

AflaQuant™

Total Aflatoxin Quantitative ELISA Kit

Competitive ELISA (Enzyme-Linked Immuno-Sorbent Assay) for the quantitative determination of the **Total Aflatoxins** in Foods, Cereals, Formula Feed, Edible Oils, Milk, Nuts, Biscuits, Beer, Wine, Soy Sauce, Vinegar, Butter, Corn, Cotton Seeds, Spices, and other commodities, including Animal Feeds

Catalogue Number: TAA250626

**For Research Use Only. Not for
Diagnostic Procedures.**

(This test kit is designed for detection of Mycotoxin substances within food products. This is not a medical device and is not intended to diagnose or prevent any diseases or other conditions.)



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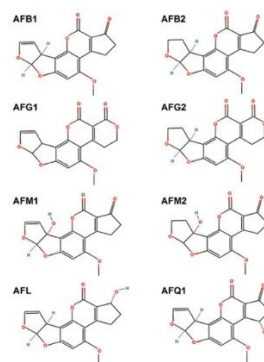
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Different Aflatoxins –



Chemical structures of major Aflatoxins - Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), Aflatoxin M1 (AFM1), Aflatoxin M2 (AFM2), Aflatoxicol (AFL), and Aflatoxin Q1 (AFQ1)

About Aflatoxins –

Aflatoxins are a group of poisonous, carcinogenic, and mutagenic mycotoxins produced by certain fungi (moulds), primarily *Aspergillus flavus* and *Aspergillus parasiticus*. These moulds can grow in soil, hay, decaying vegetation, and grains. Aflatoxins can contaminate crops during production, harvest, storage, and processing. They can be found in many foods, including Peanuts, Corn, Cottonseed, Tree nuts, Rice, Figs, Spices, Crude vegetable oils, and Cocoa beans.

Health Hazards due to Aflatoxins –

Exposure to aflatoxins can cause a variety of health issues, including:

Acute toxicity: Nausea, vomiting, abdominal pain, convulsions, and other signs of acute liver injury

Long-term toxicity: Growth retardation, cirrhosis, and hepatocellular carcinoma

Birth defects: Aflatoxins can be teratogenic in hamsters and mice

Kidney and immune system problems: Aflatoxins can cause kidney and immune system problems

Manufactured By:



BIC BIOTECH Pvt. Ltd.

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Assay Principle –

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Total Aflatoxin-specific antibodies are pre-coated onto the wells of microtiter strips of a 96-well plate. The Aflatoxin-HRP Conjugate (Conjugate), standards, and samples (Aflatoxins extracted in 70% Methanol), are added into the pre-coated wells. The Aflatoxin in standards or extracted samples compete with Aflatoxin-HRP Conjugate for binding sites available on the coated antibodies. The more is the amount of Aflatoxin in a sample the greater occupancy it will make and in turn will leave lesser binding sites for binding of Aflatoxin-HRP Conjugate during a given time interval. The unbound material is washed off in the next washing step. When chromogen substrate (peroxide and TMB) is added to wells, it produces blue colour progressively in proportion to bound Aflatoxin-HRP Conjugate by the action of enzyme HRP on the substrate hydrogen peroxide and chromogen TMB. This progression of blue colour development is stopped by Stopping Solution (an acid) after a fixed time interval. This leads to formation of yellow colour in the wells that are read at 450 nm in a Microplate Reader.

Thus, a sample containing high amounts of Aflatoxin will allow only less amount of Aflatoxin-HRP Conjugate binding and thereby less colour development. So, the more is the colour development in a well the less will be the Aflatoxin in that well and *vice versa*. So, the colour development is inversely proportional to the concentration of Aflatoxin in a sample.

Kit Storage and shelf-life –

Store entire kit at 4 – 8°C when not in use. Shelf-life would be 2 year from the date of manufacturing.

Do not freeze components of the kit.

Kit Components –

1. 96-well precoated ready-to-use microtiter plate with single-break strips – 1 No.
2. Empty 96-well microtiter plate for making dilutions – 1 No.
3. Adhesive films for sealing precoated and empty microtiter plates – 2 Nos.
4. Total Aflatoxin Standards (0.0, 0.2, 0.5, 1.0, and 2.0 ng/ml; 1.5 ml each) – 5 Glass Vials
5. Ready-to-use Diluent – 24 ml
6. Ready-to-use Aflatoxin-HRP Conjugate – 12 ml
7. 20 X Wash buffer – 50 ml
8. Ready-to-use TMB substrate – 12 ml
9. Ready-to-use Stop solution – 12 ml

