

**Intended Use**

The Beacon Saxitoxin Plate Kit is a quantitative immunoassay for the detection of Saxitoxin in shellfish samples. This product is intended for research use only.

**Principles**

Saxitoxin HRP Enzyme Conjugate is pipetted into the Goat  $\alpha$ -Rabbit (GAR) coated test wells followed by the Calibrators, Controls, and the Sample Extract(s). A soluble polyclonal Rabbit  $\alpha$ -Saxitoxin antibody solution is then added to the test wells to initiate the reaction. During an incubation, Saxitoxin and Saxitoxin HRP Enzyme Conjugate compete for binding to the soluble polyclonal Rabbit  $\alpha$ -Saxitoxin antibody which is in turn immobilized on the GAR coated test wells. Following the incubation, the wells are washed to remove any unbound reagents. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Saxitoxin concentration of the sample is derived.

**Reagents and Materials Provided**

1 Unit	Plate containing 12 test strips of 8 GAR coated test wells each that are vacuum sealed in an aluminized pouch with a desiccant.
4 X 2 mL	Vials of Saxitoxin Calibrators (0, 0.02, 0.08, and 0.32 ppb).
1 X 8 mL	Bottle of Saxitoxin HRP Enzyme Conjugate.
1 X 8 mL	Bottle of Rabbit $\alpha$ -Saxitoxin Antibody.
1 X 50 mL	Bottle of 10X Sample Extraction Buffer (dilute prior to use).
1 X 50 mL	Bottle of 10X Wash Concentrate (dilute prior to use).
1 X 14 mL	Bottle of Substrate.
1 X 14 mL	Bottle of Stop Solution.

**Reagents and Materials Required but Not Provided**

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Materials for 1X sample extraction buffer preparation.
- Reagents and materials for 10 mM Phosphate Buffered Saline (PBS) preparation.
- Reagents and materials for 10% Methanol/10 mM PBS preparation.
- Materials for sample preparation.
- Materials for 1X wash solution preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

### Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after their expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), 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).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume 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 Antibody, Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- 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.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

### Specificity

The following table shows the percent cross reactivity relative to Saxitoxin.

Compound	% Cross-Reactivity
Saxitoxin	100
Saxitoxin Dihydrochloride	80.4
Neo-Saxitoxin	0.8
Decarbamoyl Saxitoxin	18
GTX 2 & 3	12
GTX 1 & 4	< 0.1
GTX 5	25.6
Decarbamoyl GTX 2 & 3	0.4
Decarbamoyl Neo-Saxitoxin	0.7
C 1 & 2	1.4

### Sensitivity

The following table shows the Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the assay.

Type of Shellfish	LOD (µg/100g)	LOQ (µg/100g)
Blue Mussel	3.9	13
Steamer	5.2	17
Mahogany Clam	4.9	16

**1X Sample Extraction Buffer Preparation** (preparation at least one day prior to use is recommended)

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Sample Extraction Buffer bottle to the container.
3. Tightly cap and gently swirl to mix.

**10 mM Phosphate Buffered Saline (PBS) Preparation**

1. Measure 1000 mL of laboratory quality distilled or deionized water and add to a clean container with a tight fitting lid.
2. Weigh 0.34 g of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  (F.W. 137.99) and add to the container.
3. Weigh 1.08 g of  $\text{Na}_2\text{HPO}_4$  (F.W. 141.96) and add to the container.
4. Weigh 8.5 g of  $\text{NaCl}$  and add to the container.
5. Gently stir to mix until completely dissolved (pH of the solution should be  $7.2 \pm 0.1$ ).

**10% Methanol/10 mM PBS Preparation**

1. Measure 90 mL of 10 mM PBS for each 100 mL being prepared and add to the container.
2. Measure 10 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix. Store tightly sealed to minimize evaporative loss.

## Sample Preparation

### Shellfish (Eco-Friendly Acetate Acid Buffer Extraction):

1. Thoroughly clean the outside of the shellfish with laboratory quality distilled or deionized water.
2. Cut the adductor muscles of the mussel or scallop using a sharp knife.
3. Rinse off the inside of the shellfish with laboratory quality distilled or deionized water to remove sand and other foreign substances.
4. Detach the tissue from the shellfish shell by removing the tissue and adductor muscles that connect it at the hinge.
5. Weigh 120 – 150 g of shellfish tissue and transfer to a sieve.
6. Gently shake the sieve to drain the excess liquid.  
Note: For scallops, only keep the viscera and roe for testing. Exclude the scallop muscles.
7. Transfer the drained tissue to a clean, 500 mL container and homogenize to a soupy texture.
8. Weigh 2 g of homogenized tissue into a 50 mL conical tube (M (Mass) = 2 g).
9. Measure 10 mL of 1X Sample Extraction Buffer and add to the conical tube (V (Volume) = 10 mL).
10. Vigorously shake the conical tube or vortex for 2 minutes.
11. Centrifuge for 20 minutes at approximately 3,000 X g to pellet the contents.
12. Transfer the supernatant to a microcentrifuge tube and centrifuge a second time for 2 - 5 minutes at approximately 12,000 X g to pellet any remaining debris.  
Note: Avoid the top lipid layer when transferring as it may cause erroneous results.
13. Dilute the supernatant 1:50 in 10% methanol/10 mM PBS (DF<sub>1</sub> (Dilution Factor 1) = 50) and use in the assay.  
Note: Samples that contain, or are expected to contain, concentrations of Saxitoxin too high for quantification should be further diluted in 10% methanol/10 mM PBS to fit into the standard curve. This additional dilution is referred to as DF<sub>2</sub> (Dilution Factor 2).

