WARNING: Contains Toxic Components

Product Manual

Aflatoxin Competitive ELISA Kit

Catalog Number

AKR-350 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Mycotoxins are structurally diverse fungal metabolites that can contaminate the ingredients of animal feed and human food. Aflatoxin is a naturally occurring mycotoxin produced by two types of mold: Aspergillus flavus and Aspergillus parasiticus. Aspergillus flavus is common and most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation and in hay and grains undergoing microbiological deterioration. It invades all types of organic substrates whenever and wherever the conditions are favorable for growth, specifically high moisture content and high temperature. At least 13 different types of Aflatoxin are produced in nature and Aflatoxin B1 (AFB1) is considered the most toxic (Figure 1). Aflatoxin B1, which is a genotoxic hepatocarcinogen, likely causes cancer by inducing DNA adducts which leads to genetic changes in target liver cells. AFB1 is metabolized by cytochrome-P450 enzymes to the reactive intermediate AFB1-8, 9 epoxide (AFBO) which then binds to liver cell DNA, resulting in DNA adduct formation. AFBO is also capable of causing aflatoxicosis when it binds to proteins, forming amino acid adducts and resulting in liver cirrhosis, nutritional deficits, and immunological suppression.

The aflatoxins are among the most potent genotoxic agents known. Aflatoxins induce chromosomal aberrations, micronuclei, sister chromatid exchange, unscheduled DNA synthesis, chromosomal strand breaks, and form adducts in rodent and human cells.

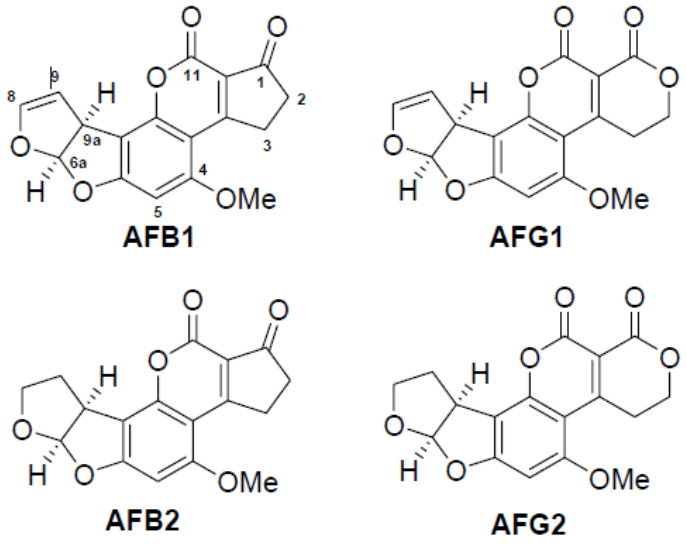

Figure 1. Structures of 4 Types of Aflatoxin Molecules.

Assay Principle

Cell Biolabs' Aflatoxin Competitive ELISA Kit provides a convenient method for the detection of total Aflatoxin B1 and Aflatoxin B2 adducts in protein samples. First, an AFB1 conjugate is coated on an ELISA plate. The unknown AFB1/2 samples or AFB1-BSA standards are then added to the AFB1 conjugate preabsorbed ELISA plate. After a brief incubation, an anti-AFB1/2 monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The total content of AFB1 plus AFB2 in unknown samples is determined by comparison with a predetermined AFB1-BSA standard curve.

Related Products

1. AKR-351: Aflatoxin DNA Adduct Competitive ELISA Kit
2. STA-301: OxiSelect™ BPDE Protein Adduct ELISA Kit
3. STA-357: OxiSelect™ BPDE DNA Adduct ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-AFB1/2 Antibody (500X) (Part No. 435001): One 15 µL vial of anti-AFB1/2 Antibody.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. AFB1-BSA Standard (Part No. 435002): One 40 µL vial of 0.22 mg/mL AFB1-BSA in PBS at 100 nmol AFB1/mg protein. The amount of conjugated AFB1 is predetermined by a spectrophotometric method as described by Chu and Ueno (See Ref. 5).
2. AFB1 Conjugate (500X) (Part No. 435003): One 60 µL vial.

