

Saxitoxin ELISA Kit

Cat. No.:DEIA6819

Pkg. Size:96T

Intended use

The Saxitoxin ELISA is an immunoassay for the quantitative and sensitive detection of Saxitoxin. This test is suitable for the quantitative and/or qualitative detection of Saxitoxin in water 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.

General Description

Saxitoxin is one of the toxins associated with paralytic shellfish poisoning (PSP). 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 ug per 100 g edible portion of fresh, frozen, or tinned shellfish.

Principle Of The Test

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 (sheep anti-rabbit) immobilized on the 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 reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Reagents And Materials Provided

1. Microtiter plate coated with a second antibody (sheep anti-rabbit).
2. Standards : 0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/ml
3. Antibody Solution (rabbit anti-saxitoxin), 6 ml
4. Saxitoxin-HRP Conjugate, 6 ml
5. Sample Diluent Concentrate (10X), 2 X 25 ml. Use to dilute samples
6. Wash Solution (5X) Concentrate, 100 ml
7. Color Solution (TMB), 12 ml
8. Stop Solution, 12 ml

Materials Required But Not Supplied

1. Micro-pipettes with disposable plastic tips (10-200, and 200-1000 ul)
2. Multi-channel pipette (10-300 ul) or stepper pipette with plastic tips (10-300 ul)
3. Microtiter plate washer

4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)

Storage

The Saxitoxin ELISA should to be stored in the refrigerator (4–8°C). The solutions have to be allowed to reach room temperature (20–25°C) before use. Reagents may be used until the expiration date on the box.

Specimen Collection And Handling

Preparation of Sample (Mussels):

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

1. Mussels are removed from their shells, washed with deionized water and homogenized.
2. Mix 10 gm of homogenized mussels with 10 ml of 0.1M HCl and boil for 5 minutes while stirring.
3. Allow to cool and centrifuge for 10 minutes at approximately 3500 g.
4. Adjust pH to < pH 4.0 with 5 N HCl.
5. Remove 10 ul and dilute to 10 ml with Sample Dilution Buffer (1:1000 dilution).
6. Run in the assay as sample.

The STX concentration in the samples is determined by multiplying the concentration of the diluted extract by a factor of 2000. Highly contaminated samples should be diluted further and re-analyzed, we recommend further dilutions of 1:10 with sample dilution buffer. The dilution factor will then be 20000. Samples with low 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.

Alternative Sample Preparation:

1. Mussels are removed from their shells, washed with deionized water, dried and homogenized using a Polytron or equivalent.
2. A 1.0 gm portion is then mixed with 6 ml methanol/DI water (80/20) using a Polytron or equivalent.
3. Centrifuge 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 um filter.
6. Remove 10 ul and dilute to 1.0 ml with sample Dilution Buffer (1:100 dilution), then analyze as samples. The STX concentration in the samples is determined by multiplying the concentration of the diluted extract by a factor of 1000.

Reagent Preparation

Micro-pipetting equipment and 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, the substrate solution and the stop solution 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 (4–8°C).
3. The standard solutions, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
4. Dilute the Wash Buffer at a ratio of 1:5. If using the entire bottle (100 ml) then add to 400 ml of deionized or distilled water.

Dilute the Sample Diluent at a ratio of 1:10 with deionized water.

5. The stop solution has to be handled with care as it contains diluted H₂SO₄.

6. 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.

Assay Steps

1. Add 50 ul of the standard solutions or the samples (water) or sample extracts (shellfish) into the wells of the test strips according to the working scheme given. The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards have to be run with each test. Never use the values of standards which have been determined in a test performed previously. We recommend using duplicates or triplicates.

Std 0-Std 5: Standards 0; 0.02; 0.05; 0.10; 0.20; 0.40 ppb

Sam1, Sam2, etc.: Samples

Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4			etc.	etc.						
B	Std 0	Std 4										
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										

2. Add 50 ul of enzyme conjugate solution to the individual wells successively using a multi- channel pipette or a stepping pipette.
3. Add 50 ul of antibody solution to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 30 min at room temperature.
5. Wash the strips four times using the washing buffer solution. Please use at least a volume of 300 ul 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 ul of substrate solution to the wells. The strips are incubated for 30 min at room temperature. Protect the strips from

direct sunlight.

7. Add 100 μ l of stop solution to the wells in the same sequence as for the substrate solution.

8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

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 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 concentration of Saxitoxin compared to standard 1 (0.02 ng/ml) are considered as negative. Samples showing a higher concentration than standard 6 (0.4 ng/ml) must be diluted further to obtain more accurate results.

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 close to the middle of the tests gives the most accurate results.

Specificity

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%

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.

Reproducibility

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

Precautions

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.

Limitations

Numerous organic and inorganic compounds commonly found in water 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 water samples, test interferences caused by matrix effects can't be completely excluded. Mistakes in handling the test also can 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 Saxitoxin ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.