Aflatoxin B1 ELISA Kit

Catalog No.: abx365121

Size: 96T

Storage: Store at 4°C for up to 6 months.

Application: For quantitative detection of Aflatoxin B1 in Grain, Feed, Peanut, Seasoning, Spice powder, Edible oil, Muscle (beef, pork), Ham sausage.

Sensitivity: 0.03 ppb (ng/ml)

Sample recovery rate: 90 ± 30%

Detection Limit: Grain, Feed, Peanut, Seasoning, Spice powder – 3 ppb; Edible oil – 1 ppb; Muscle (beef, pork), Ham sausage – 0.5 ppb; Milk – 0.1 ppb.

Cross-reactivity: Aflatoxin B1 (AFB1) – 100%; AFB2 – 39%; AFG1 – 100%; AFG2 – 13.2%; AFM1 – 6.6%; AFM2 – < 1.0%.

Introduction: Aflatoxins are toxic and carcinogenic derivatives of hexanoic acid, broadly similar in structure to cholesterol, produced by various members of the Aspergillus family of fungi on cereals and nuts. These chemicals pass through cell membranes, where they can be metabolized to epoxide derivatives by P450 enzymes. These then react with guanine bases in DNA, which leads them to their removal by the base excision and nucleotide excision repair pathways and the potential replacement with an adenine-thymine base pair. The liver is particularly susceptible due to the high expression level of various P450 enzymes. Exposure to aflatoxins in food (all types) or via the skin (B1 only) can lead to fever, weight loss, liver damage, liver cancer, genotoxicity, immunosuppression, and teratogenicity. Treatment for aflatoxin poisoning consists of treating the symptoms, including liver transplants, antibiotics, and chemotherapy.

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Aflatoxin B1 is pre-coated onto a 96-well plate. The standards and samples and a biotin-conjugated antibody specific to aflatoxin are added to the wells and incubated. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well. After TMB substrate solution is added only wells that contain aflatoxin B1 will produce a blue colour product that changes into yellow after adding the stop solution. The intensity of the yellow colour is inversely proportional to the amount of aflatoxin bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of aflatoxin B1 can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard vials – 0 ppb, 0.03 ppb, 0.09 ppb, 0.27 ppb, 0.81 ppb, 2.43 ppb: 1 ml
3. Detection Reagent A: 7 ml
4. Detection Reagent B: 6 ml
5. Substrate Reagent A: 6 ml
6. Substrate Reagent B: 6 ml
7. Stop Solution: 6 ml
8. Sample Diluent (10X): 40 ml
9. Wash Buffer (20X): 25 ml
10. Plate Sealers: 3

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. High-precision pipette and sterile pipette tips
3. Automated plate washer (optional)
4. ELISA shaker (optional)
5. Centrifuge and microfuge tubes
6. Absorbent filter papers
7. Nitrogen evaporator or water bath
8. Homogenizer
9. Vortex mixer
10. Balance

Reagents Required But Not Provided

1. Deionized water
2. Methanol (pure)
3. Ice
4. Dichloromethane
5. n-Hexane (unbranched)
6. HCl
7. $ZnSO4 \cdot 7H2O$
8. Ethyl acetate
9. Acetonitrile

Protocol

A. Preparation of sample and reagents

1. Preparation of reagents

- **1 M HCl**
  Dilute 8.6 ml of **HCl** to 100 ml with **deionized water**. Mix fully.

- **Sample Solution**
  Add 200 ml of **Methanol** and 1 ml of **1 M HCl**, mix fully.

- **1 M ZnSO4 Solution**
  Dissolve 28.8 g of **ZnSO4 · 7H2O** in 100 ml of **deionized water**.

- **EA-DCM Solution**
  Add 400 ml of **Ethyl acetate** to 100 ml of **Dichloromethane**. Mix fully.

- **Sample Diluent A**
  Dilute the **Sample Diluent (10X)** 10-fold with **deionized water** to make **1X Sample Diluent** (e.g. dilute 20 ml **Sample Diluent (10X)** in 180 ml **deionized water**).

- **Sample Diluent B**
  Add 30 ml of **Methanol** to 70 ml of **Sample Diluent A**. Mix fully.

- **Sample Diluent C**
  Dilute the **Sample Diluent (10X)** 5-fold with **deionized water** to make **2X Sample Diluent** (e.g. dilute 20 ml **Sample Diluent (10X)** in 90 ml **deionized water**).

- **Sample Diluent D**
  Dilute the **Wash Buffer (20X)** 40-fold with **deionized water** to make **0.5X Sample Diluent** (e.g. dilute 10 ml **Wash Buffer (20X)** in 390 ml **deionized water**).

- **1X Wash Buffer**
  Dilute the **Wash Buffer (20X)** 20-fold with **deionized water** to make **1X Wash Buffer** (i.e. dilute 10 ml **Wash Buffer (20X)** in 90 ml **deionized water**).

2. Sample pretreatment

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Feed:** Weigh 1 g of homogenized sample and add to a 50 ml centrifuge tube. Add 5 ml of **Sample Solution**. Vortex for 1 minute, then centrifuge at 4000 RPM for 5 minutes. Take 100 µl of the supernatant and add to a centrifuge tube. Add 0.4 ml of **Sample Diluent C** and mix thoroughly. Aliquot 50 µl of the mixture for analysis.
  Note: Sample dilution factor: 30. Minimum detection dose: 3 ppb.

