



Atrazine ELISA Kit

Catalog Number KA1414

96 assays

Version: 22

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of Atrazine in Water Sample.

Background

Atrazine is a widely used agricultural pesticide in the world. It provides the control of broad-leaved weeds and grasses in asparagus, corn, fruit orchard, and citrus groves. The widespread application, stability and relatively high solubility of atrazine in water allow it to leach from soil and be a relatively persistent environmental contaminant. United States Environmental Protection Agency (EPA) has set an enforceable Maximum Contaminant Level for atrazine at 1 part per billion (ppb).

Principle of the Assay

The enzyme immunoassay for atrazine is based on the competition between the atrazine to be assayed and the Atrazine-Alkaline Phosphatase conjugate, for binding to rabbit antibody directed against atrazine, coated onto microwells. The sample containing the atrazine, and the Atrazine-Alkaline Phosphatase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. The intensity of the color developed is inversely proportional to the concentration of atrazine in the sample. The concentration is calculated on the basis of a standard curve.

General Information

Materials Supplied

List of component

Component	Amount
96-well microtiter plate (#S). Twelve strips of 8 detachable wells, coated with Anti-Atrazine antibody.	96-wells
Positive Calibrator containing 10 µg/mL of atrazine.	0.25 mL
Atrazine-Alkaline Phosphatase conjugate (ATZ-ALP) (#3).	10.5 mL
p-Nitrophenyl Phosphate (pNPP) substrate (#5). Ready to use.	10.5 mL
Wash Buffer (10x PBS-Tween) (#6). Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution, 3 N NaOH. (#7)	5.5 mL

Storage Instruction

All reagents of the kit are stable, if stored at 2-8°C, until the expiration date stated on the kit.

Materials Required but Not Supplied

- ✓ Pipetters capable of delivering 50 µL and 100 µL
- ✓ Microtiter plate reader (wavelength 405 nm)
- ✓ Plate washer or squeezable wash bottle
- ✓ Timer
- ✓ Absorbent paper towels

Precautions for Use

Reagents are for in vitro research use only.

- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of atrazine in the samples are high (>50 ng/mL), dilute sample such that points fall in the middle range of the standard curve.
- ✓ Do not return unused reagents back into their original bottles.
- ✓ Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
- ✓ The stop solution contains NaOH. Avoid contact with skin or eyes. If exposed, flush with water.
- ✓ Dispose of all materials, containers and devices in the appropriate receptacle after use.

Assay Protocol

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 50 μ L of standard or samples to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 μ L of ATZ-ALP conjugate (#3) to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 40 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 μ L of pNPP substrate (#5) to each well and incubate at room temperature for 20 min. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add 50 μ L of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 405 nm using an ELISA reader

✓ Simplified Assay Procedure

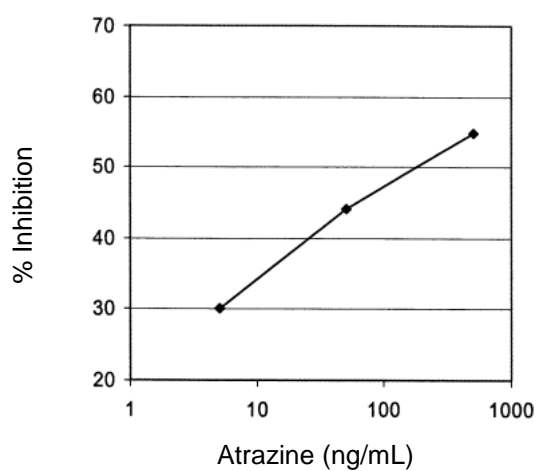
1. Add sample or standard (50 μ L).
2. Add enzyme conjugate (100 μ L) 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 μ L), wait for 20 min. at RT.
5. Add stopping solution (50 μ L) and read at 405 nm.

Data Analysis

Calculation of Results

1. Calculation
 - a. Average the absorbance (OD_s) for each standard concentration of atrazine including 0 ng/mL (OD_0)
 - b. % of Inhibition = $100 - (OD_s / OD_0) \times 100$
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} paper.
3. Calculate atrazine concentration in the sample by interpolation and multiply by the sample's dilution factor to obtain the actual quantity of atrazine.

- ✓ Atrazine Inhibition curve



Performance Characteristics

- ✓ Cross reactivity
- Do not cross react with other pesticides such as 24D, Paraquat, Parathion, and Glyphosate.

Resources

Plate Layout

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3								
2								
1								
	A	B	C	D	E	F	G	H