Materials Not Supplied

1. Protein samples containing Aflatoxin B1 and/or B2 adducts such as purified protein, cell lysates, or food samples
2. 1X PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Storage

Important Note: Both the AFB1-BSA Standard and AFB1 Conjugate are highly toxic. All handling should be performed in a fume hood, and great care should be taken to avoid any skin contact, inhalation, or ingestion.

Upon receipt, aliquot and store the AFB1-BSA Standard and AFB1 Conjugate at -20ºC to avoid multiple freeze/thaw cycles. Store all other kit components at 4ºC.

Preparation of Reagents

- AFB1 Conjugate Coated Plate:

  Important Notes:
  - ***The AFB1 Conjugate is highly toxic. All handling should be performed in a fume hood, and great care should be taken to avoid any skin contact, inhalation, or ingestion.***
  - ***The AFB1 Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.***

  1. Immediately before use, prepare 1X AFB1 Conjugate by diluting the AFB1 Conjugate stock (500X) in 1X PBS. For example, add 5 µL of the AFB1 Conjugate (500X) to 2.5 mL of 1X PBS and mix well.
  2. Add 100 µL of the 1X AFB1 Conjugate to each well and incubate overnight at 4ºC. Remove the AFB1 Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-AFB1 Antibody and Secondary Antibody: Immediately before use, dilute the Anti-AFB1/2 antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Important Note: The AFB1-BSA Standard is highly toxic. All handling should be performed in a fume hood, and great care should be taken to avoid any skin contact, inhalation, or ingestion.

Prepare a dilution series of AFB1-BSA standards in the concentration range of 0 to 800 ng/mL by diluting the AFB1-BSA Standard in Assay Diluent (Table 1).

Standard Tubes	0.22 mg/mL AFB1-BSA Standard (µL)	Assay Diluent (µL)	AFB1-BSA (ng/mL)	AFB1 Adduct (nM)
1	4	1096	800	80
2	200 of Tube #1	200	400	40
3	200 of Tube #2	200	200	20
4	200 of Tube #3	200	100	10
5	200 of Tube #4	200	50	5
6	200 of Tube #5	200	25	2.5
7	200 of Tube #6	200	12.5	1.25
8	200 of Tube #7	200	0	0

Table 1. Preparation of AFB1-BSA Standards

Assay Protocol

Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use. Each AFB1/2 sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or AFB1-BSA standard to the wells of the AFB1 Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.

   Note: If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA.
3. Add 50 µL of the diluted anti-AFB1/2 antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Aflatoxin Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

