Ochratoxin A (OTA) ELISA Kit

Catalog No.: abx364876

Size: 96T

Storage: Store at 4°C.

Application: For quantitative detection of OTA in Cereals and Feed.

Sensitivity: 1 ppb (ng/ml)

Detection Limit: Grain – 5 ppb, Feed – 10 ppb

Cross-reactivity: Ochratoxin A – 100%

Sample recovery rate: 85% ± 15%

Introduction: Ochratoxin A (OTA) is a mycotoxin produced by various *Aspergillus* and *Penicillium* species and can be found in contaminated food products. Exposure to OTA has been linked to several diseases, particularly renal diseases such as Balkan endemic nephropathy (BEN) and chronic interstitial nephropathy (CIN).

Principle of the Assay

This kit is based on indirect competitive enzyme-linked immuno-sorbent assay technology. OTA is pre-coated onto a 96-well plate. The standards and samples and a biotin conjugated antibody specific to OTA 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 OTA will produce a blue color product that changes into yellow after adding the stop solution. The intensity of the yellow color is inversely proportional to the OTA amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of OTA can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard: 1 ml each of:
   0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb
3. Detection Reagent A: 5.5 ml
4. Detection Reagent B: 11 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

Materials Required But Not Provided

1. 37°C incubator
2. Microplate reader (450 nm)
3. High-precision pipette and sterile pipette tips
4. Nitrogen evaporator or water bath
5. ELISA shaker
6. 1.5 ml EP tubes to prepare samples
7. Absorbent filter papers
8. 100 ml and 1 L graduated cylinders
9. Scale (precision 0.01 g)

Reagents Required But Not Provided

1. Methanol
2. $NaHCO_3$
3. Deionized water

Protocol

A. Preparation of sample and reagents

1. Preparation of Sample pretreatment solutions

- **Solution 1 – 70% methanol solution**
  Add 30 ml of methanol to 70 ml of deionized water. Mix thoroughly.

- **Solution 2 – 0.1 M $NaHCO_3$ solution**
  Dissolve 4.2 g of $NaHCO_3$ in 500 ml of deionized water. Mix thoroughly.

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.

- **Grain:** Weigh 4 g of crushed homogenate and add to a 50 ml tube. Add 10 ml of 70% methanol and mix thoroughly for 5 min. Centrifuge at 4000 RPM for 10 min at room temperature. Take 1 ml of the supernatant and add to a new tube. Add 1 ml of 0.1 M $NaHCO_3$ solution and mix thoroughly. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 5, minimum detection dose: 5 ppb

- **Feed:** Weigh 2 g of crushed homogenate and add to a 50 ml tube. Add 10 ml of 70% methanol solution and mix thoroughly for 5 min. Centrifuge at 4000 RPM for 10 min at room temperature. Take 1 ml of the supernatant and add to a new tube. Add 1 ml of 0.1 M $NaHCO_3$ solution and mix thoroughly. Take 50 µl for detection and analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 10 ppb

3. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 40 ml of concentrated wash buffer into 760 ml of distilled water).

B. Assay Procedure

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

1. Set standard and sample 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 prepared standards solutions into the standard wells.
3. Add 50 µl of appropriately diluted sample into test sample wells.
4. Immediately add 50 µl of Detection Reagent A into each well. Add the solution 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 in the dark at 37°C for 30 minutes.
6. Remove the cover, and wash the plate 5 times. 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 1 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this 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 min.

7. Add 100 µl of Detection Reagent B to each well. Tap the plate gently to ensure thorough mixing. Cover the plate and incubate at 37°C for 30 min.
8. Repeat the wash procedure in step 6.
9. Add 50 µl of Substrate reagent A to each well, and then 50 µl of Substrate reagent B into each well. Cover the plate and incubate at 37°C in dark conditions for 15 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.
10. Add 50 µl of Stop solution into each well (including the blank well). There should be a colour change to yellow. Gently tap the plate to ensure thorough mixing.
11. 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.

This assay is competitive, therefore there is an inverse correlation between OTA concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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 dump the residual solution back into the vial.