Importance of Saxitoxin Determination

Saxitoxin is one of the “paralytic shellfish poisons” (PSP), produced by several marine dinoflagellates and cyanobacteria (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/B0) 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 25%
- GTX 2 & 3 23%
- GTX-5B 23%
- Lyngbyatoxin 13%
- Sulfo GTX 1 & 2 2.9%
- 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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B. 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 period of the standards 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 (4-8°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 Solution 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 H2SO4.

C. Assay Procedure

1. Add 50 µL of the standard solutions, 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 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with paraffin 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 1X washing buffer solution. Please use a volume of at least 300 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of substrate solution to the wells successively using a multi-channel or stepping pipette. Cover the wells with paraffin 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 solution using a multi-channel or stepping pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

D. 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/B0 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/B0 for each standard on a vertical linear (y) axis versus the corresponding Saxitoxin concentration on horizontal logarithmic (x) axis on graph paper. %B/B0 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 this standard curve. Samples showing lower concentrations of Saxitoxin compared to 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.

E. Additional Materials

1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 µL)
2. Multi-channel pipette (10-300 µL) or stepper pipette with plastic tips (10-300 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)
6. Seawater Matrix Saxitoxin Standards (please contact Abraxis)

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.

G. Sample Preparation (Mussels)

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 1:10,000 dilution).
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 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.

H. Alternative Sample Preparation

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.