Tetrodotoxin ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of TTX

Catalog Number: EL2057-01

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

1. Background

Tetrodotoxin (TTX) and its analogues belong to a group of neurotoxins that are produced by various marine bacteria. The toxin can accumulate in certain species of fish, different marine bivalves (clams, oysters and mussels) and gastropods. Due to the worldwide increase in water temperature TTX has appeared also in the European waters. TTX has been recently detected in seafood harvested in the United Kingdom, Portugal, Spain, Greece and the Netherlands. The ingestion of contaminated seafood can have fatal consequences. On the cellular level TTX causes blockage of voltage-gated sodium channels that leads to alteration of neuronal functions and muscle paralysis. Death can occur due to heart or respiratory failure. The majority of TTX poisoning cases have been caused by the consumption of pufferfish commonly known to produce the toxin in Japan, which has led to a regulatory limit of 2000 μg/kg for the pufferfish tissue in Japan. There are no maximum limits for TTX in the European Union. According to the recent European Food Safety Authority (EFSA), if the concentration of TTX and its analogues is less than or equal to 44 μg/kg in shellfish meat, there should not be adverse effects in humans.

2. Test Principle

Attogene's TTX ELISA Kit includes the components to run a competitive enzyme-labeled immunoassay. The TTX sample extract and calibrators are pipetted into the TTX conjugate coated test wells followed by the TTX antibody to initiate the reaction. What follows is a 30-minute incubation period, during which TTX from the sample and TTX antigen compete to bind to the TTX antibody ("Antibody #1"). The Antibody#1 is captured on the walls of the test well. Following this 30-minute incubation, the contents of the wells are removed and the wells are washed to remove any unbound TTX and free antibody. 1X HRP-conjugated Antibody#2 is then added to each well for another 30-minute incubation. The wells are washed afterwards, and a colorless substrate is added to the wells and any bound enzyme conjugate causes the substrate's conversion to a blue color. This process takes place over a 15-minute incubation, then the reaction is stopped and the intensity of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the TTX concentration of the samples is derived.

3. Applications

This kit can be used for quantitative analysis of TTX in water samples.

4. Equipment and Reagents Needed (not provided)

4.1 Equipment

- ELISA Reader (450nm/630nm)
- Deionized water
- Vortex mixer
- Centrifuge
- Timer
- Wash bottle
- Polystyrene centrifuge tube: 50mL, 2mL
- Micropipettes: 20μL-200 μL, 100μL-1000μL, tips
- Multi-channel pipette: 8-channel (50, 100 & 150 μL)

5. Components Provided in This Kit

- Microtiter plate with 96 wells coated with TTX
- TTX Standards (5 vials × 0.8mL/vial): 0ppb (green cap), 5ppb (purple cap), 10ppb (yellow cap), 50ppb (blue cap), 100ppb (orange cap)
- TTX Antibody#1: 11mL
- 100X HRP-Conjugated Antibody#2: 0.25mL
- Antibody#2 Diluent: 20mL
- 20X Wash Solution: 28mL
- TMB Substrate Solution: 12mL
- Stop Solution: 14mL

6. Reagents Preparation

- 1X Wash solution: combine one volume of the 20X Wash Solution with 19 volumes of deionized water. Mix well.
- 1X HRP-conjugated Antibody#2: combine one volume of the 100X HRP-Conjugated Antibody#2 with 99 volumes of Antibody#2 Diluent. Vortex for 10 seconds to mix.
  ☛ Prepare this solution fresh before each test.

7. Notice and Precautions Before Operation

- Please use fresh tips in the process of experiment and change the tip when pipetting different reagents.
- If running more than two strips at once, the use of a multichannel pipette is recommended.
- Make sure that all experimental instruments are clean.
- Treated samples can be stored at 2-8°C for 24 hours in the dark.

8. Assay Process

9.1 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.

9.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25°C) for more than 30 minutes. Shake gently before use.
2. Set up the experimental scheme, noting all the standards and samples' positions; all standards and samples should be run in duplicate.
3. Get the microwells needed out and return the rest into the foil pouch at 2-8°C immediately.
4. The diluted wash solution should be brought to room temperature before use.
5. Dispense **50µL of TTX Standards or sample** into each well.
6. Dispense **100µL of Antibody#1** into appropriate test wells.
7. Shake the plate gently for 30 seconds using a back-and-forth motion.
8. Cover the plate. Incubate for **30 minutes** at room temperature.
9. Decant the contents of the wells into an appropriate waste container.
10. Rinse the microwells with 250µL of the 1X Wash Solution 3 times.
11. Absorb the residual solution by inverting with absorbent paper to remove the last of the 1X Wash Solution.
12. Add **150µL of 1X HRP-Conjugated Antibody#2** (freshly prepared) to each well.
13. Shake the plate gently for 30 seconds using a back-and-forth motion.
14. Cover the plate. Incubate for **30 minutes** at room temperature.
15. Decant the contents of the wells into an appropriate waste container.
16. Rinse the microwells with 250µL of the 1X Wash Solution 3 times.
17. Absorb the residual solution by inverting with absorbent paper to remove the last of the 1X Wash Solution.
18. Add **100µL TMB Substrate Solution** to each well, and mix by shaking the plate gently and incubate for **15 minutes** at 25°C with cover.
19. Add **100µL of Stop Solution** to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5 minutes of adding the Stop Solution).

10. Results

10.1 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

10.2 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 (100 ppb), the concentration of TTX 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 (5ppb to 100ppb). Results must then be multiplied by the dilution factor used.

11. Sensitivity, Accuracy and Precision

11.1 Detection limit:

- Water 5ppb

11.2 Accuracy:

- Water ..... 80±10%

11.3 Precision:

- C.V. of the ELISA kit ..... less than 10%

12. General Instructions

12.1 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).

12.2 Microwells

- Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tapping the microwells holder.

12.3. Shaking of Reagents

- Shake each reagent gently before use.

12.4. Skin Protection

- The Stop Solution is 0.75N HCl, so avoid contact with skin.

12.5 Out of Date Kits

- Don't use kits that are expired. Don't exchange the reagents of different batches, as the effectiveness and sensitivity of each kit is calibrated with one set of components only and is variable otherwise.

12.6 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 plate is recommended.

12.7 Special Issues Concerning Solutions and Reagents

- TMB Substrate should be discarded if it turns a blue hue before testing, as it should be clear. An absorbance value of the zero standard less than 0.5 (A450nm<0.5) indicates

that one or more of the reagents went bad.

12.8 Special Issues Concerning Color

- The coloration reaction takes 15 minutes after the addition of TMB Substrate, but you can prolong the incubation time up to 35 minutes or more if the color is too light to be determined. Do not exceed 40 minutes.

12.9 Incubation Temperatures

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

13. Storage

- Storage conditions: 2-8°C, without light
- Storage period: 12 months or until expiration date
    - For most accurate results, only use reagents and standards from the same kit. The kit's expiration date is thereby that of the earliest expiring component.

Customer Notes:

Attogene
3913 Todd Lane, Suite 310 Austin, TX 78744
Phone: 512- 333-1330
Email: sales@attogene.com
Web: www.attogene.com
EL2057-01.v3