Instructions for Use

Version: 1.0.1
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Total Aflatoxin (AF) ELISA Kit

Catalog No.: abx365147

Size: 96 tests

Storage: Store at 2- 4°C for up to 12 months.

Application: Aflatoxin in samples, such as cereals, formula feed and edible oil.

Detection limit: Serum---0.2 ppb; Liver, Muscle---0.4 ppb

Sample Recovery Rate: Serum, Liver and Muscle -- 85 ± 25%

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology quantifying the total Aflatoxin (AF) in various samples, including cereals, formula feed, and edible oil. It includes an ELISA microtiter plate, HRP conjugate, antibody working solution, standards, and other supplementary reagents. The microtiter plate is pre-coated with a coupled antigen. During the assay, AF in the samples or standards competes with the coupled antigen on the solid-phase support for binding sites on the anti-AF antibody. Horseradish Peroxidase (HRP) conjugate is then added to each well, followed by a substrate reagent for color development. The optical density (OD) of the samples is inversely proportional to the concentration of AF, which is determined by comparing the sample OD values to a standard curve.

Kit Components

1. 96-well microplate
2. Standards (1 ml each): 0 ppb, 0.02 ppb, 0.04 ppb, 0.08 ppb, 0.16 ppb, 0.32 pp
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 sealer: 3
10. Hermetic bag: 1

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. High-precision pipette and sterile pipette tips
3. ELISA shaker
4. 50 ml centrifuge tubes
5. Absorbent filter papers
6. Centrifuge
7. Vortex mixer
8. 100 ml and 1 L graduated cylinders
9. Nitrogen evaporator
10. Water bath
11. Mechanical homogenizer
12. Deionized water
13. Balance

Reagents Required But Not Provided

1. Methanol
2. N-hexane (C_6H_{14})
3. Trichloromethane

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Protocol

Bring all reagents and samples to room temperature before use.

A. Preparation of Samples and Reagents

1. Reagents

Prepare a sufficient quantity of the following solutions for the number of wells being assayed.

- **Solution 1 – 70% Methanol**

  Mix 100% methanol with double distilled water in a ratio of 7:3 (for example, add 7 ml of methanol to 3 ml of double distilled water).

- **Solution 2 – 35% Methanol**

  Mix 70% methanol (solution 1) with double distilled water in a ratio of 1:1 (for example, add 3 ml of methanol to 3 ml of double distilled water).

- **Solution 3 – Wash Buffer**

  Dilute the 20X Concentrated Wash Buffer with double distilled water in a ratio of 1:19 (for example, add 1 ml of 20X Concentrated Wash Buffer to 19 ml of double distilled water).

2. Sample Pretreatment

- **Serum:** Collected serum samples must be clear; avoid using hemolysed samples. Dilute the serum sample by a factor of 10. Take 0.5 ml of sample and add 0.5 ml of 70% Methanol (Solution 1) and centrifuge at 4000 rpm for 5 minutes at room temperature. Take 200 µl of the supernatant and add 800 µl of 35% Methanol (Solution 2). Mix thoroughly. Take 50 µl of the solution for analysis.

- **Liver and Muscle samples:** Homogenize the sample using a mechanical homogenizer. Weigh 1 g of the homogenate sample into a clean sterile 50 ml centrifuge tube and add 10 ml of 100% methanol. Vortex for 5 minutes then centrifuge at 4000 rpm for 5 minutes at room temperature. Transfer 2 ml of supernatant to another centrifuge tube and dry with a nitrogen evaporator or a water bath at 50-60°C. Add 2 ml of deionized water to the dried sample to dissolve thoroughly, then add 6 ml of trichloromethane. Vortex for 5 minutes, then centrifuge at 4000 rpm for 5 minutes at room temperature. Remove the upper layer of the solution, and transfer 3 ml of the lower fraction to another centrifuge tube and dry with a nitrogen evaporator or a water bath at 50-60°C. Add 1 ml of N-hexane to the dried materials to dissolve thoroughly, vortex for 5 minutes and add 2 ml of 35% Methanol (Solution 2) and vortex for 2 minutes. Centrifuge at 4000 rpm for 5 minutes at room temperature, then take 50 µl of the lower fraction of liquid for detection and analysis.

B. Assay Procedure

Bring all reagents to room temperature prior to use. All reagents should be mixed thoroughly by swirling before pipetting. Avoid foaming.

1. Number the sample and ordered standard wells, and record their positions. *All samples and standards should be tested in duplicate.*
2. Add 50 µl of standard into the standard wells.
3. Add 50 µl of sample into the sample wells.
4. Add 50 µl of Detection Reagent A to each well,
5. Add 50 µl of Detection Reagent B to each well.
6. Cover the plate with a plate sealer, tap the plate gently to mix, and incubate at 25°C for 30 minutes in the dark.
7. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting.
8. Invert the plate and blot it against clean absorbent paper towels. Add 50 µl of Substrate Reagent A to each well.
9. Add 50 µl of Substrate Reagent B to each well. Shake gently for 5 seconds to ensure thorough mixing, and incubate at 25°C for 15 minutes in the dark.
10. Add 50 µl of Stop Solution to each well, and tap the plate gently to ensure thorough mixing.
11. Within 10 minutes of introducing the Stop Solution, determine the optical density of each well with a microplate reader at 450 nm.

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C. Analysis of Results

1. Calculating Sample Concentration from Standard Curve

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 on the y-axis, and absorbance measured on the x-axis (a semi-logarithmic plot). 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.

D. Precautions

Bring all reagents to room temperature prior to use.

1. 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.
2. Do not allow the wells to dry fully during the washing procedure. Ensure the Wash Buffer is thoroughly mixed, and the wells are washed completely.
3. Do not use the Substrate Reagent if it has begun to turn blue without being added to the wells.
4. Do not use any reagents that are expired, or use reagents from other kits with this assay.
5. The Stop Solution is caustic. Avoid contact with the skin or eyes, and thoroughly wash any spilled solution with water.
6. It is recommended that the same operator perform the experiment throughout, to avoid inconsistencies between operator procedures.

D. Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit: https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.

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