Importance of Okadaic Acid Determination

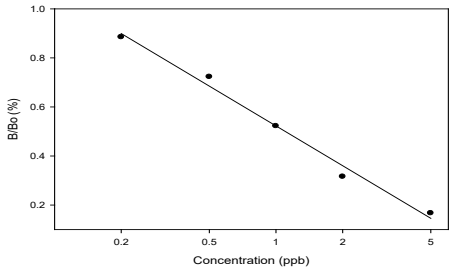
Okadaic Acid is one of the "diarrheic shellfish poisons" (DSP) produced by the dinoflagellate species *Dinophysis* and *Prorocentrum*. Contamination of shellfish with okadaic acid has been associated with harmful algal blooms throughout the world.

In man, DSP causes dose-dependent symptoms of diarrhea, nausea, and vomiting. The action level established by the FDA is 0.2ppm. The EU has established a level of 160ug OA eq (OA, DTXs, PTXs)/kg.

The ABRAXIS® Okadaic Acid ELISA allows the determination of 40 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of detection for Okadaic Acid is calculated as: Xn +/- 3SD (n=20) and is equal to 0.1 ug/g in shellfish when using a dilution factor of 100. The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is at approximately 1.7 ng/mL. Determinations closer to the middle of the calibration range of the test yields the most accurate results.



Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity: This ELISA recognizes Okadaic Acid and other DSP toxins with varying degrees:

Cross-reactivities:

Okadaic Acid (OA)	100%
Dinophysistoxins DTX-1	117%
Dinophysistoxins DTX-2	13%
C8 Diol Metabolite*	52%

* After hydrolysis in clam matrix.

No cross-reactivity was shown with any of the following common PSP shellfish toxins: saxitoxin, neosaxitoxin, dc-STX, gonyautoxins-1/4, gonyautoxins-2/3, B-2; B-1; C-1/2 and domoic acid.

Samples: Water and shellfish samples were tested for matrix effects in the ELISA. No matrix effects were determined.

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Okadaic Acid (DSP) ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Okadaic Acid (DSP) in Water and Contaminated Samples

Product No. 520021

1. General Description

The ABRAXIS® Okadaic Acid ELISA is an immunoassay for the quantitative and sensitive detection of Okadaic Acid. Okadaic Acid is one of the toxins associated with diarrheic shellfish poisoning (DSP). This test is suitable for the quantitative and/or qualitative detection of Okadaic Acid in water samples as well as shellfish samples. For shellfish samples a sample preparation is required. If necessary, positive samples can be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in this test kit contain small amounts of Okadaic Acid. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Okadaic Acid ELISA should to be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Okadaic Acid by specific antibodies. Okadaic Acid, when present in a sample and an okadaic acid-enzyme-conjugate compete for the binding sites of rabbit anti-okadaic acid antibodies in solution. The okadaic acid antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Okadaic Acid present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the ABRAXIS® Okadaic Acid ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

No matrix effects have been observed with seawater samples (salinity up to 38 parts per thousand).

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, exposure to direct or indirect sunlight during the substrate reaction, or extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The ABRAXIS® Okadaic Acid ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.

6. Working Instructions

A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-rabbit).
2. Standards (7): 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ng/mL, 1.5 mL each
3. Antibody Solution (rabbit anti-okadaic acid), 6 mL
4. Okadaic Acid Conjugate, 6 mL
5. Sample Diluent Concentrate (10X), 25 mL, must be diluted before using to dilute samples, see Test Preparation (Section E)
6. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
7. Substrate (Color) Solution (TMB), 16 mL
8. Stop Solution, 12 mL

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 µL)
2. Multi-channel pipette (10-250 µL), stepper pipette with plastic tips (10-250 µL), or electronic repeating pipette with disposable plastic tips
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)
6. Deionized or distilled water
7. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section E)
8. Glass vials with Teflon caps
9. Methanol/deionized water (80/20)
10. Polytron or equivalent (see Preparation of Samples, Section C)
11. Centrifuge capable of rotating at 3000 x g
12. 0.45µm syringe filter (Millex HV, Millipore or equivalent) with 10 mL syringe
13. Vortex
14. For hydrolysis of sample extract (if required, see Section D):
    a. 1.25 N NaOH
    b. 1.25 N HCl
    c. Heat block capable of maintaining 80ºC

