Aflatoxin B1 (AFB1) ELISA Kit

Catalog No.: abx365146

Size: 96T

Storage: Store at 4°C.

Application: For quantitative detection of Aflatoxin B1 in serum and animal tissue (liver, muscle).

Detection Limit: Serum – 0.3 ng/ml (ppb); Liver, Muscle – 0.6 ng/ml (ppb).

Sample Recovery Rate: Serum, Liver Muscle – 85 ± 15%

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, samples and a biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the pre-coated AFB1 and the AFB1 in the sample with the biotin-labelled antibody. The HRP-conjugated reagent is added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient AFB1 will produce a blue colored product that changes into yellow after adding the acidic stop solution. The intensity of the yellow color is inversely proportional to the amount of AFB1 bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of AFB1 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 (100%)
3. Trichloromethane
4. n-Hexane

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 (100%) 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.

- **Serum:** Collect using convention methods (Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Avoid hemolytic samples. Samples should be clear with no visible particles, turbidity, or bacterial pollution. After collection, serum samples can be stored between 2-8°C for up to 1 week or at -20°C for long-term storage). Take 0.5 ml of serum sample and add 5 ml of 70% methanol. Centrifuge at 4000 rpm for 5 minutes at room temperature. Take 0.2 ml of the supernatant and add to a centrifuge tube. Add 0.8 ml of 35% methanol and mix thoroughly. Aliquot 50 µl of the mixture for analysis.
  Note: Sample dilution factor: 10. Detection limit: 0.3 ng/ml.

- **Liver (pig) and muscle (pig, cow goat, sheep):** Weigh 1 g of homogenized sample and add to a 50 ml centrifuge tube. Add 10 ml of pure (100%) methanol. Vortex for 5 minutes, then centrifuge at 4000 rpm for 5 minutes at room temperature. Take 2 ml of homogenate supernatant and dry at 50-60°C in a nitrogen evaporator or water bath. Dissolve the residue with 2 ml of deionized water and add 6 ml of trichloromethane. Vortex for 5 minutes, then centrifuge at 4000 rpm for 5 minutes at room temperature. Discard the upper liquid layer and aliquot 3 ml of the lower liquid layer to a new centrifuge tube. Dry at 50-60°C in a nitrogen evaporator or water bath. Dissolve the residue with 1 ml of n-hexane and add 2 ml of 35% methanol. Vortex for 2 minutes, then 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.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Steps requiring a water bath or nitrogen evaporator should be carried out in a ventilated environment.
- 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 corresponding 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. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 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. Invert the plate and blot it against clean absorbent paper towels.

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. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
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.

C. Calculations

This assay is competitive, therefore there is an inverse correlation between aflatoxin concentration in the sample and the absorbance measured. It is recommended to create a semi-logarithmic graph with the Concentration (x-axis) and Absorbance (%) measured (y-axis). Draw a best fit trendline through the standard points. Use this graph to 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.

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

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.

E. 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.