

**WARNING: Contains
Toxic Components**

Product Manual

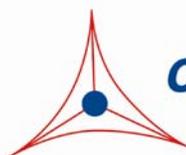
Aflatoxin DNA Adduct Competitive ELISA Kit

Catalog Number

AKR-351

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.

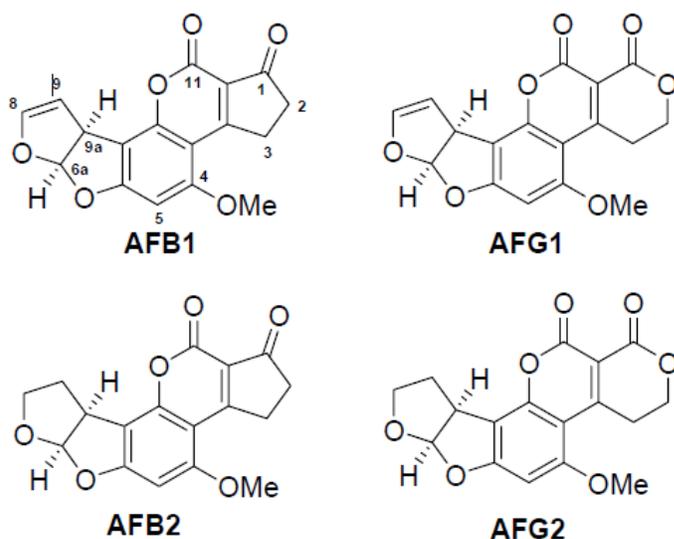


Figure 1. Structures of 4 Types of Aflatoxin Molecules.

Assay Principle

Cell Biolabs' Aflatoxin DNA Adduct Competitive ELISA Kit provides a convenient method for the detection of total Aflatoxin B₁-DNA adducts (ring-opened and ring-closed forms). First, the unknown AFB₁-DNA samples or AFB₁-DNA standards are added to the AFB₁-DNA conjugate preabsorbed ELISA plate. After a brief incubation, an anti-AFB₁-DNA antibody is added, followed by an HRP conjugated secondary antibody. The total content of AFB₁-DNA in unknown samples is determined by comparison with a predetermined AFB₁-DNA standard curve.

Related Products

1. AKR-301: Rapid Qualitative E. coli O157:H7 Test Kit
2. AKR-302: Rapid Qualitative Salmonella Test Kit
3. AKR-350: Aflatoxin Competitive ELISA Kit
4. STA-301: OxiSelect™ BPDE Protein Adduct ELISA Kit
5. STA-357: OxiSelect™ BPDE DNA Adduct ELISA Kit

Kit Components

1. 96-well AFB1-DNA Coated Plate (Part No. 435101): One strip well 96-well plate.
2. Anti-AFB1-DNA Antibody (500X) (Part No. 435102): One 10 µL vial of anti-AFB1-DNA Antibody.
3. AFB1-DNA Standard (Part No. 435103): One 480 µL vial of 12.5 µg/mL AFB1-DNA Standard at 1 µmole AFB1 adduct per mg DNA. The amount of conjugated AFB1 is predetermined by a spectrophotometric method as described by Lin et al. (See Ref. 5).
4. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Materials Not Supplied

1. DNA samples containing Aflatoxin B1 adducts such as cell or tissue genomic DNA
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Storage

Important Note: Both the 96-well AFB1-DNA Coated Plate Standard and AFB1-DNA Standard 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-DNA Standard at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C.

Preparation of Reagents

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

Preparation of Standard Curve

Important Note: The AFB1-DNA 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-DNA standards in the concentration range of 0 to 4 µg/mL by diluting the AFB1-DNA Standard in Assay Diluent (Table 1).

Standard Tubes	12.5 µg/mL AFB1-DNA Standard (µL)	Assay Diluent (µL)	AFB1-DNA (µg/mL)	AFB1 Adduct (µM)
1	96	204	4	3.94
2	150 of Tube #1	150	2	1.97
3	150 of Tube #2	150	1	0.99
4	150 of Tube #3	150	0.5	0.49
5	150 of Tube #4	150	0.25	0.25
6	150 of Tube #5	150	0.125	0.125
7	150 of Tube #6	150	0.0625	0.0625
8	0	150	0	0

Table 1. Preparation of AFB1-DNA Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each AFB1-DNA sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or AFB1-DNA standard to the wells of the AFB1-DNA 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-DNA 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-DNA Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

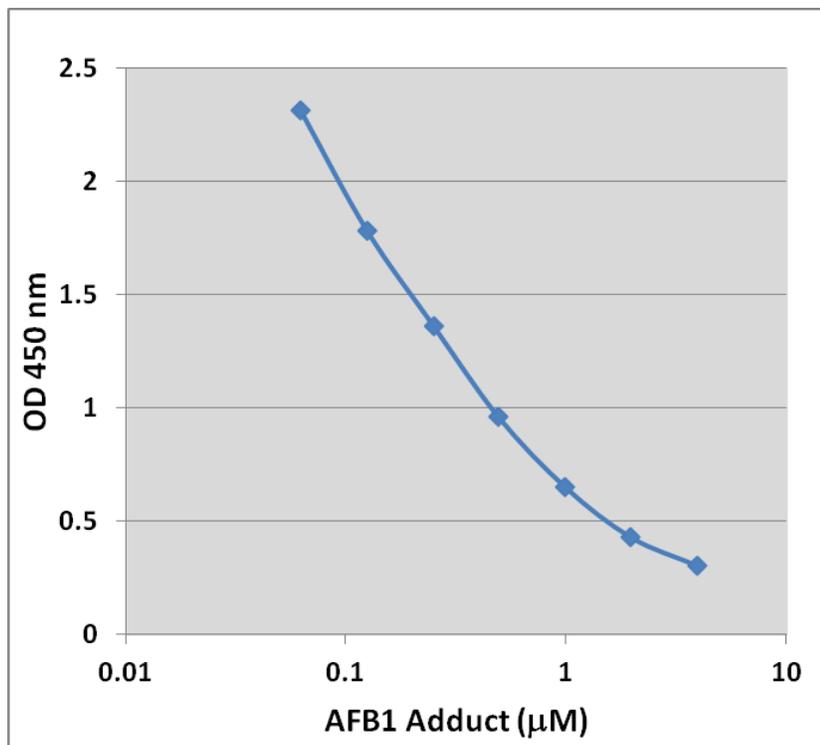


Figure 2: AFB1-DNA Standard Curve

References

1. Wang J-S, Groopman JD. (1999) *Mut Res.* **424**: 167-181

2. Moudgil V, Redhu D, Dhanda S, Singh J. (2013) *J Environ Pathol Toxicol Oncol.* **32**:165-175
3. Hamid AS, Tesfamariam IG, Zhang Y and Zhang ZG. (2013) *Oncology Letters* **5**:1087-1092
4. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. (2004) *Am J Clin Nutr.* **80**:1106-1122
5. Lin JK, Miller JA, Miller EC (1977) *Cancer Research* **37**:4430-4438

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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