

I. Evaluation

Evaluation of ELISA results may be performed using a spreadsheet macro available from Eurofins Abraxis or other commercial ELISA evaluation programs such as 4-parameter (preferred) or Log/Logit. For 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 µg/100 g 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 2 (40 µg/100 g) are considered as negative. Samples showing a higher concentration than standard 2 (40 µg/100 g) are considered positive.

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

Importance of Saxitoxin Determination

Saxitoxin, known as "paralytic shellfish poison" (PSP), is one of the toxins produced by several marine dinoflagellates and freshwater cyanobacteria. Contamination of shellfish with Saxitoxin has been associated with harmful algal blooms throughout the world.

In humans, PSP causes dose-dependent perioral numbness or tingling sensations and progressive muscular paralysis, which can result in death through respiratory arrest. The maximum guidance level established by the EU and FDA is 80 µg per 100 g of fresh, frozen, or tinned shellfish.

The PSP Shipboard ELISA kit allows for the determination of 42 samples in duplicate determination. The assay can be performed in about 1 hour.

Performance Data

Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.
Selectivity: This ELISA recognizes Saxitoxin and other PSP toxins to 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.		

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Changes made : Changed temperature for storage and added 4-parameter for evaluation

PSP Shipboard ELISA Kit, Microtiter Plate



Abraxis

Enzyme-Linked Immunosorbent Assay for the Determination
of Saxitoxin (PSP) in Shellfish Samples

Product No. 52255SB

1. General Description

The PSP Shipboard ELISA Kit 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 shellfish. A sample preparation is required (see Sample Preparation, Section E). Positive samples should be confirmed by HPLC, LC/MS, or other conventional methods as appropriate.

2. Safety Instructions

The standard solutions in the test kit contain small amounts of Saxitoxin. In addition, the color 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 PSP Shipboard 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 (see Test Preparation, Section F). 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 color 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 PSP Shipboard ELISA Kit, 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, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or color reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C), or exposure to direct or indirect sunlight during the color reaction.

The PSP Shipboard ELISA Kit provides screening results. As with any analytical technique (HPLC, LC/MS, etc.), positive samples requiring 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 (5): 0, 20, 40, 80, 120 µg/100 g, 1 mL each
3. Control at 60 µg/100 g, 1 mL
4. Reagent 1 (Saxitoxin-HRP Conjugate Solution), 6 mL
5. Reagent 2 [Antibody Solution (rabbit anti-Saxitoxin)], 6 mL
6. Wash Buffer (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section F)
7. Color Solution (TMB), 12 mL
8. Stop Solution, 12 mL

B. PSP Shipboard Accessory Pack Materials PN 530009 (available separately)

1. Diluent in dilution vials with blue stickers, 20, with labels (Dilution 1)
2. Diluent in dilution vials with red stickers, 20, with labels (Dilution 2)
3. 4 mL glass vials with caps, 20, with labels (Sample Extract)
4. Pipette tips, 1 rack of 96, 10-200 µL
5. Plastic transfer pipettes, 20
6. Microtiter plate frame with strip of blank wells (for zeroing reader)
7. Adhesive plate covers, 3
8. Simplified qualitative procedure/flow chart, data sheets (5), graph papers (5)

C. Additional Materials (not provided with the test kit)

1. Fixed volume 50 and 100 µL micro-pipettes with disposable plastic tips
2. Deionized or distilled water
3. Squeeze wash bottle with 500 mL capacity (for diluted 1X Wash Buffer, see Test Preparation, Section F)
4. Fisherbrand™ 3D Platform Rotator (Fisher catalog # 88-861-04540) for plate mixing
5. Strip/tube combo reader or microtiter plate reader (wavelength 450 nm)
6. Timer
7. Absorbent paper towels
8. Materials for sample preparation:
 - a. Shucking knife
 - b. Strainer (#10 sieve)
 - c. Plastic tablecloth (to protect work area)
 - d. Deionized or distilled water (for rinsing samples prior to homogenization)
 - e. Immersion blender or appropriate grinder
 - f. 600 mL plastic beaker (VWR 83008-810)
 - g. Permanent marker
 - h. Conical tubes with caps (VWR # 21008-169)
 - i. 125 mL plastic container with lid (VWR# 89202-838) or Ziploc bag
 - j. 25 mL disposable plastic pipettes (VWR # 89130-900) with Pipetting device (VWR # 53502-244) or 25 mL graduated cylinder (VWR # 83008-874 or 83008-870)
 - k. Paint filters or coffee filters
9. Reagents for sample preparation:
 - a. Isopropyl alcohol/white vinegar extraction solution – Combine 5 parts rubbing alcohol (70% isopropyl alcohol) and 2 parts white vinegar (5% acetic acid). Mix thoroughly. Store in a tightly capped container at room temperature.
 - b. 10% bleach solution (for cleaning equipment between samples)

D. Sample Collection and Storage

1. Fill out all necessary collection data.
2. Harvest shellfish as follows:

Note: A minimum of 150 g of meat for each sample should be processed

 - a. Blue mussels – 30 mussels per sample
 - b. Littleneck clams – 1.5" size – 20 clams per sample
 - c. Butter clams – > 3" size – 5 per sample; < 2" size – at least 12 per sample
 - d. Surf clams – > 3" size - at least 12 per sample
 - e. Other shellfish – at least 20 per sample
3. Verify all data sheets have been completed after sampling.
4. Place shellfish into a plastic Ziploc bag with the data sheet.
5. For on-site or testing within 2 days, store shellfish in refrigerator (2-8°C). For storage greater than 2 days, samples must be homogenized and stored frozen until extraction.