- **Edible oil:** Aliquot 0.1 ml of sample into a 4 ml centrifuge tube. Add 1ml of **n-Hexane** and 1 ml of **Sample Diluent B**. Vortex for 5 min, then centrifuge at 4000 RPM for 5 minutes. Discard the upper and middle liquid layers. Aliquot 50 µl of the lower liquid layer for analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 1 ppb.

- **Grain:** Weigh 1 g of crushed homogenate and add to a 50 ml centrifuge tube. Add 5 ml of **Methanol**. Vortex for 5 minutes, centrifuge at 4000 RPM for 5 minutes. Take 100 µl of the supernatant and add to a separate centrifuge tube. Add 300 µl of **Sample Diluent D**. Vortex for 2 minutes. Aliquot 50 µl of the liquid for analysis.
  Note: Sample dilution factor: 24, minimum detection dose: 3 ppb.

- **Seasoning (soy sauce, chili powder, cumin powder), peanut:** Weigh 1 g of crushed homogenate into a 50 ml centrifuge tube. Add 5 ml of **Methanol**. Vortex for 1 minute, then centrifuge at 4000 RPM for 5 minutes. Take 100 µl of the supernatant and transfer to another centrifuge tube. Add 0.4 ml of an appropriate diluent (**Chili powder:** 0.4 ml of **Sample Diluent A**; **Soy sauce, peanut:** 0.4 ml of **deionized water**; **Cumin powder:** 0.4 ml of **Sample Diluent C**), then vortex for 5 minutes. Aliquot 50 µl of the mixture for analysis.
  Note: Sample dilution factor: 30, minimum detection dose: 3 ppb.

- **Spice powder (Illicium verum, ground pepper):** Weigh 1 g of crushed homogenate and add to a 50 ml centrifuge tube. Add 5 ml of **Acetonitrile**. Vortex for 5 minutes, centrifuge at 4000 RPM for 5 minutes. Take 100 µl of the supernatant and aliquot to a separate centrifuge tube. Add 1 ml of **n-Hexane** and 0.4 ml of **Sample Diluent C**. Vortex for 5 minutes, then centrifuge at 4000 RPM for 5 minutes. Discard the upper and middle liquid layers. Aliquot 50 µl of the lower liquid layer for analysis.
  Note: Sample dilution factor: 30. Minimum detection dose: 3 ppb.

- **Milk:** Aliquot 2 ml of milk into a 50 ml centrifuge tube and add 0.4 ml of **1 M ZnSO4 Solution** and 6 ml of **EA-DCM Solution**. Vortex for 5 minutes, centrifuge at 4000 RPM for 5 minutes. Aliquot 1.5 ml of supernatant into a separate centrifuge tube, dry in a fume hood with nitrogen evaporators or water bath at 50°C. Dissolve the residue with 2 ml of **n-Hexane** and add 1 ml of **Sample Diluent B.** Vortex until fully mixed, then centrifuge at 4000 RPM for 10 minutes. Discard the upper and middle liquid layers. Aliquot 50 µl of the lower liquid layer for analysis.
  Note: Sample dilution factor: 2, minimum detection dose: 0.1 ppb.

- **Muscle (beef, pork), Ham sausage:** Weigh 2 g of crushed homogenate and add to a 50 ml centrifuge tube. Add 8 ml of **Ethyl acetate**. Vortex for 20 minutes, then centrifuge at 4000 RPM for 10 minutes. Aliquot 2 ml of supernatant into a separate centrifuge tube, dry in a fume hood with nitrogen evaporators or water bath at 50°C. Dissolve the residue in 2 ml of **n-Hexane**, vortex for 10 seconds, add 1 ml of **Sample Diluent A**. Centrifuge at 4000 RPM for 5 minutes, discard the upper and middle liquid layers. Allow to stand for 15 minutes. Aliquot 50 µl of lower liquid layer for analysis.
  Note: Sample dilution factor: 2, minimum detection dose: 0.5 ppb.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Samples that contain sodium azide (NaN_3) cannot be detected as it interferes with HRP.

B. Assay Procedure

Bring all reagents to room temperature prior to use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Any unused wells should be stored in the original foil bag with the provided desiccant at 2-8°C.

1. Set standard and sample wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.

2. Add 50 µl of the standard solutions to the standard wells. Add each solution at the bottom of each well without touching the side wall.

3. Add 50 µl of prepared sample to the sample wells.

4. Immediately add 50 µl of **Detection Reagent B** into each well, and then add 50 µl of **Detection Reagent A**. Add the solutions at the bottom of each well without touching the side wall.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 30 minutes in dark conditions.
6. Remove the cover, and discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 300 µl of **Wash Buffer** to each well and soak for at least 30 seconds. Discard the contents and blot the plate on absorbent material. Repeat the wash procedure for a total of five times.

Note: For automated washing, discard the solution in all wells and wash five times overfilling the wells with **Wash Buffer**. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

7. Add 50 µl of **Substrate Solution A** into each well and then 50 µl of **Substrate Solution B**. Cover the plate and incubate at 25°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
8. Add 50 µl of **Stop Solution** into each well. There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
9. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.
10. OPTIONAL: measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis.
11. OPTIONAL: Calculate the absorbance percentage by dividing each OD value by the OD value of the 0 ppb standard, and multiplying by 100 to give a percentage.

This assay is competitive, therefore there is an inverse correlation between aflatoxin concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (x-axis) and absorbance measured (y-axis). (Alternatively, plot the absorbance percentage from step 13 against the standard concentration.) Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.

10. Do not use components from a different kit or expired ones.

11. **Substrate Solution A** and **Substrate Solution B** are easily contaminated; work under sterile conditions when handling these solutions. The substrate solutions should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not replace the residual solution back into the vial.