9. Dispense **100 µL of Substrate** into each well.
10. Incubate the wells for **30 minutes**.
11. Dispense **100 µL of Stop Solution** into each test well.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells. Samples containing less color than a calibrator will have a concentration of Saxitoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis) versus the log of the calibrator concentration (X axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the X axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.02 ppb or >0.32 ppb, respectively. Alternatively, Beacon can supply a spreadsheet template which can be used for data reduction. Please contact Beacon for further details.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD ± SD*	%RSD	%Bo**	STX conc. (ppb)
Negative Control	2.149 2.072	2.110 ± 0.055	2.6	100	N/A
0.02 ppb Calibrator	1.775 1.804	1.789 ± 0.020	1.1	84.8	N/A
0.08 ppb Calibrator	1.242 1.193	1.218' ± 0.035	2.9	57.7	N/A
0.32 ppb Calibrator	0.489 0.482	0.486 ± 0.005	1.0	23.0	N/A
Sample	0.491 0.511	0.501 ± 0.014	2.8	23.7	0.309

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

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.

BEACON ANALYTICAL SYSTEMS, INC.
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
Fax (207) 602-6502
www.beaconkits.com

Beacon Analytical Systems Inc.

Saxitoxin Plate Kit

Cat.# 20-0173

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Saxitoxin Plate Kit is a competitive ELISA for the quantitative analysis of Saxitoxin in contaminated samples

USE PRINCIPLES

The Beacon Saxitoxin plate kit is a competitive enzyme-labeled immunoassay. The Saxitoxin HRP conjugate, sample extract and calibrators are pipetted into the test wells followed by Saxitoxin antibody into the test wells to initiate the reaction. During the 30 minute incubation period, Saxitoxin from the sample and Saxitoxin HRP conjugate compete for binding to Saxitoxin antibody. The Saxitoxin antibody is captured on the walls of the test well. Following this 30 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound Saxitoxin, Saxitoxin HRP conjugate and free Saxitoxin antibody. After wash, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 30 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 Saxitoxin concentration of the samples is derived.

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 – 8ºC.

1 plate containing 12 test strips of 8 wells each vacuum-packed in aluminized pouch with indicating desiccant.

1 vial of Negative control (0.0 ppb Saxitoxin)

3 vials each containing 2 mL of Saxitoxin calibrators corresponding to 0.02, 0.08 and 0.32 µg/L (ppb) of Saxitoxin.

1 vial containing 7 mL Saxitoxin HRP Enzyme Conjugate.

1 vial containing 7 mL of Polyclonal anti-Saxitoxin antibody.

1 bottle containing 50 mL 10X Wash solution concentrate.

1 vial containing 14 mL of Substrate.

1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

1 Instructional Booklet

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Pipet with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8-channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer
- Wash bottle

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The following table shows the % cross reactivity of Saxitoxin. All concentrations are in parts per billion (ppb).

Compound	% CR
Saxitoxin dihydrochloride	**100 %**
Neosaxitoxin	**0.8 %**
Decarbamoyl STX	**18 %**
GTX 2& 3	**12 %**
GTX 1 & 4	**<0.1 %**
Decarbamoyl GTX 2& 3	**0.4 %**
Decarbamoyl NeoSTX	**0.7 %**

PRECAUTIONS

- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Each reagent is optimized for use in the Beacon Saxitoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Saxitoxin 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 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Saxitoxin is a toxin and should be treated with care.
- 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.
- Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.

SAMPLE PREPARATION (MUSSELS)

1. Thoroughly clean the outside of the mussels with laboratory grade water.
2. Cut the adductor muscles of the mussels using a sharp knife.
3. Rinse off the inside of the mussels with laboratory grade water to remove sand and other foreign substances.
4. Detach the tissue from the mussel shells by removing the tissue and adductor muscles that connect it at the hinge.
5. Transfer 120-150 g of mussel tissue to a sieve and gently shake the sieve to drain the excess liquid.
6. Remove the drained tissue into a 500 mL clean container and homogenize to a soupy texture.
7. Tare a 50 mL conical tube and add 10 grams of homogenized tissue.
8. Add 20 mL of methanol and vigorously shake tube for 5 minutes.
9. Centrifuge tube for 20 minutes at 5,000 rpm.
10. Transfer the clear supernatant to a clear glass vial for storage.
11. Dilute extracts 1:40 in 10 mM Phosphate buffer before running in assay.*

*Proper dilution is based on level of contamination.

SAMPLE PREPARATION (LOBSTER TOMALLEY)

1. Collect the tomalley from the cooked lobster into a clean beaker.
2. Mix the greenish tomalley thoroughly with a spatula until it turns to a homogenous green paste.
3. Weigh out 5 grams of the mixed tomalley and add 40 mL of 0.1 N HCl. Vortex vigorously for 2 minutes.
4. Filter 10-15 mL of the upper layer of the extract through a Whatman #4 paper filter and transfer 1.5 mL of the filtered extract into a microcentrifuge tube.
5. Centrifuge for 5 minutes at 10,000 rpm.
6. Dilute the supernatant into 10 mM PBS buffer before running assay.
7. Due to the unknown concentration of toxin in the samples, a range of dilutions is suggested (i.e., 1:10, 1:20, and 1:40).

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Prepare the 1X wash solution by adding the contents of the 10X wash concentrate bottle to 450 mL Lab grade water in a wash bottle.
2. Allow reagents and sample extracts to reach room temperature prior to running the test.
3. 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 desiccant.
4. Using a pipet with disposable tips, add **50 µL enzyme conjugate** to the appropriate test wells. Be sure to use a clean pipet tip for each. Add **50 µL of Calibrators or Sample** extract to each well.
5. Dispense **50 µL of Antibody Solution** into each test well.
6. Shake the plate gently for 30 seconds and incubate the test wells for **30 minutes**.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with wash solution and dump. Repeat 3X for a total of four washes.
8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.