Okadaic Acid Plate Kit

Cat. # 20-0184

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Okadaic Acid Plate Kit is a competitive ELISA for the quantitative analysis of okadaic acid (DSP) in samples.

ASSAY PRINCIPLES

The Beacon Okadaic Acid plate kit is a competitive enzyme-labeled immunoassay. The Okadaic Acid HRP conjugate is pipetted into the test wells followed by calibrators or sample extracts. Okadaic Acid Antibody solution is then added to the test wells to initiate the reaction. During the 30 minute incubation period, Okadaic Acid from the sample and the Okadaic Acid HRP conjugate compete for binding to Okadaic Acid antibody. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Okadaic Acid, Okadaic Acid HRP conjugate and free Okadaic Acid antibody. A clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 minute 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 Okadaic Acid concentration of the samples is derived.

SPECIFICITY

The following table shows the % cross reactivity of Okadaic Acid and related toxins.

Compound	% CR
Okadaic Acid	**100 %**
Dinophysistoxins DTX-1	**120%**
Dinophysistoxins DTX-2	**20%**
Domoic Acid	**< 1%**
Neosaxitoxin	**< 1%**

Saxitoxin	**<1%**

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Okadaic Acid Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Okadaic Acid Plate Kits with different Lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. The Stop Solution is 1N hydrochloric acid. 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.

REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

- Plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.
- 4 vials each containing 2 mL of Okadaic Acid calibrators corresponding to 0, 0.2, 0.5, 1, 2, and 5 µg/L (ppb) of Okadaic Acid.
- 1 vial containing 8 mL Okadaic Acid HRP Enzyme Conjugate.
- 1 vial containing 8 mL of Polyclonal anti-Okadaic Acid antibody.
- 1 vial containing 14 mL of Substrate.
- 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- Instructions

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Pipet with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Micro well plate or strip reader with 450nm filter.
- Methanol
- Timer
- Wash bottle

SAMPLE PREPARATION

SHELLFISH EXTRACTION PROTOCOL:

1. Homogenize the shellfish tissue.
2. Extract 1.0 g of the homogenized tissue with 10mL of 80%Methanol/water.
3. Vortex vigorously for 5 minutes.
4. Centrifuge for 5 minutes at 3,000rpm at room temperature.
5. Filter the supernatant using a glass fiber filter.
6. Dilute the filtered extract 1:50 into 10%Methanol/10mM PBS (ex: 40ul of filtered extract/1.96 mL of 10%Methanol/10mM PBS).

Dilution factor is 1:500

TEST PROCEDURE

(NOTE: Running Calibrators and sample extracts in duplicates will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Using a pipet with disposable tips, add **50 µL of enzyme conjugate** to each test well. Add **100 µL Calibrators or Sample** to the appropriate test wells. Be sure to use a clean pipet tip for each.
4. Dispense **50 µL of Antibody Solution** into each test well.
5. Shake the plate gently for 30 seconds and incubate the test wells for **30 minutes**.
6. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory grade water and dump. Repeat 3X for a total of four washes.
7. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Incubate the wells for **30 minutes**.
10. Dispense **100 µL of Stop Solution** into each test well.
11. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells. Samples containing less color than a calibrator well have a concentration of Okadaic Acid greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration of Okadaic Acid less than the concentration of the calibrator.

2. Quantitative interpretation requires graphing the absorbances of the calibrators (y axis) versus the log of the calibrator concentration (x axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the x axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as <0. 2 ppb or >5 ppb, respectively.

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SAMPLE CALCULATIONS

Well Contents	OD_{450}	Average OD ± Std. Dev.	%RSD	%Bo*
0 ppb Calibrator	2.08 2.11	2.09 ± 0.025	1.18	100
0.2 ppb Calibrator	1.68 1.72	1.70 ± 0.032	1.89	81
0.5 ppb Calibrator	1.31 1.28	1.29 ± 0.016	1.23	62
1.0 ppb Calibrator	0.9 0.92	0.91 ± 0.011	1.22	43
2.0 ppb Calibrator	0.56 0.55	0.56 ± 0.012	2.17	27
5.0 ppb Calibrator	0.32 0.3	0.31 ± 0.013	4.33	15

This data is for example purposes only.

* %Bo = (OD_{450} / 0 ppb OD_{450})*100

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

Safety

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

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