Version 02-09/25

User's Manual

Neosaxitoxin ELISA Kit

REF **DEIASL216**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Neosaxitoxin Plate Kit is an immunoassay for the detection of Neo-Saxitoxin in shellfish samples. This product is intended for research use only.

Principles of Testing

Calibrators and the Sample Extract(s) are pipetted into the mixing wells followed by Neo-Saxitoxin HRP Enzyme Conjugate. The reagents are mixed and transferred to the test wells to initiate the reaction. During an incubation, Neo-Saxitoxin in the calibrator/sample and Neo-Saxitoxin HRP Enzyme Conjugate compete for binding to the polyclonal Neo-Saxitoxin antibody immobilized on the test wells surface. Following the incubation, the wells are washed to remove any unbound Neo-Saxitoxin and Neo-Saxitoxin HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Neo-Saxitoxin concentration of the sample is derived.

Reagents And Materials Provided

1. 1 Unit Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
2. 1 Unit Plate containing 12 strips of 8 mixing wells each that are packaged in a zip-loc bag.
3. 6 X 2 mL Vials of Neo-Saxitoxin Calibrators (0, 0.03, 0.08, 0.2, 0.5, and 1.5 ppb).
4. 1 X 12 mL Bottle of Neo-Saxitoxin HRP Enzyme Conjugate.
5. 1 X 50 mL Bottle of 10X Wash Concentrate (dilute prior to use).
6. 1 X 14 mL Bottle of Substrate.
7. 1 X 14 mL Bottle of Stop Solution.

Materials Required But Not Supplied

1. Pipette(s) with disposable tips capable of dispensing the required volume(s).
2. Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s).
3. Laboratory quality distilled or deionized water.
4. Reagents and materials for sample preparation.
5. Materials for 1X wash solution preparation.
6. Personal protective equipment.
7. Paper towels or equivalent absorbent material.
8. Timer.
9. Microtiter plate or strip reader capable of reading at 450 nm.

Storage

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 to 8°C.

Specimen Collection And Preparation

Freshwater:

If there are any visible particulates in the water sample, filtration is recommended by using Whatman #1 filter paper or a syringe filter.

Shellfish:

1. Thoroughly clean the outside of the mussels with laboratory quality distilled or deionized water.
2. Cut the adductor muscles of the mussel using a sharp knife.
3. Rinse off the inside of the mussel with laboratory quality distilled or deionized water to remove sand and other foreign substances.
4. Detach the tissue from the mussel shell by removing the tissue and adductor muscles that connect it at the hinge.
5. Weigh 120 – 150 g of mussel tissue and transfer to a sieve.
6. Gently shake the sieve to drain the excess liquid.
7. Transfer the drained tissue to a clean, 500 mL container and homogenize to a soupy texture.
8. Weigh 10 g (M) of homogenized shellfish tissue into a conical tube.
9. Measure 10 mL (V) of 0.1N HCl and add to the conical tube.
10. Vigorously shake the conical tube for 2 minutes.
11. Gently boil for 5 minutes.
12. Centrifuge for 20 minutes at 3,000 X g or 5 minutes at 12,000 X g.
13. Transfer the supernatant to a clean glass vial.
14. Dilute the extract 1:50 (DF1) in 10% methanol/0.001N HCl and use in the assay.

Reagent Preparation

1X Wash Solution Preparation

1. Measure 450 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Wash Concentrate bottle to the container.
3. Gently swirl to mix.
4. Transfer the 1X Wash Solution to a wash bottle to use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.

2. Place the appropriate number of mixing wells and test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense 100 µL of Calibrators and Sample Extract(s) into the appropriate mixing well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense 100 µL of Enzyme Conjugate into each mixing well.
5. Mix the contents of each well by gently pipetting up and down with a multichannel pipette and transfer 100 µ L of the mixture to the test wells.
6. Incubate the test wells for 30 minutes at room temperature. Discard the mixing wells.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Solution and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution. Alternatively, the last wash can be done using laboratory quality distilled or deionized water to reduce interference associated with wash solution bubbles.
8. Dispense 100 µL of Substrate into each well.
9. Incubate for 30 minutes at room temperature.
10. Dispense 100 µL of Stop Solution into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used test wells in an appropriate waste container.

Quality Control

• The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99.

• The average absorbance of the zero calibrator replicates must be ≥ 1.0.

• The average absorbance of calibrator replicates must have a coefficient of variation (%CV) < 15%.

• The average absorbance of sample replicates must have a coefficient of variation (%CV) < 20%.

Interpretation Of Results

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact for further details.

To ensure the validity of the results, please adhere to the following:

• Ensure QC criteria are met.

• The concentration of Neo-Saxitoxin in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by any dilution factor used.

• In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the

sample extract to fit into the standard curve (DF_2) and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

• The Neo-Saxitoxin (NEO-STX) concentration in the shellfish sample can be converted to µg of NEO-STX equivalent/100 g by using the following equation:

$$\frac{\mu g \text{ of NEO-STX equiv.}}{100\ g} = \text{NEO-STX Conc.} \times (DF_1 \times DF_2) \times \frac{(M+V)}{10 \times M}$$

NEO-STX Conc.	=	Neo-Saxitoxin concentration in the sample in ppb
DF_1	=	Dilution of the supernatant (e.g. 50)
DF_2	=	Any additional dilution of the sample
V	=	Volume of 0.1N HCl added to the homogenized sample in mL (e.g. 10 mL)
10	=	$\frac{1000\ g}{100\ g}$
M	=	Mass of homogenized shellfish tissue in grams (e.g. 10 g)

Detection Range

0.3 ppb - 1.5 ppb

Sensitivity

Recreational Water (Ponds, Rivers, Lakes, etc.):

LOD (ppb): 0.0181

LOQ (ppb): 0.0597

Drinking Water:

LOD (ppb): 0.0205

LOQ (ppb): 0.0675

Specificity

The following table shows the percent cross reactivity of Neosaxitoxin.

Neosaxitoxin 100%

Saxitoxin Dihydrochloride 36.3%

GTX 5 ..... 18.4%

Decarbamoyl Saxitoxin 2.4%

GTX 1 & 4 .....0.8%

Decarbamoyl Neo-Saxitoxin 0.7%

Decarbamoyl GTX 2 & 3 .....0.4%

GTX 2 & 3 .....0.3%

C 1 & 2 .....0.1%

Precautions

• Read the product brochure in its entirety prior to use.

• The kit, in its original packaging, can be used until the end of the month indicated on the box label.

• Do not use reagents after expiration date.

• Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.

• Reagents should be brought to room temperature, 20°C to 28°C (68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.

• Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).

• Running Calibrators and Samples in duplicate will improve assay precision and accuracy.

• Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.

• If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.

• All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.

• Each reagent is optimized for use in the Neo-Saxitoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Neo-Saxitoxin Plate Kits with different lot numbers.

• Do not reuse test wells.

• Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.

• Damage to or obstruction of the optical surface may cause unsatisfactory results.