E. Shellfish Sample Preparation, Extraction, and Dilutions

Note: Thoroughly clean the immersion blender and beaker with the 10% bleach solution between samples to prevent contamination.

1. Thoroughly rinse the outside of the shellfish with deionized or distilled water to remove any sand or mud.
2. Open the shellfish with the shucking knife by cutting the adductor muscles. Remove the desired tissue and place in the strainer.
3. Rinse tissue with fresh water to remove any grit or shell fragments. Drain thoroughly (about 5 minutes).
4. Transfer the sample to a 600 mL beaker and puree with immersion blender for 1 minute or until the entire sample is homogenized.
5. Using a 25 mL disposable plastic pipette and pipetting device, transfer 10 mL of the homogenized sample to an appropriately labeled 50 mL conical tube. Transfer the remaining sample to an appropriately labeled plastic container, cap tightly, and freeze.
6. Using a clean pipette or graduated cylinder, add 10 mL of the isopropyl alcohol/white vinegar extraction solution to the 10 mL of sample in the conical tube (1:1 ratio). Cap tightly and shake vigorously for 30 seconds.

Note: If the pipette or graduated cylinder comes in contact with any sample, obtain a new pipette or thoroughly clean the graduated cylinder with the 10% bleach solution before using for additional samples to avoid contamination.

7. Filter the sample extract through the paint/coffee filter into a clean, appropriately labeled plastic container or measuring cup. This extract can then be diluted and tested immediately, stored refrigerated (2-8°C) up to 2 days, or frozen for long-term storage.

Note: The following steps use the materials contained in the PSP Shipboard Accessory Pack.

8. Using a disposable plastic transfer pipette, transfer about 1 mL of the filtered extract to an appropriately labeled 4 mL glass vial.
9. Add 100 µL of the extract to an appropriately labeled blue stickered dilution vial (Dilution 1). Cap and shake well.
10. Add 100 µL of Dilution 1 (from step 9) to an appropriately labeled red stickered dilution vial (Dilution 2). Cap and shake well. Analyze Dilution 2 as the sample (see Assay Procedure, Section H, Step 1)

F. Test Preparation

Micro-pipetting equipment and disposable pipette tips for pipetting the standards, samples, Reagent 1, Reagent 2, color, and stop solutions are necessary. Use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. **Remove the foil bag containing the microtiter plate and all reagents from the kit box. Remove all reagent bottles and vials from the protective foam. Allow the microtiter plate and reagents to sit at room temperature (20-25°C) for at least 2 hours 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. After analysis, store the remaining kit in the refrigerator (2-8°C).
3. The standard solutions, Reagent 1, Reagent 2, color, 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. Empty the entire contents of the 5X Wash Buffer Concentrate into the squeeze wash bottle and fill to the neck with deionized or distilled water.
5. The Stop Solution must be handled with care as it contains diluted H₂SO₄.

G. 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.

	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	Contr.										
D	Std 1	Contr.										
E	Std 2	Sam 1										
F	Std 2	Sam 1										
G	Std 3	Sam 2										
H	Std 3	Sam 2										

Std 0-Std 4: Standards
0, 20, 40, 80, 120 µg/100 g

Cont.: Control - 60 µg/100 g

Sam 1, Sam 2, etc.: Samples

H. Assay Procedure

1. Add **50 µL of the standards, control, or sample extract (Dilution 2)** into the wells of the test strips using a fixed volume 50 µL micropipette according to the working scheme above. We recommend using duplicate or triplicate wells for each standard, control, and sample.
2. Add **50 µL of Reagent 1** to the individual wells successively using a fixed volume 50 µL micropipette.
3. Add **50 µL of Reagent 2** to the individual wells successively using a fixed volume 50 µL micropipette. 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. **Place the strip holder on the Fisherbrand™ 3D Platform Rotator and mix at 80 RPM for 30 minutes at room temperature.** Protect from direct or indirect sunlight.
5. Remove the covering, decant the contents of the wells into an appropriate waste container. Blot the inverted plate on a stack of absorbent paper towels. Wash the strips **four times** using the **diluted 1X wash buffer. Blot the inverted plate after each wash step** on a stack of paper towels. For each washing step, flood the wells with the diluted 1X wash buffer using a squeeze wash bottle. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. Add **100 µL of color solution** to the wells successively using a fixed volume 100 µL micropipette. 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.
7. **Place the strip holder on the Fisherbrand™ 3D Platform Rotator and mix at 80 RPM for 30 minutes at room temperature.** Protect from direct or indirect sunlight.
8. Add **100 µL of stop solution** to the wells in the same sequence as for the color solution using a fixed volume 100 µL micropipette.
9. Read the absorbance at 450 nm using a microplate or manual well strip ELISA photometer within 15 minutes after the addition of the stop solution.