Materials needed but not provided in the kit –

1. 70% Methanol
2. *n*-Hexane
3. Chloroform or Dichloromethane
4. De-Ionized water
5. Grinder / Mortar and Pestle / Homogenizer
6. Vortex Mixer
7. Orbital Shaker and refrigerated Centrifuge
8. Graduated Glass Pipettes
9. Gloves
10. Absorbent-paper Towels
11. Whatman # 1 Filter Paper
12. Glass Funnels
13. Glass Test-tube (5, 15, 25, and 50 ml capacity)
14. Measuring Glass Cylinders
15. 20, 50, 200, and 1000 µl Single Channel Adjustable Volume Micropipettes and Tips
16. 300 µl 8-Channel Adjustable Volume Micropipette and Tips
17. Plastic Troughs
18. Balance 0 – 150 g having least count of 0.001 g
19. 96-Well Microplate Reader (ELISA Reader; Variable wave-length or having 450 nm filter)
20. Printer for Microplate Reader
21. Printed Microplate Well Layout in the form of 12 vertical x 8 horizontal table
22. Calculator



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Precautions and Safety Measures –

Read precautions carefully, follow them and strictly adhere to safety measures

1. Always store kit components at 4 – 8°C before and remainder after use
- 2. Do not freeze the kit components at any point of time**
3. Bring the entire kit to room temperature (25-30°C) at least half-an-hour before use
4. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming
5. Use only very clean glass and plastic containers wherever needed
6. Use separate plastic tips for each pipetting to avoid cross-contaminations
7. After washing wells with wash solutions remove the solution in the wells completely either by aspiration or by decanting and then patting the plate against absorbent paper towel
8. Read absorbance just after the determined time interval

9. After addition of stop solution mix the contents of the well onto an Orbital Shaker
10. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers
11. All specimens and standards should be run at the same time, so that all conditions of testing are the same
12. Do not use reagents after expiration date
13. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits
14. Do not mix components from different batches
15. Use at least duplicates for blanks, standards, samples (each dilution)
16. Ensure plates are properly sealed or covered during incubation steps
17. Use protective gloves while handling Aflatoxin standards, extracted samples, and Stop Solution
18. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity

- hazard). In case of contact, rinse the affected zone with plenty of water
19. Make a good record of all calculations, additions, dilution, etc. as per GLP
20. Even the same operator might get different results in two separate experiments. So, in order to get reproducible results within permissible error limits, all the steps of assay should be controlled
21. Avoid spillage of various solutions. In case of spillage use 70% methanol mop followed by water mop

Preliminaries before beginning of assay –

1. Bring the entire kit to room temperature (25-30°C). Keep for least half-an-hour before use
2. Determine the total number of samples to be tested and accordingly fill details in the printed plate layout
3. Determine the number of pre-coated strips
4. While the kit components are brought to room temperature, prepare samples and standard dilutions as given below



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5. Reagent preparation –

- a. 70% Methanol – As per requirement mix de-ionized water with methanol in the ratio 3:7
- b. 1X dilution of wash buffer – As per requirement mix 1 part of 20X Wash buffer with 19 parts of de-ionized water

6. Sample preparations –

Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results. It is recommended to store samples to be used for 5 days at 4°C, for 1 month at -20°C and for 2 months at -80°C. Extracted samples must be appropriately diluted so that the expected concentration falls within the detection range of the kit. Samples that contain NaN₃ (sodium azide) cannot be analysed by this kit as it interferes with HRP.

- a. **Cereals (Grains), Corn, Cotton Seeds, etc.**
– Take 2 g of the material and finely pulverized in a mortar and pestle or a homogenizer. Take the homogenate into a 50 ml Eppendorf tube and add 10 mL of 70% methanol, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 2 mL of supernatant, add 4 mL of trichloromethane or dichloromethane, shake for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take the upper phase liquid to another tube; keep the lower phase

liquid for use (lower liquid A). Add 4 mL of trichloromethane or dichloromethane to the upper liquid, shake well for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Discard the upper liquid and keep the lower liquid (lower liquid B) (4) Mix lower liquid A and lower liquid B thoroughly. Take 2 mL of mixed lower liquid and blow-dry with nitrogen evaporators/water bath at 50-60°C. Add 0.5 mL of 70% methanol to dissolve thoroughly, add 0.5 mL of deionized water, mix fully. Take 50 µl for detection and analysis. Note: Sample dilution factor is 10.

