Saxitoxin ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of Saxitoxin

Catalog Number: EL2048-04

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

The Saxitoxin Plate Kit is a competitive ELISA for the quantitative analysis of saxitoxin in shellfish samples.

2. Test Principle

The Saxitoxin Plate Kit uses a polyclonal antibody that binds both saxitoxin and a Saxitoxin-enzyme conjugate. Saxitoxin in the sample competes with the Saxitoxin-enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

- Add Saxitoxin-enzyme conjugate and calibrator or sample containing Saxitoxin to a test well, followed by antibody solution. The conjugate competes with any saxitoxin in the sample for the same antibody binding sites. The test well is coated with anti-rabbit IgG to capture the rabbit anti-Saxitoxin added.
- Wash away any unbound molecules, after you incubate this mixture for 30 minutes.
- Add colorless substrate solution to each well. In the presence of bound Saxitoxin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.
- Since the same number of antibody binding sites are available in every well, and each well receives the same number of Saxitoxin-enzyme conjugate molecules, a sample containing a low concentration of saxitoxin allows the antibody to bind many Saxitoxin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of saxitoxin allows fewer Saxitoxin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to saxitoxin concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

3. Applications

This kit can be used for rapid test of Saxitoxin in samples such as mussels and lobster tomalley.

4. Components Provided in This Kit

- Microtiter plate with 96 wells coated with second antibody
- Saxitoxin Standards: (4 vials × 0.8mL/vial): 0ppb (green cap), 0.02ppb (purple cap), 0.08ppb (yellow cap), 0.32ppb (blue cap)
- Conjugated HRP: 5mL
- Saxitoxin Antibody: 5mL
- 20X Wash Solution: 28mL
- TMB Substrate: 12mL
- Stop Solution: 14mL
- 10X Sample Extraction Buffer: 25mL

5. Equipment and Reagents Needed (not provided)

- ELISA reader (450nm)
- Deionized water
- Methanol
- Homogenizer
- Vortex mixer
- Centrifuge
- Timer
- Wash bottle
- Absorbent material
- Polystyrene centrifuge tube: 50ml, 2ml
- Micropipettes: 20μl-200μl, 100μl-1000μl
- Multi-channel pipette: 8-channel (50 & 100 μl)

6. Specificity

The % cross reactivity of saxitoxin relative to related compounds is shown in the table below.

Compound	% CR
Saxitoxin dihydrochloride	100%
Neosaxitoxin	0.8%
Decarbamoyl STX	18%
GTX 2 & 3	12%
GTX 1 & 4	<0.1 %
Decarbamoyl GTX 2 & 3	0.4 %
Decarbamoyl NeoSTX	0.7 %

7. Reagents Preparation

- Prepare 1X wash solution by diluting the 20X Wash Solution: Mix 1 volume of the 20X Wash Solution with 19 volumes of deionized water.
- Prepare 1X Sample Extraction Buffer by mixing 1 volume of 10x Sample Extraction Buffer with 9 volumes of deionized water.

8. Sample Preparation

For Mussels

- Thoroughly clean the outside of the mussels with laboratory grade water.
- Cut the adductor muscles of the mussels using a sharp knife.
- Rinse off the inside of the mussels with laboratory grade water to remove sand and other foreign substances.
- Detach the tissue from the mussel shells by removing the tissue and adductor muscles that connect it at the hinge.
- Transfer 120-150 g of the mussel tissue to a sieve and gently shake the sieve to drain the excess liquid.

- Transfer the drained tissue to a clean container and homogenize until it resembles a soupy texture.
- Tare a 50 mL conical tube and add 10 grams of the homogenized tissue.
- Add 20 mL of 100% methanol and vigorously shake the tube for 5 minutes.
- Centrifuge the tube for 20 minutes at 5,000 rpm.
- Transfer the clear supernatant to a clear glass vial for storage.
- Dilute extracts 1:40 in 10 mM phosphate buffer before running in the assay.*

  *Proper dilution is based on level of contamination.

