Importance of Saxitoxin Determination

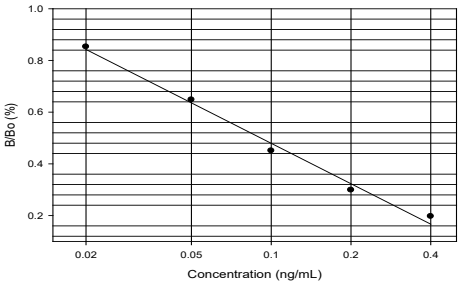
Saxitoxin is one of the "paralytic shellfish poisons" (PSP), produced by several marine dinoflagellates and freshwater algae. Contamination of shellfish with saxitoxin has been associated with harmful algal blooms throughout the world.

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 ABRAXIS® 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_0) is at approximately 0.09 ng/mL Determinations closer to the middle of the calibration curve give the most accurate results.



Test reproducibility: The standard curve R^2 must be ≥ 0.98. The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples and Control should be ≤15%. The Control should be within its acceptable range and Standard 0 absorbance value should be between 0.8 - 3.000.

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

Cross-reactivities:

Saxitoxin (STX) (as STX.diHCl)	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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ABRAXIS® Saxitoxin (PSP) ELISA Microtiter Plate

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

Product No. 52255B

1. General Description

The ABRAXIS® 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 as well as other contaminated samples. For shellfish and benthic mat samples, a sample preparation is required. Please see section C for the sample preparation procedure for shellfish. For benthic mat samples, please see the Gold Standard Diagnostics website for the appropriate technical bulletins for the sample preparation. 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 stop solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The ABRAXIS® Saxitoxin ELISA Kit should to 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. Consult state, local, and federal regulations for proper disposal of all reagents.

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 ABRAXIS® 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 cannot 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 include: 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, and extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The 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.

6. 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 STX-diHCl, 1.5 mL each
3. Control at 0.075 ± 0.015 ng/mL STX-diHCl, 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. ABRAXIS® 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 µL)
2. Multi-channel pipette (10-300 µL), stepper pipette with plastic tips (10-300 µL), 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 (wavelength 450 nm)
7. Shaker for microtiter plates (optional)
8. Materials and reagents for sample preparation
9. Seawater Matrix Saxitoxin Standards (please contact Gold Standard Diagnostics)

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 µL 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).

Please note that assay results are in STX eq., based on the STX.diHCl calibration standards.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 µm filter (Millex HV, Millipore or equivalent).
6. Remove 10 µL 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.

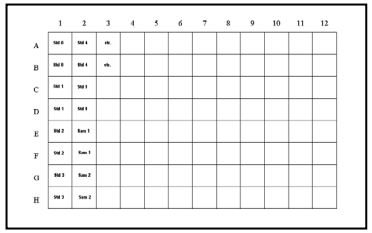
1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, enzyme conjugate, antibody, substrate, and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the ABRAXIS® Wash Buffer (5X) 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 H_2SO_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 Gold Standard Diagnostics) 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



G. Assay Procedure

1. **Add 50 µL of the standards, control, samples (preserved freshwater), or sample extracts (shellfish or benthic mats)** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. **Add 50 µL of conjugate solution** to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. **Add 50 µL 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. Remove the covering and decant the contents of the wells into a sink. Blot the inverted plate on a stack of paper towels. **Wash the strips four times using the diluted wash buffer.** Please use a volume of at least 250 µL of 1X wash buffer for each well and each washing step. Remaining buffer in the wells should be removed by blotting the inverted plate on a stack of paper towels after each washing step.
6. **Add 100 µL 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 µL 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

Please note that assay results are in STX eq., based on the STX.diHCl calibration standards.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 $\%B/B_0$ 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 $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding Saxitoxin concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ 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 each sample matrix (see Sample Preparation, Sections C and D, for shellfish, the Anatoxin-a and Saxitoxin in Benthic Mats technical bulletin for benthic mat samples, and the Saxitoxin in Freshwater Sample Preparation technical bulletin for preserved freshwater samples).

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