# Saxitoxin ELISA Kit

*Competitive enzyme immunoassay kit for quantitative analysis of Saxitoxin*

## Catalog Number: EL2048-01

**For Research Use Only. Not for use in Diagnostic Procedures.**

## 1. Background

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

## 2. Test Principle

The 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

## 3. Applications

This kit can be used for rapid test of Saxition in samples such as mussels and lobster tomalley.

## 4. Components Provided in This Kit

- Plate (1 ea) – containing 12 strips of 8 wells coated with sheep anti-rabbit antibodies
- Negative Control (1 x vial) – containing 2 mL of 0.0 ppb (µg/L) saxitoxin
- Saxitoxin Calibrators (3 x vials) – containing 2 mL with concentrations of 0.02, 0.8 and 0.32 ppb of saxitoxin
- Saxitoxin HRP Enzyme Conjugate (1 BTL) – containing 8 mL
- Anti-Saxitoxin Antibody Solution (1 BTL) – containing 8 mL
- Substrate (1 BTL) – containing 14 mL
- Stop Solution (1 BTL) – containing 14 mL
- 10X Wash Solution (1 BTL) – containing 50 mL
  *Must be diluted before use. See Assay Procedure Step 3.

## 5. Equipment and Reagents Needed (not provided)

- ELISA reader (450nm)
- Deionized water
- Vortex mixer
- Timer
- Wash bottle
- Absorbent material
- Micropipettes: 20µl-200µl, 100µl-1000µl
- Multi-channel pipette: 8-channel (50 & 100 µl)

## 6. Specificity

The % cross reactivity 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 % |

## 7. Sample Preparation

### For Mussels

- Thoroughly clean the outside of the mussels with laboratory grade water.
- Cut the adductor muscles of the mussels using a sharp knife.
- Rinse off the inside of the mussels with laboratory grade water to remove sand and other foreign substances.
- Detach the tissue from the mussel shells by removing the tissue and adductor muscles that connect it at the hinge.
- Transfer 120-150 g of the mussel tissue to a sieve and gently shake the sieve to drain the excess liquid.
- Transfer the drained tissue to a clean container and homogenize until it resembles a soupy texture.
- Tare a 50 mL conical tube and add 10 grams of the homogenized tissue.
- Add 20 mL of methanol and vigorously shake the tube for 5 minutes.
- Centrifuge the tube for 20 minutes at 5,000 rpm.
- Transfer the clear supernatant to a clear glass vial for storage.

- Dilute extracts 1:40 in 10 mM phosphate buffer before running in the assay.*

  *Proper dilution is based on level of contamination.

**For Lobster Tomalley**

- Transfer the tomalley from the cooked lobster into a clean beaker.
- Mix the greenish tomalley thoroughly with a spatula until it turns to a homogenous green paste.
- 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.
- 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.
- Centrifuge for 5 minutes at 10,000 rpm.
- Dilute the supernatant into 10 mM PBS buffer before running assay.
- 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).

## 8. Assay Procedure

- Bring all kit reagents and samples to room temperature.
- 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.
- 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).
- Dispense **50 µL of the Enzyme Conjugate** into each well.
- 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.
- Dispense **50 µL of the Antibody Solution** into each well.
- Shake the plate gently for 30 seconds using a back and forth motion.
- Incubate the wells for **30 minutes** at room temperature.

- 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. Invert the plate onto absorbent paper and tap out as much of the wash solution as possible.
- Add **100 µL of the Substrate** to each well.
- Shake the plate gently for 30 seconds using a back and forth motion.
- Incubate the wells at room temperature for **30 minutes**.
- 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 carefully.**
- 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.
- For a microtiter plate reader that has data reduction capabilities, use a 4-parameter curve fit. Alternatively, a semi-log fit can also be used if a 4-parameter curve fit is not available.
- 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.

## 9. Results

- 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.

- 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. 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.

## 10. General Instructions

- **Temperature of Reagents and Samples**
  - The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃). The antibody solution should be stored at 4℃, which will be used immediately after taking out. If the antibody solution is return to room temperature before assay, the OD values will be higher, and the result of the assay will not be right.
- **Microwells**
  - Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
- **Shaking of Reagents**
  - Shake each reagent gently before use.
- **Skin Protection**
  - Keep your skin away from the Stop Solution for it is the 1 N HCl solution.
- **Out of Date Kits**
  - Don't use kits that are out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
- **General Comments**
  - Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
- **Special Issues Concerning Solutions and Reagents**
  - Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).
- **Incubation Temperatures**
  - Incubation temperature should be at room temperature (20-28℃). Higher or lower temperature on day of testing will lead to experiment-to-experiment changes.

## 11. Safety

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

### Who we are

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

Contact Us

3913 Todd Lane, Suite 310
Austin, TX 78744

Phone: 512- 333-1330

Email: sales@attogene.com

Web: www.attogene.com

EL2048-01.V1_20220118