Aflatoxin (total) ELISA Kit

Enzyme Immunoassay for the quantification of Aflatoxin (total) in food (extraction, dilution, centrifugation).

Catalog number: ARG81030

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INTRODUCTION

Aflatoxins are poisonous carcinogens and mutagens that are produced by certain molds (*Aspergillus flavus* and *Aspergillus parasiticus*) which grow in soil, decaying vegetation, hay, and grains. They are regularly found in improperly stored staple commodities such as cassava, chili peppers, cottonseed, millet, peanuts, rice, sesame seeds, sorghum, sunflower seeds, sweetcorn, tree nuts, wheat, and a variety of spices. When contaminated food is processed, aflatoxins enter the general food supply where they have been found in both pet and human foods, as well as in feedstocks for agricultural animals. Animals fed contaminated food can pass aflatoxin transformation products into eggs, in the findings of high percentages of samples of aflatoxin-contaminated chicken meat and eggs in Pakistan.

Children are particularly affected by aflatoxin exposure, which is associated with stunted growth, delayed development, liver damage, and liver cancer. An association between childhood stunting and aflatoxin exposure has been reported in some studies but could not be detected in all. Furthermore, a causal relationship between childhood stunting and aflatoxin exposure has yet to be conclusively shown by epidemiological studies, though such investigations are under way. Adults have a higher tolerance to exposure, but are also at risk. No animal species is immune. Aflatoxins are among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1. [Provide by Wikipedia: Aflatoxin]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. The samples or Standards (Aflatoxin Standards) are first added to the Antibody-binding Protein coating microplate. Then Aflatoxin Conjugate and Antibody specific for Aflatoxin is added to each well and incubate. The Aflatoxin Conjugate competes with the samples / Standards for the limited number of Antibody sites. After washing away any unbound substances, the TMB Substrate is added to the wells and color develops in inversely proportion to the amount of Aflatoxin content bound with the Antibody. The color development is stopped by the addition of STOP Solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Aflatoxin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-Binding Protein coating microplate	8 X 12 strips	4°C
Standards (0, 0.05, 0.1, 0.25, 0.5, 1.5 ng/mL Aflatoxin Standards, dyed red)	1 mL each (ready to use)	4°C
Diluent Buffer (dyed red)	60 mL X 2 (ready to use)	4°C
Aflatoxin Conjugate (Aflatoxin-Peroxidase, dyed red)	6 mL (ready to use)	4°C
Antibody (Anti-Aflatoxin Antibody, dyed blue)	6 mL (ready to use)	4°C
10X Wash Buffer	60 mL	4°C
TMB Substrate	15 mL (ready to use)	4°C (protect from light)
STOP Solution	15 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Methanol
- Hexan (Spices only)
- Votex mixer or Ultra-Turrax
- Plastic bag to store unused microtiter strips.
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Solid Samples (Cereals, Nuts, Dry Fruit):

A. Grind sample to pass through a 20 mesh sieve and thoroughly mix prior to sub-sampling.
B. Suspend 20 g of sample in 100 mL of 70% methanol.
C. Mix suspension for 5 minutes.
D. Centrifuge at a minimum of 3000 g for 5 minutes.
E. Dilute 100 µL of supernatant with 600 µL of Diluent Buffer and test the samples in the ELISA.

Dilution factor = 35

Spices:

A. Grind sample to pass through a 20 mesh sieve and thoroughly mix prior to sub-sampling.
B. Suspend 20 g of sample in 100 mL of 70% methanol.
C. Mix suspension for 5 minutes.
D. Centrifuge at a minimum of 3000 g for 5 minutes.
E. To 1 mL of supernatant add 2 mL of Hexan and mix for 5 min, then separate the upper hexan layer.
F. Dilute 100 µL of the lower layer with 600 µL of Diluent Buffer and test the samples in the ELISA.