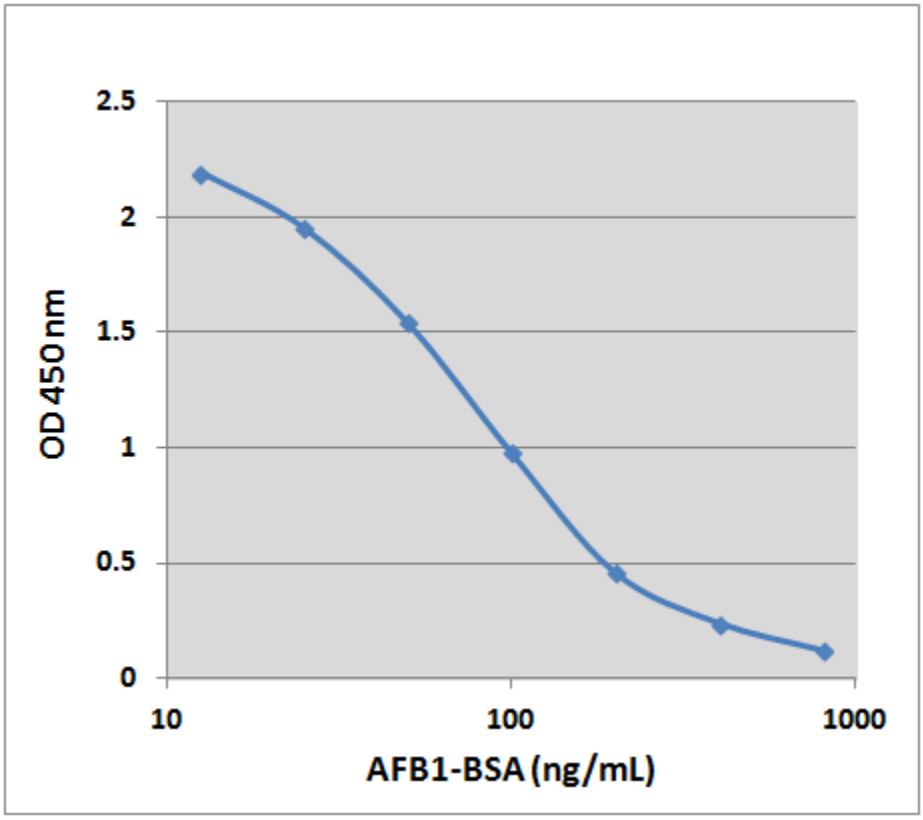

Figure 2: AFB1-BSA Standard Curve.

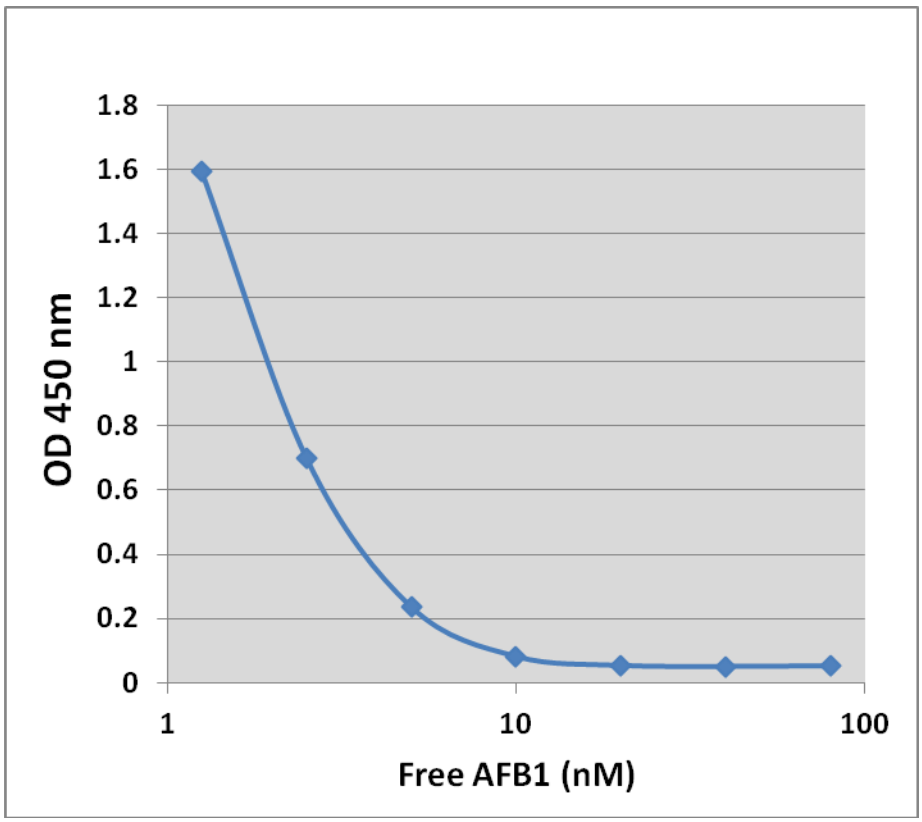

Figure 3: Free AFB1 Competition in Aflatoxin Competitive ELISA.

References

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Warranty

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