Beacon Analytical Systems Inc.

Domoic Acid Plate Kit

Cat. # 20-0249

Product Insert

Please read completely before use

INTENDED USE

The Beacon Domoic Acid Plate Kit is a competitive ELISA for the quantitative analysis of domoic acid in shellfish samples.

ASSAY PRINCIPLES

The Beacon Domoic Acid plate kit is a competitive enzyme-labeled immunoassay for the biotoxin which causes Amnesic Shellfish Poisoning (ASP). Shellfish sample extract(s) or calibrator solution(s) are pipetted into a test well followed by Domoic Acid HRP Enzyme Conjugate to initiate the reaction. During a 30-minute incubation period, domoic acid from the sample and Domoic Acid HRP Enzyme Conjugate compete for binding to the domoic acid antibody coated on the plate wells. Following this incubation, the wells are washed to remove any unbound domoic acid and enzyme conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following another 30-minute incubation, the reaction is stopped with the addition of Stop Solution and the amount of yellow color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the domoic acid concentration of the sample is derived. The color intensity is inversely proportional to the amount of domoic acid present.

NOTE: Color is inversely proportional to domoic acid concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

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 to 8ºC.

1	Plate containing 12 test strips of 8 wells each vacuum-sealed in an aluminized pouch with a desiccant.
6	Vials Domoic Acid Calibrators (0, 0.5, 1.5, 5, 15 and 50 µg/L (ppb))
1	Bottle Domoic Acid HRP Enzyme Conjugate
1	Bottle Substrate
1	Bottle Stop Solution (Caution! Contains 1N HCl. Handle with care.)

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MATERIALS REQUIRED BUT NOT PROVIDED

Sample Dilution Buffer and Extraction Solution Preparation:

- Laboratory quality distilled or deionized water
- Acetonitrile, ACS grade
- Methanol, ACS grade
- Glass storage container with a tight-fitting lid

Sample Extraction:

- Glassware for sample dilution
- Glass ware capable of measuring 1.9, 3.9 and 16 mL
- Balance
- Timer
- High-speed blender
- Vortex mixer
- Disposable microcentrifuge tubes
- Centrifuge capable of reaching 10,000 X g

Assay Procedure:

- Laboratory quality distilled or deionized water
- Pipette with disposable tips capable of dispensing 100 µL
- Multi-channel pipette; 8 channels capable of dispensing 100 µL (recommended if running more than two strips at once.)
- Paper towels or equivalent absorbent material.
- Wash bottle
- Timer
- Orbital shaker (optional)
- Microwell plate or strip reader with 450 nm filter.

SPECIFICITY

Domoic Acid is an amino acid similar in structure to kainic acid which naturally occurs in some seaweed. The % cross reactivity (%CR) of several compounds relative to domoic acid is shown in the table below.

Compound	% CR
Domoic acid	100
Kainic acid	< 0.1
Glutamine	< 0.1
Glutamic acid	< 0.1
Saxitoxin	< 0.1
Neo-saxitoxin	< 0.1
Okadaic acid	< 0.1
Brevetoxin	< 0.1

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Running Calibrators and samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using an appropriate and calibrated pipette is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- Each reagent is optimized for use in the Beacon Domoic Acid Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Domoic Acid Plate Kits with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Do not use reagents after expiration date.
- Reagents should be brought to room temperature, 20 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Domoic Acid Calibrators contain 10% acetonitrile and should be kept tightly capped to minimize evaporation.
- 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.

SAMPLE DILUTION BUFFER PREPARATION – 10% Acetonitrile/water

1. Mix 1 part ACS grade acetonitrile with 9 parts laboratory quality distilled or deionized water.
2. Transfer to a clean glass container with a tight-fitting lid.
3. Swirl to mix completely. Store tightly sealed to minimize evaporation.

EXTRACTION SOLUTION PREPARATION – 50% Methanol/water

1. Mix 1 part methanol with 1 part laboratory quality distilled or deionized water.
2. Transfer to a clean glass container with a tight-fitting lid.
3. Swirl to mix completely. Store tightly sealed to minimize evaporation.

