Zearalenone ELISA Kit

Catalog Number KA1428

96 assays

Version: 22

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of Zearalenone in Sample Extract.

Background

Zearalenone is a Mycotoxin produced by *Fusarium graminearum* on corn, wheat and barley. Zearalenone induced hyperestrogenism in animals and can exert estrogenic effects on the mammalian reproductive system. The extensive biological effects of zearalenone require that its presence be routinely monitored in human foods and animal feeds. The Zearalenone ELISA Kit is intended for the quantitative detection of zearalenone levels in grains and milk.

Principle of the Assay

The enzyme immunoassay for zearalenone is based on the competition between the zearalenone to be assayed and the zearalenone-alkaline phosphatase conjugate, for binding to rabbit antibody directed against zearalenone, coated onto microwells. The sample containing the zearalenone, and the zearalenone-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 zearalenone 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-Zearalenone antibody.	96 (8x12) wells
Calibrators containing 0, 1, 3 and 10 ng/mL of zearalenone	0.6 mL x 4
Zearalenone-Alkaline phosphatase conjugate (ZLN-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 stores at 2 - 8°C, until the expiration date stated on the kit.

Materials Required but Not Supplied

- ✓ Pipetters capable of delivering 25 µL, 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 zearalenone 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 25 µL 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 µL of ZLN-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 µL 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 µL 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

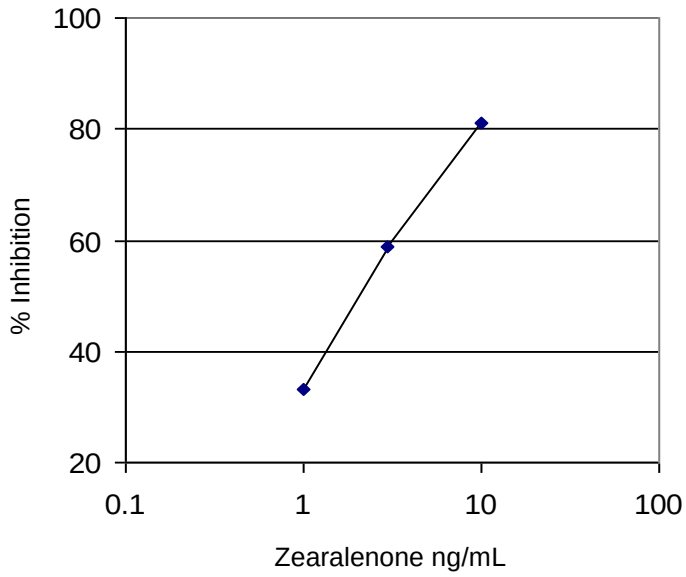
1. Add sample or standard (25 µL).
2. Add enzyme conjugate (100 µL). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µL), wait for 20 min. at RT.
5. Add stop solution (50 µL) and read at 405 nm.

Data Analysis

Calculation of Results

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

✓ Zearalenone Inhibition curve



Performance Characteristics

✓ Cross reactivity

Do not cross react with other mycotoxins.

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								