Beacon Analytical Systems Inc.

Saxitoxin Plate Kit

Cat. # 20-0173

Instructional Brochure

READ COMPLETELY BEFORE USE

INTENDED USE

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

USE PRINCIPLES

The Beacon Saxitoxin Plate Kit uses a polyclonal antibody that binds both saxitoxin and a saxitoxin-enzyme conjugate. Saxitoxin in the sample competes with the saxitoxin-enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

- Add saxitoxin-enzyme conjugate and calibrator or sample containing saxitoxin to a test well, followed by antibody solution. The conjugate competes with any saxitoxin in the sample for the same antibody binding sites. The test well is coated with anti-rabbit IgG to capture the rabbit anti-saxitoxin added.
- Wash away any unbound molecules, after you incubate this mixture for 30 minutes.
- Add colorless substrate solution to each well. In the presence of bound saxitoxin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of saxitoxin-enzyme conjugate molecules, a sample containing a low concentration of saxitoxin allows the antibody to bind many saxitoxin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of saxitoxin allows fewer saxitoxin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to saxitoxin concentration.
Darker color = Lower concentration
Lighter color = Higher concentration

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 strips of 8 wells coated with sheep anti-rabbit antibodies
1 Vial Negative Control – containing 2 mL of 0.0 ppb (µg/L) saxitoxin
3 Vials Saxitoxin Calibrators – containing 2 mL with concentrations of 0.02, 0.08 and 0.32 ppb of saxitoxin
1 Bottle Saxitoxin HRP Enzyme Conjugate – containing 8 mL
1 Bottle Anti-Saxitoxin Antibody Solution – containing 8 mL
1 Bottle Substrate – containing 14 mL
1 Bottle Stop Solution – containing 14 mL (Caution! 1N HCl. Handle with care.)
1 Bottle 10X Wash Solution – containing 50 mL (Must be diluted before use. See Assay Procedure Step 3.)

MATERIALS REQUIRED BUT NOT PROVIDED

- Clean running water or a wash bottle containing tap or deionized water
- Wash bottle
- Orbital shaker (optional)
- Pipette with disposable tips capable of dispensing 50 and 100 µL.
- Multi-channel pipette with disposable tips; 8-channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microtiter well plate or strip reader with a 450 nm filter
- Timer

*Additional materials may be required for sample preparation. See Sample Preparation Protocols.

SPECIFICITY

The % cross reactivity (% CR) of saxitoxin relative to related compounds is shown in the table below.

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.
- Do not freeze the plate kit components or expose them to temperatures greater than 37°C (99°F).
- Reagents should be brought to room temperature, 20 to 28ºC (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- 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.
- If running more than two strips at once, the use of a multichannel pipette is recommended.
- Use approved methodologies to confirm and positive results.

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 grams of the mussel tissue to a sieve and gently shake the sieve to drain the excess liquid.
6. Transfer the drained tissue to a clean container and homogenize until it resembles a soupy texture.
7. Tare a 50 mL conical tube and add 10 grams of the homogenized tissue.
8. Add 20 mL of methanol and vigorously shake the tube for 5 minutes.
9. Centrifuge the 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 the assay.*

*Proper dilution is based on level of contamination.

SAMPLE PREPARATION (LOBSTER TOMALLEY)

1. Transfer 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. Tare a 50 mL conical tube and add 5 grams of the mixed tomalley. 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. 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. Bring all kit reagents and samples to room temperature.
2. Remove the required number of antibody coated strips from the re-sealable foil bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
3. Prepare 1X wash solution by diluting the 10X concentrate (i.e. 50 mL of the 10X plus 450 mL of deionized water in a 500 mL wash bottle).
4. Dispense **50 µL of the Enzyme Conjugate** into each well.
5. Add **50 µL of the Calibrators, Control and Samples** into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
6. Dispense **50 µL of the Antibody Solution** into each well.
7. Shake the plate gently for 30 seconds using a back and forth motion.
8. Incubate the wells for **30 minutes** at room temperature.
9. Decant the contents of the wells into an appropriate waste container. Flood the wells completely with 1X wash solution, then decant. Repeat this wash step four times for a total of five washes.
10. Following the last wash, invert the plate onto absorbent paper and tap out as much of the wash solution as possible.
11. Add **100 µL of the Substrate** to each well.
12. Shake the plate gently for 30 seconds using a back and forth motion.
13. Incubate the wells at room temperature for **30 minutes.**
14. Add **100 µL of the Stop Solution** to each well in the same order of addition as the Substrate.

    WARNING: Stop Solution is 1N hydrochloric acid. Handle with care.
15. Measure and record the absorbance on a microtiter plate reader at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

CALCULATE RESULTS

1. It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation such as a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available.
2. 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 will have a concentration less than the concentration of the calibrator.
3. 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.
4. If the absorbance of a sample is lower than the highest calibrator (0.32 ppb), the concentration of saxitoxin is too high and out of range of the standard curve. Dilute the sample in 10% methanol/PBS and rerun. Samples should be diluted to fit into the standard curve (0.02 ppb to 0.32 ppb). Results must then be multiplied by the dilution factor used.

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 or contact us at info@beaconkits.com.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request 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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