For Lobster Tomalley

- Transfer the tomalley from the cooked lobster into a clean beaker.
- Mix the greenish tomalley thoroughly with a spatula until it turns to a homogenous green paste.
- Tare a 50 mL conical tube and add 5 grams of the mixed tomalley. Add 40 mL of 0.1 N HCl. Vortex vigorously for 2 minutes.
- Filter 10-15 mL of the upper layer of the extract through a Whatman #4 paper filter. Transfer 1.5 mL of the filtered extract into a microcentrifuge tube.
- Centrifuge for 5 minutes at 10,000 rpm.
- Dilute the supernatant into 10 mM PBS buffer before running assay.
- Due to the unknown concentration of toxin in the samples, a range of dilutions is suggested (i.e., 1:10, 1:20, and 1:40).

9. Assay Procedure

Instructions Prior to Beginning Assay

1. Ensure that all reagents and microwells are at room temperature (20-25°C).
2. Return all reagents to 2-8°C immediately after their use.
3. Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
4. Avoid direct sunlight during the incubation.

Steps in the Assay Process

- Take all reagents out at room temperature (20-25°C) for more than 30 minutes. Shake gently before use.
- Take out the needed microwells and return the rest into the zip-lock bag at 2-8°C immediately.
- The diluted wash solution should be brought to room temperature before use.
- Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
- Dispense **50 µL of the Saxitoxin Standards, positive control, or sample** into each well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
- Add **50 µL of the Conjugated HRP** into each well.
- Dispense **50 µL of the Saxitoxin Antibody** into each well.
- Shake the plate gently for 30 seconds using a back and forth motion.
- Cover the plate. Incubate for **30 minutes** at room temperature.
- Decant the contents of the wells into an appropriate waste container. Rinse the microwells with 250µL of the 1X Wash Solution 3 times.
- Absorb the residual solution by inverting with absorbent paper to remove the last of the wash solution.
- Add **100 µL of the TMB Substrate** to each well.
- Shake the plate gently for 30 seconds using a back and forth motion.
- Cover the plate. Incubate for **30 minutes** at room temperature.
- Add **100 µL of the Stop Solution** to each well in the same order of addition as the Substrate. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5min after addition of Stop Solution).

10. Results

Calculating the Percentage absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

  Absorbance (%) = $B / B_0 * 100$

B = the mean absorbance value of each standard or each sample
B_0 = absorbance value of zero standard

11.2 Drawing a Standard Curve

- To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.
- The concentration of each sample (ppb), which can be read from the standard curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.
- Sample dilution factor: If the absorbance of a sample is lower than the highest calibrator (0.32 ppb), the concentration of Saxitoxin is too high and out of range of the standard curve. Dilute the sample and rerun. Samples should be diluted to fit into the standard curve (0.02 ppb to 0.32 ppb). Results must then be multiplied by the dilution factor used.

11. General Instructions

- **Temperature of Reagents and Samples**
  - The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been restored to room temperature (20-25°C).
- **Microwells**
  - Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tapping the microwells holder.
- **Shaking of Reagents**
  - Shake each reagent gently before use.
- **Skin Protection**
  - The Stop Solution is 0.75N HCl, keep your skin away from it.
- **Out of Date Kits**
  - Don't use kits that are expired. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
- **General Comments**
  - Keep the ELISA kits at 2-8°C, do not freeze. Store the unused microwell plates back to the foil pouch. Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
- **Special Issues Concerning Solutions and Reagents**
  - Substrate solution should be abandoned if it turns color. The reagents may turn bad if the

absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

- **Incubation Temperatures**
  - Incubation temperature should be at room temperature (20-25°C). Higher or lower temperature on day of testing will lead to experiment-to-experiment changes.

12. Storage

- Storage condition: 2-8°C
- Storage period: 12 months

Customer Notes

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