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Saxitoxin (PSP) Extraction Kit for Shellfish

For Use With
Saxitoxin (PSP) Strip Test (520044/520045)

Product No. 520047

1. General Description

The extraction procedure detailed in this insert is a process in which toxins are released from prepared shellfish tissue and converted into a liquid sample matrix prior to analysis. The PSP Extraction Kit for Shellfish is intended to extract Saxitoxin from shellfish samples prior to testing with the Eurofins Abraxis PSP (Saxitoxin) Strip Test (not included).

2. Safety Instructions

After analysis, unused shellfish samples and extracts should be discarded according to local, state, and federal regulations.

3. Storage and Stability

The PSP Extraction Kit for Shellfish should be stored between 2-30°C when not in use. The extraction kit and shellfish samples should be allowed to come to room temperature before use. For testing within 2 days of collection, store unprocessed shellfish in a refrigerator (2-8°C). For storage greater than 2 days after sample collection, samples must be homogenized and stored frozen (-20°C) until extraction.

4. Limitations of the PSP Extraction Kit

This procedure is intended for use with shellfish samples. Other matrices should be thoroughly validated before use with this procedure.

5. Warnings and Precautions

- For testing within 2 days of harvesting, store shellfish in refrigerator (2-8°C). For storage greater than 2 days, shellfish samples must be homogenized (as described in Section D, steps 1-7) and stored frozen (-20°C) until extraction.
- The extraction kit is designed to be used with fresh shellfish, shellfish that has been stored refrigerated for less than two days or homogenized shellfish tissue that has been stored frozen for greater than 2 days. Reagents as well as refrigerated and frozen samples need to be allowed to reach room temperature before extraction.
- Verify the extraction kit is within expiration by inspecting the date on the kit box prior to use.
- For test strips packaged in a desiccated vial, the vial should be kept completely closed except for opening to remove test strips. When re-closing, snap lid firmly.
- Thoroughly clean the immersion blender and beakers with 10% bleach solution between samples to prevent cross-contamination that could produce inaccurate results.
- As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. 50 mL Conical Tubes
2. Gauze Pads and Weighing Dishes
3. Saxitoxin Extraction Solution, 60 mL bottle
4. Extraction Collection Bottles
5. Sample Dilution Bottles, labeled “A”, “B”, and “C”, 9 mL each (1 set for each sample to be tested)
6. Transfer Pipettes

B. Materials and Reagents Required (and not provided)

1. Shucking knife
2. Strainer (#10 sieve)
3. Plastic tablecloth (for protecting work area)
4. Deionized or distilled water (for rinsing samples prior to homogenization)
5. Immersion blender or equivalent electronic blender
6. 600 mL plastic beaker
7. Permanent marker
8. 10% Bleach solution (for cleaning equipment between samples)
9. PSP Strip Test for Water Kit (PN 520044/520045)

C. Sample Collection and Storage

1. Record collection data as necessary.
2. Harvest shellfish as follows:

Note: A minimum of 150 g of shellfish tissue should be collected and processed for each sample. Below are the approximate quantities of each species that will provide a 150 g sample.