SAMPLE PREPARATION

Shellfish Tissue: Total Dilution Factor (TDF) = 4,000

1. Shuck shellfish tissue from the shell. Wash, drain, dry, and homogenize using a high-speed blender.
2. Weigh 4 g of homogenized tissue and add 16 mL of Extraction Solution (5X dilution).
3. Mix for 3 minutes using a vortex mixer.
4. Transfer 1 mL into a microcentrifuge tube and centrifuge at 10,000 X g for 5 minutes.
5. Prepare a 1:800 dilution of the supernatant with Sample Dilution Buffer using the following procedure.
    a. 1:40 dilution – 100 µL of supernatant layer avoiding any particulates, into 3.9 mL of Sample Dilution Buffer. Mix.
    b. 1:20 dilution – 100 µL of dilution A into 1.9 mL Sample Dilution Buffer. Mix.
6. Use B in the assay procedure.

EU Screening Level = 20 ppm (20 mg/kg). The assay dilution factors are set to detect 20 ppm domoic acid. An adjustment of the secondary dilution can be made to adjust the range of tissue detection levels. A minimum of 40-fold secondary dilution is suggested to provide the lowest level of detection at 0.1 mg/kg (ppm).

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate 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 the desiccant to limit exposure to moisture.
3. Dispense **100 µL of Calibrators and sample extract** into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **100 µL of Enzyme Conjugate** into each well.
5. Gently shake the plate for 30 seconds using a back-and-forth motion and incubate the wells for **30 minutes** at room temperature.
6. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory grade distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
7. Dispense **100 µL of Substrate** to each well. Shake the plate gently for 30 seconds using a back-and-forth motion. If running more than two strips at once, the use of a multi-channel pipette is recommended.
8. Incubate for **30 minutes** at room temperature.
9. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate. If running more than two strips at once, the use of a multi-channel pipette is recommended.
10. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm using a strip or plate reader.

RESULTS INTERPRETATION

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Samples with a lower absorbance (less color) than a calibrator well have a concentration of domoic acid greater than the concentration of the calibrator. Samples with a higher absorbance (more color) than a calibrator well have a concentration less than the concentration of the calibrator.

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculation is available upon request. Please contact Beacon for further details.

Samples with absorbances lower than the highest calibrator, the concentration of domoic acid is too high for quantification. Further dilute the sample extract in Sample Dilution Buffer and retest along with the Calibrators. If the sample falls within the curve, multiply the results by the Total Dilution Factor (TDF).

EXAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**	Domoic Acid Concentration	Adjusted Domoic Acid Concentration***
0 ppb Calibrator	1.817 1.755	1.786 ± 0.044	2.5	100	0 ppb	0.0 ppm
0.5 ppb Calibrator	1.510 1.500	1.505 ± 0.007	0.5	84	0.5 ppb	2.0 ppm
1.5 ppb Calibrator	1.230 1.240	1.235 ± 0.007	0.6	69	1.5 ppb	6.0 ppm
5 ppb Calibrator	0.833 0.821	0.827 ± 0.008	1.0	46	5.0 ppb	20.0 ppm
15 ppb Calibrator	0.520 0.510	0.515 ± 0.007	1.4	29	15.1 ppb	60.0 ppm
50 ppb Calibrator	0.319 0.317	0.318 ± 0.001	0.4	18	49.5 ppb	200.0 ppm
Sample	0.801 0.810	0.806 ± 0.006	0.80	45	5.4 ppb	21.5 ppm

This data is for example purposes only.

* Standard Deviation

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

*** Adjusted domoic acid concentration for shellfish tissue. Multiply the domoic acid concentration by the 4000 TDF.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302 or contact us at info@beaconkits.com.

SAFETY

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

GENERAL LIMITED WARRANTY

Beacon Analytical Systems, Inc. (“Beacon”) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon’s sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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