Domoic Acid ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Domoic Acid in Water, Seawater and Shellfish Samples

Product No. 520505

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 and seawater 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 sulphuric 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 kit should be stored in the refrigerator (2-8°C). All components of the kit must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Consult state, local, and federal regulations for the proper disposal of all reagents.

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-protein analogue immobilized on the microtiter plate compete for the binding sites of the anti-Domoic Acid HRP Conjugate in solution. After a washing step and addition of the substrate solution, a color signal is generated. 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 cannot be completely excluded.

A minimum dilution factor of 1:50 is required for seawater samples and water samples with high 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 (or reagents), incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, 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 Domoic Acid conjugated to a protein, in a resealable foil pouch with desiccant.
2. Domoic Acid Standards (6): 0, 6.5, 22, 254, 865, and 10 000 ppt (parts per trillion) and Control (1) 75 ppt ± 15 ppt, 1.0 mL each.
3. 10X Sample Diluent, 30 mL, must be diluted prior to use, see Test Preparation (Section C).
4. Anti-Domoic Acid HRP Conjugate Concentrate (2), must be diluted prior to use, see Test Preparation (Section C).
5. Anti-Domoic Acid HRP Conjugate Diluent.
6. Wash Solution (5X) Concentrate, 100 mL, must be diluted prior to use, see Test Preparation (Section C).
7. Color (Substrate) Solution (TMB), 13.5 mL.
8. Stop Solution, 13.5 mL.

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

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Stepper/electronic repeating pipette with plastic tips (10-250 µL) or multi-channel pipette (50-250 µL).
3. Microtiter plate reader with wave length 450 nm
4. Timer
5. Large sized bottles (500 mL sized or larger)
6. Parafilm or adhesive film microplate cover
7. Deionized water
8. Methanol
9. 50 mL Centrifuge tubes
10. Centrifuge capable of 3000 X g
11. Analytical balance

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a repeater/stepping pipette for adding the conjugate, substrate and stop solutions in order to equalize the incubations periods of the solutions 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 should be stored in the foil bag and zip-locked closed.
3. The anti-Domoic Acid HRP Conjugate Concentrate must be diluted prior to use. To dilute, add 10 mL of the HRP Conjugate Diluent to the amber glass vial. Close the vial with the rubber stopper and shake well before use. Once diluted, the HRP Conjugate is stable for one (1) week, refrigerated.
4. To prepare 1X Sample Diluent, dilute the 10X Sample Diluent at a ratio of 1:10. If using the entire bottle (30 mL), add 270 mL of deionized water to 30 mL of 10X Sample Diluent into a large sized clean bottle. Once diluted, the Sample Diluent is stable for one (1) week, refrigerated.
5. 100% Methanol **must** be added to the 1X Sample Diluent prepared in Step 4 prior to dilution of samples to create a 10% Methanol Sample Diluent solution. **For reference, 1 mL of 100% Methanol is required for every 9mL of 1X Sample Diluent used.**

NOTE: The Standards, Color solution and stop solution are all ready to use and **DO NOT** require any further dilutions.

6. Dilute the wash buffer (5X) concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized water to 100 mL wash buffer (5X) concentrate into a large sized clean bottle. Once diluted, the wash buffer is stable for one (1) week, refrigerated.

7. The stop solution should be handled with care as it contains diluted sulfuric acid (H_2SO_4).
8. 50% Methanol is required for extracting and analysing shellfish samples. To prepare, add equal volumes of methanol and deionized water (example: add 10 mL Methanol to 10 mL deionized water).

D. Preparation of Samples

Samples should be analyzed immediately after preparation to prevent adsorption/degradation of the analyte.

Shellfish

1. Weigh 4 g of shellfish sample into a 50 mL plastic centrifuge tube.
2. Add 16 mL of 50% Methanol, vortex thoroughly for 30 seconds.
3. Centrifuge sample for at least 10 minutes at 3000 X g.
4. Collect 50 µL of the supernatant and dilute by adding to 950 µL 1X Sample Diluent.
5. Dilute the sample further by adding 50 µL from the previous step to 950 µL 1X Sample Diluent with 10% Methanol.

   NOTE: The Domoic Acid concentration contained in the samples is determined by multiplying the concentration of the diluted extract by a factor of 2000 to account for extraction (Step 2) and dilution (Step 5). Highly contaminated samples outside the range of the curve should be further diluted and re-analyzed.

Water Samples

Seawater samples and samples with high salinity (~38 ppt) must be diluted by a minimum factor of 1:50 to account for matrix interference (20 µL water sample added to 980 µL 1X Sample Diluent with 10% Methanol). Highly contaminated samples outside the range of the curve should be further diluted and re-analyzed.

E. Working Scheme

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Control										
F	Std 2	Control										
G	Std 3	Samp1										
H	Std 3	Samp1										

Std 0-Std 5: Standards
Control: Control
Samp1, Samp 2, etc.: Samples

F. Assay Procedure

1. Add **50 µL** of each standard, control, and sample in duplicate into the wells of the test strips according to the working scheme provided in this insert
2. Add **50 µL** of the diluted anti-Domoic Acid HRP concentrate (see Section C. Test Preparation) to the individual wells successively using a stepper/electronic repeating or multi-channel pipette.
3. Cover the wells with parafilm or microplate cover and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents. Incubate the strips for 60 minutes at room temperature.
4. After incubation, remove the covering and decant the contents of these wells into a sink. Wash the strips **four times** using the 1X washing buffer solution. Use 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
5. Add 100 µL of color substrate solution to the wells using a stepper/electronic repeating or multi-channel pipette. Cover the wells with parafilm or microplate cover and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Incubate the strips for 15 minutes at room temperature. Protect the strips from direct sunlight.

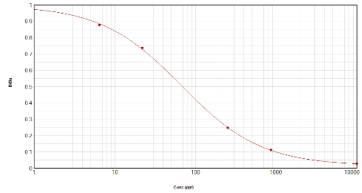
6. Remove the covering and add 100 µL of stop solution to the wells in the same sequence as for the substrate solution using a stepper electronic repeating or multi-channel pipette. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds
7. Read the absorbance at 450 nm using a microplate ELISA microtiter plate reader 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 [4-Parameter – if not available, please contact Eurofins Abraxis]. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 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/B0 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/B0 for samples will then yield levels in ppb of Domoic Acid by interpolation using the standard curve.
Samples showing a concentration lower than Standard 1 (6.5 ppt) should be reported as < 6.5 ppt (parts per trillion) of Domoic Acid. Samples showing a higher concentration than Standard 5 (10 000 ppt) can be reported as > 10 000 ppt (parts per trillion) or diluted further and re-analyzed to obtain an accurate quantitative result. Results for shellfish samples must be multiplied by a factor 2000 to account for extraction and dilution. Seawater samples/water samples with high salinity must be multiplied by a factor of 50 to account for dilution as well.

H. Performance Data

The Domoic Acid ELISA has an estimated detection limit (90% B/B0) of 6.5 ppt (parts per trillion). The middle of the test (50% B/B0) is approximately 75 ppt (parts per trillion) (or 3.75 ppb for seawater or 150 ppb for shellfish after sample dilution). Determinations closer to the middle of the calibration curve give the most accurate results.



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

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

Cross-reactivities: A number of various toxins and compounds including Saxitoxin (10 ppb), PbTx-2 (10 ppb), Okadaic Acid (10 ppb), Glutamine (100 ppb), Glutamic Acid (100 ppb), and Proline (100 ppb) were tested with this ELISA and shown to have no cross-reactivity.

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