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 samples

D. Sample Preparation and Extraction

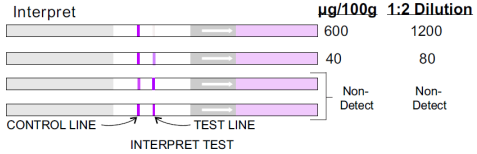
1. Thoroughly rinse the outside of the shellfish with deionized or distilled water to remove any sand or mud.
2. Open the shellfish with the shucking knife and rinse contents with deionized or distilled water to remove any sand and other foreign material. Cut the adductor muscle to remove the desired tissue and collect into a 600 mL beaker.
3. Repeat steps 1 and 2 until 120-150 mL of desired tissue is collected.
4. Transfer the tissue to a #10 sieve to drain the sample. Allow to drain for 5 minutes.
5. Remove any remaining shell pieces and fragments and discard along with the drainage.
6. Transfer the sample to a 600 mL beaker and puree with the immersion blender or equivalent electronic blender for 1 minute or until the entire sample is homogenized.
7. Using a clean shucking knife, transfer the homogenized sample to a 50 mL conical tube until the sample fills up to the 10 mL gradation mark. Transfer the remaining sample to an appropriately labeled plastic container, and cap tightly. This homogenized sample can then be diluted and tested immediately, stored refrigerated (2-8°C) for up to 2 days, or frozen (-20°C) for long-term storage.
8. When ready to extract samples, use the gradations on the 50 mL conical tube containing the sample to fill the conical tube to the 20 mL gradation with Saxitoxin Extraction Solution (10 mL of Saxitoxin Extraction Solution in addition to the 10 mL sample). Cap and shake well.
9. Filter the diluted sample extract through the filter provided into a clean, appropriately labeled plastic container or measuring cup.
10. When samples are to be analyzed:
    - 10.1 Using a new graduated disposable pipette for each sample, draw the filtered extract to the 1 mL line (graduation mark slightly below bulb) and add 1 mL of filtered extract into Sample Dilution Bottle “A”. Cap, label bottle with sample number/ID and shake well.
    - 10.2 Using a new graduated disposable pipette, draw the diluted extract from Sample Dilution Bottle “A” to the 1 mL line (graduation mark slightly below bulb) and add to Sample Dilution Bottle “B”. Cap, label bottle with sample number/ID and shake well.
    - 10.3 Using a new graduated disposable pipette, draw the diluted extract from Sample Dilution Bottle “B” to the 1 mL line (graduation mark slightly below bulb) and add to Sample Dilution Bottle “C”. Cap, label bottle with sample number/ID and shake well.
    - 10.4 Proceed immediately with **Section E. Sample Preservation Procedure** in the PSP (Saxitoxin) Strip Test insert (PN 520044/520045). Following analysis using the procedure described in the PSP (Saxitoxin) Strip Test insert, results for shellfish tissue should be evaluated as described below.

E. Evaluation of Results

Sample concentrations are determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the control line indicates a result which is below the limit of detection of the test. Test strips with a test line which is lighter than the control line indicates a result which is between 40 µg/100 g and 600 µg/100 g total PSP concentration in sample. Test strips with a very faint test line or no test line visible indicates a result which is > 600 µg/100 g. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	Very light intensity or no test line present	≥600 ng/mL (ppb)
Control line present	Test line intensity ≥control line	≤40 ng/mL (ppb)

The appearance of test strips may also be compared to the illustration below to determine approximate sample concentration ranges. *Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents.* Do not use strips run previously to determine sample concentrations, as test line intensities may vary once strips are completely dry.



Performance Data

The Eurofins Abraxis Paralytic Shellfish Poison (PSP) Strip Test for shellfish samples will detect in the range of 40-600 µg/100 g, as the sample is diluted 2000 fold during extraction. At this level, the test line exhibits moderate intensity. At levels greater than 600 µg/100 g, the test line is faint or not visible. For sample screening requiring a higher concentration range (greater than 40 – 600 µg/100 g) or for retesting of samples which exceed the standard assay detection range (≥ 600 µg/100 g) that require a more definitive result, samples can be diluted pior to addition to the Sample Preservation Vials, as mentioned in the PSP (Saxitoxin) Strip Test insert. The detection range for the shellfish extraction kit is then determined by multiplying the standard concentration range by the dilution factor of the samples. For example, a sample that has been extracted up to Step 10.4 of **Section D. Sample Preparation and Extraction** which tests positive (> 40 µg/100 g) can be diluted 1:2 (1 mL of Sample Dilution Bottle “C” into 1 mL of distilled or deionized water) before proceeding to the PSP (Saxitoxin) Strip Test Insert (PN 520044/520045). Test results for the 1:2 dilution similar to the 40 µg/100 g result pictured above would then indicate a positive sample with a saxitoxin concentration of 80 µg/100 g. Any furhter dilution of the sample would be outside of the detection range of the strip test and will produce a negative result.

Importance of Saxitoxin Determination

Saxitoxin is one of the toxins associated with paralytic shellfish poisoning (PSP). It is 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 in fresh, frozen, or tinned shellfish.