- b. **Formula feed** – Take 2 g of the material and finely pulverized in a mortar and pestle or a homogenizer. Take the homogenate into the 50 mL tube, add 10 mL 70% of methanol, shake for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Take 0.5 mL of supernatant, add 0.5 mL of deionized water, mix well. Take 50 µL for analysis. Note: Sample dilution factor is 10 (If aflatoxin content is higher in the sample, take the mixed liquid from step 2, diluted with 35% methanol, the sample dilution multiple is the actual dilution multiple in that case.
- c. **Beer** – Stir beer thoroughly to remove CO₂, take 2 mL of beer sample and add 1 mL of deionized water, then add 7 mL methanol, vortex for 5 min. Take 0.5 mL of mixed sample liquid and add 0.5 mL of deionized water, mix well. Take 50 µL for detection

and analysis. Note: Sample dilution factor is 10

- d. **Wine, Soy Sauce, Vinegar** – Take 2 ml of the sample and add 2 ml of deionised water, and then add 10 ml of chloroform or dichloromethane. Vortex for 5 min and centrifuge at 4000 RPM for 10 min at room temperature. Take 1 ml of the lower phase liquid and blow-dry with nitrogen evaporators at 50-60°C. Add 0.5ml of 70% methanol to the dried materials and mix with 0.5 ml deionized water. Following this, take 50 µl of the sample for detection and analysis. Note: the sample dilution factor is 5
- e. **Edible oil, Peanut, Butter and high fat food** – Weigh 2 g of crushed homogenate into the 50 mL tube, add 8 mL of *n*-hexane and 10 mL 70% of methanol, vortex for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Discard the upper liquid, and take 0.5 mL of lower liquid, add 0.5 mL of deionized water, mix well. Take 50 µL for detection and analysis. Note: Sample dilution factor is 10
- f. **Biscuit** – Add 2 g of crushed biscuit homogenate into a 50 ml Eppendorf tube and add 10 ml of 70% methanol. Vortex the sample for 5 min and then centrifuge at 4000 RPM for 10 min at room temperature. Take 2 ml of the supernatant, add 4 ml of chloroform or dichloromethane, vortex for 5 min and



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then centrifuge at 4000 RPM for 10 min at room temperature. Transfer the upper phase liquid to another vessel, keep the lower phase liquid for use (lower liquid A). Add 4 ml of chloroform or dichloromethane to the upper phase liquid, vortex for 5 min and centrifuge at 4000 RPM for 10 min at room temperature. Discard the upper phase liquid and keep the lower phase liquid (lower liquid B). Mix lower liquid A and lower liquid B thoroughly. Take 2 ml of the mixed lower liquid and blow-dry with nitrogen evaporators at 50-60°C. Add 0.5 ml of 70% methanol to dissolve the residue and mix with 0.5 ml deionized water. Following this, take 50 µl of the sample for detection and analysis. Note: the sample dilution factor is 10

- g. Spices like turmeric, cardamom, ginger, etc.** - Grind 20–50 g of the dried spice sample into a fine powder. Mix 5 g of the ground sample with 25 mL of 70% methanol in water. Vortex or blend vigorously for 3 minutes, then filter through Whatman No. 1 paper or centrifuge at 3000 x g for 5 minutes to collect a clear supernatant. Turmeric and ginger contain intense natural pigments (curcumin/gingerols) and secondary metabolites that can cause non-specific background signals in competitive ELISA. Dilute the clear filtrate with an appropriate volume of PBS-Tween buffer (typically a 1:2 to 1:5 dilution). Use it for assay.

Assay Procedure –

1. Prepare a layout as given below
2. Add 200 µl of diluent into respective wells of empty microtiter plate
3. To each of the above wells add 50 µl of respective standards and sample extracts and mix the contents well
4. Transfer 100 µl of diluted standards and samples from the above wells into respective wells of pre-coated strip plate wells in duplicate
5. Add 100 µl of Conjugate (Aflatoxin-HRP Conjugate) into each of the above wells except wells marked A1 and A2
6. Incubate the pre-coated wells at room temperature (cover the wells with sealing film) for 15 minutes
7. After incubation, decant the contents of the wells by inverting the wells into sink and pat the inverted plate wells on to absorbent paper towel to remove traces of adherent liquid in the wells
8. Add 300 µl of 1X wash buffer into each of the wells. Swirl gently for 30 seconds. Decant the contents as in step 7 above
9. Repeat Step 8 three more times
10. Add 100 µl TMB substrate into each well except wells marked B1 and B2 and incubate the contents for 5 min at room temperature (keep the wells covered with sealing film)
11. Add 100 µl of stop solution to all the wells and swirl the contents thoroughly but gently for 30 seconds
12. Read the absorbance of the contents of the wells in a microplate reader at 450 nm and take a print out of the absorbance value
13. From the average values of standards (minus the average values of blank) make a plot against concentration of standards
14. The average absorbance values of samples can be read from the above plot or calculated using following formula:

$$\frac{\text{Absorbance of the Sample (B)} \times 100}{\text{Absorbance of standard (B}_0\text{)}}$$



Sensitivity and range of the kit

The lowest detection limits – 0.15
ng / ml or 0.15 ppb

The highest detection limits – can detect
up to 500 ppb (Please make appropriate
dilution of the extracted sample)

A	Conj. Blank	Conj. Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	Subs. Blank	Subs. Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	Air Blank	Air Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	Std 1	Std 1	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	Std 2	Std 2	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	Std 3	Std 3	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	Std 4	Std 4	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	Std 5	Std 5	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample



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