Aflatoxin B ELISA Kit

Catalog Number KA1412

96 assays

Version: 15

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of Aflatoxin in Sample Extract.

Background

Aflatoxins are produced primarily by some strains of *Aspergillus flavus* and *Aspergillus parasiticus*. There are four principle aflatoxins B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the most frequently encountered and the most toxic of the group. The primary products contaminated with aflatoxins include peanuts, corn, wheat, rice, cottonseed, copra, and nuts. Because aflatoxins are naturally occurring compounds, precautions must be taken to control the quality of the food and feed in which they may occur. Aflatoxins are potent liver toxins in all animals in which they have been tested and carcinogens in some species. The United States Food and Drug Administration have set the guidelines of aflatoxin level in animal feeds and human foods at 20 parts per billion (20 ppb).

Principle of the Assay

The enzyme immunoassay for aflatoxin is based on the competition between the aflatoxin to be assayed and the aflatoxin-alkaline phosphatase conjugate, for binding to rabbit antibody directed against aflatoxin, coated onto microwells. The sample containing the aflatoxin, and the aflatoxin-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 aflatoxin in the sample. The concentration is calculated on the basis of a standard curve.

General Information

Materials Supplied

List of component

Component	Amount
96-wells microtiter plate (#S). Twelve strips of 8 detachable wells, coated with rabbit Anti-Aflatoxin antibody.	96 (8x12) wells
Calibrator containing 0, 0.5, 2.0 and 8.0 ng/mL of Aflatoxin $B_{1.}$	0.6 mL x 4
Aflatoxin-Alkaline Phosphatase conjugate (AFX-ALP). (#3)	10.5 mL
p-Nitrophenyl Phosphate (pNPP) substrate. Ready to use. (#5)	10.5 mL
Wash Buffer (10xPBS-Tween). Dilute 10 fold with distilled or deionized water to 150 mL prior to use. (#6)	15 mL
Stop Solution, 3 N NaOH. (#7)	6 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 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 Aflatoxin in the samples are high (>10 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

Sample Preparation

✓ Preparation and Extraction of samples

1. For preparation of sample, refer to AOAC Official Methods of Analysis 977.16 (see 49.2.01).
2. Extraction - Accurately weigh 5.0 g of ground sample and transfer to a 50 mL flask with glass stopper. Add 25 mL of methanol-deionized water (7+3), and shake well for one minute. Filter the mixture through Whatman No.1 filter paper, and collect enough extract for the assay.
3. Preparation of negative control - For negative control, follows the same procedure as Step 2 of Preparation and Extraction of samples using a 5.0 g aflatoxin-free sample.

Assay Procedure

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

1. Carefully add 25 µL of standard or sample 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 AFX-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

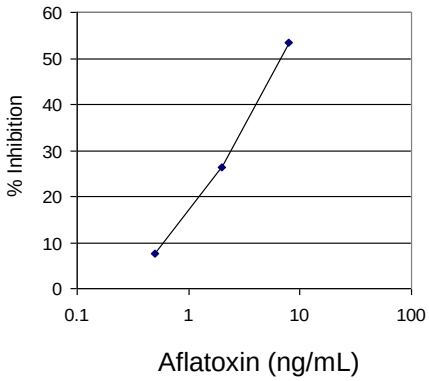
1. Add sample or standard (25 µL).
2. Add enzyme conjugate (100 µL). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µL), wait for 20 min. at RT.
5. Add stopping solution (50 µL) and read at 405 nm.

Data Analysis

Calculation of Results

1. Calculation
   (a) Average the absorbance (OD_s) for each standard concentration of aflatoxin 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 aflatoxin B_1 concentration of sample by interpolation and multiply by dilution factor to obtain the actual quantity of aflatoxin B_1.

✓ Aflatoxin Inhibition curve



Performance Characteristics

- Cross Reactivity
  By the assay, the following compounds tested at the stated levels are found to give results not greater than a level of 0.5 ng/mL of aflatoxin B_1.

Compound	Conc. (ng/mL)	% Inhibition
Gentamicin	10,000	<10
Neomycin	10,000	<10
Tylosin	10,000	<10
Sulfamethazine	10,000	<10
Sulfadimethoxine	10,000	<10
Zearalenone	10,000	<10
Narasin	10,000	<10
Chloramphenicol	10,000	<10
Progesterone	10,000	<10

There is the possibility that other substances and/or factors not listed above may interfere with the test.

Resources

Plate Layout

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