

### Importance of Saxitoxin Determination

Saxitoxin is one of the "paralytic shellfish poisons" (PSP), produced by several marine dinoflagellates and fresh water algae. Contamination of shellfish with saxitoxin has been associated with harmful algal blooms throughout the world.

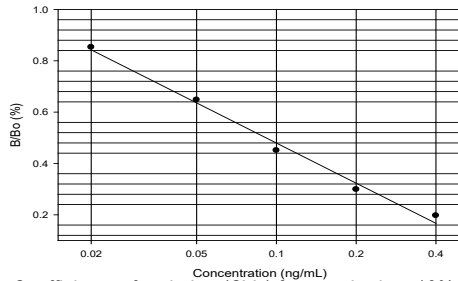
In man, PSP causes dose-dependent perioral numbness or tingling sensations and progressive muscular paralysis, which can result in death through respiratory arrest. The maximum tolerance levels established by the EU and FDA are 40-80 µg per 100 g edible portion of fresh, frozen, or tinned shellfish.

The Saxitoxin ELISA allows the determination of 42 samples in duplicate determination. Only a few milliliters of sample are required. The test can be performed in about 1 hour.

### Performance Data

Test sensitivity:

The detection limit for Saxitoxin is 0.015 ng/mL (mean of 6 blank determinations minus 3 SD). The middle of the test (50% B/B<sub>0</sub>) is at approximately 0.09 ng/mL. Determinations closer to the middle of the calibration curve give the most accurate results.



Test reproducibility:

Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity:

This ELISA recognizes Saxitoxin and other PSP toxins with varying degrees:

Cross-reactivities:

Saxitoxin (STX)	100% (per definition)
Decarbamoyl STX	29%
GTX 2 & 3	23%
GTX-5B	23%
Lyngbyatoxin	13%
Sulfo GTX 1 & 2	2.0%
Decarbamoyl GTX 2 & 3	1.4%
Neosaxitoxin	1.3%
Decarbamoyl Neo STX	0.6%
GTX 1 & 4	<0.2%

Cross-reactivities with other classes of algal toxins have not been observed.

Samples:

Drinking water, ground water, and surface water were tested for matrix effects in the ELISA. No matrix effects were determined.

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## Saxitoxin (PSP) ELISA, Microtiter Plate



Abraxis

Enzyme-Linked Immunosorbent Assay for the Determination of Saxitoxin (PSP) in Water and Contaminated Samples

Product No. 52255B

### 1. General Description

The Saxitoxin ELISA is an immunoassay for the quantitative and sensitive detection of Saxitoxin. Saxitoxin is one of the toxins associated with paralytic shellfish poisoning (PSP). This test is suitable for the quantitative and/or qualitative detection of Saxitoxin in water samples (please refer to the appropriate technical bulletins for freshwater and seawater samples) as well as other contaminated samples. For shellfish samples, a sample preparation is required. If necessary, positive samples can be confirmed by HPLC, GC/MS, or other conventional methods.

### 2. Safety Instructions

The standard solutions in the test kit contain small amounts of Saxitoxin. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

### 3. Storage and Stability

The Saxitoxin ELISA Kit should be stored in the refrigerator (2-8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the last day of the month as indicated by the expiration date on the box.

### 4. Test Principle

The test is a direct competitive ELISA based on the recognition of Saxitoxin by specific antibodies. Saxitoxin, when present in a sample, and a saxitoxin-enzyme conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (anti-rabbit) immobilized on the microtiter plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the Saxitoxin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA plate reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

### 5. Limitations of the Saxitoxin ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded.

Samples containing methanol must be diluted to a concentration < 20% methanol to avoid matrix effects.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Eurofins Abraxis Saxitoxin ELISA Kit provides screening results. As with any analytical technique (GC/MS, HPLC, etc.), positive samples requiring some action should be confirmed by an alternative method.

### Working Instructions

#### A. Materials Provided

1. Microtiter plate coated with a second antibody (anti-rabbit)
2. Standards (6) and Control: 0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL, 1.5 mL each
3. Control at 0.075 ± 0.015 ppb, 1.5 mL
4. Antibody Solution (rabbit anti-Saxitoxin), 6 mL
5. Saxitoxin-HRP Conjugate Solution, 6 mL
6. Sample Diluent (10X) Concentrate, 2 X 25 mL, must be diluted prior to use for shellfish or freshwater sample dilutions, see Test Preparation (Section E)
7. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section E)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 12 mL

## B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000  $\mu\text{L}$ )
2. Multi-channel pipette (10-300  $\mu\text{L}$ ), stepper pipette with plastic tips (10-300  $\mu\text{L}$ ), or electronic repeating pipette with disposable plastic tips
3. Deionized or distilled water
4. Container with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section E)
5. Microtiter plate washer (optional)
6. Microtiter plate reader (wave length 450 nm)
7. Shaker for microtiter plates (optional)
8. Materials and reagents for sample preparation
9. Seawater Matrix Saxitoxin Standards (please contact Eurofins Abraxis)

## C. Sample Preparation (Mussels and other shellfish)

**NOTE:** If a 100 g sample is needed for regulatory purposes, extraction solution volume should be adjusted accordingly.

