10x concentrated | 1 vial |
| 125 mL | Dilution buffer (DB) , r.t.u. * | 1 vial |
| 15 mL | Chromogenic substrate (TMB substrate), r.t.u. * | 1 vial |
| 30 mL | Stop solution, r.t.u. * | 1 vial |
| Sealable pouch for unused strips | | 1 piece |
| Instruction manual | | |
| Certificate of quality | | |

**Material Required but Not Provide With the Kit**

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).

**Preparation of Reagents and Samples**

a. **Allow all kit components to reach room temperature.**
b. **Vortex Diluting solution (DB) r.t.u. and TMB r.t.u. in order to ensure homogeneity**.
c. Just before use thoroughly mix tested of the standards and samples. If needed, the samples may be diluted with distilled water 2 times, 5x or 10x to increase the clarity. The grade of dilution is important to estimate the exact concentration of the probe.
d. Prepare Wash buffer by diluting the concentrate 10 times with an appropriate volume of distilled or deionised water (100 mL of the concentrated Wash buffer + 900 mL of distilled water). If there are crystals of salt presented in the concentrated Wash buffer, warm up the vial to 32 to 37℃ in a water bath. Diluted Wash buffer is stable for one week if stored at 2 to 10℃.
e. 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 crosscontamination 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 to each well that will be used. Then add Standards stock solution of MC (STA, STB, ST..) and tested samples (S1, S2, S..). If you want to exclude a possible laboratory error, two parallels may be employed for each sample. *See Figure 1.*
d. Incubate for **45 (+/- 5) minutes** at room temperature, **240rpm**.
e. Add **20 µL** of MC-LR-Px conjugate to each well. Do not empty the well contents or 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 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 nm.

Figure.1.: Samples and Standards pipetting scheme

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | STA | S2 | | | | | | | | | | |
| b | STB | S3 | | | | | | | | | | |
| c | STC | S3 | | | | | | | | | | |
| d | STD | S… | | | | | | | | | | |
| e | STE | | | | | | | | | | | |
| f | S1 | | | | | | | | | | | |
| g | S1 | | | | | | | | | | | |
| h | S2 | | | | | | | | | | | |

**Processing of Results**

Calculate the concentration for each sample:

a. calculate the average values of OD 450/620 if you pipetted in parallels
ST A / ST B/ ST C / ST D / ST E / samples

b. calculate the %A (towards ST A=negative control) for each sample and standard
**%A = ( Asample/ANeg. control ) x 100**

c. in Excel use X/Y Graph to set up the calibration curve A%450/620 = ax+b

d. by substitution of value % A into the formula we obtain the value of concentration of MC-LR in the sample.

e. If the sample has been diluted, the outcome has to be multiplied by the grade of dilution of the sample.

f. If %A of the sample is lower than %A of ST E is necessary to dilute the sample for obtain the accurate concentration of MC in the sample.

**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 ug/L.

**Validity, Specificity and Sensitivity of the Test**

**A. Validity of the test**

The results of the test are valid if:

The mean absorbance (%A) of MC-LR standard ST E is around 30 %

The mean absorbance (%A) of MC-LR standard ST B is around 90%

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

**B. Intraassay variability**

(N = number of parallels):

| **n** | **A** | **±δ** | **min – max** | **CV repro.** |
|---|---|---|---|---|
| 8 | 0.271 | 0.02 | 0.229 – 0.294 | 7.4% |
| 20 | 0.124 | 0.011 | 0.106 – 0.148 | 8.8% |

**C. Interassay variability**

(N = number of paralells):

| **n** | **%A** | **±δ** | **min – max** | **CV repro.** |
|---|---|---|---|---|
| 8 | 58.26 | 5.59 | 52.3-66.35 | 9.6 % |
| 8 | 88.8 | 3.6 | 83.5-93.1 | 4 % |

**Safety Precautions**

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. However 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.

Chromogenic substrate (TMB substrate) contain 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 or Anti- mouse IgG Px-conjugate
* Use of identical pipette tip for different solutions

**Storage and Expiration**

Store the kit reagents at +2 to +10℃, 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.

**Flow Chart**

**Step 1.** **Prepare reagents and samples, dilute it**

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**Step 2.** **Wash 3 times (250 µL/ well), aspirate**

↓

**Dispense 40 µL/ well of dilution buffer**

↓

**Add 40ul/well of standards and samples**

↓

**Incubate 45 min at room temperature, 240rpm**

↓

**Step 3.** **Dispense 20 µL/ well of MC-LR-Px conjugate**

↓

**Incubate 15 min at room temperature, 240rpm**

↓

**Wash 5 times (250 µL/well), aspirate**

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**Step 4.** **Dispense 100 µL/well TMB substrate**

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**Incubate 20 minutes at room temperature, dark**

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**Step 5.** **Dispense 100 µL/well of Stop solution**

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**Step 6.** **Read optical density for 450 nm (ref. 690nm) till 20 min**

**References**

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