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User's Manual

Tetrodotoxin ELISA Kit

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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.

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PRODUCT INFORMATION

Intended Use

The Tetrodotoxin indirect competitive ELISA is an immunoassay for the quantitative and sensitive detection of Tetrodotoxin in water samples and puffer fish samples.

General Description

Tetrodotoxin (TTX) is a powerful neurotoxin, which is tolerance to heat, salt and cooking. Minimum lethal dose for human is about 0.5mg/60 kg of body weight, the toxicity is 1000 times great than the sodium cyanide. Every year many people are ill due to improper eating or eating puffer fish. Therefore, it is significant to accurate detection of tetrodotoxin in puffer fish in order to prevention and control of tetrodotoxin poisoning. The detection method is sensitive, fast, simple and specific, only need a small amount of fish sampling.

Principles of Testing

Tetrodotoxin (TTX) ELISA Test Kit is based on indirect competitive ELISA principle. Antigen is coated in test wells, the sample extract and calibrator are pipetted into the test wells followed by TTX monoclonal antibody into the test wells to initiate the reaction. After 30 minutes incubation the wells are washed with wash solution. Then add enzyme labeled anti-mouse IgG antibody, incubation, then adding the TMB Substrate solution, terminate the reaction by the stop solutions, measured the amount of color in each well with a microplate reader at OD450 nm. The color of the unknown samples is compared to the color of the calibrators and the TTX concentration of the sample is derived.

Reagents And Materials Provided

1. Micro-well strips: 12 strips with 8 removable wells each
2. Standard solution (1 mL each): 0 ng/mL, 3.3 ng/mL, 10 ng/mL, 30 ng/mL, 90 ng/mL, 270 ng/mL
3. Enzyme conjugate (12 mL), red cap
4. Antibody solution (7 mL), green cap
5. TMB Substrate solution (6 mL×2), red cap
6. Stop solution (7 mL), yellow cap. (Caution! 2M H_2SO_4. Handle with care.)
7. 20× Wash solution (30 mL), transparent cap
8. 2× Sample diluent (50 mL), blue cap

Materials Required But Not Supplied

1. Microplate Reader (including 450 nm)
2. Centrifuge
3. Electric stove
4. Micropipette

5. Magnetic stirrer
6. Graduated cylinder
7. Funnel
8. Flask
9. Acetic acid
10. deionized water or distilled water
11. Extraction-solvent (See reagent preparation)

Storage

Store the kit at 2- 8°C.The shelf life is 12 months when the kit is properly stored.

Specimen Collection And Preparation

1. Weigh 2 g of homogenized globefish (muscle/skin/viscera) sample with a beaker, add 10 mL of Extraction-solvent, heat to boil by electric furnace, stir 10 min.
2. Cooling to room temperature, supernatant was filtered in to centrifuge tube.
3. Centrifuge the sample at 4,000×g for 5 minutes, pipette 100µL of the supernatant and diluted with 1× sample diluent.
4. Use 50 µL the sample per well for the assay and 4°C save backup.

Optional:

1. Measure volume of supernatant, then put the supernatant in a separating funnel.
2. Adding equal volume of ether defatted, shock 1min, standing layer, release the water level. Measure volume of the water level then put in to another separatory funnel, then add an equal volume of ether, shock 1min, standing layer.
3. Made water layer inpouring in a graduated cylinder, diluted with 1× sample diluent.

Reagent Preparation

1. **Extraction solvent**

   The formulation of Extraction solvent is as follow: 0.01M PBS (pH7.4) +0.1% acetic acid

2. **1× sample diluent**

   Mix 1 volume of 2× Sample diluent with 1 volume of distilled water.

3. **1× Wash Solution**

   Mix 1 volume of 20× Wash Solution with 19 volumes of distilled water.

Assay Procedure

1. Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use. Put the required micro-well strips into plate frames, re-sealed the unused microplate, stored at 2-8°C, not frozen. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the

original stock tubes/bottles. Using disposable reservoirs when handling reagents is recommended thus can minimize the risk of contamination.

2. **Number:** Number every micro-well position and all standards and samples should be run in duplicate. Record the standards and samples positions.
3. **Add standard solution/sample and antibody solution:** Add 50 µL of standard solution or prepared sample to corresponding wells. Add 50µL of antibody solution to each well, mix gently by shaking the plate manually and incubate for 30min at 37°C with cover.
4. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 300µL of diluted wash solution at interval of 10s for 5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
5. **Add enzyme conjugate:** Add 100 µL of enzyme conjugate to each well, shake properly, and incubate for 30min at 37°C with cover.
6. **Wash:** Repeat wash as in step 4.
7. **Coloration:** Add 100 µL of the substrate solution and incubate at 37°C for 15 min in the dark for coloration.
8. **Determination:** Add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well. (Recommend to read the OD value at the dual-wavelength 450/620nm within 5 min).

Calculation

The mean values of the absorbance values is equivalent to the percentage of the average OD value (B) of the testing sample and the standard solution divided by the OD value (B_0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is:

Percentage of absorbance value = B/B_0 x 100%

B - the average (double wells) OD value of the testing sample or the standard solution

B_0 - the average OD value of the 0ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Tetrodotoxin standard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the testing sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Tetrodotoxin concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples.

Precision

Coefficients of variation (CVs) for standards: <10%

CVs for samples: <15%

Detection Range

0-270ng/ml

Sensitivity

≤3.3ng/ml

Recovery

70-120%

Precautions

1. The standard solutions of the test kit contain small amounts of TTX, Handle with care.
2. The Stop Solution is 2 M sulphuric acid. Avoid contact with skin and mucous membranes.
3. Please put all the reagents back into the 2-8 °C after every Reagent addition steps.
4. Every reagent addition steps should be complete in 5 minutes.