1. Remove mussels from shells, wash with deionized water and homogenize.
2. Mix 10 g of homogenized mussels with 10 mL of 0.1M HCl and boil for 5 minutes while stirring.
3. Allow to cool. Centrifuge for 10 minutes at approximately 3500 g.
4. Collect supernatant. Adjust pH to < pH 4.0 with 5 N HCl.
5. Remove 10  $\mu\text{L}$  and dilute in 10 mL of 1X Sample Diluent (this will be a 1:1,000 dilution). Vortex.
6. Analyze as sample (Assay Procedure, step 1).

The STX concentration in the samples is determined by multiplying the ELISA result for the diluted extract by a factor of 2,000. Highly contaminated samples (those outside of the calibration range of the assay), must be diluted further and re-analyzed. We recommend further dilutions of 1:10 with 1X Sample Diluent. The dilution factor will then be 20,000. Samples with low levels of contamination of STX or samples that contain STX congeners with low cross-reactivity (see chart) can be detected in the assay by diluting samples 1:250 before analysis. The assay has low cross-reactivity against GTX 1 & 4, therefore food samples containing these congeners at low concentrations might be underestimated by this ELISA.

## D. Alternative Sample Preparation (Mussels and other shellfish)

1. Remove mussels from shells, wash with deionized water and homogenize using a Polytron or equivalent.
2. Mix 1.0 g of homogenized mussels with 6 mL of methanol/DI water (80/20) using a Polytron or equivalent.
3. Centrifuge the mixture for 10 minutes at 3000 g. Collect supernatant.
4. Add 2 mL methanol/deionized water (80/20) to the mussel tissue residue. Re-centrifuge the mixture for 10 minutes. Add supernatant to first portion.
5. Bring the volume of the collected supernatant to 10 mL with methanol/deionized water (80/20). Filter extract through a 0.45  $\mu\text{m}$  filter (Millex HV, Millipore or equivalent).
6. Remove 10  $\mu\text{L}$  and dilute to 1.0 mL with 1X Sample Diluent (1:100 dilution). Vortex. Analyze as sample (Assay Procedure, step 1).

The STX concentration in the samples is determined by multiplying the ELISA result by a factor of 1,000.

## E. Test Preparation

Micro-pipetting equipment and disposable pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the antibody, conjugate, substrate and stop solutions in order to equalize the incubation periods of the standard solutions and the samples on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, enzyme conjugate, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the 5X Wash Buffer Concentrate at a ratio of 1:5. If using the entire bottle (100 mL) add to 400 mL of deionized or distilled water.
5. Dilute the 10X Sample Diluent Concentrate at a ratio of 1:10 with deionized or distilled water (i.e. 1 mL of 10X Sample Diluent Concentrate into 9 mL of deionized water) as needed for sample dilutions.
6. The Stop Solution must be handled with care as it contains diluted  $\text{H}_2\text{SO}_4$ .
7. Freshwater samples must be preserved immediately upon collection to prevent loss of saxitoxin from the samples. Please refer to the Saxitoxin in Freshwater Sample Preparation Bulletin for details.
8. Seawater samples must be analyzed using Seawater Matrix Saxitoxin Standards (available separately, please contact Eurofins Abraxis) and an alternate Assay Procedure. Please refer to the Saxitoxin in Seawater Sample Analysis Bulletin for details.

## F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards

0; 0.02; 0.05; 0.10; 0.20; 0.40 ppb

Sam1, Sam2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	etc.									
B	Std 0	Std 4	etc.									
C	Std 1	Std 5										
D	Std 1	Std 5										
E	Std 2	Sam 1										
F	Std 2	Sam 1										
G	Std 3	Sam 2										
H	Std 3	Sam 2										

## G. Assay Procedure

1. Add **50  $\mu\text{L}$  of the standards, control, samples (preserved freshwater), or sample extracts (shellfish)** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add **50  $\mu\text{L}$  of enzyme conjugate solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add **50  $\mu\text{L}$  of antibody solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 to 60 seconds. Be careful not to spill the contents.
4. Incubate the strips for **30 minutes** at room temperature.
5. Decant the contents of the wells into an appropriate waste container. Wash the strips **four times** using the diluted wash buffer. Please use a volume of at least **250  $\mu\text{L}$  of 1X wash buffer** for each well and each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
6. Add **100  $\mu\text{L}$  of substrate (color) solution** to the wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 to 60 seconds. Be careful not to spill the contents. Incubate the strips for **30 minutes** at room temperature, protected from direct sunlight.
7. Add **100  $\mu\text{L}$  of stop solution** to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

## H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B<sub>0</sub> for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B<sub>0</sub> for each standard on a vertical linear (y) axis versus the corresponding Saxitoxin concentration on horizontal logarithmic (x) axis on graph paper. %B/B<sub>0</sub> for the control and samples will then yield levels in ppb of Saxitoxin by interpolation using the standard curve.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing lower concentrations of Saxitoxin than standard 1 (0.02 ng/mL) are considered as negative. Samples showing a higher concentration than standard 5 (0.4 ng/mL) must be diluted further to obtain accurate results. Results must be multiplied by the appropriate dilution factor for the sample extract (see Sample Preparation, Sections C and D) or preserved freshwater sample (see Saxitoxin in Freshwater Sample Preparation technical bulletin).

As with any analytical technique (GC/MS, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.