### Shellfish (Methanol Extraction):

1. Thoroughly clean the outside of the shellfish with laboratory quality distilled or deionized water.
2. Cut the adductor muscles of the mussel or scallop using a sharp knife.
3. Rinse off the inside of the shellfish with laboratory quality distilled or deionized water to remove sand and other foreign substances.
4. Detach the tissue from the shellfish shell by removing the tissue and adductor muscles that connect it at the hinge.
5. Weigh 120 – 150 g of shellfish tissue and transfer to a sieve.
6. Gently shake the sieve to drain the excess liquid.  
Note: For scallops, only keep the viscera and roe for testing. Exclude the scallop muscles.
7. Transfer the drained tissue to a clean, 500 mL container and homogenize to a soupy texture.
8. Weigh 5 g of homogenized tissue into a 50 mL conical tube (M (Mass) = 5 g).
9. Measure 10 mL of 100% methanol and add to the conical tube (V (Volume) = 10 mL).
10. Vigorously shake the conical tube or vortex for 2 minutes.
11. Centrifuge for 20 minutes at approximately 3,000 X g.
12. Transfer the supernatant to a clean glass vial.
13. Dilute the supernatant 1:10 in 10 mM PBS followed by a 1:5 dilution in 10% Methanol/10 mM PBS and use in the assay (DF<sub>1</sub> (Dilution Factor 1) = 50).  
Note: Samples that contain, or are expected to contain, concentrations of Saxitoxin too high for quantification should be further diluted in 10% methanol/10 mM PBS to fit into the standard curve. This additional dilution is referred to as DF<sub>2</sub> (Dilution Factor 2).

### **1X Wash Solution Preparation** (preparation at least one day prior to use is recommended)

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Wash Concentrate bottle to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

### **Assay Procedure**

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **50  $\mu$ L of Enzyme Conjugate** into each well.
4. Dispense **50  $\mu$ L of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50  $\mu$ L of Antibody** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution 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. Alternatively, the last wash can be done using laboratory quality distilled or deionized water to reduce interference associated with wash solution bubbles.
8. Dispense **100  $\mu$ L of Substrate** into each well.
9. Incubate for **30 minutes** at room temperature.
10. Dispense **100  $\mu$ L of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used test wells in an appropriate waste container.

### **Quality Control (QC) Criteria**

- The correlation coefficient ( $R^2$ ) of the calibration curve, analyzed using a 4-parameter logistic regression, must be  $\geq 0.99$ .
- The average absorbance of the zero calibrator replicates must be  $\geq 1.0$ .
- The average absorbance of calibrator replicates must have a coefficient of variation (%CV)  $< 15\%$ .
- The average absorbance of sample replicates must have a coefficient of variation (%CV)  $< 20\%$ .

## Result 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 calculations is available upon request. Please contact Beacon for further details.

To ensure the validity of the results, please adhere to the following:

- Ensure QC criteria are met.
- The concentration of Saxitoxin in a sample is determined by comparing the average sample absorbance to the standard curve.
- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in 10% methanol/10 mM PBS to fit into the standard curve and retest alongside the calibrators. This additional dilution is referred to as DF<sub>2</sub> (Dilution Factor 2).
- The Saxitoxin (STX) concentration in the sample can be converted to µg of STX diHCl equivalent/100 g by using the following equation:

$$\frac{\mu\text{g of STX diHCl equiv.}}{100\text{ g}} = 1.244 \times \text{STX Conc.} \times \frac{(DF_1 \times DF_2) \times (M + V)}{10 \times M}$$

$$1.244 = \frac{\text{Molecular weight of Saxitoxin diHCl equivalent (372.2 g/mol)}}{\text{Molecular weight of Saxitoxin (299.29 g/mol)}}$$

STX Conc. = Saxitoxin concentration in the sample in ppb

M = Mass of the homogenized sample in grams

V = Volume of extraction solution added to the homogenized sample

DF<sub>1</sub> = Dilution of the supernatant

DF<sub>2</sub> = Any additional dilution of the sample

$$10 = \frac{1\text{ Kg}}{100\text{ g}}$$

**Technical Assistance**

For questions regarding this kit or for additional information about Beacon products, contact us.

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**Safety**

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

**General Limited Warranty**

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