C. Preparation of Samples (Mussels and other shellfish)

1. Mussels are removed from their shells, washed with deionized water, thoroughly dried and homogenized using a Polytron or equivalent.
2. A 1.0 g portion of the homogenized mussels is then mixed with 6 mL methanol/deionized water (80/20) using a Polytron or equivalent.
3. Centrifuge mixture for 10 minutes at 3000 x g. Collect the supernatant.
4. Add 2 mL methanol/deionized water (80/20) to the mussel tissue residue. Re-centrifuge mixture for 10 minutes. Collect supernatant and add to the first portion.
5. Bring final volume of collected supernatant to 10 mL with methanol/deionized water (80/20). Filter the extract through a 0.45µm syringe filter (Millex HV, Millipore or equivalent).
6. Add 990 µL of 1X Sample Diluent to a 4 mL glass vial with Teflon-lined cap. Add 10 µL of the filtered extract to the diluent (1:100 dilution). Vortex.
7. Analyze diluted extracts as samples (Assay Procedure, Section G, Step 1)

The Okadaic Acid concentration contained in the samples is determined by multiplying the concentration of the diluted extract by a factor of 1000. Highly contaminated samples outside the range of the curve should be diluted further and re-analyzed. Samples with low concentrations of Okadaic Acid or samples which must meet specific regulatory levels may be analyzed at lesser dilutions.

D. Hydrolysis (To be performed when required)

1. Add 500 µL of the sample extract (from Section C, Step 5) to a glass vial with Teflon lined cap.
2. Add 100 µL of 1.25 N NaOH. Cap vial and vortex for 15-20 seconds.
3. Incubate in a heat block set at 80°C for 40 minutes.
4. Allow to cool at room temperature. Add 100 µL of 1.25 N HCl. Cap vial and vortex for 15-20 seconds.
5. Add 990 µL of 1X Sample Diluent to a 4 mL glass vial with Teflon-lined cap. Add 10 µL of the hydrolyzed extract to the diluent (1:100 dilution). Vortex.

When performing the hydrolysis procedure, all results obtained by the ELISA needs to be multiplied by 1,400 to obtain the final DSP concentration in the sample. The multiplication factor accounts for the sample extraction, hydrolysis, and dilutions.

E. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette, stepping pipette, or electronic repeating pipette for adding the antibody, substrate (color), and stop solutions in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

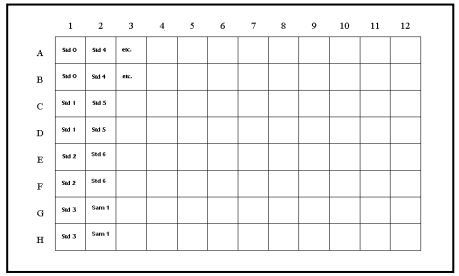
1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, enzyme conjugate, antibody solution, substrate, and stop solution are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
5. Dilute the Sample Diluent (10X) Concentrate at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of Sample Diluent (10X) Concentrate into 9 mL of deionized water) as needed for sample dilutions.
6. The stop solution should be handled with care as it contains diluted H_2SO_4.

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 6: Standards
0; 0.1; 0.2; 0.5; 1.0; 2.0; 5.0 ppb

Sam1, Sam2, etc.: Samples



G. Assay Procedure

1. **Add 100 µL of the standard solutions or the samples (water) or sample extracts (shellfish)** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. **Add 50 µL of conjugate solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. **Add 50 µL of antibody solution** to the individual wells successively using a using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. **Incubate the strips for 60 minutes at room temperature.**
5. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips three times using the diluted wash buffer.** Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. **Add 150 µL of substrate (color) solution** to the wells successively using a using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents. **Incubate the strips for 20-30 minutes at room temperature.** Protect the strips from direct sunlight.
7. **Add 100 µL of stop solution** to the wells in the same sequence as for the substrate solution using a using a multi-channel, stepping, or electronic repeating pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 4-Parameter (preferred) or Logit/Log. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Okadaic Acid concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Okadaic Acid by interpolation using the standard curve.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing lower concentrations of Okadaic Acid compared to standard 1 (0.1 ng/mL) are considered as negative. Samples showing a higher concentration than standard 6 (5.0 ng/mL) must be diluted further to obtain more accurate results. Results must be multiplied by the appropriate dilution factor for the sample extract (see Sections C and D).