Atrazine ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of Atrazine

Catalog Number: EL2056-01

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

1. Background

Atrazine is frequently applied within agricultural landscapes to kill weeds and is rated a Restricted Use Pesticide (RUD). Like many pesticides, a part of the active substance does not reach the target plant but evaporates during application or remains in the soil. Due to its wide application and relatively high persistence, atrazine can be detected in rain, surface water, and groundwater, and poses risk for public drinking water. There is a risk of exposure usually via drinking water; studies on this have shown risk of carcinogenicity and human reproductive systems and development. Atrazine is prohibited in over 60 countries, and in the U.S., the USEPA Safe Drinking Water Act (SDWA) guidelines state that the MCL for atrazine in drinking water is 3 ppb. For this reason, it is desirable to test water samples and food matrices for potential residues of triazines as these herbicides frequently occur in waterways and soil.

2. Test Principle

Attogene's Atrazine ELISA Kit includes the components to run a competitive enzyme-labeled immunoassay. The atrazine sample extract and calibrators are pipetted into the test wells followed by the atrazine antibody to initiate the reaction. What follows is a 30-minute incubation period, during which atrazine from the samples/calibrators and atrazine antigen compete to bind to the atrazine 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 atrazine and free antibody. 1X HRP-conjugated Antibody#2 is then added 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 atrazine concentration of the samples is derived.

3. Applications

This kit can be used for quantitative analysis of Atrazine 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 Atrazine
- Atrazine Standards (6 vials × 0.8mL/vial): 0ppb, 0.5ppb, 1.5ppb, 3ppb, 6ppb, and 10ppb
- Atrazine 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. Keep standards and samples out of light as well, as **atrazine is light sensitive**.
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. Take out the microwells needed and return the rest into the foiled pouch 2-8°C immediately.
4. The diluted wash solution should be brought to room temperature before use.
5. Dispense **50µL of Atrazine 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

(10 ppb), the concentration of Atrazine 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 (0.5ppb to 10ppb). Results must then be multiplied by the dilution factor used.

11. Sensitivity, Accuracy and Precision

11.1 Detection limit:

- Water 0.5ppb

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

- Substrate solution 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 temperature on the day of testing will lead to experiment-to-experiment changes.

13. Storage

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

Customer Notes:

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