Competitive enzyme immunoassay kit for quantitative analysis of Okadaic Acid

Catalog Number: EL2050-01

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

1. Background

Okadaic Acid (OA) is a one of the diarrhetic shellfish poisons (DSP) produced by dinoflagellate genera Dinophysis and Prorocentrum. There are several chemically different toxins associated with DSP. They are lipophilic and polyether compounds and can be divided into three main groups:

1. Acidic toxins
2. Neutral toxins
3. Other toxins

Contamination of shellfish with OA has been associated with harmful algae blooms throughout the world. In humans, DSP causes dose-dependent symptoms of diarrhea, nausea, and vomiting. The action levels established by the FDA for OA is 200ppb. The EU has established a level of 160 ppb of OA or its equivalent.

The Attogene Okadaic acid ELISA kit enables international and government regulatory agencies, food manufacturers and processors, as well as quality assurance organizations to detect OA in food, feed, fish, and environmental samples of concern.

2. Test Principle

The Okadaic Acid test kit is a competitive enzyme-labeled immunoassay. The sample extract and calibrators are pipetted into the test wells followed by the Okadaic Acid monoclonal antibody. During the 30 minutes incubation period, any free Okadaic Acid from the sample or calibrators competes with the Okadaic Acid antigen bound to the ELISA plate compete for binding to the Okadaic Acid antibody in solution. The Okadaic Acid antibody is captured on the walls of the test well. Following this 30-minute incubation, the contents of the wells are removed, and the wells are washed to remove any unbound Okadaic Acid and free Okadaic Acid antibody. After wash, 1X HRP-conjugated Antibody#2 is added for 30 minutes incubation. The wells are washed afterwards, and a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 15-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.

3. Applications

This kit can be used for rapid test of Okadaic Acid in liquid or solid samples such as water, food, shellfish, wastewater, and numerous environmental samples.

4. Cross Reactions

Okadaic Acid	100%
Dinophysistoxin DTX-1	120%
Dinophysistoxin DTX-2	20%
Domoic Acid	<1%
Neosaxitoxin	<1%

5. Components Provided in This Kit

- Microtiter plate with 96 wells coated with Okadaic Acid
- Okadaic Acid standard solutions (6 vials × 0.8mL/vial):
    - 0ppb, 0.2ppb, 0.5ppb 1ppb, 2ppb, 5ppb
- Okadaic Acid Antibody#1 11mL
- 100X HRP-Conjugated Antibody#2 0.25mL
- Antibody#2 Diluent 20mL
- 20X Wash solution 28mL
- TMB Substrate solution 12mL
- Stop solution 14mL

6. Equipment and Reagents Needed (not provided)

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

7. Reagents Preparation

- 1X Wash solution: combine one volume of the 20X Wash Solution with 19 volumes of deionized water. Mix well.
- 1X HRP-conjugated Antibody#2: combine one volume of the 100X HRP-Conjugated Antibody#2 with 99 volumes of Antibody#2 Diluent. Vortex for 10 seconds to mix. Prepare this solution fresh before each test.

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 1N 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 EXTRACTION PROTOCOL:

- Homogenize the shellfish tissue.
- Extract 1.0 g of the homogenized tissue with 10mL of 80% Methanol/water.
- Vortex vigorously for 5 minutes.
- Centrifuge for 5 minutes at 3,000rpm at room temperature.
- Filter the supernatant using a glass fiber filter.

- 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

10. Assay Process

10.1 Instructions Prior to Beginning Assay:

1. Ensure that all reagents and microwells are at room temperature (20-25℃).
2. Return all 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.

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.
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 Okadaic Acid standards, positive control, or sample into each well.
6. Dispense 100μL of the Antibody#1 into appropriate test wells.
7. Shake the plate gently for 30 seconds using a back-and-forth motion.
8. Cover the plate. Incubate for 30 minutes at room temperature.
9. Decant the contents of the wells into an appropriate waste container.
10. Rinse the microwells with 250μL of the 1X wash solution for 3 times.
11. Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
12. Add 150μL of freshly prepared 1X HRP-conjugated Antibody#2 to each well.
13. Shake the plate gently for 30 seconds using a back-and-forth motion.
14. Cover the plate. Incubate for 30 minutes at room temperature.
15. Decant the contents of the wells into an appropriate waste container.

16. Rinse the microwells with 250μL of the 1X wash solution for 3 times.
17. Absorb the residual water by inverting with absorbent paper to remove the last of the wash solution.
18. Add 100μL TMB substrate solution to each well, mix gently by shaking the plate manually and incubate for 15min at 25℃ with cover.
19. 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 (5.0 ppb), the concentration of Okadaic Acid 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 (0.2 ppb to 5.0 ppb). Results must then be multiplied by the dilution factor used.

12. Sensitivity, Accuracy and Precision

12.1 Test Sensitivity:

- Overall Sensitivity.....0.2ppb

12.2 Detection limit:

- Water, wastewater, culture media 0.2ppb

12.3 Accuracy:

- Water, wastewater, culture media ..... 80±15%

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 needs 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 months

Who we are

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

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