
# Competitive enzyme immunoassay kit for quantitative analysis of Brevetoxin

# Catalog Number: EL2023-01

**For Research Use Only. Not for use in Diagnostic Procedures.**

## 1. Background

Brevetoxins (PbTx) are a class of cyclic polyether compounds produced by certain algae such as *Karenia brevis*. *K. brevis* can produce Brevetoxins in large quantities during an algae bloom which then pose a major health threat and are important to monitor and mitigate.

## 2. Test Principle

The Brevetoxin plate kit is a competitive enzyme-labeled immunoassay. The brevetoxin HRP conjugate, sample extract and calibrators are pipetted into the test wells followed by the brevetoxin antibody into the test wells to initiate the reaction. During the 30 minutes incubation period, Brevetoxin from the sample and brevetoxin HRP conjugate compete for binding to the brevetoxin antibody. The Brevetoxin antibody is captured on the walls of the test well. Following this 30 minutes incubation, the contents of the wells are removed and the wells are washed to remove any unbound brevetoxin, brevetoxin HRP conjugate and free brevetoxin antibody. After wash, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 minutes incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the brevetoxin concentration of the samples is derived.

## 3. Applications

This kit can be used for rapid test of brevetoxins in liquid samples such as water, wastewater and algal cultures.

## 4. Cross Reactions

| | |
|---|---|
| Brevetoxin PbTx-3 | 100% |
| PbTx-2 | 162% |
| Neo Saxitoxin | < 1% |
| Okadaic Acid | < 1% |
| Saxitoxin | < 1% |

## 5. Equipment and Reagents Needed (not provided)

### 5.1 Equipment

- ELISA reader (450nm/630nm)
- Deionized water
- Vortex mixer
- Timer
- Wash bottle
- Polystyrene centrifuge tube: 50ml, 2ml
- Micropipettes: 20μl-200μl, 100μl-1000μl
- 300μl-multipipette

## 6. Components Provided in This Kit

- Microtiter plate with 96 wells coated with antibody.
- Brevetoxin (PbTx-3) standard solutions (6 bottles×2ml/bottle) 0ppb, 0.1ppb, 0.25ppb 0.5ppb, 1ppb, 2.5ppb.
- Brevetoxin enzyme conjugate 8ml.
- Polyclonal anti-brevetoxin antibody 8ml.
- Substrate solution 14ml.
- Stop solution 14ml.

## 7. Reagents Preparation

- N/A

## 8. Notice and Precautions Before Operation

- Please use one tip in the process of experiment and change the tips when absorbing different reagent.
- The stop solution is 1 N hydrochloric acid, which is corrosive and an irritant. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
- If running more than two strips at once, the use of a multichannel pipette is required.
- Make sure that all experimental instruments are clean.
- Treated samples can be stored at 2-8°C for 24h in dark.

## 9. Sample Preparation

### 9.1. Liquid (water, wastewater, liquid media)

- Make sure sample is free of particles and adjusted to a neutral pH.
- If necessary, centrifuge to pellet insoluble material: 3000g / 5min / at room temperature (20-25°C);
- Take 50μl of the supernatant of the sample for assay.

### 9.2. Shellfish sample preparation (muscle, clams, oysters and scallops)

- Wash the outside of the shells and shuck the shells. Collect the viscera into a clean container. Homogenize with commercial blender for at least 3 minutes to be sure of its homogeneity.

- Add 9.0 mL of 90% Methanol/water to 1 g of this homogenate and vortex vigorously for 2 min.
- Centrifuge the content for 10 min. at 3000 Xg.
- Collect the supernatant into a clean vial.
- Dilute the extract with sample diluent, 20 ul into 980 ul (1:50 dilution).
- The diluted sample extracts are ready to test.

## 10. Assay Process

### 10.1 Instructions Prior to Beginning Assay

1. Ensure that all reagents and microwells are at room temperature (20-25℃). Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
2. Return all the rest reagents to 2-8℃ immediately after their use.
3. Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
4. Avoid direct sunlight during the incubation; use the plate cover provided in the kit to cover the plate.

### 10.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25℃) for more than 30min. Shake gently before use. Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Dispense 50μl of the brevetoxin enzyme conjugate into each well.
6. Dispense 50μl of the standards, positive control, or sample into appropriate test wells.
7. Dispense 50μl of antibody solution into each well.
8. Shake the plate gently for 30 seconds using a back and forth motion.
9. Incubate for 30 minutes at room temperature.
10. Decant the contents of the wells into an appropriate waste container.
11. Rinse the microwells with 250μl laboratory grade water at interval of 10 seconds for 4-5 times.
12. Absorb the residual water by inverting with absorbent paper to remove the last if the wash solution.
13. Add 100μl substrate solution to each well, mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.
14. Add 100μl the stop solution to each well. Mix gently by shaking the plate manually and

measure the absorbance at 450nm (Read the result within 5min after addition of stop solution).

## 11. Results

### 11.1 Calculating the Percentage absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

  Absorbance (%) = B / $B_0$ *100

  B = the mean absorbance value of each standards or each sample

  $B_0$ = absorbance value of zero standard

### 11.2 Drawing a Standard Curve

- To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.
- The concentration of each sample (ppb), which can be read from the standard curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.
- Sample dilution factor: If the absorbance of a sample is lower than the highest calibrator (2.0 ppb), the concentration of Microcystin is too high and out of range of the standard curve. Dilute the sample in laboratory grade water and rerun. Samples should be diluted to fit into the standard curve (0.1 ppb to 2.5 ppb). Results must then be multiplied by the dilution factor used.

## 12. Sensitivity, Accuracy and Precision

### 12.1 Test Sensitivity:

- Overall Sensitivity ..... 0.1ppb

### 12.2 Detection limit:

- Water, wastewater, culture media ..... 0.1ppb

### 12.3 Accuracy:

- Water, wastewater, culture media ..... 80±10%

### 12.4 Precision:

- C.V. of the ELISA kit ..... less than 10%

## 13. General Instructions

### 13.1 Temperature of Reagents and Samples

- The mean values of the absorbance values obtained for the standards and the samples

will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃). The antibody solution should be stored at 4℃, which will be used immediately after taking out. If the antibody solution is return to room temperature before assay, the OD values will be higher, and the result of the assay will not be right.

13.2 Microwells

- Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

13.3. Shaking of Reagents

- Shake each reagent gently before use.

13.4. Skin Protection

- Keep your skin away from the stop solution for it is the 1 N HCl solution.

13.5 Out of Date Kits

- Don't use kits that are out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

13.6 General Comments

- Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

13.7 Special Issues Concerning Solutions and Reagents

- Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

13.8 Special Issues Concerning Color

- The coloration reaction need 30min after the addition of solution A and solution B, but you can prolong the incubation time ranges to 35min or more if the color is too light to be determined, never exceed 40min, on the contrary, shorten the incubation time properly.

13.9 Incubation Temperatures

- Incubation temperature should be at room temperature (20-28℃). Higher or lower temperature on day of testing will lead to experiment-to-experiment changes.

## 14. Storage

- Storage condition: 2-8℃
- Storage period: 12 mon

## Who we are

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