Dilution factor = 35

Liquid Samples (Beer):

A. Depending on the number of samples. Dilute an adequate volume of Diluent Buffer with 10% methanol.
B. Carbonized samples should be preliminarily degassed by moderate

heating.

C. Cloudy samples (such as beer brewed from wheat) should preliminarily be sterile-filtered.

D. Dilute 100 µL sample with 900 µL Diluent Buffer/methanol dilution and test the diluted samples in the ELISA.

Dilution factor = 10

Oil Samples:

A. Dilute 20 mL of oil with 100 mL of 70% methanol and mix for 5 min in a separating funnel.

B. Discard the oil layer.

C. Dilute 100 µL of the methanol layer with 600 µL of Diluent Buffer and test the samples in the ELISA.

Dilution factor = 35

Note:

1. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles. In case of too highly concentrated samples, an adequate volume of Diluent Buffer is diluted with methanol to a concentration of 10% methanol. The sample extracts have to be further diluted with this dilution.
2. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 ml of 10X Wash Buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Each Samples or Standards should be assayed in duplicate or triplicate.

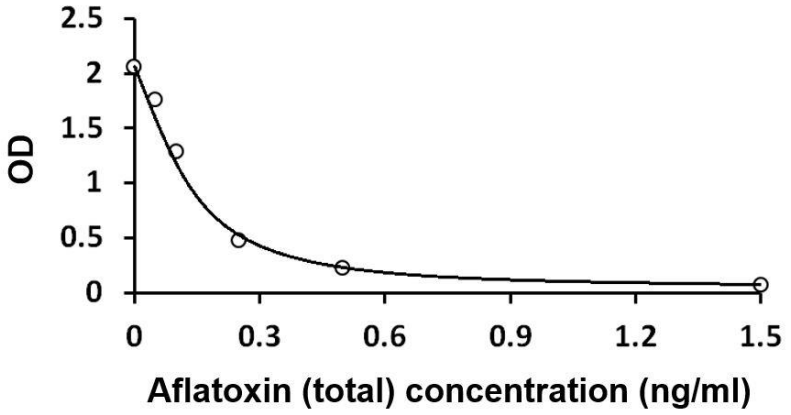
1. Add 100 µL of prepared samples or Standards to the Antibody-Binding Protein coating microplate.
2. Add 50 µL of the Aflatoxin Conjugate to each well.
3. Add 50 µL of the Antibody to each well.
4. Incubate at RT for 30 mins on a microplate shaker.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Warm TMB Substrate to RT. Add 100 µl of TMB Substrate to each well, including the blank wells. Incubate for 15 minutes at RT in the dark.
7. Add 100 µl of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.

8. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

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

Aflatoxin (ng/mL)	% binding of 0 ng/mL
0	100
0.05	74
0.1	63
0.25	32
0.5	21
1.5	6



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. The diluted samples must be further converted by the appropriate sample dilution factor for calculating the sample concentration in ppb. The factors for each sample matrix are listed in the sample preparation section.

   Example:

   A wheat sample prepared as described above results in 0.2 ng/mL

   C_{sample} = 0.2 (ng/mL) * 35 (ppb*ml/ng) = 7 ppb
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
6. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
7. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of the Aflatoxin (total) ELISA kit is 0.015 ng/mL

Intra-assay and Inter-assay precision

The CV value of intra-assay was 4.7 % and inter-assay was 6.6 %.

Recovery

Wheat	99 %	Hazelnut	87 %
Rye	92 %	Peanut	101 %
Barley	92 %	Almond	93 %
Oats	85 %	Dry Fruit-Sultana	103 %
Sorghum	101 %	Dry fruit- Fig	114 %
Rice	102 %	Chili	92 %
Corn	92 %	Oil	121 %
Beer	101 %		

Cross reactivity

Aflatoxin B1	100 %
Aflatoxin B2	73 %
Aflatoxin G1	74 %
Aflatoxin G2	49 %
Aflatoxin M1	60 %