Aflatoxin B1 ELISA Kit

Catalog No.: abx364883

Size: 96T

Storage: Store at 4°C.

Application: For quantitative detection of Aflatoxin B1 in Beer, Corn skin, Wheat bran, Edible oil, Biscuits, Cereal, Soy sauce, Wine, Vinegar.

Sensitivity: 0.03 ng/ml (ppb)

Detection Limit: Beer – 0.3 ng/ml; Corn skin, Wheat bran, Edible oil, Biscuits – 0.6 ng/ml; Cereal, Soy sauce, Wine, Vinegar – 0.15 ng/ml.

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 (0 ng/ml, 0.03 ng/ml, 0.06 ng/ml, 0.12 ng/ml, 0.24 ng/ml, 0.48 ng/ml): 1 ml each
3. Detection reagent A: 5.5 ml
4. Detection reagent B: 5.5 ml
5. Substrate reagent A: 6 ml
6. Substrate reagent B: 6 ml
7. Stop solution: 6 ml
8. Wash buffer (20X): 40 ml
9. Plate sealers: 3
10. Hermetic bag: 1

Materials Required But Not Provided

1. 37°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. Centrifuge and microfuge tubes
7. Absorbent filter papers
8. Nitrogen evaporator or water bath
9. Homogenizer
10. Vortex mixer

Reagents Required But Not Provided

1. Deionized water
2. Methanol (pure)
3. Dichloromethane or trichloromethane
4. n-Hexane (unbranched)

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Protocol

A. Preparation of sample and reagents

1. Preparation of reagents

- **Wash buffer:** Dilute the 20X wash buffer 20-fold with deionized water (i.e. dilute 40 ml of 20X wash buffer in 760 ml deionized water) to make the 1X wash buffer solution.
- **70% methanol solution:** Dilute pure methanol in a 7:3 ratio in deionized water (e.g. 70 ml methanol to 30 ml water).
- **35% methanol solution:** Dilute 70% methanol solution 2-fold in deionized water (e.g. add 50 ml 70% methanol to 50 ml 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.

- **Cereals:** Weigh 2 g of homogenized sample and add to a 50 ml centrifuge tube. Add 5 ml of 70% methanol. Vortex for 5 minutes, centrifuge at 4000 rpm for 10 minutes. Take 0.5 ml of the supernatant and add to a centrifuge tube. Add 0.5 ml of deionized water and mix thoroughly. Aliquot 50 µl of the mixture for analysis.
  Note: Sample dilution factor: 5. Detection limit: 0.15 ng/ml.

- **Corn skin and wheat bran:** Weigh 2 g of homogenized sample and add to a 50 ml centrifuge tube. Add 20 ml of 70% methanol. Vortex for 5 min, centrifuge at 4000 rpm for 10 minutes. Add 0.5 ml of the mixture to 0.5 ml of deionized water, and mix thoroughly. Aliquot 50 µl of the liquid for analysis.
  Note: Sample dilution factor: 20. Detection limit: 0.6 ng/ml. If the sample has a high concentration of aflatoxin, it can be diluted further by adding 0.1 ml of the analyte to 0.9 ml of 35% methanol solution. The sample dilution factor would be 200 and the minimum detection dose would be 6 ng/ml.

- **Edible oil:** Measure 2 g of homogenized sample or 2 ml of liquid sample and add to a 50 ml centrifuge tube. Add 8 ml of n-Hexane and 10 ml of 70% methanol. Vortex for 5 minutes, centrifuge at 4000 rpm for 10 minutes. Discard the upper liquid layer. Take 0.5 ml of the lower liquid layer and add to 0.5 ml of deionized water. Add 0.5 ml of this mixture to 0.5 ml of 35% methanol. Vortex for 30 seconds. Aliquot 50 µl of the liquid for analysis.
  Note: Sample dilution factor: 20. Detection limit: 0.6 ng/ml.

- **Biscuits:** Weigh 1 g of homogenized sample and add to a 15 ml centrifuge tube. Add 10 ml of 70% methanol. Vortex for 5 minutes, centrifuge at 4000 rpm for 10 minutes. Take 2 ml of the supernatant to another 15 ml centrifuge tube, and dry at 50-60°C in a nitrogen or water bath. Dissolve the residue with 2 ml of deionized water and vortex for 30 seconds. Add 6 ml of trichloromethane and vortex for 5 minutes. Centrifuge at 4000 rpm for 5 minutes at room temperature. Discard any remaining upper liquid layer, and 1.5 ml of the lower liquid layer and transfer to another tube. Dry at 50-60°C in a nitrogen or water bath. Dissolve the residue with 0.5 ml of N-hexane and vortex for 30 seconds. Add 1 ml of 35% Methanol and vortex for 1 minute. Centrifuge at 4000 rpm for 5 minutes at room temperature. Discard any remaining upper liquid layer and aliquot 50 µl of the lower liquid layer for analysis.
  Note: Sample dilution factor: 20. Detection limit: 0.6 ng/ml.

- **Beer:** Stir beer vigorously to remove CO_2. Take 2 ml of beer and add 1 ml of deionized water and 7 ml of pure methanol. Vortex for 5 minutes, aliquot 0.5 ml of the mixture and add to 0.5 ml of deionized water. Mix thoroughly, and aliquot 50 µl of the mixture for analysis.
  Note: Sample dilution factor: 10. Detection limit: 0.3 ng/ml.

- **Soy sauce, wine, vinegar:** Measure 2 ml of sample and add 2 ml of deionized water. Add 10 ml of trichloromethane. Vortex for 5 minutes, centrifuge at 4000 rpm for 10 minutes. Take 1 ml of the lower liquid layer and dry at 50-60°C in a nitrogen or water bath. Add 0.5 ml of 70% methanol, resuspend the solid, and add 0.5 ml of deionized water to dissolve thoroughly. Aliquot 50 µl of the mixture for analysis.
  Note: Sample dilution factor: 5. Detection limit: 0.15 ng/ml.

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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.
- Bring samples 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.

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. Add 50 µl of Detection Reagent B and then add 50 µl of Detection Reagent A to all wells.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 25°C for 30 minutes in the dark.
6. Remove the cover, and discard the solution without touching the side walls. Blot the plate on an absorbent material. Add 300 µl of 1X 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 reagent A into each well and then 50 µl of Substrate reagent B. Gently mix for 5 seconds. Cover the plate and incubate at 25°C in the dark for 10-20 minutes. *Optimal reaction time should be determined by the end user.*
8. Add 50 µl of stop solution into each well. 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 within 10 minutes of adding the stop solution.

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C. Calculations

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 Concentration (x-axis) and Absorbance (%) measured (y-axis). 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.

$$Absorbance\ (\%) = \frac{A}{A_0} \times 100\%$$

where:

A	Average absorbance of standard or sample
A_0	Average absorbance of 0 ng/ml of standard

D. Precautions

1. Equilibrate all reagents to room temperature (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, 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, and samples in duplicate.
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. Do not use components from a different kit or expired ones.
10. Substrate reagent A and Substrate reagent 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 unused solution back into the vial.

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