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User's Manual

Okadaic Acid (DSP) ELISA Kit

REF **DEIA6822**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Competitive enzyme immunoassay kit for quantitative analysis of Okadaic Acidn in liquid or solid samples such as water, food, shellfish, wastewater, and numerous environmental samples.

General Description

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 200 ppb. The EU has established a level of 160 ppb of OA or its equivalent.

The 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.

Principles of Testing

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 antibody. During the 30 minutes incubation period, any free Okadaic Acid from the sample or calibrators competes with the Okadaic Acid HRP 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. 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.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with Okadaic Acid
2. Okadaic Acid Standards: (6 vials × 0.8 mL/vial): 0 ppb (green cap), 0.3 ppb (purple cap), 0.9 ppb (yellow cap), 2.7 ppb (blue cap), 5.4 ppb (orange cap), 16.2 ppb (red cap)
3. Conjugated HRP: 6 mL
4. Okadaic Acid Antibody: 6 mL

5. 20× Wash Solution: 28 mL
6. TMB Substrate Solution: 12 mL
7. Stop Solution: 14 mL
8. 10× Sample Extraction Buffer: 25 mL

Materials Required But Not Supplied

1. ELISA Reader (450 nm/630 nm)
2. Deionized water
3. Methanol
4. Vortex mixer
5. Timer
6. Wash bottle
7. Polystyrene centrifuge tube: 50 ml, 2 ml
8. Micropipettes: 20 µl-200 µl, 100 µl-1000 µl

Storage

• Storage condition: 2-8°C

• Storage period: 12 months

Specimen Collection And Preparation

Notice and Precautions Before Operation

• Please use a fresh tip in the process of experiment and change the tips when absorbing different reagent.

• 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 24 hours in the dark.

Liquid (water, wastewater, liquid media):

1. Make sure sample is free of particles and adjusted to a neutral pH.
2. If necessary, centrifuge to pellet insoluble material: 4000 g / 5min / at room temperature (20-25°C)
3. Take 50 µl of the supernatant of the sample for assay.
4. NOTE: Based on the detection range, the dilution factor can be adjusted higher by diluting the water sample into 10% Methanol.

Shellfish Extraction:

1. Remove tissue from shells, remove excess liquid and homogenize the meat.
2. Extract 0.5 g of the homogenized tissue with 2 mL of 50% Methanol/water.
3. Vortex vigorously for 5 minutes.
4. Centrifuge for 5 minutes at 4,000 rpm at room temperature.

5. Transfer 0.5 mL of supernatant to a new tube and heat at 75°C for 5 minutes.
6. Centrifuge for 5 minutes at 4,000 rpm at room temperature.
7. Transfer 50 µl of supernatant to a new tube, add 950 µl of 10% Methanol mix well.
8. Dilution factor is 1:100.

Plate Preparation

Reagent Preparation

1. 1× Wash Solution: combine one volume of the 20× Wash Solution with 19 volumes of deionized water. Mix well.
2. 1× Sample Extraction Buffer: Mix 1 volume of 10× Sample Extraction Buffer with 9 volumes of deionized water.
3. 10% Methanol: Mix 9 volumes of 1× Sample Extraction Buffer with 1 volume of methanol.

Assay Procedure

Instructions Prior to Beginning Assay:

1. Ensure that all reagents and microwells are at room temperature (20-25°C).
2. Return all reagents to 2-8°C 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.

Steps in the Assay Process:

1. Take all reagents out at room temperature (20-25°C) 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°C.
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 50 µL of the Okadaic Acid Standards, positive control, or sample into each well.
6. Dispense 50 µL of Conjugated HRP into each well.
7. Dispense 50 µL of Okadaic Acid Antibody to each well.
8. Shake the plate gently for 30 seconds using a back-and-forth motion.
9. Cover the plate. Incubate for 30 minutes at room temperature.
10. Decant the contents of the wells into an appropriate waste container.
11. Rinse the microwells with 250 µL of the 1X Wash Solution 3 times.
12. Add 100 µL TMB Substrate Solution to each well, mix gently by shaking the plate manually and incubate for 15min at 25°C with cover.
13. Add 100 µL Stop Solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5 min after addition of Stop Solution).

Calculation

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 / B0 *100

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

   B0 = absorbance value of zero standard

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 (16.2 ppb), the concentration of Okadaic Acid is too high and out of range of the standard curve. Dilute the sample and rerun. Samples should be diluted to fit into the standard curve (0.3 ppb to 16.2 ppb). Results must then be multiplied by the dilution factor used.

Precision

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

Detection Limit

Water, wastewater, culture media .....0.6 ppb

Sensitivity

Overall Sensitivity.....0.3 ppb

Specificity

Okadaic Acid.....100%

Dinophysistoxin DTX-1.....40%

Dinophysistoxin DTX-2.....20%

Domoic Acid.....<1%

Neosaxito.....<1%

Precautions

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 restored to room temperature (20-25°C).

Microwells

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

Shaking of Reagents

• Shake each reagent gently before use.

Skin Protection

• The Stop Solution is 0.75 N HCl, keep your skin away from it.

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.

General Comments

• Keep the ELISA kits at 2-8°C, Do Not Freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

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 (A450 nm < 0.5).

Incubation Temperatures

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