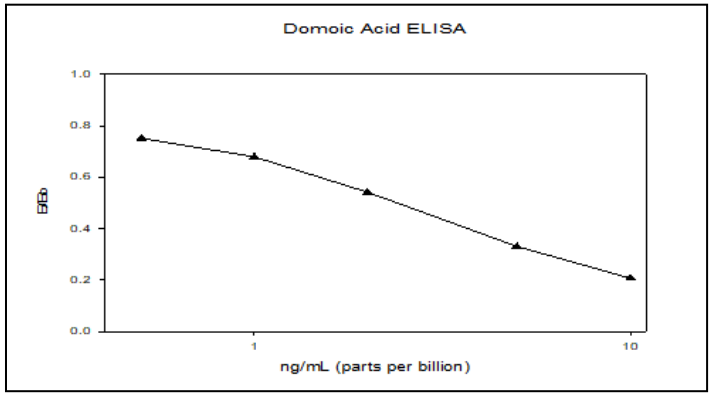
## Importance of Domoic Acid Determination

Domoic Acid is one of the "amnesiac shellfish poisons" (ASP) produced primarily by the diatom *Pseudo-nitzschia multiseries* as well as other diatoms. Contamination of shellfish with domoic acid has been associated with harmful algal blooms throughout the world.

In man, ASP causes dose-dependent symptoms of diarrhea, nausea, and vomiting. The action level established by the FDA is 20 ppm. The EU has established a level of 20 mg DA/kg.

The Domoic 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




For demonstration purposes only. Not for use in sample interpretation.

Test sensitivity: The limit of detection for Domoic Acid is calculated as 90% B/Bo and is equal to 0.17 ng/mL (ppb). The concentration of toxin necessary to cause 50% inhibition (50% $B/B_0$) is at approximately 2.2 ng/mL. Determinations closer to the middle of the calibration curve 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 Domoic Acid (DA).
No cross-reactivity was shown with any of the following marine algal toxins: Saxitoxin, Okadaic Acid, and PbTx-2.

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

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R042418

# Domoic Acid (ASP) ELISA, Microtiter Plate

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

**Product No. ON0021**

## 1. General Description

The Domoic Acid ELISA is an immunoassay for the quantitative and sensitive detection of Domoic Acid. Domoic Acid is one of the toxins associated with amnesiac shellfish poisoning (ASP). This test is suitable for the quantitative and/or qualitative detection of Domoic Acid in water samples as well as shellfish samples. For shellfish samples a sample preparation is required (see Preparation of Samples, Section C.). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

## 2. Safety Instructions

The standard solutions in this test kit contain small amounts of Domoic Acid. In addition, the substrate solution contains tetramethylbenzidine (TMB) 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 Domoic Acid ELISA should to be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

## 4. Test Principle

The test is a direct competitive ELISA based on the recognition of Domoic Acid by specific antibodies. Domoic Acid, when present in a sample, and a domoic acid-enzyme-conjugate compete for the binding sites of mouse anti-domoic acid antibodies in solution. The domoic acid antibodies are then bound by a second antibody (goat anti-mouse) 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 Domoic 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 Domoic 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 can not 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, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Domoic 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.

## Working Instructions

### A. Materials Provided

1. Microtiter plate coated with a second antibody (goat anti-mouse)
2. Standards (5): 0, 0.5, 1.0, 2.0, 5.0, 10.0 ng/mL (ppb), 1 mL each
3. Control, 3.5 ng/mL, 1 mL
4. Antibody Solution (mouse anti-domoic acid), 6 mL
5. Domoic Acid-HRP Conjugate, 6 mL
6. Sample Diluent, 25 mL. Use to dilute samples
7. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 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 (10-250 µL), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section D)
5. Graduated cylinder
6. Paper towels or equivalent absorbent material
7. Timer
8. Tape or parafilm
9. Microtiter plate reader (wave length 450 nm)
10. Microtiter plate washer (optional)
11. Materials for sample preparation, if necessary (see Preparation of Samples, Section C)

### C. Preparation of Samples (Mussels and other shellfish)

1. Mussels are removed from their shells, washed with deionized water, thoroughly dried and homogenized (Polytron or equivalent).
2. A 0.5 g portion of the homogenized mussels is then mixed with 2 mL Methanol/deionized water (50/50) (Polytron or equivalent). Vortex ~1 min.
3. Centrifuge mixture for 15 minutes at 4000 g. Collect the supernatant.
4. Remove 20 µL and dilute to 1.0 mL with Sample Dilution Buffer (1:50).
5. Analyze diluted extracts as samples (see Assay Procedure, Section F, Step 1).

The Domoic Acid concentration contained in the samples is determined by multiplying the concentration of the diluted extract by a factor of 200 to account for extraction (step 2) and dilution (step 4). Highly contaminated samples outside the range of the curve should be diluted further and re-analyzed. Samples with low concentrations of Domoic Acid or samples which must meet specific regulatory levels may be analyzed at lesser dilutions.

### D. 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.

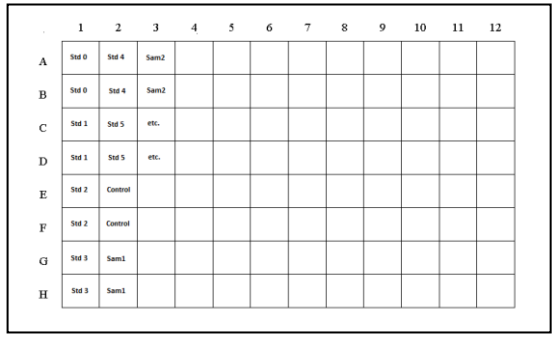
1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions,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

### E. 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 5: Standards
0; 0.5; 1.0; 2.0; 5.0; 10.0 ppb

Sam1, Sam2, etc.: Samples



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Std 0 | Std 4 | Sam2 | | | | | | | | | |
| B | Std 0 | Std 4 | Sam2 | | | | | | | | | |
| C | Std 1 | Std 5 | etc. | | | | | | | | | |
| D | Std 1 | Std 5 | etc. | | | | | | | | | |
| E | Std 2 | Control | | | | | | | | | | |
| F | Std 2 | Control | | | | | | | | | | |
| G | Std 3 | Sam1 | | | | | | | | | | |
| H | Std 3 | Sam1 | | | | | | | | | | |

### F. Assay Procedure

1. Add **50 µL of the standards, control, 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 enzyme 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 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. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Blot the inverted plate on a stack of paper towels. Wash the strips **three times** using the diluted wash buffer. 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 remaining buffer in the wells and, if necessary, remove by additional blotting.
6. Add **100 µL of substrate (color) solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Incubate the strips for **30 minutes** at room temperature. Protect the strips from direct sunlight.
7. Add **50 µL of stop solution** to the wells in the same sequence as for the substrate solution 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.

### G. 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 Domoic Acid concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Domoic Acid by interpolation using the standard curve. Results can also be determined by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing lower concentrations of Domoic Acid compared to standard 1 (0.50 ng/mL) should be reported as containing < 0.5 ppb of Domoic Acid. Samples showing a higher concentration than standard 5 (10.0 ng/mL) must be diluted further to obtain accurate results. Results for prepared shellfish samples must be multiplied by 200 to account for the sample preparation (see Section C).

As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.